SCREENING AND EVALUATION OF FUNGAL ENDOPHYTES MODULATING PLANT GROWTH UNDER HEAT STRESS

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PALB 6030

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY

UNIVERSITY OF AGRICULTURAL SCIENCES

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Thesis submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfillment of the requirements for the
Degree of

DOCTOR OF PHILOSOPHY
IN
AGRICULTURAL MICROBIOLOGY

BENGALURU **AUGUST, 2022**

Affectionately Dedicated

To

My Beloved Parents,

Smt. Tilothame

Shri. Shivaramu, P. k

DEPARTMENT OF AGRICULTURAL MICROBIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

CERTIFICATE

This is to certify that the thesis entitled **"SCREENING AND EVALUATION OF FUNGAL ENDOPHYTES MODULATING PLANT GROWTH UNDER HEAT STRESS"** submitted in partial fulfilment of the requirements for the degree of **DOCTOR OF PHILOSOPHY** in Agricultural Microbiology to the University of Agricultural Sciences Bangalore is a record of *bonafide* research work carried out by **Ms. ARPITHA P. S., PALB 6030** during the period of her study in this University under my guidance and supervision. This Thesis has not previously formed the basis for award of any degree, diploma, associateship, fellowship or other similar titles.

Place: Bengaluru
Month & Year: August, 2022

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"Gratitude is the memory of the heart"

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SCREENING AND EVALUATION OF FUNGAL ENDOPHYTES MODULATING PLANT GROWTH UNDER HEAT STRESS

ARPITHA, P. S.

ABSTRACT

Global temperature increases day by day due to climate change. The raised temperature significantly affects physiological processes of rice that results in reduced productivity. Endophytes are one of the biological strategies used to sustain the productivity. They are beneficial asymptomatic microbial partners and mediate imparting abiotic stress tolerance through habitat adapted symbiosis. In this study, thirty fungal endophytes of Thar Desert and Himalayan cold desert plants were screened against temperatures stress. Among 30 fungal endophytes, five isolates (ACT 2, LAS 4, LAS 6, PRC 2 and SAP 3) of Thar desert and three fungi (A2, A7 and X5) of Himalayan cold desert sustained the growth from 40 to 44 ^{o}C. These eight endophytes were identified as *Aspergillus flavus* (ACT 2), *Aspergillus nidulans* (SAP 3), *Aspergillus terrus* (PRC 2), *Chaetomium* sp. (LAS 4 and LAS 6), *Ceriporia lacerate* (A7), *Endomelanoconiopsis endophytica* (X5) and *Penicillium feniculosum* (A2) by ITS region sequences. The identified endophytes were evaluated for their ability to impart thermotolerance in rice by exposing to heat stress (45 ^{o}C for 7h/ day) for 10 days. The endophytes inoculated plants increased growth and biomass compared to un-inoculated plants. Based on stress tolerance index of growth attributes, the four endophytes (*A. flavus*, *A. nidulans*, *E. endophytica* and *P. funiculosum*) inoculated plants were analysed for physiological attributes. These plants showed increased phytohormones, antioxidants and osmolytes production compared to un-inoculated plants. They also improved membrane stability and photosynthetic efficiency of plants indicating that these endophytes can impart thermotolerance in rice.

August, 2022

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ಶಾಖದ ಒತ್ತಡದಲ್ಲಿ ಸಸ್ಯದ ಬೆಳವಣಿಗೆಯನ್ನು ಮಾರ್ಪಡಿಸುವ ಅಂತರ್ಜೀವಿ ಶಿಲೀಂಧ್ರಗಳ ತಪಾಸಣೆ ಮತ್ತು ಮೌಲ್ಯಮಾಪನ

ಅರ್ಪಿತ, ಪಿ. ಎಸ್.

ಪ್ರಬಂಧ ಸಾರಾಂಶ

ಹವಮಾನ ಬದಲಾವಣೆಯಿಂದ ಜಾಗತಿಕ ತಾಪಮಾನವು ದಿನದಿಂದ ದಿನಕ್ಕೆ ಹೆಚ್ಚುತ್ತಿದೆ. ತಾಪಮಾನದಲ್ಲಿನ ಈ ಏರಿಕೆಯು ಭತ್ತದ ಶಾರೀರಿಕ ಪ್ರಕ್ರಿಯೆಗಳ ಮೇಲೆ ಗಮನಾರ್ಹವಗಿ ಪರಿಣಾಮ ಬೀರಿ, ಆಹಾರ ಉತ್ಪಾದನೆಯ ಇಳಿಕೆಗೆ ಕಾರಣವಾಗುತ್ತದೆ. ಅಂತರ್ಜೀವಿಗಳು ಸಸ್ಯಗಳ ಲಕ್ಷಣರಹಿತ ಪ್ರಯೋಜನಕಾರಿ ಸೂಕ್ಷ್ಮಜೀವಿ ಪಾಲುದಾರರಾಗಿದ್ದು, ಆವಾಸಸ್ಥಾನ ಅಳವಡಿಕೆ ಸಹಜೀವನದ ಮೂಲಕ ಅಜೀವಕ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆಯಲ್ಲಿ ಮಧ್ಯಸ್ಥಿಕೆವಹಿಸುತ್ತವೆ. ಭತ್ತದಲ್ಲಿ ಶಾಖದ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆಗಾಗಿ ಅಂತರ್ಜೀವಿ ಶಿಲೀಂಧ್ರಗಳನ್ನು ಪರೀಕ್ಷಿಸುವುದು ಮತ್ತು ಮೌಲ್ಯಮಾಪನ ಮಾಡುವುದು ಈ ಅಧ್ಯಯನದ ಉದ್ದೇಶವಾಗಿದೆ. ಥಾರ್ ಮರುಭೂಮಿ ಮತ್ತು ಹಿಮಾಲಯ ಶೀತ ಮರುಭೂಮಿ ಸಸ್ಯಗಳ ಮೂವತ್ತು ಶಿಲೀಂಧ್ರ ಪ್ರತ್ಯೇಕತೆಗಳನ್ನು ಅಧಿಕ ತಾಪಮಾನಗಳಿಗೆ ಒಡ್ಡಿ ಪರೀಕ್ಷಿಸಲಾಯಿತು. ಮೂವತ್ತು ಪ್ರತ್ಯೇಕತೆಗಳಲ್ಲಿ, ಥಾರ್ ಮರುಭೂಮಿಯ ಐದು ಪ್ರತ್ಯೇಕತೆಗಳು (ಎ.ಸಿ.ಟಿ.೨, ಎಲ್.ಎ.ಎಸ್.೪, ಎಲ್.ಎ.ಎಸ್.೬, ಪಿ.ಆರ್.ಸಿ.೨ ಮತ್ತು ಎಸ್.ಎ.ಪಿ.೩) ಮತ್ತು ಹಿಮಾಲಯ ಶೀತ ಮರುಭೂಮಿಯ ಮೂರು ಪ್ರತ್ಯೇಕತೆಗಳಾದ ಎ೨, ಎ೭ ಮತ್ತು ಎಕ್ಸ್-೫ಗಳ ಬೆಳವಣಿಗೆಯು ಹೆಚ್ಚಿನ ತಾಪಮಾನದಲ್ಲಿಯೂ (೪೦-೪೪ ಡಿಗ್ರಿ ಸೆಂಟಿಗ್ರೇಡ್) ದಾಖಲಾಗಿದೆ. ಈ ಎಂಟು ಅಂತರ್ಜೀವಿಗಳನ್ನು *ಆಸ್ಪರ್‌ಜಿಲ್ಲಸ್ ಫ್ಲೇವಸ್* (ಎ.ಸಿ.ಟಿ.೨), *ಆಸ್ಪರ್‌ಜಿಲ್ಲಸ್ ನಿಡುಲನ್ಸ್* (ಎಸ್.ಎ.ಪಿ.೩), *ಆಸ್ಪರ್‌ಜಿಲ್ಲಸ್ ಟೆರಸ್* (ಪಿ.ಆರ್.ಸಿ ೨), *ಕೆಟೋಮಿಯಮ್* ಪ್ರಭೇದ (ಎಲ್.ಎ.ಎಸ್.೪ ಮತ್ತು ಎಲ್.ಎ.ಎಸ್೬), *ಸೆರಿಪೊರಿಯ ಲ್ಯಾಸರೆಟಾ* (ಎ೭), *ಎಂಡೋಮೆಲನೊಕೊನಿಯೊಪಸ್ಸಿಸ್ ಎಂಡೋಫೈಟಿಕಾ* (ಎಕ್ಸ್೫) ಮತ್ತು *ಪೆನಿಸಿಲಿಯಂ ಫೆನಿಕೈಲೊಸಂ* (ಎ೨) ಎಂದು ಐ.ಟಿ.ಎಸ್. ಅನುಕ್ರಮಗಳ ಆಧಾರದ ಮೇಲೆ ಗುರುತಿಸಲಾಗಿದೆ. ಈ ಅಂತರ್ಜೀವಿಗಳನ್ನು ಭತ್ತದ ಸಸ್ಯಗಳಿಗೆ ಉಪಚರಿಸಿ ಶಾಖದ ಒತ್ತಡಕ್ಕೆ (೪೫ ಡಿಗ್ರಿ ಸೆಂಟಿಗ್ರೇಡ್ /೭ ಗಂಟೆ/ದಿನಕ್ಕೆ, ೧೦ ದಿನ) ಒಡ್ಡುವ ಮೂಲಕ, ಶಾಖ ಸಹಿಷ್ಣುತೆ ಸಾಮರ್ಥ್ಯವನ್ನು ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಯಿತು. ಅಂತರ್ಜೀವಿ ಉಪಚರಿಸಿದ ಸಸ್ಯಗಳಲ್ಲಿ ತೆಂಡೆಗಳು, ಎಲೆಗಳು, ಬೇರಿನ ಸಾಂದ್ರತೆ ಮತ್ತು ಸಸ್ಯದ ಒಟ್ಟಾರೆ ತೂಕವನ್ನು ಹೆಚ್ಚಿಸಿರುವುದು ಕಂಡುಬಂದಿದೆ. ಬೆಳವಣಿಗೆಯ ಗುಣಲಕ್ಷಣಗಳ ಒತ್ತಡ ಸಹಿಷ್ಣುತೆಯ ಸೂಚ್ಯಾಂಕವನ್ನು ಆಧರಿಸಿ, ಶಾಖ ಒತ್ತಡದ ವಿರುದ್ಧ ಅತ್ಯುತ್ತಮವಾಗಿ ಕಾರ್ಯನಿರ್ವಹಿಸಿದ ನಾಲ್ಕು ಅಂತರ್ಜೀವಿಗಳು (*ಆಸ್ಪರ್‌ಜಿಲ್ಲಸ್ ಫ್ಲೇವಸ್*, *ಆಸ್ಪರ್‌ಜಿಲ್ಲಸ್ ನಿಡುಲನ್ಸ್*, *ಎಂಡೋಮೆಲನೊಕೊನಿಯೊಪಸ್ಸಿಸ್ ಎಂಡೋಫೈಟಿಕಾ* ಮತ್ತು *ಪೆನಿಸಿಲಿಯಂ ಫೆನಿಕೈಲೊಸಂ*) ಭತ್ತದಲ್ಲಿನ ಶಾರೀರಿಕ ಗುಣಲಕ್ಷಣಗಳಾದ ಸಸ್ಯಹಾರ್ಮೋನ್, ಉತ್ಕರ್ಷಣ ನಿರೋಧಕ, ಆಸ್ಮೋಲೈಟ್ ಮತ್ತು ಭಾಷ್ಪವಿಸರ್ಜನೆಗಳನ್ನು ನಿಯಂತ್ರಿಸಿ ತನ್ಮೂಲಕ ಪೊರೆಯ ಸ್ಥಿರತೆ, ದ್ಯುತಿಸಂಶ್ಲೇಷಕ ದಕ್ಷತೆ ಮತ್ತು ವರ್ಣದ್ರವ್ಯಗಳನ್ನು ಸುಧಾರಿಸಿ, ಭತ್ತದ ಶಾರೀರಿಕ ಕ್ರಿಯೆಯನ್ನು ಮಾರ್ಪಡಿಸಿವೆ. ಈ ಸಂಶೋಧನೆಯಿಂದ ಅಂತರ್ಜೀವಿ ಶಿಲೀಂಧ್ರಗಳು ಭತ್ತದ ಬೆಳೆಯಲ್ಲಿ ಶಾಖ ಒತ್ತಡವನ್ನು ತಗ್ಗಿಸಬಲ್ಲವೆಂದು ತಿಳಿದುಬಂದಿದೆ.

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ಕೃಷಿ ಸೂಕ್ಷ್ಮಜೀವಿಶಾಸ್ತ್ರ ವಿಭಾಗ
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(ಎನ್. ಈರಣ್ಣ)
ಮುಖ್ಯ ಸಲಹೆಗಾರರು

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I INTRODUCTION

Global climatic changes have intensified the induction of abiotic stresses to crops, which ultimately reduce the growth and yield and consequently limit global agricultural production (Meena *et al*., 2017). Climatic prediction models describe that the increase in temperature due to climatic change is synergistically accompanied by drought stress due to demand for soil moisture by plants. In addition, rising in mean global temperature also results in saline conditions through moisture evaporation from the soil (Suzuki, 2016). Therefore, raised temperatures exert an adverse impact on plant growth and physiology by inducing multiple abiotic stresses.

Intergovernmental Panel on Climate Change (IPCC, 2014) has predicted that the mean atmospheric temperature will increase by 1-3.2 ℃ by the end of 21st century. Earlier studies revealed that, for every 1 ℃ increase in temperature, there is a 4–14 % reduction in grain yield (Lobell *et al*., 2011). The increased temperature adversely affects tropical and subtropical areas where rice is widely cultivated (Fischer and Knutti, 2015). Rice is an important cereal crop, feeding approximately four billion people worldwide and sensitive to high temperature. Therefore, rise in temperature beyond the threshold level causes irreversible damages on growth and development of crop which is called heat stress.

Moreover, heat stress could alter different physiological and molecular processes that affect growth and development process from germination to maturity (Bahuguna and Jagadish, 2015). Increase in reactive oxygen species (ROS) is one of the primary events under heat stress, which result in peroxidation of lipids affecting the membrane integrity. Heat stress affects the photosynthetic system by changing the photochemical reactions in thylakoid lamellae and carbon metabolism in the stroma of chloroplast (Wahid *et al*., 2007; Mathur *et al*., 2011). Heat stress exposure during post anthesis stage could affect reproductive success, seed set and grain filling in crop plants by altering hormonal regulation and carbon metabolism (Bahuguna and Jagadish, 2015; Shi *et al*., 2017).

To overcome such problems, the plants itself developed some mechanisms such as activation of antioxidant system, accumulation of osmolytes, homeostasis of phytohormones, production of heat shock protein, and activation of stress related

genes. Even though, absence or inadequacy of these mechanisms can be seen in sensitive plants which lead to adverse effect on plant growth and productivity.

In this view, climate resilient agriculture is only the solution to stabilized food production. Therefore, thermotolerant plants have been achieved through genetic engineering, breeding programs and tissue culture techniques which are time consuming, expensive and sometimes involve environmental concern. Therefore, one of the eco-friendly approaches is exploring plant-microbe symbiotic interaction to mitigate adverse effects of climate change on crop production.

All plants in natural ecosystems are thought to be symbiotic with endophytic microbes. An endophyte is a microorganism (fungi, bacteria, protozoa, virus or algae) that live part of its life or complete its life cycle inside living plant without causing negative symptoms (DuPorte, 1925; Correa and McLachlan, 1994; Wennstrom, 1994; Saikkonen *et al*., 2004). They are able to promote plant growth and impart resistance to environmental constraints in return for carbohydrates fixed by the plant during photosynthesis. Fossil evidence indicates that the relationship among plants and symbionts has been persistent throughout the evolutionary history of land plants. Moreover, close association of plants and microorganisms is known from both aquatic and terrestrial environments (Harman, 2011).

The intimate associations of endophytes play a pivotal role in imparting fitness and survival of plants thriving in stressful habitats. For example, *Curvularia protuberata* provide thermal protection for *Dichianthelium lanuginosum* plants growing in geothermal soils of Yellowstone National Parks (Redman *et al*., 2002). Without this endophyte, plant do not survive in the habitat, indicating that the stress adaptation involve symbiotic process. Existence of plant-fungal endophyte symbiosis follows three stages such as (1) invasion of fungi into the tissues of plants (2) colonization of plant tissue by invaded fungi and (3) expression of symbiotic lifestyle by plant-fungal interaction (Singh *et al*., 2011).

The fungal endophytes provide stress tolerance to their host plant either by activation of host plant stress response system when they are exposed to stress (Redman *et al*., 1999) or by production of anti-stress biochemical compounds by the fungi itself (Bacon and Hill, 1996). In addition, fungal endophytes can regulate plant growth, development, and reproduction (Rodriguez *et al*., 2009).

Many studies documented that the fungal endophytes enhance the osmolytes accumulation such as proline, glycine betaine, sugar alcohols, sorbital, mannitol, organic acids and amino acids in the plant system to improve the thermotolerance in plants by protein stabilization, osmotic adjustment and ROS detoxification during heat stress (Khan *et al*., 2010; Chen *et al.*, 2018; Ali *et al*., 2019; Ismail *et al*., 2020a). Fungal endophytes have been enhances the phytohormones in plant system to maintain cytosolic ion homeostasis to ameliorate stress. In addition, endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus- assimilating enzymes or release of organic acids and improvement in absorption of water or supporting the growth of other bacteria/fungi near the rhizosphere or in plant system.

Fungal endophytes are able to colonize other than primary host and provide similar benefit to the compatible secondary host. In addition, fungal endophyte confer a tolerance to the secondary host plant in a habitat specific manner, For example, *Fusarium culmorum,* isolated from dune grass of coastal habitat provided salt tolerance in tomato. Similarly an endophyte (*Curvularia protuberata*) isolated from panic grass of geothermal soil conferred only heat tolerance but not salt tolerance in tomato. The fungal endophytes provide an intergenomic epigenetic mechanism of plant adaptation to habitat stresses through a phenomenon called habitat-adapted symbiosis (Redman *et al*., 2011). Fungal endophytes have diversified habitat and are present in every plant species in the ecosystem. They have been reported from plants adapted to a wide range of ecosystems including hot deserts, arctic tundra, mangroves, temperate and tropical forests, grasslands and savannahs, and cropland (Lugtenberg *et al.,* 2016); extreme arctic, alpine and xeric environments (Ali *et al.,* 2018b). In the light of the above information, the present study was hypothesised to explore the possibilities of using desert endophytes to mitigate heat stress in rice with the following objectives.

1. Screening and characterization of fungal endophytes isolated from hot and cold desert plants for thermotolerance.
2. Evaluation of selected fungal endophytes imparting thermotolerance in model plant system (rice).
3. Determination of antioxidants and phytohormones produced in endophyte inoculated rice grown under heat stress.

II REVIEW OF LITERATURE

Raising temperature is one of the long lasting and challenging threats faced by the globe especially in arid, semiarid and tropics. Intergovernmental Panel on Climate Change (IPCC, 2014) has predicted that the mean atmospheric temperature will increase by 1-3.2 ℃ by the end of 21st century. Increase in temperature from 3 to 4 ℃ can affect crop productivity up to 15–35 % in Asia and Africa and 25–35 % in Middle East (Ortiz *et al*., 2008). IPCC also reported that India would likely suffer from 10–40 % loss in crop production by 2080–2100. Thus, raising temperature has become a serious problem in agriculture production in different localities around the world.

Increase in temperature beyond threshold level referred to as heat stress which causes irreversible damages to plant growth and development (Hemmati *et al.*, 2015). Heat stress is an imperative environmental stress that restricts the plant growth, disturb its metabolic activities and productivity (Bakhtavar *et al*., 2015). It affects all developmental stages of plant growth such as germination, vegetative growth as well as fruit and seed development resulting in reduced crop yield.

To overcome heat stress, plants develop some of the mechanisms such as activation of antioxidant system, accumulation of osmolytes, protecting photosynthetic systems by heat shock protein production *etc*. Thermo tolerance of plants is achieved through genetic engineering, breeding programs and tissue culture which are time consuming, expensive and harmful to environment (Queitsch *et al*., 2000; Jan *et al.*, 2018 and Lamaoui *et al*., 2018). One of the eco-friendly alternative approach is to explore endophyte mediated heat stress tolerance to sustain agricultural crop productivity (Rodriguez and Redman, 2005; White and Torres, 2010 and Torres *et al.*, 2012).

2.1 Endophytes

The term Endophyte is derived from Greek words, *endo* = within, *Phyte*= plant. It was originally postulated by De Bary (1866). Endophytes are microorganisms (fungi, bacteria, protozoa, virus or algae) that live part of its life or complete its life cycle inside living plant without causing negative symptoms (DuPorte, 1925; Correa and McLachlan, 1994; Wennstrom, 1994; Saikkonen *et al*., 2004). The distribution of endophytes is ubiquitous and has been reported in all most all tissues of plant

including leaves, stems, roots, flowers and fruits. There is a large biodiversity among endophytes and it is not rare for some plant species to host more than 100 different endophytic species.

Endophytic fungal transmission is mainly active through stomata, cell wall or wounds unlike endophytic bacterial passive transmission through wounds and other tissue openings or active with enzymes or vectors, *e. g.* insects (Schulz and Boyle, 2005) thus, making fungal endophytes more competent over bacterial endophytes. There is an evidence that fungal endophytes can protect plants exposed to various biotic (Prestidge and Gallagher, 1988; Latch, 1993) and abiotic stresses (Rodriguez *et al*., 2008; Hahn *et al*., 2008).

Endophytes colonization has been shown to enhance plant tolerance for many abiotic stresses such as heat (Marquez *et al*., 2007; Khan *et al*., 2012a), drought (Hahn *et al*., 2008; Gibert *et al*., 2012), freezing, UV-B radiation (Draggen, 2007), nutrient deprivation, salinity (Maggio *et al*., 2003; Waller *et al*., 2005; Rodríguez and Redman, 2008; Khan *et al*., 2012b), alkaline conditions (Bu *et al*., 2012) and heavy metal contamination (Zhang *et al*., 2006; Li *et al*., 2012).

2.2 The relationship between plant and fungal endophytes

The existence of fungal endophytes from fossil records suggests that endophyte-host associations might evolved at the time of development of first higher plants on earth. A complex relationship exists between an endophyte and its host, ranging from mutualistic or symbiotic to antagonistic or slightly pathogenic. The symbiotic relationship between the endophyte and host plant vary depending on the host genotypes and the environmental factors (Rodriguez and Redman, 1997). Besides some saprophytic fungi found in senescent plant have been isolated as endophytes inhabiting healthy tissues (Promputtha *et al*., 2007). These entophytic fungi behave as latent saprophytes and are asymptomatic and spatially restricted during the host development, but can grow and unrestrictedly reproduce when the tissue dies (Zabalgogeazcoa, 2008). Plant-fungus relationship has been proclaimed a pivotal source for plant growth and development (Rodriguez and Redman, 2008). Symbiotic fungi may be present as differential intracellular or intercellular symbiotic colonization structures in response to plant health or stress status (Abdellatif *et al*., 2009).

Successful symbiosis of plant-fungal endophyte follows three stages such as (1) invasion of fungi into the tissues of plants (2) colonization of plant tissue by invaded fungi and (3) expression of symbiotic lifestyle by plant-fungi interaction (Singh *et al*., 2011).

Mutualistic symbiosis induces between the endophytes and plant system by various mechanisms to avoid host defense mechanisms and maintain intercalary growth within the host. For example, *Epichloe festucae* maintain a symbiotic interaction in perennial rye grass by regulating the reactive oxygen species (ROS) production with the expression of *RacA* and *NoxA* genes, which are involved in hyphal morphogenesis (Tanaka *et al.,* 2008) and remodelling of cell wall by conversion of chitin to chitosan with the help of chitin deacetylases and hence this hyphae switch from free living to endophytes (Noorifar *et al*., 2021).

2.3 Classification of fungal endophytes

Fungal endophytes are found in phyla Basidiomycota, Ascomycota and Glomeromycota and can be classified in a variety of ways. There are two major classes of fungal symbionts associated with plants such as fungal endophytes which reside entirely within plant tissues and may be associated with roots, stems and/or leaves and mycorrhizal fungi that reside only in roots but extend out into the rhizosphere (Rodriguez *et al.*, 2004)

Rodriguez *et al*. (2009) proposed a novel system for classification of the endophytic fungi such as clavicipitaceous (C-endophytes) and non-clavicipitaceous endophytes (NC-endophytes). This approach is based on breadth or narrowness of host range, plant organs/tissue colonized, level of plant colonization, biodiversity of endophytes within individual host and method of transmission between the hosts.

Class-1, C- endophytes are systemic and vertically transmitted through seeds from one generation to other and exclusively restricted to grass species. They live their entire life cycle within the aerial portion of the grass, forming non-pathogenic and usually intercellular association. They normally increase plant biomass, confer drought tolerance and produce chemicals which are toxic to animals. Non-clavicipitaceous endophytes are taxonomically diverse, horizontally transmitted from

plant to plants and colonize almost all the plants such as non-vascular plants, ferns and angiosperms in the ecosystem.

NC- endophytes were further separated into three subclasses, class-2, class-3 and class-4. Class-2 endophytes are unique compared to others because they provide habitat-specific stress tolerance to the host; it could be a disease, drought, heat or saline tolerance and is defined as habitat-adapted symbiosis. For example, an endophyte *Curvularia protuberata* colonized in *Dichanthelium lanuginosm* plant growing at Yellowstone National Park, USA is able to tolerate high temperature and thus helps its host plant to survive under hot environment (Redman *et al*., 2002). Similarly, *Fusarium culmorum* which colonized *Leymus mollis* provide fitness to the host plant to tolerate seawater salinity (Rodriguez *et al*., 2008). However, when growing the host plants and endophytes separately, neither one is capable of tolerating temperature and saline stress (Marquez *et al*., 2007; Rodriguez *et al*., 2008). Therefore, it plays a significant role in helping their host to survive in hostile environment.

Class-3 endophytes have broad host range, transmit horizontally and are restricted to shoot for colonization. Class-4 endophytes are also transmitted horizontally from plant to plant and restricted to roots.

This system of classification exclude the mycorrhizal fungi because this fungi occupy the rhizosphere along with plant tissues like roots whereas some authors define endophytes as being found solely within their plant hosts prior to host senescence or mortality (Sherwood and Carroll, 1974; Carroll, 1988). However, more recent evidence suggests that other endophytic fungi do sometimes extend into the rhizosphere and/or soil (Taniguchi *et al.,* 2012), further blurring the lines between mycorrhizae and fungal endophytes.

2.4 Habitat of fungal endophytes

Fungal endophytes have diversified habitat and are present in every plant species in the ecosystem. Diversity and number of endophytes in host plants are known to vary with altitude, humidity, precipitation, temperature, host plant species and communities (Spellerberg and Fedor, 2003; Kumar and Hyde, 2004; Yuan *et al*., 2011). Biodiversity of endophytes depends on environmental factors, while the

species diversity is dependent upon the nature of the host plant and their ecological location (Ramesh *et al*., 2017).

Endophytes have been reported from plant adapted to a wide range of ecosystems including hot deserts, arctic tundra, mangroves, temperate and tropical forests, grasslands and savannahs, and cropland (Lugtenberg *et al.,* 2016); extreme arctic, alpine and xeric environment (Ali *et al.,* 2018b).

Thar Desert exhibits a broad array of habitats and biodiversity of flora and fauna. During summer, the ambient air temperature ranges between 32 - 49 °C and the soil temperature often rise beyond 50 °C (Sangamesh *et al.,* 2017). The cold deserts of western Himalayan region are characterized by wide range of fluctuated temperature from winter (-40 ^{o}C) to summer (40 ^{o}C) with low annual precipitation (80-300 mm mostly in the form of snow) (Butola *et al.,* 2012). The other climatic stress factors in this region are least fertile sandy soils with very less water holding capacity, sparse plant density, dry humidity (<30 %), intense solar radiation, low oxygen content, low atmospheric pressure, high wind velocity and rugged terrain. This desert has immense plant diversity including a plethora of medicinal and aromatic plants and most of the plants belong to xerophytes (Qadri *et al.,* 2013).

These hot and cold desert plants have adapted to such adverse conditions by various morphological and physiological modifications. These plants serve as habitat for fungal endophytes which plays a significant role in helping their host to survive in hostile environment. Several reports have been documented that these plants serve as an untapped source of novel endophytes for use in agriculture (Rodriguez *et al*., 2008; Morsy *et al.,* 2010; Zhou *et al*., 2015).

2.5 Isolation, screening and identification of fungal endophytes

Fungal endophytes could be isolated from every part of plant such as root, stem, petiole, leaves, flowers, fruits and seeds. A single tropical leaf may harbour approximately 90 endophytic species and 50 distinct genera in grassland species (Bayman, 2006; Porras-Alfaro *et al*., 2008). The endophytic fungi have been isolated from various types of plants including conifers, grasses, marine algae, lichens, mosses, ferns and teridophytes (Li *et al*., 2007; Melo *et al*., 2014; Eo and Park, 2019; Gao *et al*., 2019).

Thermotolerant/thermophilic fungi were found in diversified areas such as hot springs, geo thermal soil, pile of hey, herbivore dung, coal spoil tips, municipal refuge *etc*. (Salar, 2014). Zhou *et al.* (2015) reported that 60.22 per cent of fungal endophytes isolated from the plants of geothermal ecosystem in China were thermo-tolerant and also suggested that many endophytes in geothermal ecosystem have close relationship with soil fungi.

Sangamesh *et al.* (2017) evaluated thermotolerant endophytes of the Thar desert by culturing the fungi at 40 °C and 45 °C on a temperature controlled shaker. Out of 82 OTUs (operational taxonomic units), six endophytes, namely, ACJ-2, ACJ-5 (*Aspergillus flavus*), SAP-3 (*Aspergillus* sp.), SAP-6, LAS-4 (*Aspergillus* sp.) and LAS-6 (*Chaetomium* sp.) were tolerant to as high as 45 °C. Rest of the OTUs (operational taxonomical units) did not survive in temperature beyond 35 °C.

Traditional classification and identification of endophytic fungi depends on microscopic features, colony characteristics on artificial media and biochemical reactions (Sutton and Cundell, 2004). This kind of methods have served in the past but they have major drawbacks as they cannot be applied to non-cultivatable organisms, non-sporulating group of fungi and occasionally, biochemical characteristic of some organisms do not fit into the patterns of any known genus and species besides they are time consuming. Hence, in recent years, these fungal genes have been sequenced with the help of molecular tools that enabled many of the endophytic fungal taxa to be identified up to species level (Lacap *et al*., 2003; Promputtha *et al*., 2005; Jeewon *et al*., 2013; Doilom *et al*., 2017).

Amplification and sequencing of target regions within the ribosomal DNA gene complex has emerged as a useful adjunctive tool for the identification of endophytic fungi and does not depend on fungus sporulation for identification (Buzina *et al.,* 2001; Iwen *et al.,* 2002; Rakeman *et al.,* 2005; Schwarz *et al.,* 2006). The ribosomal DNA (rDNA) is present in all organisms and its evolution is rapid, so it is used to discriminate related species or even varieties of the same species. The ITS (Internal Transcribed Spacer) regions are flanked by preserved segments (18S, 5.8S and 28S genes) which provide information about the phylogeny and the taxonomic level, since their evolution is slow and they are highly similar within different taxa. The ITS region of rDNA sequences is widely used to examine phylogenetic positions

or relationship of a species (Ramesh *et al*., 2017). Manasa *et al*. (2020) identified the fungal OTUs by amplifying the 18s ribosomal region of the internal transcribed spacer (ITS) of the genomic DNA using ITS1 and ITS4 as forward and reverse primers respectively.

2.6 Effects of high temperature on rice and mechanisms of thermotolerance

2.6.1 Impact of high temperature on rice growth and production

Rice is an internationally vital cereal crop and a stable food for approximately half of the world's population. An analysis of historical data showed that the rice grain yield decreased by 7–8 % for 1^{o} C rise in day temperature from 28 to 34 ^{o} C (Baker *et al*., 1992). Similarly, the results obtained from irrigated field trails executed at the International Rice Research Institute from 1992-2003 demonstrated that each degree centigrade increase in minimum temperature may cause 10 % reduction in rice yield (Peng *et al*., 2004). The rice grain yield decreased by 14 % for every 1°C increase in daily average temperature (Aggarwal, 2009). Lobell *et al*. (2011) reported that high temperatures reduce grain yield per plant by 70 % for each 1 ^{o}C increase in temperature, resulting in 4–14 % yield loss in rice. Although rice can still maintain normal growth at temperatures ranging from 27 to 32 ^{o}C without significant reduction in grain yield, temperatures above 32 ^{o}C negatively affect all stages of plant growth and development (Aghamolki *et al.*, 2014).

Frequent heat waves have had serious impacts on rice production (Zhang *et al*., 2014). The extremes of high temperature on daily and seasonal time scales may occur more frequently in near future, especially in tropical and subtropical areas where rice is widely cultivated (Fischer and Knutti, 2015). The optimum temperature for rice growth varies from 25 to 30 °C during the day and from 20 to 22 °C for night time (Chen *et al*., 2017). A rise in temperature beyond threshold level causes irreversible damages to plant growth and development and termed as heat stress.

Rice responds differently to heat stress at different growth stages in its life cycle. Plants sensitivity to temperature depends on variety, physiological status and time of exposure to heat stress (Fahad *et al.*, 2018). High temperature affects almost all the growth stages of rice, *i.e*. from emergence to ripening and harvesting as follows.

Growth stage	Threshold temperature (°C)	Symptoms	References
Germination	40	Delay and decrease in emergence	Satake and Yoshida (1978)
Seedling	35	Poor growth of the seedling	Yoshida *et al*. (1981)
Tillering	32	Reduced tillering and height	Satake and Yoshida (1978)
Booting	–	Decreased number of pollen grains	Shimazaki *et al*. (1964)
Anthesis	33	Poor anther dehiscence and sterility	Jagadish *et al*. (2007)
Flowering	35	Floret sterility	Satake and Yoshida (1978)
Grain formation	34	Yield reduction	Morita *et al*. (2004)
Grain ripening	29	Reduced grain filling	Yoshida *et al*. (1981)

2.6.2 Effect of high temperature on tillering of rice plant

Tillers are branches that develop from the leaf axils at each un-elongated node of main shoot or from other tillers during vegetative growth, growing independently by means of its own adventitious roots. Tillering is a two-stage process: the formation of axillary buds at each leaf axil and its subsequent growth. Tiller number is an important attribute for grain yield. Chaudhary and Ghildyal (1970) demonstrated that the tillering is harshly affected by temperatures exceeding 33 ^{o}C. The best day and night temperatures for tillering are 25 ^{o}C and 20 ^{o} C respectively (Sato, 1972). However, increase in temperature from 15 ^{o}C to 33 ^{o}C may increase number of tillers (Yoshida, 1973).

Rice plants with more tillers can show a greater inconsistency in mobilizing assimilates and nutrients among tillers, resulting in variations in grain development and yield among tillers. Differences in yield and grain development among tillers are major consequences of heat stress due to disturbed mobilization of assimilates and nutrients between tillers (Yoshida *et al*., 1981). De Datta (1981) observed a considerable decrease in rice plant height, tiller number and dry weight as a result of exposure to high temperatures during the vegetative stage. Manalo *et al.* (1994) reported that a temperature rise from 29/21 ^{o}C to 37/29 ^{o}C decreased tiller number by

10 %. Similarly, Mitra and Bhatia (2008) observed that the decreased number of tillers, plant height and biological yield in rice due to heat stress.

2.6.3 Effects of high temperature on physiology of rice plant

Photosynthesis is one of the most susceptible physiological processes to heat stress in rice plants. The leaf photosynthesis of rice increased from lowest (22 ^{o}C) to the intermediate temperature (32 ^{o}C) and then decreased in the plants grown at 42 ^{o}C (Egeh *et al.,* 1992). In rice, maximum peaks of photosynthesis at approximately 30 ^{o}C and CO_2 assimilation may decrease significantly after suffering from heat stress (Yamori *et al.,* 2011). Mathur *et al.* (2014) concluded that high temperature stress mainly inhibited various redox and metabolic reactions that occurs in Photosystem II, Photosystem I and cytochrome complex eventually resulting in decreased photosynthetic rate. Huang *et al*. (2017) indicated that the Rubisco activity, regeneration capacity of RuBP (Rubilose bisphosphate), rate of electron transport and CO_2 diffusion capacity are sensitive to temperature and are negatively impacted by high temperature. Sonjaroon *et al.* (2018) observed a reduction of 57 % photosynthesis in rice plants subjected to a moderate period (7 days) of high temperature during the day (40 °C).

Chlorophyll status is a key index for evaluating plant photosynthetic efficiency. A reduction in photosynthetic rate was linked to the decrease in chlorophyll content due to impaired biosynthesis or accelerated pigment degradation and also increased chlorophyllase activities under heat stress (Todorov *et al.,* 2003; Camejo *et al*., 2006). Injury of the thylakoid membrane and reduction in chlorophyll content was observed in rice and maize under heat stress (Ristic *et al*., 2007). The chlorophyll a/b ratio is associated with the photosynthesis capability of plants under biotic or abiotic stresses. A decreased chlorophyll ratio in crop plants under drastic factor conditions shows increased resistance to heat and *vice-versa* (Rana *et al*., 2011).

High temperatures may also affect membrane stability through lipid peroxidation leading to the production of peroxide ions and melondialdehyde (MDA) and the peroxidation was accompanied by electrolyte leakage (Liu and Huang, 2000). Increased MDA content and electrolyte leakage percentage was observed in rice under temperature stress at 37 °C /30 °C (day/night) (Zhang *et al.,* 2009; Liu *et al*.,

2013). Sanchez-Reinoso *et al*. (2014) stated that the change in concentration of MDA is a good indicator of membrane structural integrity. According to Hussain *et al.* (2019), an increase in MDA content and decrease in chlorophyll content indicate negative impact on the photosynthetic efficiency of plants under heat stress.

2.6.4 Mechanisms of plant system to tolerate high temperature

Plants acclimate to sub lethal heat stress by altering metabolism at physiological, biochemical and molecular levels. Changes in the membrane physical state and composition, production of heat shock proteins, transcription factors, osmolytes and augmented levels of antioxidant defence are key processes to maintain cellular redox homeostasis under heat stress (Krasensky and Jonak, 2012).

Under high temperature conditions, plants exhibit short-term avoidance or acclimation mechanisms such as transpiration cooling, stomatal closure, and so on (Mathur *et al.,* 2014). This means a close relationship exist between CO_2 delivery and water transportation in leaf. Stomata, through which CO_2 and water vapour diffuse into and out of the leaf, are involved in the regulation and control of photosynthesis and transpiration responses (Farquhar and Sharkey, 1982; Jones, 1998).

2.7 Mechanisms of endophytic microbes confer heat stress tolerance to plants

The endophytic fungi provide stress tolerance to their host plant by different mechanisms such as activation of host plant stress response system when they are exposed to stress (Redman *et al*., 1999) and production of anti-stress biochemical compounds by endophytic fungi itself or through the stimulation of plant by fungal endophytes (Bacon and Hill, 1996).

2.7.1 Production/induction of antioxidants by endophytes to confer stress tolerance

Reactive oxygen species (ROS) play an important role in signal transduction when the plant is subjected to stress. ROS such as hydrogen peroxide, hydroxyl radical, superoxide anion radicals and singlet oxygen can be generated and accumulated in plants under heat stress (Suzuki *et al.*, 2012). Uncontrolled ROS accumulation can damage cell membrane, protein and DNA which leads to oxidative stress and cause programed cell death. In order to negate this effect, plants employed

antioxidant defense system (ADS) to tolerate heat stress. This is achieved by production of antioxidant enzymes such as CAT (catalase), POX (peroxidase), SOD (super oxide dismutase), APX (ascorbate peroxidase), PAL (phenylalanine ammonialyase), DHAR (dehydroascorbate reductase), MDHAR (monodehydroascorbate reductase) and GR (glutathione reductase) (Alici and Arabaci, 2016). ADS is responsible for deactivation of ROS by reducing their vitality or distressing their chain of oxidizing reactions (Choudhury *et al*., 2017).

The endophytes may protect their host/secondary plant from oxidative stress by production of antioxidant enzymes or stimulate the plant to produce antioxidants, thereby alleviating the detrimental impacts of stress on plant tissues (Rodriguez and Redman, 2005). Waller *et al*. (2005) demonstrated that the *Piriformospora india* enhances the ratio of reduced to oxidized ascorbate and induces DHAR activity due to activation of host glutathione-ascorbate cycle in colonized barley and mitigate the saline stress. Similarly, Baltruschat *et al.* (2008) point out that the antioxidant enzyme activities are maintained at high level in *Piriformospora indica*-infected plants but decrease gradually in uninfected plants under salinity. White and Torres (2010) suggested that oxidative stress avoidance through production of antioxidative compounds could account for *Epichlioe* endophyte enhanced stress tolerance in host grasses.

Piriformospora indica colonized cabbage plants were exposed to polyethylene glycol, it mimicked drought stress by up regulation of peroxidase, catalase and superoxide dismutase activities within 24 hours and improved the shoot and root growth of plant (Sun *et al*., 2010). Thermotolerant endophyte, *Thermomyces lanuginosus* provide thermotolerance to *Cullen plicata* plants by maximizing the activities of PAL, SOD, CAT and POX (Ali *et al*., 2019).

2.7.2 Production of osmolytes by endophytes to confer stress tolerance

Under high temperature, plants accumulate or synthesize compatible solutes known as osmolytes such as proline, glycine betaine, sugar alcohols, sorbital, mannitol, organic acids and amino acids (Ahmad and Umar, 2011). These compatible solutes are neutrally charged, low molecular weight compounds, playing important role in the stabilization of proteins, osmotic adjustment and detoxification of ROS during stress (Khan *et al.*, 2015; Hasanuzzaman *et al*., 2019).

2.7.2.1 Proline

Proline plays a vital role in relieving cytoplasmic acidosis, protein compatible hydrotrope and keep suitable ratio of NADP+/NADPH harmonious with metabolism under stress (Hare and Cress, 1997). On overcoming of stress conditions, rapid breakdown of accumulated proline releases sufficient amount of strong reducing agents that enhance process of ATP synthesis and mitochondrial oxidative phosphorylation. These ATP molecules are used to repair damages induced by stress (Hare *et al*., 1998; Ashraf and Foolad, 2007). It acts as free radicals scavenger and as buffer for cellular-redox potential under stress (Balal *et al*., 2017).

Several reports have been documented that proline concentration is maximized in endophytes inoculated plants under stress conditions. Vimal *et al.* (2018) stated that *Curtobacterium albidum* SRV4 elevated proline production (4 µM g/FW) in rice when subjected to 300 mM NaCl concentration which was significant compared to control (3 µM g/FW). ABD-Allah *et al.* (2018) isolated endophytic bacterium *Bacillus subtilis* BERA 71 that produced a total proline content of 70 mg g/FW. High concentration of proline was observed in *Rhizopus oryzae* associated with sunflower and soybean as compared to non-inoculated seedlings at 40 ^{o}C and clearly indicating thermal alleviating capability of endophytic fungus (Ismail *et al*., 2020c)

2.7.2.2 Phenols and flavonoids

Phenols and flavonoids act as defensive compounds against abiotic stresses. These compounds accumulated in plant tissues could help to protect themselves from damaging effects by acting as a free radical scavenger because of the presence of hydroxyl groups in their structure (Heim *et al*., 2002). Daidzein and genistein are important chemical constituents for isoflavonoids biosynthetic pathway. Khan *et al.* (2010) found higher concentration of daidzein and genistein in *Aspergillus fumigatus* sp. LH02 associated soybean plants and suggested that endophytic fungus provide salt stress tolerance.

Similarly few reports documented the role of endophytic fungi in enhancing accumulation of phenols or flavonoids in heat stressed plants. Ali *et al.* (2019) reported that an endophyte *Thermomyces lanuginosus* inoculated *Cullen plicata* plant showed higher amount of flavonoids compared to un-inoculated plants when exposed

to 45 ^{o}C. *Aspergillus violaceofuscus* is able to produce phenolics (6.6 mg/mL) and flavonoids (2 µg/mL) and could help to mitigate the accretion of ROS and lower the heat stress effects on sunflower plants by accumulation of phenols (Ismail *et al.,* 2020b).

2.7.2.3 Glycine Betaine (GB)

GB is one of the quarternary ammonium compounds which can mitigate heat stress in plant system. It is synthesized in chloroplast and maintains the photosynthetic efficiency by protecting the thylakoid membranes and PSII which are sensitive to heat stress (Allakhverdiev *et al.,* 2007 and Gupta and Thind, 2018). A very few reports are described that endophytes might involve in production/ accumulation of glycine betaine in host to reduce osmotic potential and maintain the cell turgor under osmotic stress. For example, an endophyte *Epichloe bromicola* maintains osmotic balance by accumulation of higher glycine betaine in wild barley and enhance the photosynthetic efficiency under hyperosmotic stress (Chen *et al.,* 2018).

2.7.3 Regulation of phytohormones by endophytes to confer stress tolerance

High temperature modulates phytohormone levels, thereby affecting plant organ growth and yield. Levels of phytohormone in target organs are associated with processes involved in phytohormone homeostasis, *e.g*., biosynthesis, catabolism, deactivation, and transport (Sakakibara, 2010). They are signaling molecules and play a vital role not only in regulating plant growth and development, but also in mediating the physiological processes responding to a variety of biotic and abiotic stresses. Naturally occurring phytohormones such as indole acetic acid (IAA), gibberellins (GAs), abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA), can yield either positive or negative effects on plant growth during exposure to abiotic stresses (Kosova *et al.,* 2012). IAA and GAs function as plant growth promoters, whereas ABA, JA, and SA are categorized as stress-related hormones because these mediate abiotic and biotic stress responses such as heat, cold, drought and pathogens (Miura and Furumoto, 2013).

These phytohormones are produced by fungal endophytes which are helpful to mitigate the abiotic stress. Various novel endophytic fungal species like

Piriformospora indica, Neotyphodium sp., *Curvularia protuberate* and *Colletotrichum* sp. *etc* have been known to improve plant growth during abiotic stress conditions by balancing the phytohormones (Khan *et al*., 2012). Moreover, exogeneous application of these phytohormones has been proven to alleviate abiotic stress (Bita and Gerats, 2013; Hasanuzzaman *et al.,* 2013; Claeys *et al.,* 2014).

2.7.3.1 Auxin

Auxin play an important role in cell enlargement, cell division, tissue differentiation, apical dominance, root initiation, *etc*. (Glick, 1995; Fu *et al*., 2015). Heat stress suppressed the biosynthesis of IAA by down regulating genes involved in IAA biosynthesis and reduced IAA by upregulating the IAA-amino acid synthetase gene (GH3) in rice (Du *et al.,* 2012). It plays a vital role in heat stress-induced thermo morphogenesis, including stem (hypocotyl) elongation and leaf hyponasty. These morphological changes are considered to enhance leaf cooling capacity by increasing the leaf transpiration rate, thereby helping plants survive under heat stress (Kupers *et al.,* 2020).

Mattos *et al.* (2008) reported that the endophytic *Burkholderia kururiensis* promoted rice plant growth by production of the plant auxin, IAA. Using transgenic rice plants containing an auxin-responsive reporter (DR5-GUS), they showed that the plantlets inoculated with the endophyte had strong DR5-GUS activity in the roots compared to the non-infected control plants, indicating the IAA-induced gene expression.

Wild-type Arabidopsis seedlings inoculated with either *Trichoderma virens* or *T. atroviride* showed characteristic auxin-related phenotypes such as increased biomass production and stimulated lateral root development. *T. virens* produced the auxin-related compounds indole-3-acetic acid (13.48 µg/L), indole-3-acetaldehyde (59.4 µg/L), and indole-3-ethanol (72.33 µg/L) under axenic conditions (Contreras-Cornejo *et al*., 2009).

Mei and Flinn (2010) reported that IAA producing fungal and bacterial endophytes can improve rice growth under drought, salinity, and high temperature stress. Sun *et al*. (2014) reported that the fungi isolated from leaf samples of the

carnivorous plant *Drosera indica* L., produced IAA that can induce lateral root formation and root hair growth.

2.7.3.2 Gibberllins (GA)

Gibberellins play an essential role in regulation of growth and development of plants (Bahalla *et al.,* 2010) and are associated with several plant growth and development process such as seed germination, stem elongation, flowering, and fruit development (Bilkay *et al.,* 2010; Lu *et al.,* 2016). The historical reports documented reveal that heat stress suppressed the biosynthesis and promoted the deactivation of GA, but few studies to date have documented the effects of heat on catabolism and the transport of GA in rice plant (Wu *et al.,* 2019).

Hasan (2002) screened fungi, mostly *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium corylophilum, Penicillium cyclopium, Penicillium funiculosum,* and *Rhizopus stolonifer,* for their ability to produce gibberellin and IAA. All fungal species had the ability to produce GA but *F. oxysporum* was found to produce both GA and IAA. The optimum period for GA and IAA production by *F. oxysporum* was 10 days in the mycelium and 15 days in the filtrate at 28 ^{o}C.

Penicillium citrinum isolate IR- 3-3 from the sand dune flora, produced much higher physiologically active gibberellins than the wild type *Gibberlla fujikuroi,* a strain that has been used to produce a biologically active gibberellic acid GA3 commercially and stimulated Waito-c rice seedling growth (Khan *et al.,* 2008). Ahmad *et al*. (2010) reported that *Penicillium* sp. strain M5.A and *Aspergillus* sp. strain M1.5 produced different GAs such as GA3 GA4 GA7 GA9 and GA24 which were isolated from roots of *Monochoria vaginalis*, a serious weed of rice paddy in Korea.

Khan *et al*. (2010 and 2011) confirmed that GA and IAA producing endophytic fungal strains such as *Penicillium funiculosum* LHL06 (GA1, GA4, GA8 and GA9) and *Aspergillus fumigatus* LH02 (GA4, GA9 and GA12) can ameliorate soybean plant growth under moderate and high salinity stress.

The endophytic fungi *Phoma glomerata* LWL2 and *Penicillium* sp. LWL3 significantly promoted the shoot and allied growth attributes of GAs-deficient dwarf mutant Waito-C and Dongjin-beyo rice. Analysis of the pure cultures of these

endophytic fungi showed biologically active GAs (GA1, GA3, GA4 and GA7) in various quantities and also IAA (Waqas *et al*., 2012).

An endophyte *Paecilomyces formosus* LWL1 improved the growth of rice plant by production of secondary metabolites such as gibberellins (GA3, GA4, GA7 and GA9), auxin (IAA) and organic acids including oxalic acid, quinic acid, tartaric acid and malic acid (Waqas *et al*., 2014). Hamayun *et al.* (2017) reported that *Porostereum spadiceum* AGH786 is the first ever GAs producing basidiomycetous fungus capable of producing six types of GAs such as GA1, GA3, GA4, GA5, GA7 and GA19. This endophyte boosted NaCl tolerance and growth in soybean, by modulating seedling endogenous phytohormones and isoflavones.

The phytohormone producing endophytes, *Paecilomyces formosus* LHL10 and *Penicillium funiculosum* LHL06 synergistically produced higher amounts of gibberellins such as GA1 (4.69 ± 0.12 ng/mL), GA3 (2.78 ± 0.13 ng/mL), GA4 (32.54 ± 1.13 ng/mL), GA7 (1.28 ± 0.25 ng/mL), GA9 (31.36 ± 1.54 ng/ mL), GA24 (5.31 ± 1.91 ng/mL), and GA12 (2.63 ± 0.12 ng/ mL) and IAA (20.80 ± 1.54 μg/mL) in a co-cultured medium and their co-inoculation significantly mitigated the metal toxicity (Ni, Al, Cu, Cr and Pb) in soybean plants and hence improved the seed production (Bilal *et al*., 2020).

2.7.3.3 Abscisic acid (ABA)

The accumulated ABA is expressed in the plant in ways which can be agronomically regarded as plant stress, namely growth inhibition and reproductive failure (Trewavas and Jones, 1991). High temperature causes up-regulation of ABA biosynthesis genes while, down-regulates those genes which are responsible for ABA catabolism (Toh *et al*., 2008). ABA has a defensive role in the amelioration of heat stress by regulating opening and closing of stomata as well as restoration of plant growth and development (Yoon *et al.,* 2009). ABA enhances heat tolerance by mediating HSP (Heat shock proteins) and RBOH (respiratory burst oxidase homologs) genes expression and sucrose metabolism (Li *et al.,* 2021).

It is widely accepted that ABA contents in plant increase under gradient heat stress. The fungal endophytes might involve in down regulation of genes responsible for ABA synthesis or acceleration of ABA degradation. Hence, lower amount of ABA

was found in sunflower and soybean inoculated with *Aspergillus niger* under heat stress as compared to endophyte-free seedlings (Ismail *et al.*, 2020a).

Soybean co-inoculation with *Paecilomyces formosus* LHL10 and *Penicillium funiculosum* LHL06 induced ABA-independent pathway by up regulating the expression of ABA-independent GmERD1 and GmRD20 and down-regulating the expression of the ABA-dependent DRE-binding transcription factors GmDREB2 and GmDREB1B for withstanding combined high temperature and drought stress (Bilal *et al.*, 2020).

2.7.3.4 Cytokinin (CK)

CK play a vital role in increasing number of branches and spikelets in rice panicle (Ashikari *et al*., 2005 and Kurakawa *et al*., 2007). CK moving acropetally from roots promotes axillary bud growth in rice plants resulting in increased tiller production (Mohapatra *et al*., 2011). Numerous studies provide evidence that temperatures modulate CK responses and CK levels are involved in plant adaptive mechanisms to temperature stress (O'Brien and Benkova, 2013; Pavlu *et al.*, 2018). The heat stress severely decreases the xylem sap flow rate and CK transportation rate, which results in reduction of tiller due to inhibition of axillay bud growth. Heat-induced CK activates transcription of genes involved in photosynthesis and carbohydrate metabolism (Dobra *et al.*, 2015). Under high temperature, CK enhances antioxidant metabolism, by inducing activities of antioxidant enzymes superoxide dismutase, ascorbate peroxidase, and guaiacol peroxidase in roots of plants. (Li *et al.*, 2021).

Liu and Wei (2019) showed that the IAA/ABA ratio of DSE *Acrocalymma vagum* inoculated seedlings was higher whereas, the ratios of GA/ABA, ZR/ABA, and ZR/IAA were lower than those of non-inoculated seedlings. They suggested that the *A. vagum* may coordinate the balance of host hormones through adjusting hormone ratios after symbiosis with the host, slowing down plant growth and development to reduce the excessive consumption of water, maintaining normal cell swelling pressure to maintain normal water balance, promoting stomatal closure to reduce water transpiration and increasing cell solute concentration to enhance plant water absorption capacity, thereby enhancing plant drought resistance. Waqas *et al.* (2014) reported that the *Paecilomyces formosus* LWL1endophyte protected the rice

plants from heat stress compared to controls by reducing the endogenous level of stress-signalling compounds such as abscisic acid (25.71 %) and jasmonic acid (34.57 %) and increasing the total protein content (33.22 %).

Shahzad *et al.* (2017) studied the enhancement of salinity stress tolerance in *Oryza sativa* by inoculation of abscisic acid-producing endophytic bacteria. The results revealed that stress-sensitive endogenous ABA levels were significantly reduced, whereas the levels of endogenous SA were significantly higher in *Bacillus amyloliquefaciens* RWl-1-inoculated plants than in control plants exposed to the same level of salinity stress.

Arkhipova *et al.* (2020) studied the inoculation effects of Rhizobacteria on phytohormone status of potato microclones cultivated *in vitro* under osmotic stress. The results revealed that the inoculation of *Azospirillum brasilense* Sp245 or *Ochrobactrum cytisi* IPA7.2 promoted the growth in terms of leaf mass accumulation under osmotic stress. The effects were associated with increased concentrations of auxin (IAA) and CK (zeatin, isopentenyl adenine and isopentenyl adenosine) hormones in the leaves and stems and with suppression of ABA increase in leaf.

2.7.3.5 Jasmonates

Jasmonates (jasmonic acid, JA and methyl jasmonate, MeJA) are a class of polyunsaturated fatty acid-derived phytohormones and are ubiquitous in higher plant species. JA helps in the biosynthesis of defensive proteins, secondary metabolites, as well as control senescence, pollen development and root growth (Lorenzo *et al*., 2004).

The physiological mechanism by which JAs ameliorate tolerance to abiotic stresses especially to high temperature stress could be mainly explained in two aspects. Firstly, JAs could be enhancing the activities of antioxidative enzymes including CAT, SOD, guaiacol peroxidase, glutathione peroxidase and increasing other defensive compounds such as ascorbic acid (AsA) and HSPs, thereby suppressing ROS generation and reducing programmed cell death under stresses. Secondly, JAs could enhance osmotic regulation by increasing synthesis of some osmoregulators, such as proline, phenolic compounds, and soluble carbohydrates, and accordingly, reduce the harmful effects of stresses to plant organs, stimulate plant cell

expansion, and promote the spikelet opening in rice (Chen *et al.,* 2020 and Yang *et al.,* 2020).

A high level of JA may reduce growth at the cost of defence, as reported in rice and arabidopsis, by delaying DELLA protein degradation (Yang *et al.,* 2020). Production of phytohormones (GAs and IAA) and organic acids by *Paecilomyces formosus* LWL1 may promote growth under heat stress by maintaining the delicate balance of endogenous JA to produce the priming effect but not reduce the growth or resistance to heat stress (Waqas *et al.,* 2014).

2.7.3.6 Salicylic acid (SA)

SA is a novel phytohormone that has been suggested to promote basal thermotolerance and induce membrane thermoprotection (Kotak *et al.,* 2007). SA pre-treatment alleviates the decrease of the net photosynthesis rate by protecting photosystem II function and maintaining higher rubisco activities under heat stress (Wang *et al*., 2010). SA significantly increases the activities of proline biosynthesis enzymes while inhibiting the activities of proline-metabolizing enzymes (Lv *et al*., 2011).

SA application prior to heat stress generally improves the plant growth and physiological activities such as plant height, biomass and photosynthetic efficiency (Wassie *et al*., 2020). SA reduces heat stress-induced membrane damage and modulates the activities of antioxidant enzymes including CAT, SOD, and POD. SA application ameliorates heat stress-induced oxidative stress apparently through maintaining higher proline accumulation (Li *et al*., 2021).

The *Sphingomonas paucimobilis* ZJSH1 was found to produce various phytohormones, including salicylic acid (SA), indole-3-acetic acid (IAA), Zeatin and abscisic acid (ABA). Significantly higher contents of SA, ABA, IAA and c-ZR were detected in the inoculated seedlings of *Dendrobium officinale* (Yang *et al*., 2014). Ismail *et al*. (2018) reported that *Aspergillus japonicus* culture filtrate displayed higher concentrations of SA (63.11μg/mL) and IAA (19.19 μg/mL) and this filtrate helps to promote the growth of rice seedlings.

2.7.3.7 Ethylene

Ethylene, a stress hormone, is involved in the regulation of many physiological properties. Studies have reported that ethylene is involved in the regulation of basal thermotolerance in Arabidopsis under heat stress (Larkindale and Knight, 2002; Larkindale *et al*., 2005). However, ethylene concentration may be responsible for profuse tillering because, ethylene mediated inhibition of IAA biosynthesis allows promotion of tillering (Assuero and Tognetti, 2010) by release of tiller bud growth from apical dominance (Mohapatra *et al*., 2011). Ethylene-mediated signaling was involved in the reduction of oxidative damage, maintenance of chlorophyll content, and also regulates the mRNA transcripts of Hsfs and ethylene-signalling related genes during heat stress in rice seedlings (Wu and Yang, 2019).

Sebacina vermifera, an endophyte closely related to *Piriformspora indica*, down-regulates ethylene production in *Nicotiana attenuata* (Barazani *et al.,* 2007). However, Baltruschat *et al.* (2008) reported that *Piriformspora indica* induces ethylene biosynthesis in barley roots to confer saline stress. The endophytic bacterium *Enterobacter* sp. SA187 induces salt stress tolerance in *Arabidopsis* via production of al 2-keto-4-methylthiobutyric acid (KMBA) to activate the ethylene pathway (De Zelicourt *et al*., 2018).

2.7.3.8 Brassinosteroids

Brassinosteroids (BR) are polyhydroxylated steroidal plant hormones that are involved in plant growth regulation through their participation in metabolic processes such as photosynthesis, antioxidant activity, osmolyte accumulation, nitrogen metabolism and plant water relations under stress conditions (Chauhan *et al*., 2011; Zhang *et al*., 2014; Jin *et al*., 2015). Exogenous applications of BR have been reported to help mitigate the adverse effects of high day time temperatures in rice (Thussagunpanit *et al*., 2015a and b), maize (Yadava *et al*., 2016) and tomato (Zhou *et al*., 2014) by modulating the components of the antioxidant defence system and inducing heat shock proteins (HSP).

2.7.4. Endophytes confer stress tolerance *via* improving nutrient acquisition

High temperatures adversely affect mineral nutrition acquisition or uptake, shoot growth, pollen development and disturb the photosynthesis and respiration

processes resulting in low yield. However, endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus-assimilating enzymes or release of organic acids and improve the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system. Hence, endophytes have a vital role in plant growth promotion.

The organic acids secreted by endophytic microbes helps to solubilize plant phosphorus nutrient by conversion of mineral phosphate into inorganic phosphate through acidifying the surrounding soil and make it available for plant uptake (Rodriguez and Fraga, 1999). Malinowski *et al*. (1999) showed that endophyte infection increased root hair length and decreased root diameter in tall fescue which have role in water and mineral acquisition.

Endophytes enhance the absorption of essential minerals such as calcium, phosphorus, potassium, magnesium and sulphur of their host plant (Yuan *et al*., 2010). As *Piriformospora indica* helps in transport of inorganic phosphate to maize plants by increased expression of high affinity phosphate transporter (PiPT) and hence improved the growth of seedlings under P-deprived condition (Kumar *et al*., 2011).

Xie *et al.* (2017) reported that an endophyte, *Phomopsis liquidambari* increases the Peanut-*Bradyrhizobium* interaction via enhanced H_2O_2/NO - dependent singling cross talk and symbiotic gene *SymRK* and *CCaMK*, which intern improve the nodulation and nitrogen fixation. Endophytes also have a role in the improvement of plants nutritious value like total proteins, carbohydrates and lipids under drought, cold and salt stresses (Ikram *et al*., 2019).

Fungal endophytes have been shown to alter root architecture, increase lateral root development and number of root hairs. Changes in root architecture affect the plant's ability to uptake water from soil.

2.7.5 Expression of plant stress related genes by endophytes to confer stress tolerance

Piriformspora indica confers drought-stress tolerance to Arabidopsis by the expression of a quite diverse set of stress-related genes such as dehydration 29A, dehydration 1, phospholipase Dδ, the transcription factor gene *ANAC072*, dehydration response element binding protein 2A, salt- and drought induced ring finger 1,

calcineurin B-like protein (CBL) 1, CBL-interacting protein kinase 3 and the histone acetyltransferase (HAT) in the leaves (Sherameti *et al*., 2008).

Xu *et al*. (2013) reported that the *GmHsp90* genes were significantly up-regulated in response to heat, salt, and osmotic stresses in soybean and their overexpression in Arabidopsis enhanced growth attributes and reduced abiotic stress. Bilal *et al*. (2020) demonstrated that the soybean plant co-inoculated with *Paecilomyces formosus* LHL10 and *Pencillium funiculosum* LHL06 exponentially induced heat shock protein 90 (Hsp90) genes such as *GmHsp90A2* and *GmHsp90A1* under both high temperature and drought conditions. *Piriformospora indica*, an endophytic fungus co-cultivated with Arabidopsis plants showed earlier upregulation of mRNAs and proteins involved in drought stress from leaves of colonized seedlings than the uncolonized seedlings (Sun *et al*., 2010).

A higher level of colonization with arbuscular mycorrhizal fungus *Bromus setifolius* in grass roots was found in plants infected with the endophyte *Neotyphodium* sp. compared with non-endophyte infected plants, and a positive interaction between endophytes and arbuscular mycorrhizal fungi increased the plant growth (Novas *et al*., 2005). Hence, endophyte-mediated plant responses to stress may be associated with (a) increase or decrease in plant growth (b) enhanced photosynthesis (c) osmotic balance, (d) increased gaseous exchange and water-use efficiency and (e) enhanced antioxidant activities and (f) altered expression of stress related genes.

2.8 Influence of fungal endophytes on growth and physiology of crop plants under high temperature

Redman *et al*. (2002) reported that the endophytic fungi *Curvularia protuberata* isolated from *Diachathelium langugiosum* growing in geothermal soils could impart thermotolerance. When root zones were heated with thermal tape, nonsymbiotic plants became shriveled and chlorotic at 50 °C. In contrast, symbiotic plants tolerated constant 50 °C soil temperature for 3 days and intermittent soil temperatures as high as 65 °C for 10 days. All nonsymbiotic plants died at 65 °C heat regime and symbiotic plants survived.

Fungal endophytes can also control plant responses to stress through the production of specific molecules. *Paraphaeosphaeri aquadriseptata* produces a heat shock protein 90 (HSP90) inhibitor, monocillin I, which enhances heat stress tolerance in Arabidopsis (Mclellan *et al*., 2007).

Hubbard *et al*. (2013) reported that the endophytic fungal isolate SMCD 2206 and 2210 promoted heat stress tolerance in wheat by lowering photosynthetic stress and increasing Fv/Fm values. These isolates resulted in significantly higher plant height, average seed weight (ASW), total seed weight (TSW) and water-use efficiency (WUE) compared to un-inoculated plants.

Waqas *et al*. (2015) isolated fungal endophyte *Paecilomyces formosus* LWL1 (NCBI accession number JQ013813) from the roots of cucumber. This endophytic association with japonica variety Dongjin rice significantly improved the plant growth attributes, such as plant height, fresh weight, dry weight, and chlorophyll content under heat stress conditions.

Xu *et al*. (2017) studied on the improvement of drought and heat tolerance of tall fescue by *Epichloe* endophyte infection through altered antioxidant enzyme activity. Results revealed that the endophyte infected plants showed higher levels of CAT, guaiacol peroxidase (POD) and APX activity under heat stress. *Epichloe* endophyte improved the cell membrane stability of tall fescue through lower level of electrolyte leakage, malondialdehyde content, and hydrogen peroxide (H_2O_2) content.

Ismail *et al*. (2018) isolated an endophytic fungus *Aspergillus japonicus* EuR-26 with heat stress alleviation potential from wild plant *Euphorbia indica* L. *A. japonicus* association with soybean and sunflower had improved plant biomass and other growth features under high temperature stress (40 ^{o}C) when compared to endophyte-free plants.

Ali *et al*. (2019) isolated a thermophilic fungal endophyte, *Thermomyces lanuginosus* from a hot desert-adapted plant, *Cullen plicata*. Endophyte inoculated *Cullen plicata* plants showed significant differences in soil water content, leaves number, fruits number, fruits weight, root length, and root fresh and dry weight than un-inoculated plants under heat stress (45 ^{o}C).

Bilal *et al*. (2020) reported that the synergistic association of endophytic fungi enhances *Glycine max* L. resilience to combined abiotic stresses: heavy metals, high temperature and drought stress. Results revealed that the co-inoculation of *Paecilomyces formosus* LHL10 and *Pencillium funiculosum* LHL06 promoted plant growth attributes like shoot length, root length, seedling fresh weight and dry weight and also increased chlorophyll content, transpiration and photosynthetic rate.

Ismail *et al*. (2020a) reported that a thermo-tolerant fungal strain, *Aspergillus niger* (SonchL-7) enhanced growth in sunflower and soybean under high temperature and recorded maximum plant height, biomass and chlorophyll content in endophyte inoculated plants than control.

Ismail *et al*. (2020b) isolated a thermal stress mitigating endophytic fungus from the fern *Dryopteris filix* L. The phylogenetic study and 18S rRNA sequence similarity confirmed the potential strain as *Aspergillus violaceofuscus*. The culture filtrate of *A. violaceofuscus* exhibited higher concentration of secondary metabolites that enhanced the total chlorophyll content, plant height and biomass of sunflower and soybean seedlings under heat stress.

Ismail *et al*. (2021) reported that *Penicillium Glabrum* acted as heat stress relieving endophyte in Soybean and Sunflower. The results revealed that *P. glabrum* (DryR- 30) associated with sunflower and soybean boosted their host growth attributes like total biomass, plant height and chlorophyll contents when exposed to 40 ºC. In the light of the above investigations the present study was carried out to understand the heat stress tolerance of rice inoculated with fungal endophytes isolated from the plants growing in harsh environment.

III MATERIAL AND METHODS

The investigations on "Screening and evaluation of fungal endophytes modulating plant growth under heat stress" were carried out in the Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru and the Indian Institute of Horticulture Research (IIHR), Hesaraghatta, Bengaluru-89. Field experiment was carried out in the experimental plot K-block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru- 65. The materials used and methodology followed while conducting the experiments are presented in this chapter.

3.1 Collection of fungal endophytes

Thirty endphytic fungi were isolated from different plant species of Thar Desert, Rajasthan and Cold desert of Western Himalaya, India (Table 1) and preserved in the School of Ecology and Conservation Laboratory, University of Agricultural Sciences, GKVK, Bengaluru. They were rejuvenated on potato dextrose agar.

Table 1: List of fungal endophytic isolates used for screening of thermotolerance

Place	Host plants	Fungal Isolates
Thar Desert, Rajasthan, India	*Acacia jacquemontii*	ACJ 10
	Lasiurus scindicus	LAS 4, LAS 6
	Prosopis cineraria	PRC 2
	Salvadora persica	SAP 3, SAP 6
	Acacia tortilis	ACT 2, ACT 3
Western Himalayas	Xerophytic plants	X1, X2, X3, X4, X5, X6, X7, X8, X9 and X10
	Artimisia sp.	A1, A2, A3, A4, A5, A6, A7, A8, A9, A10, A11, A12 and A13.

3.2 Screening of fungal endophytes for thermotolerance

The fungal isolates were screened for their thermotolerance by exposing to different temperatures. Mycelial disc (5 mm) of five days old fungal colonies were placed aseptically on Potato Dextrose Agar (PDA) and incubated at 28 ^{o}C' 30 ^{o}C, 32 ^{o}C, 34 ^{o}C, 36 ^{o}C, 38 ^{o}C, 40 ^{o}C, 42 ^{o}C and 44 ^{o}C for five days. After incubation, the diameter of the radial growth of the colony was recorded.

3.3 Morphological and microscopic observation of thermotolerant fungal endophytes

Endophytic fungi were inoculated on PDA medium and incubated at 30 ºC. Color, texture, and pigmentation of the colony were documented. In addition, Slide culture technique was followed to observe fruiting body and spore characteristics. PDA agar block was mounted on a glass slide and placed in a sterile petriplate with wet filter paper. Then, on all four sides of the agar block, fungal spore or mycelia were inoculated. A sterile glass slide/coverslip was placed on top of the inoculated agar block and incubated at 30 ºC until sporulation occurred. The stained lacto phenol slide was observed under light microscope (Novex, Holland). Mycelial characteristics and spore structures (like pycnidia, conidiospore conidiophore, phalidae and metullae, conidia and ascospore) were identified using standard manual (Watanabe, 2010).

3.4 Molecular characterization of thermotolerant fungal endophytes

3.4.1 Genomic DNA

Genomic DNA of endophytic fungal isolates were extracted by CTAB (Cetyl trimethyl ammonium bromide) method (Vainio *et al*., 1998). About 500 mg of fungal mycelia were macerated using sterile pestle and marter in liquid nitrogen and a pinch of Polyvinylpyrolidone (PVP) to obtain a fine powder. The powder was added to 3ml extraction buffer (50 mM Tris-HCl of pH 8.0, 50 mM EDTA, 0.7 M NaCl, pinch of PVP and 2 µl of β-mercaptoethanol), mixed gently and incubated at 65 ºC for 45 min with intermittent shaking. The lysate was extracted by adding an equal volume of chloroform: isoamyl alcohol (24:1) and centrifuging at 10,000 rpm for 10 min. The supernatant was transferred to new tube and further chloroform: isoamyl alcohol was added and centrifuged again. This step was repeated, until the middle layer got disappeared. The genomic DNA was precipitated by adding chilled isopropanol (600 µl) and incubated at -20 ºC overnight. After incubation, the tubes were centrifuged at 10,000 rpm for 10 min and the aqueous layer was discarded and the pellet was washed by using 500 µl of 70 % chilled ethanol. The pellet so obtained was air dried and dissolved in 20 µl of deionized sterile water. The dissolved pellet was treated with five µl RNase A enzyme and incubated at 37 ºC for one hour.

3.4.2 Agarose gel electrophoresis

Agarose gel (0.8 %) was prepared by dissolving 0.34 g of agarose in 40 ml of 1X TAE (Tris acetate EDTA) buffer. The intercalating dye ethidium bromide (2.5 µl) was added and poured into the gel-casting tray placing single comb at one side and allowed for solidification. Then the comb was removed and the gel was transferred to gel tank containing 1X TAE buffer. The gel loading dye was added to the DNA and the electrophoresis was carried out at 100 V for 30 min. The DNA band in the gel was visualized under UV trans-illuminator and documented using a gel documentation unit (Vilber, E-Box CX5.TS, France). The DNA was quantified using a Nano Drop (BioSpec-nano, Shimadzu).

3.4.3 Polymerase Chain Reaction (PCR)

The internal transcribed spacer (ITS) region of genomic DNA was amplified using universal primer ITS1-F (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4-R (5′ TCCTCCGCTTATTGATATGC 3′) by polymerase chain reaction (PCR). PCR amplification was performed using thermocycler (Mater cycler Nexus gradient, Eppendrof, India) with a 20 µL reaction mixture that comprised of 2 µL 1X taq buffer with $MgCl_2$ (1.5 mM), 2µL dNTP's (10 mM), 0.5 µL each primer (10 pmol), 0.3 µL Taq DNA polymerase (3U) and 1µL template DNA (100 ng). The PCR was carried out with an initial denaturation at 94 °C for 4 min, followed by 35 cycles at 94 °C for 30s, 55 °C for 1 min and 72 °C for 30s, and final extension at 72 °C for 12 min. Then the amplified product of DNA was electrophoresed using one per cent agarose gel. The DNA band was visualized under UV and documented using a gel documentation unit (Vilber, E-Box CX5.TS, France). The amplified DNA was eluted by using gel extraction kit to obtain purified PCR product.

3.4.4 Elution of the amplified DNA (Gel extraction)

The Gene JET™ Gel Extraction Kit (Thermo Scientific) was used for rapid and efficient separation of DNA fragments from agarose gel. The gel slice containing the DNA was excised using a sterilized razor blade and was placed into a pre-weighed 1.5 ml tube and an equal volume of binding buffer was added (100 mg gel slice: 100 µL of binding buffer). The mixture was incubated at 60 °C till the gel slice was completely dissolved. The solubilized gel solution was added to the Gene JET™

purification column and centrifuged at 10,000 rpm for 60 sec. The flow-through was discarded and the column was placed back into the same collection tube. Further, 700 µl of wash buffer was added and washed by centrifugation at 10,000 rpm for 60 sec. The tube was again centrifuged for 60 sec to remove the residual wash buffer. Purification column was placed into a clean 1.5 ml micro centrifuge tube and 20 µl of elution buffer was added and centrifuged at 10,000 rpm for 60 sec. The eluted DNA was checked for its concentration using nano drop instrument and the eluted DNA was sequenced by SciGenome labs *Pvt. Ltd*., Cochin, Kerala, India. The sequence data received from the company was analysed for homology using NCBI GenBank.

3.4.5 Sequence analysis and homology search

Sequence results were analysed using the online software National Centre for Biotechnology Information (NCBI), USA. The BLAST (Basic Local Alignment Search Tool) search was done for partial length sequence homology with NCBI data (http//www. Ncbi.nlm.nih.gov/BLAST/) (Altschul *et al*., 1990).

3.4.6 Phylogenetic analysis

Phylogenetic analysis was performed to know the relationship between identified species and the sequence of the same species deposited in the NCBI GenBank. Phylogenetic analysis was performed using MEGA10 software and a phylogenetic tree was generated using neighbour-joining algorithm (Kumar *et al*., 2016).

3.4 Evaluation of selected fungal endophytes imparting thermo-tolerance in Rice

Evaluation of fungal endophytes on their ability to impart heat tolerance in thermo sensitive rice variety IR-64 was carried out in plant growth chamber at Indian Institute of Horticulture Research (IIHR), Hesaraghatta, Bengaluru. There were two sets of experiments. 1. Heat stress (45 ^{o}C for 7 h per day for 10 days) and 2. Without heat stress (normal temperature conditions, 30±0.5 ^{o}C). Each set comprised the following treatments with three replications.

1. Control (uninoculated plants)
2. *Aspergillus flavus*
3. *A. nidulans*

4. *A. terrus*

5. *Chaetomium* sp. L4

6. *Chaetomium* sp. L6

7. *Ceriporia lacerate*

8. *Endomelanconiopsis endophytica*

9. *Penicillium funiculosum*.

Rice seeds were surface sterilized using 3 per cent sodium hypochlorite followed by 70 per cent alcohol. The surface sterilized seeds were repeatedly washed with sterile water and soaked for overnight. These seeds were kept for germination for two days. The pre-germinated seeds were sown in pots filled with soil and FYM (1:1w/w). Three seedlings were maintained per pot. The plants were grown for fifteen days under greenhouse. The thermotolerant endophytes were inoculated by stem prick method (Bhunjun *et al.*, 2020) and allowed to colonize for 10 days. After colonization, set-1 seedlings were exposed to 45 ^{o}C for 7 hours per day for 10 days in growth chamber and set-2 seedlings were grown under normal conditions in greenhouse. The growth parameters *viz.*, plant height, number of tillers, number of leaves, root volume, fresh and dry weight of shoot and root were recorded. The inoculated endophytes were re-isolated.

3.6 Determination of physiological parameters induced by fungal endophytes due to temperature stress

Based on the evaluation of the eight thermotolerant fungal endophytes, the best four endophytes viz., *Aspergillus flavus, A. nidulans, E. endophytica* and *P. funiculosum* were selected and further evaluated as detailed in the previous experiment (3.5) for physiological parameters using 35 days old seedlings. The growth attributes such as plant height, number of tillers, number of leaves and dry weight of shoot and root and physiological attributes such as Melondialdehyde (MDA), Relative water content (RWC), gas exchange parameters, photosynthetic pigments, osmolytes, antioxidants and phytohormones were recorded. There were five replications for each treatment.

3.6.1 Determination of Heat Scorch Index

At the end of experiment, calculated heat scorch by recording the per cent scorch occurring in a leaves of rice and given rating as follows,

Grade	Scorch scale
0	0 % burn, healthy leaves
3	30 % burn, leaves becomes yellowish, start to drying of tips
6	60 % burn, half of the leaves dried
9	100 % burn, completely leaves are dried

$$\text{Heat Scorch Index (\%)} = \frac{Sum\ of\ all\ scorch\ rating}{Total\ number\ of\ leaves \times Maximum\ scorch\ grade} \times 100$$

Scorch Scale: 100 % 60% 30% 0%

3.6.2 Determination of Melondialdehyde (MDA)

MDA content was determined by tribarbitaric acid (TBA) method (Hodges *et al*., 1999). The leaf tissue (0.5 g) was homogenized in a mixture of 5 ml (5 % w/v) trichloroacetic acid and 0.5 ml of beta hydroxytoulene (0.5 % in methanol). The mixture was kept in boiling water bath for 30 minutes. After then 1 ml of supernatant was taken and 1 ml of thiobarbituric acid was added into it. Then the mixture was again placed in boiling water bath for 30 minutes. After 30 minutes, the reaction was stopped by placing immediately on ice bath. Optical density (OD) was measured by using spectrophotometer (UV-VIS, Systronics Ltd., India) at 532 nm and 600 nm. The MDA content was calculated using the formula given below.

$$\text{MDA equivalent (µmol/g)} = \frac{(OD532 - OD600) \times 4.69 \times V1 \times V2}{\varepsilon \times W \times A}$$

Where,
V1= Grinding volume, V2= Volume made up of extractant

ε = Molar extinction coefficient of MDA = 155 Mm/cm

W = Weight of tissue taken

A= Aliquot taken

3.6.3 Relative water content

Relative water content (RWC) was measured by following the method described by Barrs and Weatherley (1962). The leaf samples were collected and the fresh weight was recorded immediately (FW). Later the leaves were soaked in water for 24 h, surface water was removed by tissue paper and the turgid weight was recorded. Then the leaf samples were dried at 60 ^{o} C for two days and the dry weight (DW) was recorded. The RWC of the leaves was calculated by the following formula.

RWC % = [(FW – DW) / (TW - DW)] × 100

3.6.4 Gas exchange parameters

The gas exchange parameters such as net photosynthetic rate, stomatal conductance and transpiration rate were measured from the youngest fully opened leaves using Portable Photosynthesis System, LICOR LI-6400XT (Biosciences, USA). Photosynthetically active radiation of 1000 µmol m^{-2} s^{-1} and the ambient CO_2 concentration of 400 µmol mol^{-1} were maintained during the measurements.

3.6.5 Estimation of photosynthetic pigments

The chlorophylls and carotenoid pigments were estimated by modified method described by Lichtenthaler and Buschmann (2001). Leaf tissue (100 mg) was taken from fully expanded leaf. The leaf samples were incubated in 10 ml Acetone: DMSO (Dimethyl sulfoxide) (1:1 v/v) mixture for overnight under dark condition. Absorbance of the extractants were read in spectrophotometer, (UV-VIS, Systronics Ltd., India.) at 663, 645, 652 and 480 nm for estimation of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content respectively.

Chlorophyll a (mg/g FW) = $[12.7(A663) - 2.69\,(A645) \times V] \div (1000 \times W \times a)$

Chlorophyll b (mg/g FW) = $[22.9(A645) - 4.68(A663) \times V] \div (1000 \times W \times a)$

Total Chlorophyll (mg/g FW) = $[27.8(A652) \times V] \div (1000 \times W \times a)$

Carotenoid (mg/g FW= $\{[A480 + (0.114 \times A663) - (0.638 \times A645)] \times V\} \div (1000 \times W \times a)$

Where, A = Absorbance at specific wavelengths,

V = Final volume of the extract (mL),

FW = Fresh weight of the sample (g),

A = Path length of light (1 cm).

3.6.6 Determination of Osmolytes

3.6.6.1 Estimation of Proline

Proline estimation was done by using colorimetric method (Bates *et al*., 1973). Leaf tissue (0.1g) was homogenized in 3 % sulphosalicyclic acid and centrifuged at 5000 rpm for 15 min. Supernatant (2 ml) was mixed with acid ninhydrin (2 ml) and glacial acetic acid (1 ml) in a test tube and incubated for 1 h at 100 °C. After incubation, the sample was placed on ice cubes to stop the reaction. Proline was extracted from the reaction mixture by adding 4 ml of toluene. Extracted proline appeared red in colour which was separated and transferred to new tubes. The absorbance was measured at 520 nm using spectrophotometer (UV-VIS, Systronics Ltd., India). A standard curve was made using different proline concentrations. The result was expressed as µmol of proline g^{-1} fresh weight of leaf sample.

Proline (µmol/g) = (P × T ×5)/(115.5 ×S)

Where, P-Proline (µg/mL)

T-Toluene (mL)

S- Weight of sample (g)

3.6.6.2 Estimation of Glycine betaine

Glycine-betaine in fresh leaf sample was estimated by the method described by Greive and Grattan (1983). Leaf sample (0.25 g) was homogenized with 5 ml of 0.05 % toluene and mechanically shaken for 24 h at 25 °C. The homogenized sample was centrifuged at 5000 rpm for 15 min. Aliquot of 1 ml was taken in test tube and mixed with 1 ml of 2 N HCl followed by 0.2 ml of potassium tri-iodide. This mixture was kept in ice water bath for 15 min. The precipitate formed was dissolved in 5 ml of 1, 2-dichloro ethane and mixed vigorously. The supernatant was carefully aspirated with 1 ml micropipette and transferred to new tube which was kept on ice. The absorbance was measured at 365 nm with a UV – visible spectrophotometer (UV-VIS, Systronics Ltd., India). Reference standards of glycine betaine (0.05 – 0.25

µg/mL) were prepared in 2 N sulphuric acid and a standard curve was prepared. The results were expressed as µmol g^{-1} of leaf tissue.

Glycine betaine (µmol/g) = (GB ×DCE ×V1)/(117.14 ×S×V2)

Where, GB- Galic betaine (µg/mL)

DCE- Dichloroethane (mL)

V1- Total volume of Extract (mL)

S- Weight of sample (g)

V2- Volume of extract taken (mL)

3.6.6.3 Total phenols

Total phenol content was estimated by spectrophotometric method using Folin Ciocalteu Reagent (FCR) as described by Madaan *et al.* (2011). The leaf tissue (0.5 g) was homogenized in 5 ml of 80 % ethanol using pestle and mortar. The homogenized sample was centrifuged for 15 min at 5000 rpm. The aliquot of 0.5 ml was taken in test tube and added 0.5 ml of FCR reagent followed by 2 ml of distilled water and mixed well. After 2 min, 2 ml of sodium carbonate solution was added and mixed thoroughly. The reaction mixture was allowed to stand at room temperature for 30 min and the blue color developed was read in spectrophotometer (UV-VIS, Systronics Ltd., India) at 650 nm. Gallic acid was used to develop standard curve and calculated total phenol content.

Total phenol content (mg gallic acid equivalents/g) = (GAE ×V1 ×V2)/(S ×A)

Where, GAE- Galic acid equivalents (µg/mL)

V1- Total volume of Extract (mL)

V2- Volume made (mL)

S- Weight of sample (g)

A- Assay volume (mL)

3.6.6.4 Total Flavonoids

Total flavonoid content was determined as per Madaan *et al.* (2011). Leaf tissue (0.5 g) was homogenized in 5 ml of 80 % ethanol using pestle and mortar. The homogenized sample was centrifuged for 15 min at 5000 rpm. The extract of 0.5 ml was taken in a test tube and 0.15 ml of 5 % $NaNO_2$ followed by 1 ml of distilled water

was added. The mixture was incubated for 5 min at room temperature. After incubation, 0.3 ml of 10 % $AlCl_3H_2O$ was added and again the mixture was incubated for 6 min. Then, 1 ml of 1 M NaOH was added and the absorbance of pink color was recorded at 415 nm using spectrophotometer (UV-VIS, Systronics Ltd., India) against blank. The flavonoid content was calculated using rutin compound as a standard.

Total flavonoid content (mg Rutin equivalents/g) = (RE ×V1 ×V2)/(S ×A)

Where, RE- Rutin equivalents (µg/mL)

V1- Total volume of Extract (mL)

V2- Volume made (mL)

S- Weight of sample (g)

A- Assay volume (mL)

3.6.7 Enzyme assay

Enzyme extract was prepared by following the method of Chempakam *et al.* (1993). The leaf tissue (0.5 g) was homogenized in pre-chilled motor and pestle with 6 mL potassium phosphate buffer (50 mM, pH 7) and subsequently transferred to centrifuge tubes. Further, the volume was made up to10 ml and the mixture was centrifuged 5000 rpm for 15 min at 4 ºC using a refrigerated centrifuge. The resulted enzyme extract was used for the assay of protein and enzymes activities *viz*., superoxide dismutase (SOD) and catalase (CAT).

3.6.7.1 Super Oxide Dismutase (SOD) activity

Two sets of reaction mixture containing buffer, methionine, NBT, riboflavin, EDTA and enzyme extract were prepared in identical test tubes and one set was kept in light and other set in dark. The light set tubes were exposed to fluorescent light for 30 min and the absorption was measured at 560 nm using spectrophotometer (UV-VIS, Systronics Ltd., India). Test tubes without enzyme extracts were served as blank and assay protocol was follwed as given below (Du and Bramlage, 1994).

	Blank		Enzyme	
	Blank- Dark	Blank- Light	Sample -Dark	Sample-Light
Buffer (pH 7.8)	0.97 ml	0.97 ml	0.94 ml	0.94 ml
EDTA	0.03 ml	0.03 ml	0.03 ml	0.03 ml
Methionine	1.00 ml	1.00 ml	1.00 ml	1.00 ml
Enzyme extract	-	-	0.03 ml	0.03 ml
Riboflavin	0.50 ml	0.50 ml	0.50 ml	0.50 ml
NBT	0.50 ml	0.50 ml	0.50 ml	0.50 ml

Enzyme activity was calculated as follows and expressed as units/g protein.

SOD activity (units/g protein) = (A× V1)/(V2×S×P)

Where,

V1- Total volume (mL)

V2 – Volume of sample taken for assay (mL)

S- Weight of sample (g)

P- Protein (mg/g)

A= 1×Z unit/50

Z = {[(LB-DB)-(LS-DS)]/(LB-DB)×100};

LB- Light blank, SB- Dark blank, LS- light sample, DS- Dark sample,

50% reduction of colour = 1 unit of SOD, 50% = 1 unit, 1%=1unit/50

3.6.7.2 Catalase activity

Catalase activity was estimated as described by Masia (1998). The assay mixture comprised of 50 Mm potassium phosphate buffer, 0.3 % H_2O_2 (prepared immediately before use) and enzyme extract. The assay protocol was followed as given below. The activity was measured by monitoring the degradation of H_2O_2 using UV-visible Spectrophotometer (UV-VIS, Systronics Ltd., India) at 240 up to 5 min for 1 min interval.

	Substrate blank	Enzyme blank	Enzyme
50 mM Buffer	2.7 ml	2.9 ml	2.6 ml
0.3 % H_2O_2	0.3 ml	-	0.3 ml
Enzyme extract	-	0.1 ml	0.1 ml

The catalase activity was calculated by using the formula

Catalase activity (units/g protein) = (OD ×F ×V)/(S × W× P)

Where, OD - Optical density (For one OD change/min =187.528 units of enzyme)

F- Factor

V- Total volume

S - Sample taken for assay

W- Weight of sample

P- Protein (mg/g)

3.6.8 Profiling of phytohormones by Liquid Chromatogram-tandem mass spectrometry (LC-MS/MS)

Sample Extraction

The extraction procedure for different plant hormones was followed according to Pan *et al.* (2008). About 2 g of leaf sample was completely homogenized using 1-propanol/H_2O/concentrated HCl (2:1:0.002, v/v/v), sonicated for 30 minutes and kept overnight at 4°C. The following day, dichloromethane was added to the homogenate, sonicated for 30 min and then centrifuged at 12,000 rpm for 10 min. After centrifugation, the bottom layer was transferred to the conical flask containing sodium sulphate to remove the water traces and evaporated using flash evaporator. After completely dried, the sample was dissolved in 80% methanol and passed through C18 solid-phase extraction (SPE) cartridges.

LC-MS/MS conditions

The initial gradient was composed of 85 % solvent A (Water/Acetonitrile/acetic acid -95/5/0.05, v/v/v) and 15 % of solvent B (Acetonitrile/water/acetic acid - 95/5/0.05, v/v/v), held for 1 minute. At 12 minutes, the gradient was changed to 15 % of solvent A and 85 % of solvent B, held for 1 minute and a linear gradient followed by 85 % of solvent A and 15 % of solvent B in 14 minutes held for 0.5 minute. The system was then returned to the initial conditions at 15 minutes and equilibrated for 1 minute before the next injection. The flow rate was 0.2 ml/minute. The analytical column was 2.1 X 50 mm UPLC BEH-C18 column (Waters, USA) with 1.7µm particles. Guard column (Waters, USA) was used with a column temperature maintained at 25°C. The eluted hormones were identified and quantified by using a TQD-UPLC MS/MS (Tandem quadrupole – Ultra performance liquid chromatography, Waters, USA) system, optimized for the hormone analysis.

3.6.9 Confirmation of fungal endophytes present in inoculated rice plant

Rice plant inoculated with fungal endophytes (*A. flavus, A. nidulans, E. endophytica* and *P. feniculosum*) were collected at the end of experiment and processed for endophyte fungal isolation. Fungal colonies emerging from cut ends of plant shoot bits and were compared with respective mother cultures. Later, re-confirm the colonization of fungi by sequencing the re-isolated one (Somashekhar, 2018 and Manasa, 2019).

3.7 Study of plant growth promoting and stress alleviation traits in fungal endophytes

3.7.1 Phosphate Solubilisation

The inorganic phosphate solubilizing potential of fungal endophytes was evaluated *in- vitro* according to Jasim *et al.* (2013). The fungal plugs were inoculated on the plates of Pikovskaya medium (Appendix II) and incubated at 30 ^{o}C for 7 days. The diameter (mm) of clear zones around fungal plugs was measured.

3.7.2 Potassium solubilisation

The ability of potassium solubilisation by fungal endophytes was determined by growing on Aleksandrow media (Hu *et al*., 2006). Freshly grown 5 mm fungal mycelia was transferred on plates containing Aleksandrow medium (Appendix II) incubated at 30 ^{o} C for 7 days. After incubation, the clear zones formed around the colonies were measured.

3.7.3 Zinc solubilisation

Zinc solubilisation ability of fungal endophytes was determined by growing on minimal media containing ZnO as a zinc source (Fomina *et al.*, 2005). Fungal inoculation was carried out with 5 mm diameter discs of mycelium excised from actively-growing cultures which were then placed on the surface of zinc compound-amended plates (Appendix II). These were incubated at 30 ^{o}C for 7 days. The diameter (mm) of clear zones around fungal colony was measured.

The solubilization index (SI) was determined for all the above elements (P, K and Zn) according to Premono *et al.* (1996) using the formula:

$$SI = \frac{\text{width of the colony} + \text{halozone}}{\text{width of the colony}}$$

3.7.4 Siderophore production

The siderophore production was assessed by placing 5 mm freshly grown mycelial plugs on Chrome Azurol S (CAS) solid medium (Schwyn and Neilands, 1987). The fungi-inoculated plates were incubated at 30^{o}C for 7 day and measured diameter of the colony and orange colour zone surrounding the colony. Siderophore producing index (SPI) was calculated as the ratio of (colored zone+colony)/colony diameters (Desai *et al*., 2012).

3.7.5 Determination of phytohormones in fungal filtrate using High Performance Liquid Chromatogram (HPLC)

Endophytic fungi were inoculated on potato dextrose agar medium for 7 days at 30 ^{o}C. One disc (5 mm) of each fungal culture was inoculated in 100 mL of potato dextrose broth and incubated at two different temperatures 30 ^{o}C and 40 ^{o}C for 14 days. Non-inoculated media were considered as control. For IAA quantification, the CD broth was supplemented with 5 mg/ml of tryptophan (Tien *et al*., 1979).

After 14 days, culture filtrate of each flask was filtered through Whatman No. 42 filter paper. The pH of filtrate was adjusted to 2.5–3.0 by adding 0.1 N HCl or KOH. Extraction was done by the method followed by Rachev *et al*. (1993). Culture filtrate was extracted using nearly three times ethyl acetate in a separating funnel. The organic layer was separated and passed through anhydrous sodium sulphate. The solvent was evaporated in a rotary vacuum evaporator at 40^{o}C and 10 rpm (Hasan *et al*., 2002). The residue was dissolved in HPLC-grade acetonitrile/methanol.

The HPLC analyses were carried out on a Shimadzu instrument (Prominence – I, LC-2030C) equipped with a UV detector (LC-2030 UV detector) and fitted with a C18 reverse phase HPLC column (Shim-pack GIST C18, Dimension 250 X 4.6 mm, particle size 5 µm). The column temperature, 30^{o}C was maintained for all the samples with other specific conditions as given below.

Phytohormone	Solvent	Wavelength (nm)	Flow rate (mL/min)	Retention time
IAA	Methanol:water (80:20)	270	1.0	2.1
GA	Methanol:water (70:30)	208	0.8	3.1
SA	Acetonitrile:Acetic acid 0.5 % (90:10)	302	1.0	2.7
ABA	Acetonitrile:Acetic acid 0.5 % (80:20)	254	0.8	3.4

3.7.6 Total phenolic and flavonoid contents

Endophytic fungi were inoculated on PDA agar for 7 days at 30 ^{o}C. One disc (5 mm) of each fungal culture was inoculated in 100 mL of PDA broth and incubated at two different temperatures 30 ^{o}C and 40 ^{o}C for 14 days. After 14 days, filtrate and biomass of fungi were separated by filtration using sterile filter paper. The filtrate was extracted three times with ethyl acetate using separated funnels and concentrated using a rotary evaporator until dried (Salini *et al*., 2015). The dried extract was dissolved in ethanol and stored at -40^{o}C for estimation of phenols and flavonoids.

The total phenol content of fungal extract was determined by making the standard solution of gallic acid (Pekal and Pyrzynska, 2014). One mL of extract (test sample) and gallic acid (standard) were put into separate test tubes and later 0.1 mL of Folin-Ciocalteu reagent and 0.9 mL of distilled water were added into each tube. Each mixture was incubated at room temperature for 5 minutes. one mL of 7 % sodium carbonate and 0.4 ml of distilled water were added into the test tube and incubated for 30 minutes at room temperature. The reaction mixture was measured at 765 nm using spectrophotometer (UV-VIS, Systronics Ltd., India). Total phenolic content was expressed as mg of gallic acid equivalent to per gram of dried extract (mg GAE/g dry weight).

The total flavonoid content of fungal extract was estimated by using quercetin compound as standard. Extract (500 µl), quercetin (standard), and ethanol (blank) were put into separate test tubes and later 1.5 mL of ethanol was added into each tube. Then, 0.1 mL of aluminum chloride solution (0.1 g/ ml), 0.1 mL of sodium acetate (1 M) and 2.8 mL of distilled water were added to each tube. Reaction mixture was incubated for 30 minutes at room temperature and absorbance was measured at 415

nm. Total flavonoid content is expressed as mg quercetin equivalent to per gram of dried extract (mg QE/g dry weight) (Kaur and Singh, 2015).

3.7.7 Total antioxidants

The antioxidant activity assay was performed by free radical scavenging method using ABTS [2, 2'-azino-bis (3-ethyl-benzthiazolin-6-sulfonicacid)] (Salini *et al.,* 2015). A stock solution of ABTS, 7 mM (Sigma- Aldrich, United States) was prepared by co-dissolving ABTS and potassium persulfate ($K_2S_2O_8$, Merck KGaA, Darmstadt, Germany) and keeping the resulting mixture for 12–16 h in the dark to form stable radical cations (ABTSC). The solution was diluted with ethanol to obtain an absorbance of 0.706 at 734 nm. Endophytic fungal extracts (50 µL) were dissolved in 3 mL of diluted $ABTS^+$ solution. The scavenging activity of the fungal extracts was assessed from the percentage of decolorization at 734 nm after 2 min of reaction at room temperature. The $ABTS^+$ scavenging activity (%) was calculated using the equation as follows:

$$\text{ABTS+ scavenging activity} = [(OD_{734control}-OD_{734sample})/OD_{734control}]*100.$$

3.8 Field evaluation of fungal endophytes on growth and yield of Paddy during summer

The field experiment was conducted to assess the field performance of efficient endophytes in aerobic rice (Variety IR -64) during summer, February-June 2021 at experimental plot of K-block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru. During crop growth period, the average rainfall was about 48.5 mm, mean relative humidity was 86 per cent and the average maximum and minimum temperature was 31.06 ^oC and 18.28 ^oC, respectively. The experiment comprised of five treatments *viz.,* T1: Control, T2: *A. flavus*, T3: *A. nidulans*, T4: *E. endophytica*, and T5: *P. funiculosum* with four replications under Randomized Complete Block Design (RCBD). All the treatments received 100 % RDF (Recommended Dose of fertilizer), FYM (Farm yard manure) and NPK.

3.8.1 Mass production of selected fungal endophytes

Fungal endophytes were cultured on PDA medium supplemented with 100 µg/mL of ampicillin and grown at 30 ^{o} C. After 20 days of growth, conidia were harvested from plates by adding 10 mL of sterile water and gently scraping off conidia with a sterile glass slide. The suspension was filtered through four layers of sterile cotton cheese cloth gauze. The spore load was approximately 10^5 spores mL^{-1} in the suspension by using haemocytometer and the suspension was used for field application.

3.8.2 Land preparation

The land was brought to fine tilth with one deep ploughing and three harrowings. The residues of the previous crop and weeds were removed from the experimental area. The land was levelled with plank and divided into 20 plots, each plot with a size of 2.0 m X 1.5 m. Small raised bunds were formed around each plot to mark individual treatment and irrigation channels were formed between the replications. Further, individual plots were leveled and the flat beds were converted into ridges and furrows at 30 X 10 cm distance.

3.8.3 Sowing and endophytic fungal application

The standard agronomic practices for aerobic rice were followed for sowing of paddy in the experimental field. The prepared fungal spore suspension was sprayed at 25 and 40 days after sowing (DAS)

3.8.4 Fertilizer Application

The crop was raised by following recommended package of practices. The fertilizers were applied at the rate of 120:40:40 kg N, P_2O_5, K_2O per hectare. One third dose of nitrogen and entire dose of P_2O_5 and 50 % of the K_2O were applied as basal dose. The remaining nitrogen was applied in two equal split doses, one at 30 days after sowing (DAS) and another at 60 DAS, remaining 50 % of the K_2O applied along with last dose of N. The intercultural operations like weeding was done at regular intervals and necessary plant protection measures were adopted during the crop growth period.

3.8.5 Irrigation

For ensuring proper germination and plant stand, irrigation was given immediately after sowing. Subsequent irrigation was provided as and when required.

3.8.6 Harvesting

Harvesting was done by manually when grains turn golden yellow color.

3.8.7 Observations recorded

Observations for growth and yield attributes were recorded. Five randomly selected plants from each treatment leaving first two border rows from all the four sides in order to avoid sampling error were chosen for recording data of various traits. Average data from the sampled plants with respect to different traits were used for various statistical analysis. The following were the observations recorded.

Plant height (cm)

The plant height was measured in centimeter from ground level to the tip of the plant. After emergence of panicle, the height was measured from base of plant to tip of the panicle.

Tiller number/hill

In each plot, number of tillers per hill was counted at 35, 70 and 120 days after sowing (DAS).

Panicle length (cm)

Panicle length was measured at the time of maturity from the base of panicle to the tip of last spikelet prior to harvesting.

Panicle number/ hill

Total number of panicles per hill was counted at the time of maturity.

Grain and straw yield (t/ha)

The grains were separated by threshing separately from each net plot and were dried under sun for three days. Later winnowed and cleaned and then weight of the grains per net plot was recorded and converted to t/ha. Likewise, yield of straw was calculated by converting straw yield per plot.

3.9 Statistical analysis

The statistical analysis was done by using WASP 2.0 (Web Agri Stat Package 2) (www.icargoa.res.in/wasp2/index.php) and the means were separated by Duncan's Multiple Range Test (DMRT).

IV RESULTS AND DISCUSSION

Plant stress tolerance is regulated by multiple factors. Since heat or high temperature stress is one of the important factors, regulating growth and development of crops. There are reports on endophytes modulating the plants to alleviate heat stress (Shekhawat *et al*., 2021). In this study, efforts are being made to explore the fungal endophytes mediated heat tolerance in rice. The fungal endophytes were screened for tolerance under laboratory conditions. The selected fungi were identified using ITS region sequences and evaluated for thermotolerance. The results obtained were presented and discussed in this chapter.

4.1 Screening of fungal endophytes for thermotolerance

Thirty fungal isolates (eight fungi from the plants growing in Thar Desert and 22 from the Himalayan cold desert) were screened against different temperatures (28, 30, 32, 34, 36, 38, 40, 42 and 44 ^{o}C) on potato dextrose agar (Table 2). Among thirty isolates, five isolates, ACT 2, LAS 4, LAS 6, PRC 2 and SAP 3 of Thar desert and three isolates A2, A7 and X5 of Himalayan cold desert showed growth up to 40 ^{o}C. This indicated that these isolates can withstand heat stress up to 40 ^{o}C. However, the four isolates of Thar Desert (LAS 4, LAS 6, PRC 2 and SAP 3) showed growth even at 44 ^{o}C indicating their degree of tolerance to high temperature (Plate 1). Rest of the 22 fungal isolates were found mesophylls as they could grow in the temperature up to 32 ^{o}C. In addition, LAS-4 and LAS 6 isolates showed profuse growth within five days of incubation.

Thar and Himalayan cold deserts have higher temperature during the summer (50 °C and 40 ^{o} C respectively). The plants growing in such environment harbour a huge array of fungal endophytes (Tewari and Kapoor, 2013; Sangamesh *et al*., 2017). The current study revealed that the five of Thar and three of cold desert isolates could sustain growth up to 40 ^{o} C. This may be due to habitat adapted phenomenon. However, the radial growth varied among the isolates which may be attributed to genetic factors of the fungal species (Yang *et al*., 2016).

Habitat adapted phenomenon occurred by modification of genetics and physiology of fungi (Cooney and Emerson, 1964; Dix and Webester, 1995; Aguilar, 1996; Stetter, 1999). Thermotolerant or thermophilic fungi accumulated variou

Table 2: Effects of different temperatures on colony growth of fungal isolates

	Fungal isolates	Diameter of the fungal colony (cm) at different temperature								
		28 °C	30 °C	32 °C	34 °C	36 °C	38 °C	40 °C	42 °C	44 °C
Thar desert	ACJ-10	8.50±0.13	8.50±0.14	8.50±0.20	3.06±0.03	-	-	-	-	-
	ACT-2	6.26±0.26	7.06±0.12	6.86±0.42	4.49±0.08	3.33±0.08	2.80±0.05	1.43±0.01	-	-
	ACT-3	4.16±0.13	4.86±0.06	5.23±0.03	6.00±0.20	2.83±0.08	2.50±0.05	-	-	-
	LAS-4	8.50±0.09	8.50±0.01	8.50±0.03	8.50±0.03	8.50±0.01	8.50±0.01	5.23±0.03	4.00±0.45	1.33±0.09
	LAS-6	8.13±0.23	8.50±0.42	8.50±0.12	8.50±0.03	8.50±0.01	8.50±0.01	8.50±0.03	4.43±0.03	1.16±0.09
	PRC-2	4.96±0.08	5.33±0.33	5.50±0.15	5.16±0.08	4.70±0.03	3.43±0.06	3.23±0.06	2.76±0.12	1.70±0.06
	SAP-3	5.56±0.33	5.83±0.08	6.33±0.08	6.06±0.18	5.03±0.08	4.60±0.20	3.33±0.03	2.3±0.05	1.13±0.06
	SAP-6	5.63±0.12	4.43±0.03	1.83±0.06	0.46±0.03	0.26±0.06	-	-	-	-
Himalayan cold desert	A1	6.13±0.23	5.80±0.10	5.76±0.09	2.73±0.12	1.83±0.03	0.6±0.05	-	-	-
	A2	4.36±0.09	5.67±0.09	4.5±0.17	3.46±0.12	2.83±0.09	1.80±0.06	1.43±0.03	-	-
	A3	6.03±0.15	5.20±0.12	4.13±0.09	2.80±0.06	3.00±0.12	0.73±0.03	-	-	-
	A4	4.50±0.06	3.70±0.25	1.73±0.03	-	-	-	-	-	-
	A5	5.37±0.09	4.00±0.12	2.90±0.06	2.00±0.06	1.32±0.15	-	-	-	-
	A6	3.76±0.09	2.90±0.06	1.86±0.09	1.20±0.03	-	-	-	-	-
	A7	5.33±0.33	4.36±0.09	4.03±0.09	3.80±0.12	2.83±0.09	2.03±0.20	1.40±0.06	-	-
	A8	4.8±0.06	3.03±0.03	1.76±0.09	-	-	-	-	-	-
	A9	2.30±0.06	1.90±0.06	0.63±0.09	-	-	-	-	-	-
	A10	3.56±0.21	2.73±0.15	1.86±0.12	1.56±0.21	1.43±0.12	0.86±0.06	-	-	-
	A11	4.03±0.12	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-	-	-
	A12	1.90±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-	-	-
	X1	3.50±0.17	2.93±0.09	2.26±0.09	1.36±0.09	-	-	-	-	-
	X2	3.66±0.03	2.40±0.06	1.80±0.06	1.33±0.09	-	-	-	-	-
	X3	5.96±0.09	5.50±0.06	5.00±0.06	4.43±0.03	1.43±0.07	-	-	-	-
	X4	1.76±0.09	1.26±0.07	-	-	-	-	-	-	-
	X5	8.76±0.03	8.66±0.03	8.16±0.09	8.06±0.18	3.46±0.09	1.93±0.09	1.13±0.06	-	-
	X6	3.96±0.09	2.9±0.06	1.86±0.09	1.26±0.07	-	-	-	-	-
	X7	4.00±0.12	2.93±0.09	2.26±0.09	1.26±0.03	-	-	-	-	-
	X8	3.93±0.09	2.90±0.06	1.63±0.09	-	-	-	-	-	-
	X9	1.9±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-	-	-
	X10	3.63±0.09	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-	-	-

Note: ACT *Acacia jacquemontii,* ACT*Acacia tortilis,* LAS *Lasiurus scindicus,* PRC *Prosopis cineraria,* SAP *Salvadora persica, A Artimisia* sp and X xerophytic plant.

Data shown above are the means of three replication with ± standard error.

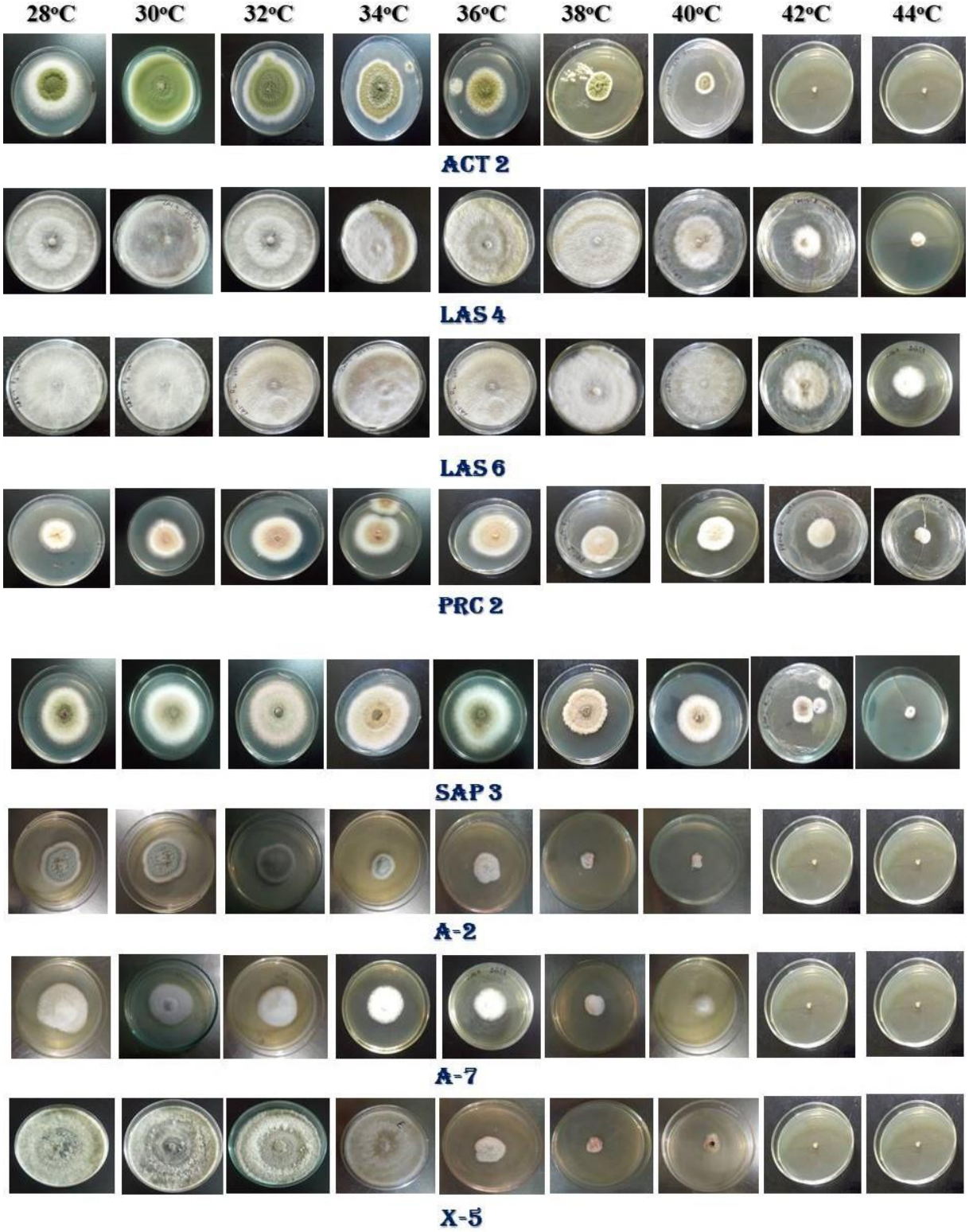


Plate 1: Growth of the selected fungal endophytes at different temperatures on fifth day.

compounds such as mannitol, trehalose, putrescine, spermidine, spermine *etc*. in their mycelia or spores at high temperature (Singhania *et al*., 1991). The *Thermomyces lanuginosus* synthesizes trehalose in spores as well as mycelia when grown at 50 ^{o}C. This suggested that the trehalose accumulation is related to increased temperature (Johri, 1980).

High temperature induces melanin pigment production in microorganisms (Kuluncsics *et al*., 1999; Cockell and Knowland, 1999). The three endophytes namely, SAP 3, LAS 4 and PRC 2 produced pigments in mycelia and spores when exposed to high temperature. This could help for survival of the organism (Gupta *et al*., 2015). In this study, SAP 3 produced dark red cleistothecia and PRC 2 produced yellow conidia with more spores at 40 ^{o} C. The isolate LAS 4 produced deep red pigment at 40 ^{o} C (Plate 2). This revealed that these isolates can tolerate to higher temperature by pigment production. However, remaining 22 isolates though isolated from the plants of the same environment did not grow at elevated temperature. Because these fungi may sustain increased temperature only if they are present in their host plant which is also known as habitat adapted symbiosis. For example, *Curvularia protuberata* isolated from *Dichanthelium lanuginosum* growing at geothermal soil (>50 ^{o} C) could sustain high temperature when it was present in host plant. Neither of the organisms (fungus or plant) can survive individually when exposed to heat stress >38 °C (Redman *et al*., 2002).

Previous researchers documented that Thar desert has habitat of thermotolerant fungi such as *Aspergillus niger, A. flavus, A awamori, Epicoccum nigrum, Neosartorya glabra*, and *Phoma* sp. (Korejo *et al*., 2017; Saket and Arnold, 2019). Similarly, Salar and Aneja (2006) documented thermophilic (*Aspergillus fumigatus, Chaetomium senegalense, Emericella nidulans, Penicillium chrysogenum*) and thermotolerant (*Chaetomium thermophile and Stilbella thermophile*) molds in temperate soils of North India. Thermophiles are more frequently isolated from temperate soils than tropical soils (Ellis and Keans, 1981). This study suggested that the endophytes isolated from hot as well as from cold deserts (ACT 2, LAS 4, LAS 6, PRC 2, SAP 3, A2, A7 and X5) could sustain heat stress.

4.2 Identification of selected thermotolerant fungal endophytes

4.2.1 Morphological and microscopic observation of thermotolerant fungal endophytes

The eight selected thermotolerant fungal endophytes were observed for their colony characters, fruiting bodies and spore characters (Plate 3). Isolate ACT-2 formed granular, flat colonies with radial grooves. Initially colony was yellow in colour gradually turned in to dark green on potato dextrose agar (PDA). Similarly, SAP-3 isolate produced yellow with light green colony and form dark reddish on reverse side of the plate. PRC-2 produced suede-like and cinnamon-buff to sand-brown colonies with yellow to deep brown reverse. All these three isolates had biseriate conidiophore with slight changes in conidial head. Conidial head of the ACT-2 was typically radiate, loose columns with globose to subglobose head and pale green conidia. PRC-2 had compact columnar with globose to ellipsoidal head with yellow coloured conidia. The isolate SAP-3 had short and columnar hyaline conidial head with globose in shape and it also produces dark red cleistothecia with ascospores. These three isolates belong to *Aspergillus* species (Plate 3a, 3b and 3c).

LAS-4 and LAS-6 isolates formed white mycelial colony on PDA. LAS- 4 produced dark reddish globose to elongated ascomata with pear shaped ascospores whereas LAS-6 produced globose to ovate ascomata with fusiform ascospores. These two isolates were identified as *Chaetomium* sp (Plate 3d and 3e).

The colony of A-2 isolate was greyish green with funiculose texture on PDA and produced biverticillate condiophore with subterminal branches and ellipsoidal conidia. It belongs to *Penicillium* genera (Plate 3f). In case of A-7, white fluffy colony was observed (Plate 3h). The isolate, X-5 produced brownish to black colour mycelia and formed pycnidial conidiomata with ellipsoidal conidia and it identified as *Endomelanconiopsis* sp (Plate 3g).

All isolates (ACT-2, LAS-4, LAS-6, SAP-3, PRC-2, A-2 and X-5) belong to ascomycota except A-7 which belong to basidiomycota. In addition, ACT-2, PRC-2, SAP-3 and A-2 isolates belongs to the class eurotiomycetes. The isolates, LAS-4 and LAS-6 belongs to the class sordariomycetes and the X-5 belong to the class saccharomycetes.

SAP 3

PRC 2

LAS 4

Plate 2: Growth of thermotolerant fungal isolates in PDA broth *i.e.*, SAP-3, PRC 2 and LAS 4 at 30 ^{o}C (left) and 40 ^{o}C (right)

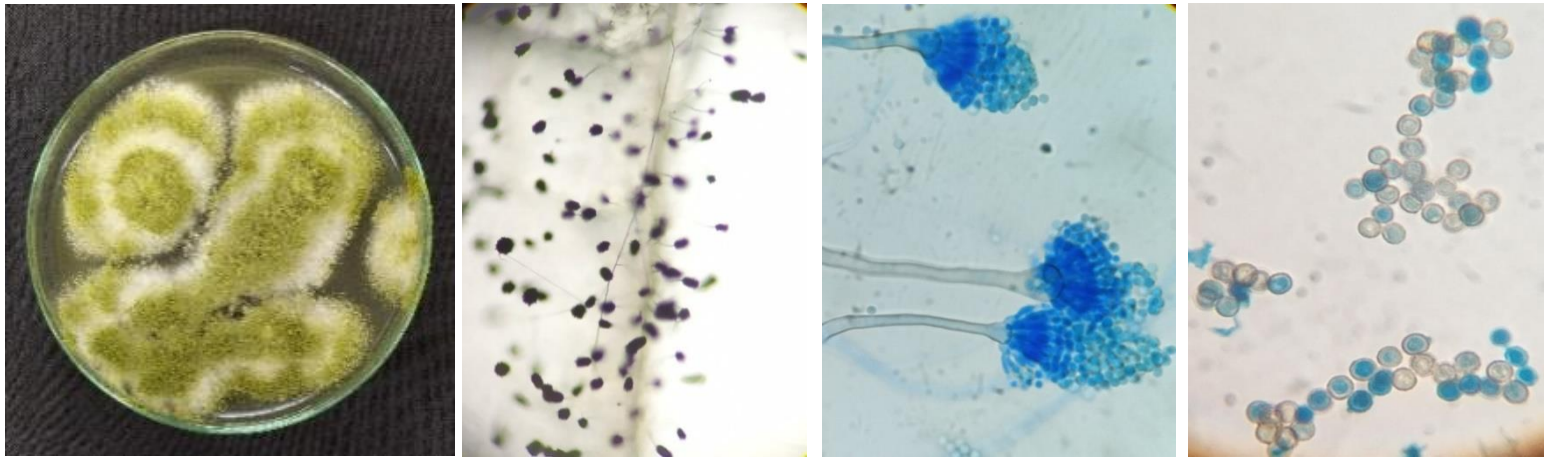


Plate 3a: ACT-2 (left to right) Colony, Conidiophore under 10X, Conidial head and stipe, 100X and Conidia

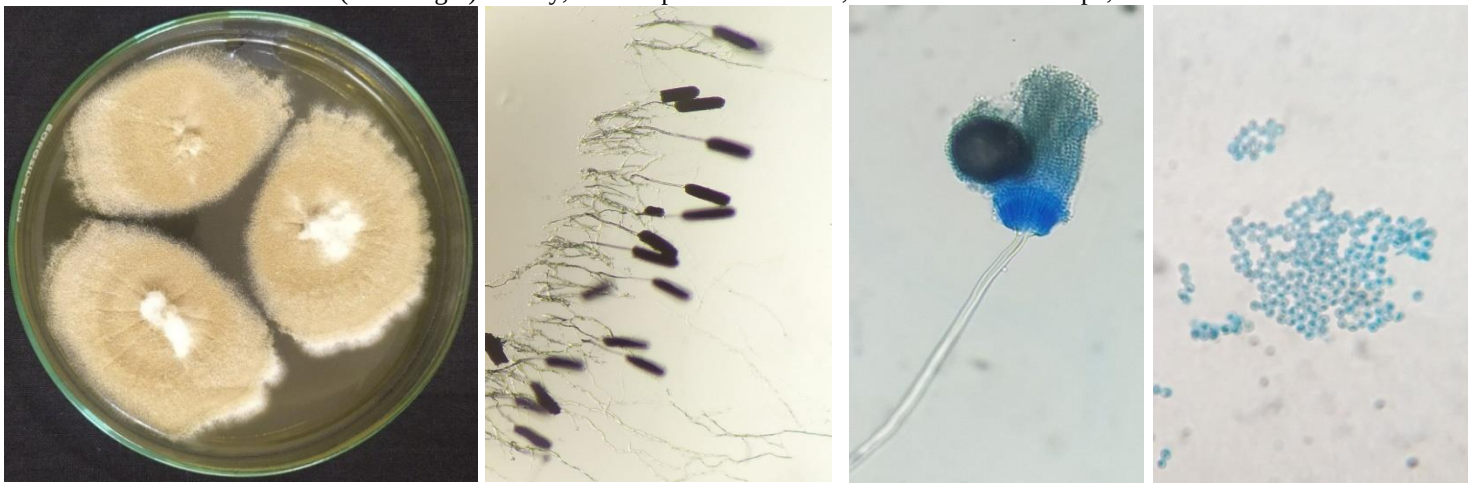


Plate 3b: PRC-2 (left to right): Colony, Conidiophore under 10X, Conidial head and stipe 100X and Conidia

Plate 3: Morphological characteristics of the thermo-tolerant fungal endophytes

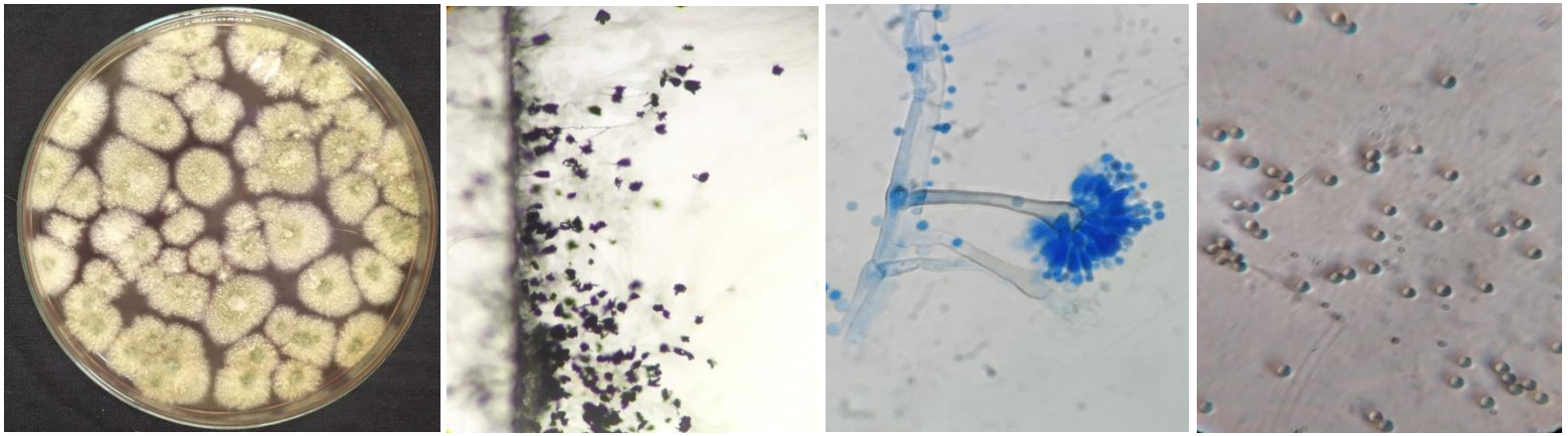


Plate 3c: SAP-3(left to right) Colony, Conidiophore under 10X, Conidial head and stipe 100X, Conidia and ascospores

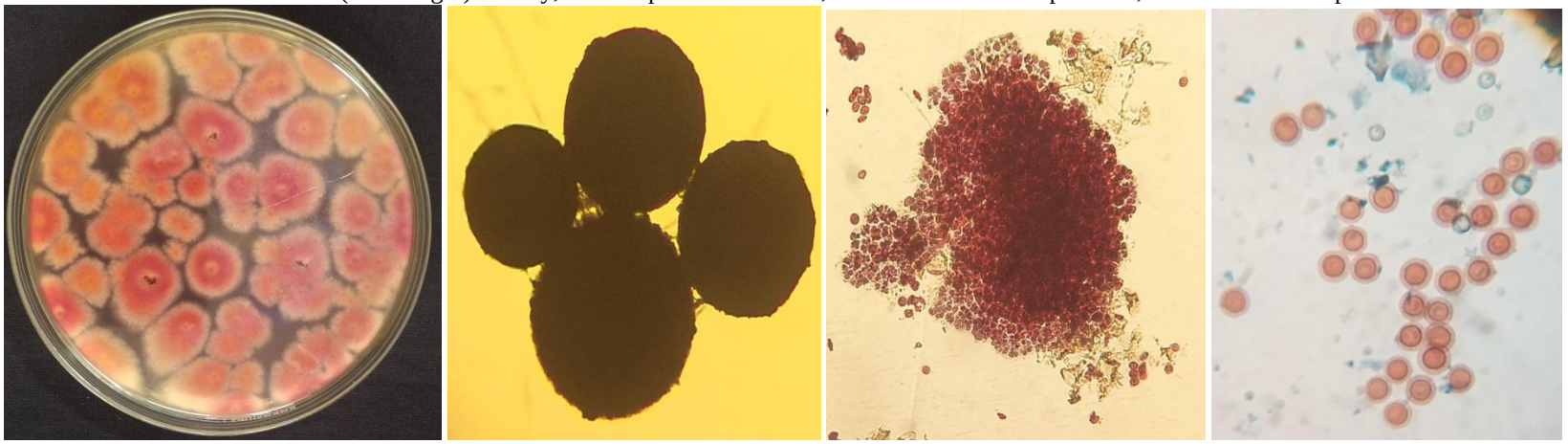


Plate 3c: SAP-3 (left to right) Reverse side of the colony, cleistothecia under 10X, ascus and ascospores

Plate 3: Morphological characteristics of the thermo-tolerant fungal endophytes

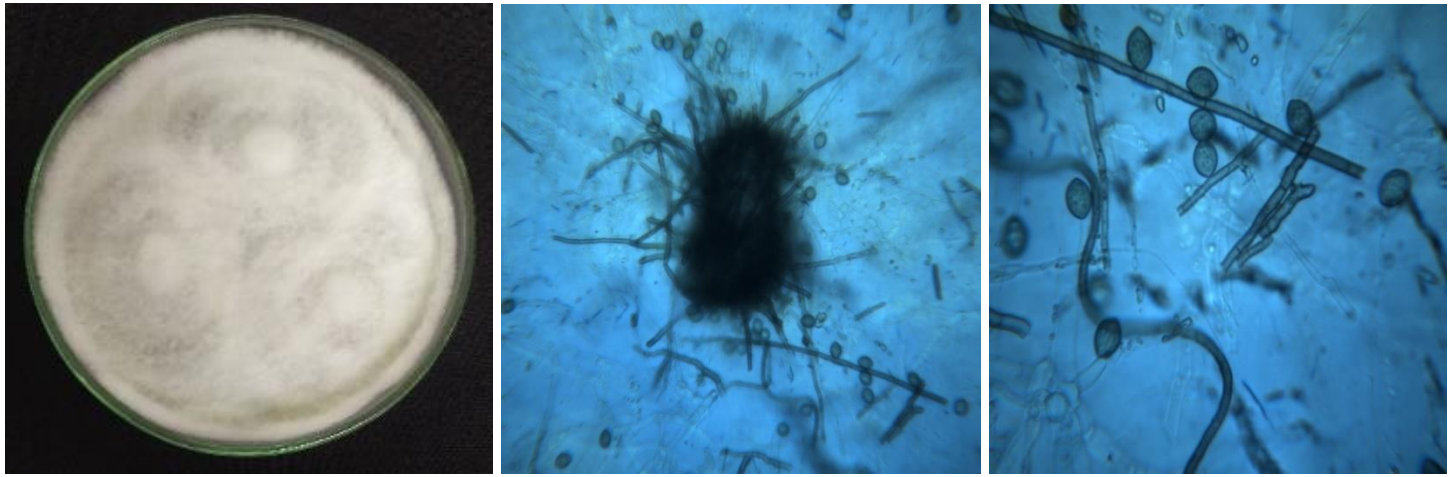


Plate 3d: LAS-4 (left to right) Colony, ascomata and ascospores

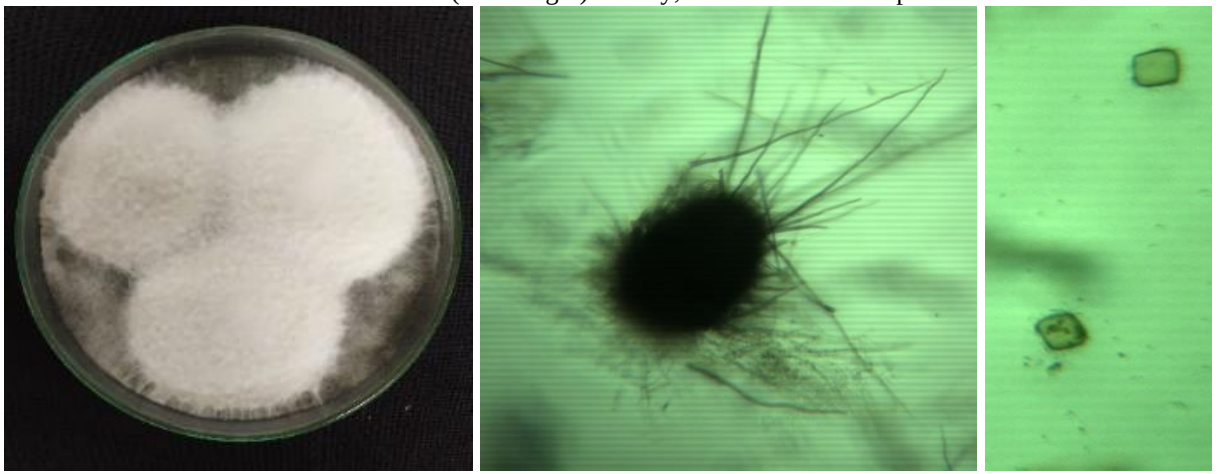


Plate 3e: LAS-6 (left to right) Colony, ascomata andaAscospores

Plate 3: Morphological characteristics of the thermo-tolerant fungal endophytes

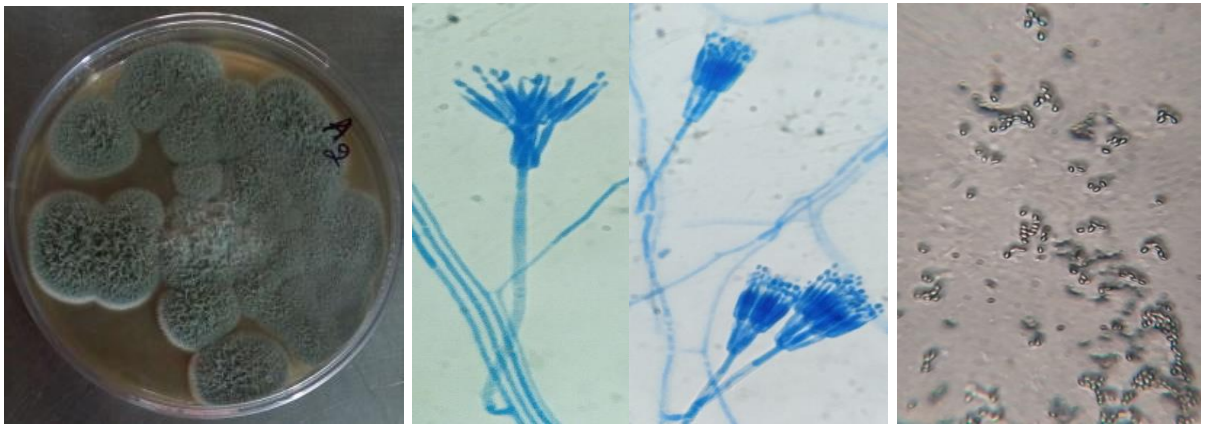


Plate 3f: A-2 (left to right) Colony, and their conidiophore

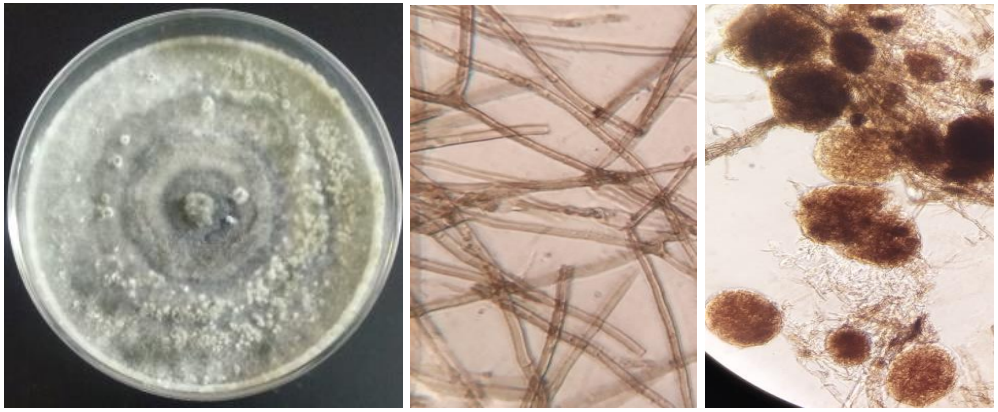


Plate 3g: *Endomelanoconiopsis endophytica* and their mycelium

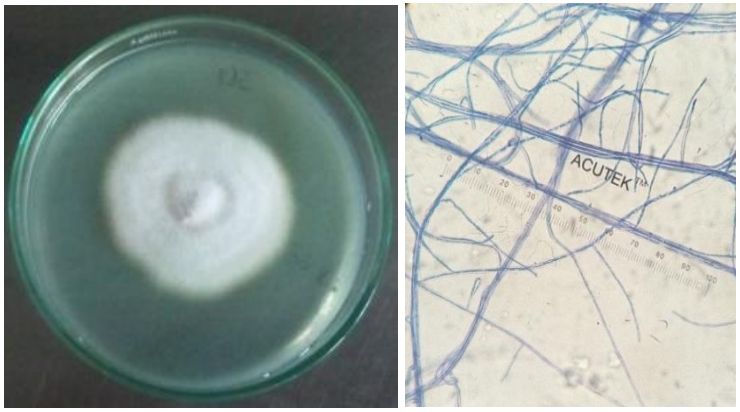


Plate 3h: A-2 (left to right) Colony and their mycelium

Plate 3: Morphological characteristics of the thermo-tolerant fungal endophytes

4.2.2 Molecular identification using ITS region sequences

Fungi can be identified by classical approach using morphological and fruiting body/spore characters. However, this approach is not useful for non-cultivatable and non-sporulating group of fungi. Therefore, in the present study, ITS (Internal Transcribed Spacer) region of rDNA sequence which is widely used to examine phylogenetic positions or relationship of a species as this region is flanked by preserved segments (18S, 5.8S and 28S genes) which provide information about phylogeny and the taxonomic level (Ramesh *et al*., 2017). Among the 8 selected isolates, the three isolates *viz*., LAS-4, LAS-6 and A-7 did not produce spores on potato dextrose agar (PDA) and czapeks dox agar (CDA) and remaining 5 isolates produced the spores. Hence, fungal isolates were identified by using ITS sequences (Table 3). The sequence size of TS1 and ITS4 range from 450 to 700 bp (Bellemain *et al*., 2010).

The partial gene sequence of the isolate LAS-4 with 586 bp sequence length showed 99 per cent homology with the sequences of the *Chaetomium* sp. strain CZPMP12 and the LAS-6 having the sequence length of 467 bp showed 99 per cent homology with *Chaetomium* sp. strain TM3 available in the NCBI data base. The phylogenetic tree constructed with ten *Chaetomium* species available in the NCBI GenBank with the sequences of these fungi showed relationship with *Chaetomium* sp (Fig. 1a). Therefore, these two isolates were confirmed as *Chaetomium* sp.

ITS partial gene sequence of the isolates ACT-2, PRC-2 and SAP-3 showed 100 per cent homology with *Aspergillus flavus*, *Aspergillus terreus* and *Aspergillus nidulans* available in the NCBI data base. The phylogenetic tree constructed with ten *Aspergillus* species available in the NCBI GenBank with the sequences of identified organisms showed that the isolate ACT-2 closely related to *Aspergillus flavus*, the PRC-2 to *Aspergillus terrus* and these two isolates formed a clade II. Similarly, SAP-3 isolate is closely related to *Aspergillus nidulans* and formed a clade III (Fig 1b). Therefore, these isolates were confirmed as *Aspergillus flavus, Aspergillus terrus* and *Aspergillus nidulans.*

The isolate A2 having 654 bp size showed 99 per cent homology with *Penicillium funiculosum* available in the NCBI data base and the phylogenetic tree constructed with ten species of *Penicillium* available in the NCBI GenBank with the

Table 3: Molecular characteristics of thermotolerant fungal endophytes

Sl. No.	Hosts	Isolates code	Closest match	Sequence length (bp)	Query coverage (%)	Percent identity (%)	Organisms identified	NCBI Accession no.
1	*Lasiurus scindicus*	LAS 6	*Chaetomium* sp. strain TM3	467	92	99	*Chaetomium* sp.	MT900577
2	*Lasiurus scindicus*	LAS 4	*Chaetomium* sp. strain CZPMP12	586	91	98	*Chaetomium* sp.	MT900575
3	*Prosopis cineraria*	PRC 2	*Aspergillus terreus* isolate Asp 7801	582	100	98	*Aspergillus terreus*	MT921660
4	*Salvadora persica*	SAP 3	*Aspergillus nidulans* strain SD531	515	100	100	*Aspergillus nidulans*	MT900566
5	*Acacia tortilis*	ACT 2	*Aspergillus flavus* isolate ACT2	495	100	100	*Aspergillus flavus*	MT899223
6	*Artimisia* sp.	A2	*Penicillium funiculosum* strain C2-20	654	98	99	*Penicillium funiculosum*	OM368442
7	*Artimisia* sp.	A7	*Ceriporia lacerate* strain BHU-MS1	479	96	98	*Ceriporia lacerate*	MT899187
8	*Xerophytic plant*	X5	*Endomelanconiopsis endophytica* strain 5345	473	98	98	*Endomelanconiopsis endophytica*	MT900590

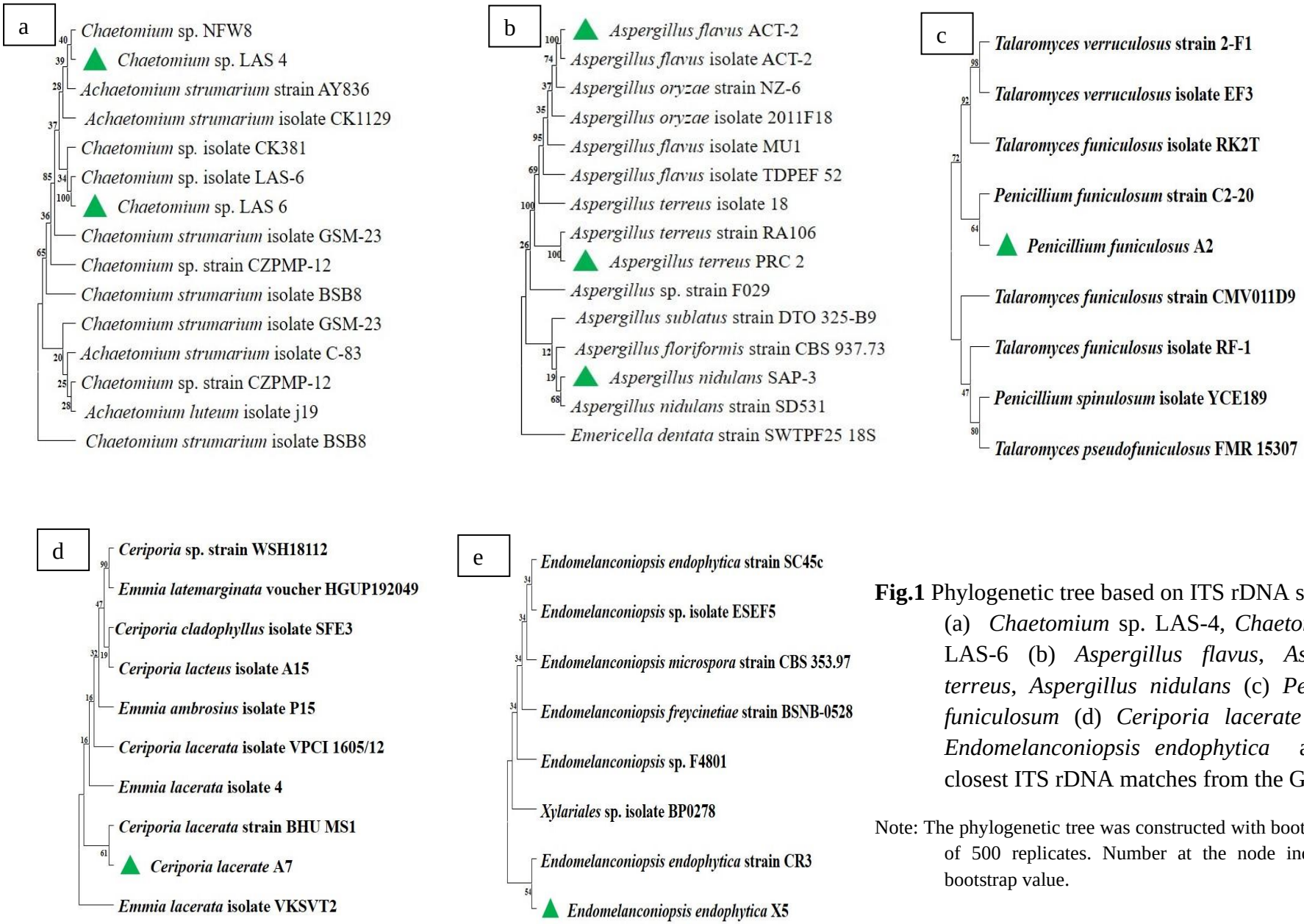


Fig.1 Phylogenetic tree based on ITS rDNA sequences (a) *Chaetomium* sp. LAS-4, *Chaetomium* sp. LAS-6 (b) *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus nidulans* (c) *Penicillium funiculosum* (d) *Ceriporia lacerate* and (e) *Endomelanconiopsis endophytica* and their closest ITS rDNA matches from the GenBank.

Note: The phylogenetic tree was constructed with bootstrap value of 500 replicates. Number at the node indicates the bootstrap value.

this organism sequence showed that it is closely related to *Penicillium funiculosum* (Fig. 1c). Therefore, the isolate A2 was confirmed as *Penicillium funiculosum.*

The ITS partial gene sequence of the isolate A7 with 479 bp showed 100 per cent homology with *Ceriporia lacerate* strain BHU-MS1 available in the NCBI data base. However, the phylogenetic tree constructed with ten species of *Ceriporia* available in the NCBI GenBank along with the sequences of identified organism showed that it is closely related to *Ceriporia lacerate* (Fig. 1d). Therefore, the isolate A7 was confirmed as *Ceriporia lacerate*

Sequences of the isolate X5 with 473 bp showed 98 per cent homology with *Endomelanconiopsis endophytica* strain 5345 available in the NCBI data base. The phylogenetic tree constructed with the 10 species of *Endomelanconiopsis* available in the NCBI GenBank and compared with the sequences of isolate X5 showed close match with *Endomelanconiopsis endophytica* (Fig. 1e). Therefore, the isolate X5 was confirmed as *Endomelanconiopsis endophytica.*

The ITS gene sequence is widely used for the identification of microorganisms. Use of ITS region sequences was proved to be a valuable source of evidence to resolve phylogenetic relationships at lower levels (Bezerra *et al*., 2012). The ITS region has the highest probability of successful identification for the broadest range of fungi with most clearly defined gap between inter and intra specific variation (Schoch *et al*., 2012), the above fungal endophytes were identified using ITS sequence homology. Manasa *et al*. (2020) identified the fungal OTUs by amplifying the 18S ribosomal region of the internal transcribed spacer (ITS) of the genomic DNA using ITS1 and ITS4 as forward and reverse primers respectively.

4.4 Evaluation of selected fungal endophytes imparting thermo-tolerance in rice

The results on the effects of fungal endophytes on shoot growth of rice at elevated temperature (45 ^{o} C) and normal conditions are presented in Table 4 and Plate 4. The shoot length of rice did not differ significantly across the treatments under normal as well as stress conditions. The *A. flavus* inoculated rice plants showed significant increase in number of tillers (5.33/plant), leaves (20.17/plant), fresh (3.42 g/p) and dry weight (0.92 g/p) of plants which is followed by *P. funiculosum* inoculated plants. This indicated that the endophytes treatment improved the growth

Table 4: Influence of fungal endophytes on shoot attributes of rice under stress [S] and without stress [WS]

Treatments	Plant height (cm)		No. of tillers (/plant)		No. of leaves (/plant)		Fresh wt. shoot (g/plant)		Dry wt. shoot (g/plant)	
	WS	S	WS	S	WS	S	WS	S	WS	S
Control	33.53 ±1.02	32.75±0.38	2.83±0.10^{e}	2.00±0.00^{bc}	12.17±0.29h	9.33±0.19^{e}	1.98±0.03^{e}	1.04±0.01^{e}	0.51±0.01^{d}	0.29±0.00^{d}
A. flavus	36.82±0.24	32.11±0.79	5.33±0.19^{a}	2.17±0.10^{ab}	20.17±0.29^{a}	12.67±0.19^{bc}	3.42±0.04^{a}	1.42±0.01^{b}	0.92±0.01^{a}	0.36±0.02^{b}
A. nidulans	35.60±0.67	33.75±1.06	3.83±0.10^{c}	2.17±0.10^{ab}	16.67±0.19^{c}	11.50±0.67^{d}	2.71±0.01^{d}	1.56±0.01^{a}	0.80±0.03^{b}	0.40±0.01^{a}
A. terreus	35.11±0.93	32.31±1.07	3.33±0.00^{d}	1.83±0.10^{c}	14.17±0.10^{f}	12.17±0.29^{bcd}	2.84±0.05^{c}	1.35±0.03^{bc}	0.79±0.01^{b}	0.35±0.00^{bc}
C. lacerata	35.51±1.66	32.62±0.84	3.50±0.10^{cd}	2.00±0.00^{bc}	13.33±0.00^{g}	9.17±0.10^{e}	2.64±0.04^{d}	1.26±0.03^{d}	0.78±0.03^{b}	0.29±0.00^{d}
Cheatomium sp. L4	36.96±0.75	32.01±0.64	3.56±0.11^{cd}	2.00±0.00^{bc}	15.00±0.58^{de}	11.50±0.48^{d}	2.95±0.06^{bc}	1.41±0.01^{b}	0.76±0.00^{b}	0.33±0.02^{c}
Cheatomium sp. L6	36.41±0.88	33.31±0.13	3.67±0.00^{cd}	2.00±0.00^{bc}	14.50±0.10^{ef}	11.83±0.10^{cd}	2.72±0.00^{d}	1.28±0.00^{cd}	0.69±0.00^{c}	0.35±0.00^{bc}
E. endophytica	36.15±0.96	32.12±0.98	3.67±0.19^{cd}	2.33±0.00^{a}	15.33±0.19^{d}	12.33±0.19^{bcd}	2.96±0.02^{b}	1.50±0.05^{a}	0.77±0.00^{b}	0.36±0.00^{b}
P. funiculosum	36.62±0.27	32.79±1.08	4.83±0.10^{b}	2.17±0.10^{ab}	18.50±0.10^{b}	13.00±0.19^{a}	3.450.04^{a}	1.40±0.02^{b}	0.88±0.01^{a}	0.36±0.00^{b}
C.D.@ 5%	NS	NS	0.351	0.189	0.769	0.960	0.113	0.074	0.045	0.031
C.D.@ 1%	NS	NS	0.479	0.259	1.054	1.314	0.155	0.102	0.062	0.042
S.Em ±	1.289	1.183	0.166	0.091	0.366	0.456	0.054	0.035	0.021	0.014
C.V	4.404	4.437	5.283	5.357	2.883	4.704	2.314	3.174	3.384	4.896

Note : ± indicates standard error of mean (n = 3); Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT).

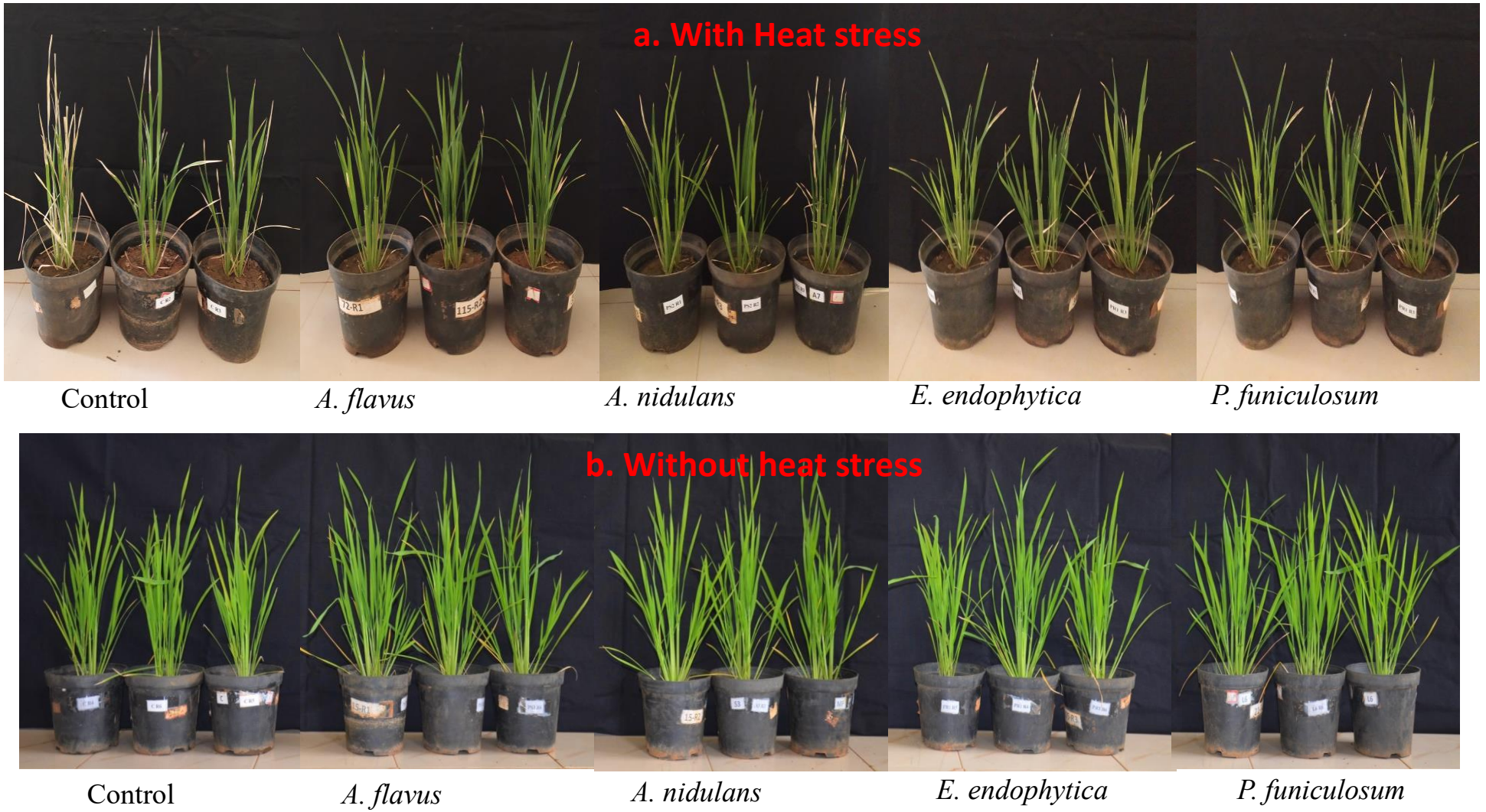


Plate 4: Influence of fungal endophytes on growth of rice under heat stress (a) and without stress (b)

of plant by producing growth promoting traits. Fungal endophytes have been shown to support plant growth and increase nutrient uptake by providing phytohormones, low molecular weight compounds, enzymes, antimicrobial substances like antibiotics and siderophores (Hamilton *et al*., 2010). The present study is in agreement with the observations of Hipol (2012) who reported that the increased growth of rice seedlings due to production of IAA and GA3 by the fungal isolates P3AL2c and P3BS1c.

High temperature is one of the major environmental stress which severely affect the growth of rice by modulating physiological and molecular process. Manalo *et al*. (1994) reported ten per cent reduction in tiller production due to increased temperature from 29/21 ^{o}C to 37/29 ^{o}C. In the present study, 46.09 % of tiller was reduced when plant exposed to high temperature. However, the extent of reduction was lesser in endophytes inoculated plants. Among the eight endophytes, *E. endophytica, A. flavus, A. nidulans* and *P. funiculosum* inoculated plants produced maximum number of tillers compared to others. This indicated that the endophytes might have positively influenced the emergence of tillers by reducing the effects of heat stress by regulating homeostasis of phytohormones (Vila-Aiub *et al*., 2005).

The *P. funiculosum* inoculated plants significantly showed highest number of leaves (13/plant) followed by *A. flavus* (12.67/plant). The least number of leaves was observed in un-inoculated plants (9.33/plant). Microbial colonization can modulate morphological adaptation to enhance the stress tolerance of host plants. The *Trichoderma harzinum* inoculated rice plants increased rolling capacity of leaves under drought condition and prevented water loses (Pandey *et al*., 2016). The present finding was confirmed with Ali *et al*. (2019) who reported that the *Thermomyces lanuginosus* colonized *Cullen plicata* plant recorded significantly increased number of leaves under heat stress.

Maximum shoot fresh weight was observed in *A. nidulans* (1.56 g/p) which is on par with the *E. endophytica* (1.50 g/p) inoculated plants. The least weight was recorded in un-inoculated plants. Colonization of endophytes in plant tissue improves relative water content and provide tolerance against drought stress in host plants (Zhang and Nan, 2007; Xu *et al*., 2017). In this study, the *A. nidulans* (1.20 g/p) inoculated plants recorded significantly higher dry weight of shoot which is followed by *E. endophytica, P. funiculosum* and *A. flavus* inoculations. Hence, the output

suggests that the endophytes might involve in enhancing the rice shoot weight by improving the production of tiller and leaves and ameliorated the effect of heat stress in plant system.

Roots play a critical role in plant survival at extreme temperatures (Huang *et al*., 2012). High temperature causes decreased root biomass, root number and total length of root due to reduction in metabolic activities (Nielsen, 1974; McMichael and Burke, 2002). The results pertaining to the effects of inoculated fungal endophytes on root growth of rice at elevated temperature (45 ^{o}C) are presented in Table 5. In general, the endophytes colonized plants showed increased growth of root compared to un-inoculated plants. Increased root volume and dry weight was observed in *A. nidulans* which is followed by *E. endophytica*. The fungal endophytes known to influence host plants growth by altering physiological process by expanding root system, stimulating root hair elongation, increasing exudation of phenolic substances around rhizosphere to resist biological or environmental stresses (Rodgriguez *et al*., 2009, Hubbard *et al*., 2012). Kandar *et al.* (2018) reported that the rice seedlings inoculated with fungal endophytes improved growth. Co-inoculation of soybean with *Paecilomyces formosus* LHL10 and *Penicillium funiculosum* LHL06 improved the root growth under heat stress (Bilal *et al*., 2021).

The higher root to shoot ratio was observed in un-inoculated plants (0.90) compared to endophytes colonized plants (0.59) under normal conditions. But, in the plants exposed to heat stress showed <0.5 root to shoot ratio. However, *A. nidulans* colonized plants recorded significantly higher root to shoot ratio which is followed by *E. endophytica* (0.44), *Cheatomium* sp. L6 (0.43), *Cheatomium* sp. L4 (0.41) and *A. terreus* (0.41). This indicated that the endophytes might involve in influencing root growth which could facilitate absorption of nutrients and water from soil. These results are in agreement with the Ali *et al.* (2019) who reported significantly higher root to shoot ratio in *Thermomyces lanuginosus* colonized *Cullen plicata* plants exposed to heat stress.

High temperature inhibit the growth of rice which is resulted in reduced biomass production due to wilting, curling and yellowing of leaves and tillers (Xu *et al.,* 2020). Though the endophyte inoculated plants showed increased biomass

Table 5: Influence of fungal endophytes on root attributes and total biomass of rice under stress [S] and without stress [WS]

Treatments	Root volume (cm^3/plant)		Dry wt. root (g/plant)		Root:Shoot		Biomass (g/plant)	
	WS	S	WS	S	WS	S	WS	S
Control	4.00 ± 0.00^{b}	1.00 ± 0.00^{f}	0.46 ± 0.02^{cd}	0.10 ± 0.00^{e}	0.90 ± 0.03^{a}	0.34 ± 0.01^{c}	0.97 ± 0.03^{d}	0.39 ± 0.00^{e}
A. flavus	3.97 ± 0.02^{bc}	1.55 ± 0.03^{b}	0.54 ± 0.00^{b}	0.12 ± 0.00^{d}	0.59 ± 0.01^{cd}	0.33 ± 0.03^{c}	1.46 ± 0.01^{a}	0.47 ± 0.02^{c}
A. nidulans	4.00 ± 0.00^{b}	1.67 ± 0.00^{a}	0.45 ± 0.00^{cde}	0.20 ± 0.01^{a}	0.57 ± 0.03^{de}	0.50 ± 0.03^{a}	1.25 ± 0.02^{b}	0.60 ± 0.00^{a}
A. terreus	2.67 ± 0.00^{f}	1.32 ± 0.01^{d}	0.43 ± 0.02^{def}	0.15 ± 0.01^{c}	0.55 ± 0.02^{de}	0.41 ± 0.02^{b}	1.22 ± 0.03^{b}	0.50 ± 0.01^{b}
C. lacerata	2.17 ± 0.00^{g}	1.42 ± 0.05^{c}	0.42 ± 0.03^{def}	0.13 ± 0.00^{d}	0.55 ± 0.02^{de}	0.45 ± 0.01^{ab}	1.20 ± 0.06^{b}	0.42 ± 0.01^{d}
Cheatomium sp. L4	3.22 ± 0.03^{e}	1.17 ± 0.00^{e}	0.39 ± 0.01^{f}	0.14 ± 0.00^{c}	0.52 ± 0.02^{e}	0.41 ± 0.01^{b}	1.17 ± 0.01^{b}	0.47 ± 0.01^{c}
Cheatomium sp. L6	3.50 ± 0.10^{d}	1.00 ± 0.00^{f}	0.41 ± 0.02^{ef}	0.15 ± 0.00^{bc}	0.59 ± 0.02^{cd}	0.43 ± 0.03^{b}	1.08 ± 0.01^{c}	0.51 ± 0.00^{b}
E. endophytica	3.83 ± 0.10^{c}	1.50 ± 0.00^{b}	0.49 ± 0.01^{c}	0.16 ± 0.01^{b}	0.64 ± 0.01^{c}	0.44 ± 0.03^{b}	1.25 ± 0.01^{b}	0.52 ± 0.00^{b}
P. funiculosum	4.63 ± 0.02^{a}	1.33 ± 0.00^{d}	0.66 ± 0.02^{a}	0.12 ± 0.00^{d}	0.75 ± 0.01^{b}	0.33 ± 0.01^{c}	1.54 ± 0.03^{a}	0.47 ± 0.01^{c}
C.D.@ 5%	0.141	0.057	0.050	0.016	0.058	0.063	0.080	0.026
C.D.@ 1%	0.194	0.078	0.068	0.022	0.079	0.086	0.110	0.035
S.Em ±	0.067	0.027	0.024	0.007	0.028	0.03	0.039	0.012
C.V.	2.303	2.475	6.116	6.191	5.401	9.054	3.831	3.049

Note: ± indicates standard error of mean (n = 3); Means with same superscript in a column do not differ significantly at $p\leq 0.05$ as per Duncan's Multiple Range Test (DMRT)

production compared to un-inoculated plants under both the conditions (Table 5), the biomass production was reduced to 60 % when the plants are exposed to high temperature. The *A. nidulans* inoculated plants produced higher biomass (0.6 g/p) which is followed by *E. endophytica* (0.5 g/p) *Cheatomium* sp. L6 (0.51 g/p), *A. terreus* (0.51g/p), *P. funiculosum* (0.47 g/p) and *A. flavus* (0.47 g/p). The least biomass was observed in un-inoculated plants (0.39 g/p). This suggested that the endophytes inoculation mitigate the temperature stress in plants Khan *et al.* (2015) reported that the *Penicillium resedanum* LK6 inoculation improved the biomass of *Capsicum* compared to control plants under heat stress. The output of this study manifestated that fungal endophytes could significantly ($p \leq 0.01$) improved all growth attributes of rice under both heat stress as well as normal conditions. The inoculation of fungal endophytes improved the growth of rice by imparting thermotolerance *via* habitat-adapted symbiosis (Redman *et al*., 2002; 2011).

4.4.2 Confirmation of endophytes in the inoculated plant tissues

The fungal endophytes were re-isolated from culm and leaves of inoculated rice seedlings and confirmed by comparing their morphology with mother culture (Plate 5). Out of eight selected fungal endophytes, only five fungi namely, *A. flavus, A. nidulans, A, terreus, E. endophytica* and *P. funiculosum* were grown on the medium. But, the remaining three endophytes (*Cheatomium* sp. L4, *Cheatomium* sp. L6 and *C. lacerate*) did not emerge from the tissue. The reason for these fungi failure to colonize is yet to be ascertained. Among the five endohytes, four fungi namely, *A. flavus, A. nidulans, E. endophytica* and *P. funiculosum* were selected for further experimentation based on stress tolerance index.

4.4.3 Stress Tolerance Index (STI) of variables

The STI values of all measured growth variables were used to develop heat map with hierarchical clustering (Fig.2). Hierarchical clustering of treatments revealed two distinct clusters based on STI namely Cluster-1 and 2. *A. flavus, A. nidulans, P. funiculosum* and *E. endophtica* belongs to cluster 1 and had higher STI. Similarly *A. terrus, Cheatomium* sp. L4, *Cheatomium* sp. L6 and *C. lacerata* belongs to cluster -2 had lower STI. The STI can be used to identify the treatment that can perform better under both stress and control conditions (Fernandez, 1992). This result reveals that the endophytes belong to cluster 1 play a role in amelioration of heat stress in rice plants. Therefore, these four endophytes were chosen for further studies.

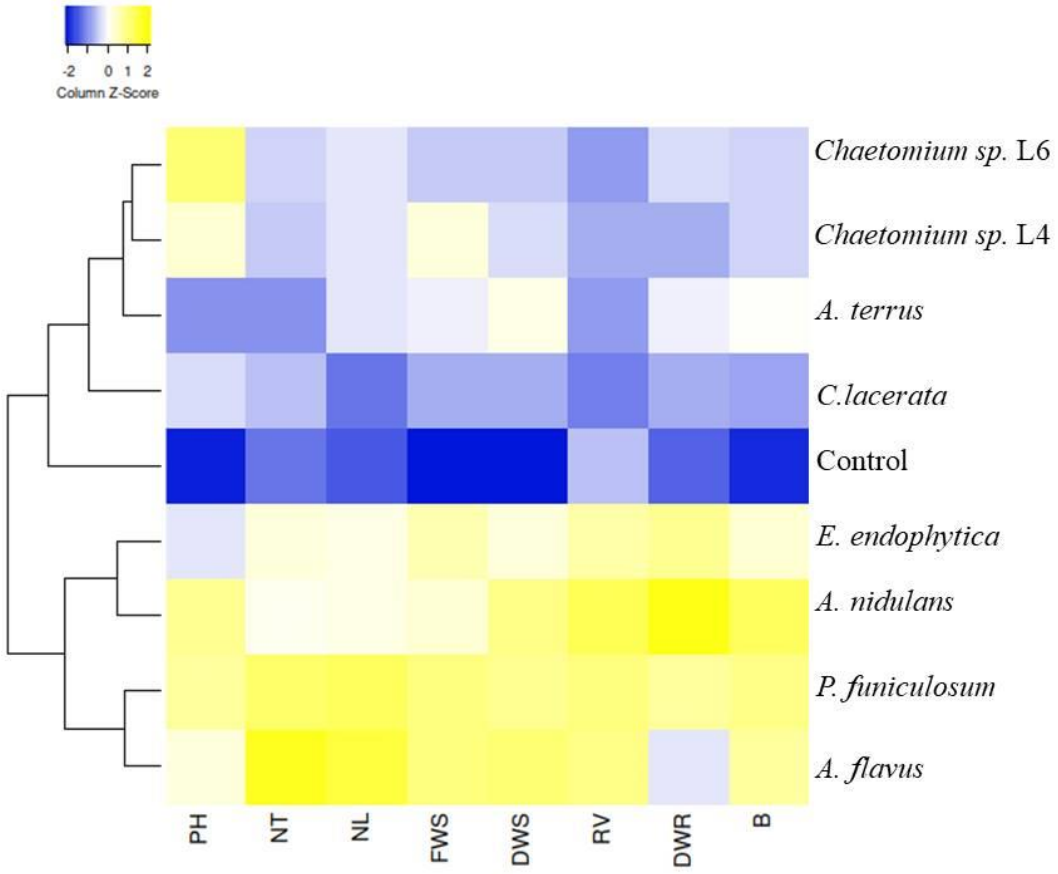


Fig. 2 Hierarchical clustering with heat map for STI of growth traits of rice inoculated with endophytes [PH plant height, NT number of tillers, NL number of leaves, FWS fresh weight of shoot, DWS dry weight of shoot, RV root volume, DWR dry weight of root, B total biomass]

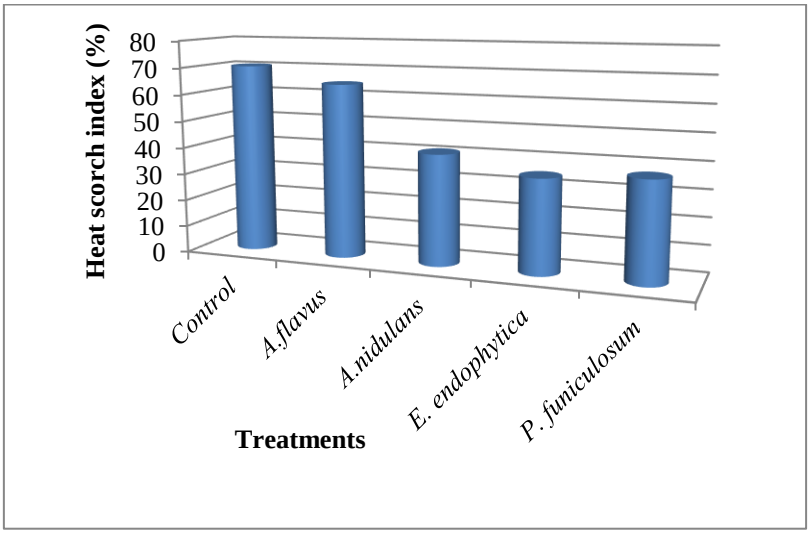


Fig. 3 Influence of fungal endophytes on heat scorch of plants under high temperature

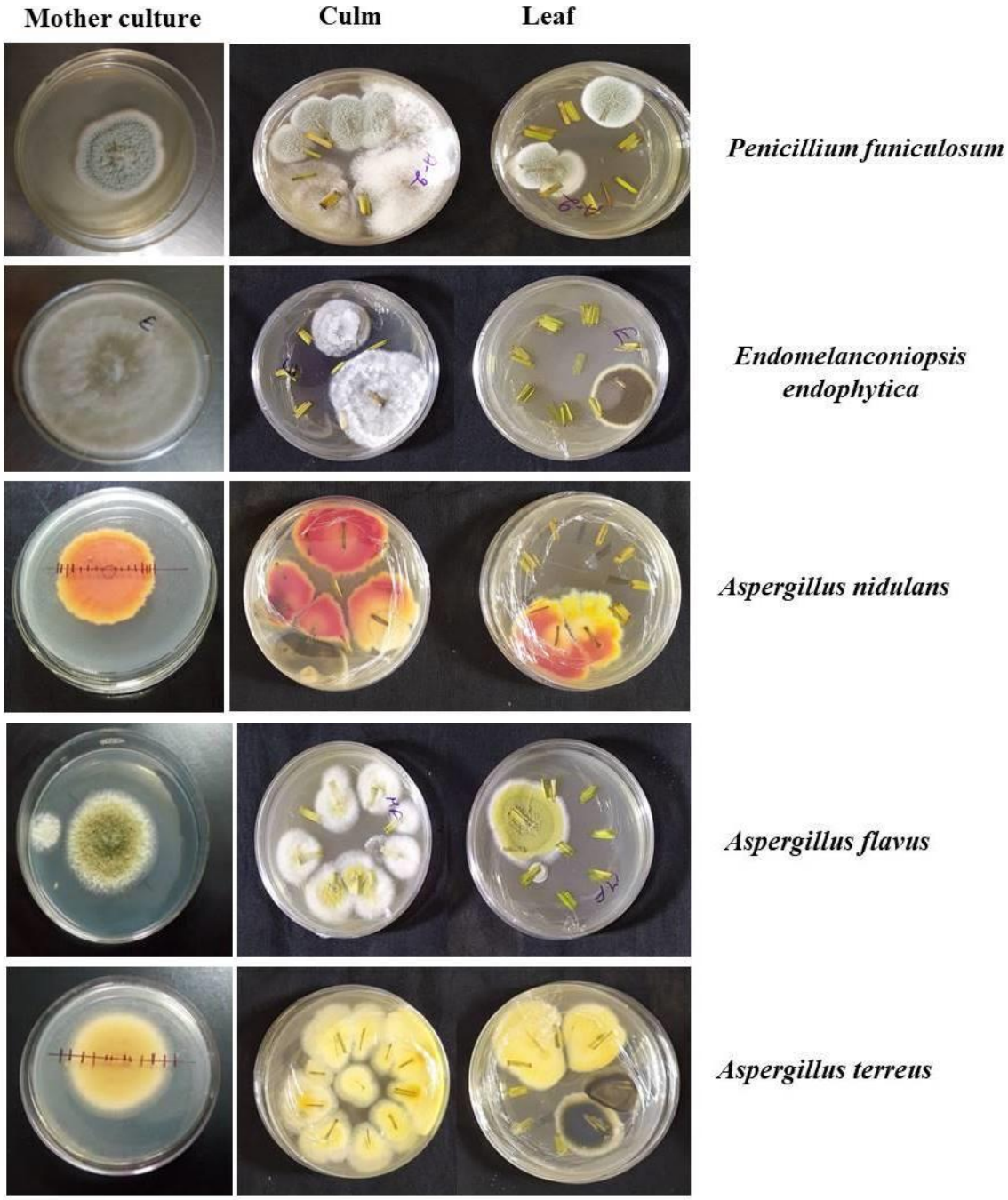


Plate 5: Comparison of re-isolated fungal endophytes with their mother culture

4.5 Analysis of phytohormone and antioxidants induced due to temperature stress

Variation in physiological attributes are influenced by fungal endophytes *viz*., *A. nidulans, A. flavus, E. endophytica* and *P. funiculosum* to ameliorate heat stress in rice plant were studied in this experiment.

4.5.1 Influence of fungal endophytes on heat scorch index of rice

High temperature causes a wilting, curling, yellowing and burning of rice leaves which results in reduction of biomass and yield. The results pertaining to scorch index caused by high temperature is presented in Fig. 3 (Plate 6). The endophyte colonized rice plants showed lesser scorch index (<65%) when exposed to 45º C compared to un-inoculated plants (70.36 %). Among four endophytes, *E. endophytica* colonized plants showed lesser scorch index (34.83 %) followed by *P. funiculosum* (36.98 %), *A. nidulans* (41.04 %) and *A. flavus* (64.63 %). This indicated that the endophytes protect rice plants by increasing plant fitness or modifying adaption methods such as leaf rolling, regulation of ROS and antioxidants, osmolyte synthesis, phytohormone balance, or expression of stress-related genes (Pandey *et al*., 2016 and Ali *et al*. 2019).

4.5.2 Influence of fungal endophytes on cell membrane stability of rice

The data pertaining to the influence of fungal endophytes on cell membrane stability under normal as well as high temperature is presented in Table 6. High temperature caused membrane damage due to peroxidation of lipid membrane resulting in accumulation of melondialdehyde (MDA) there by reduced the membrane stability (Uremura *et al*., 2006). The MDA content was decreased in endophyte inoculated plant compared to un-inoculated plants exposed to heat stress. The endophytes colonized plants showed varied MDA content. The MDA content was 2.62 µmol/g in *P. funiculosum* inoculated plants, 2.74 µmol/g in *E. endophytica,* 4.20 µmol/g in *A. nidulans* and 4.95 µmol/g in *A. flavus.* The un-inoculated plants showed increased MDA content (7.19 µmol/g). This indicated that the endophyte colonized plants protect their cell membrane and reduces the oxidative damage by alleviating the peroxidation of membrane lipids and maintaining membrane fluidity. Several studies have also demonstrated that endophytes associated plants had lesser MDA content under abiotic stress (Xu *et al*., 2017 and Ali *et al*., 2019).

Table 6: Influence of fungal endophytes on membrane stability of rice

Treatments	Melondialdehyde (µmol/g of FW)	
	Without Stress	With Stress
Control	1.80[a]	7.19[a]
A. flavus	1.63[ab]	4.95[b]
A. nidulans	1.34[c]	4.20[c]
E. endophytica	1.43[bc]	2.74[d]
P. funiculosum	1.46[bc]	2.62[d]
C.D.@ 5%	0.202	0.253
C.D.@ 1%	0.279	0.350
S.Em ±	0.067	0.084
C.V.	8.757	3.868

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT). FW=Fresh weight.

Table 7: Influence of fungal endophytes on relative water content (RWC) of rice

Treatments	RWC (%)	
	Without Stress	With Stress
Control	88.07	61.26[c]
A. flavus	91.08	76.56[b]
A. nidulans	89.71	75.98[b]
E. endophytica	90.13	81.33[a]
P. funiculosum	87.15	77.74[ab]
C.D.@ 5%	NS	4.762
C.D.@ 1%	NS	6.586
S.Em ±	1.059	1.588
C.V.	2.373	4.249

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT).

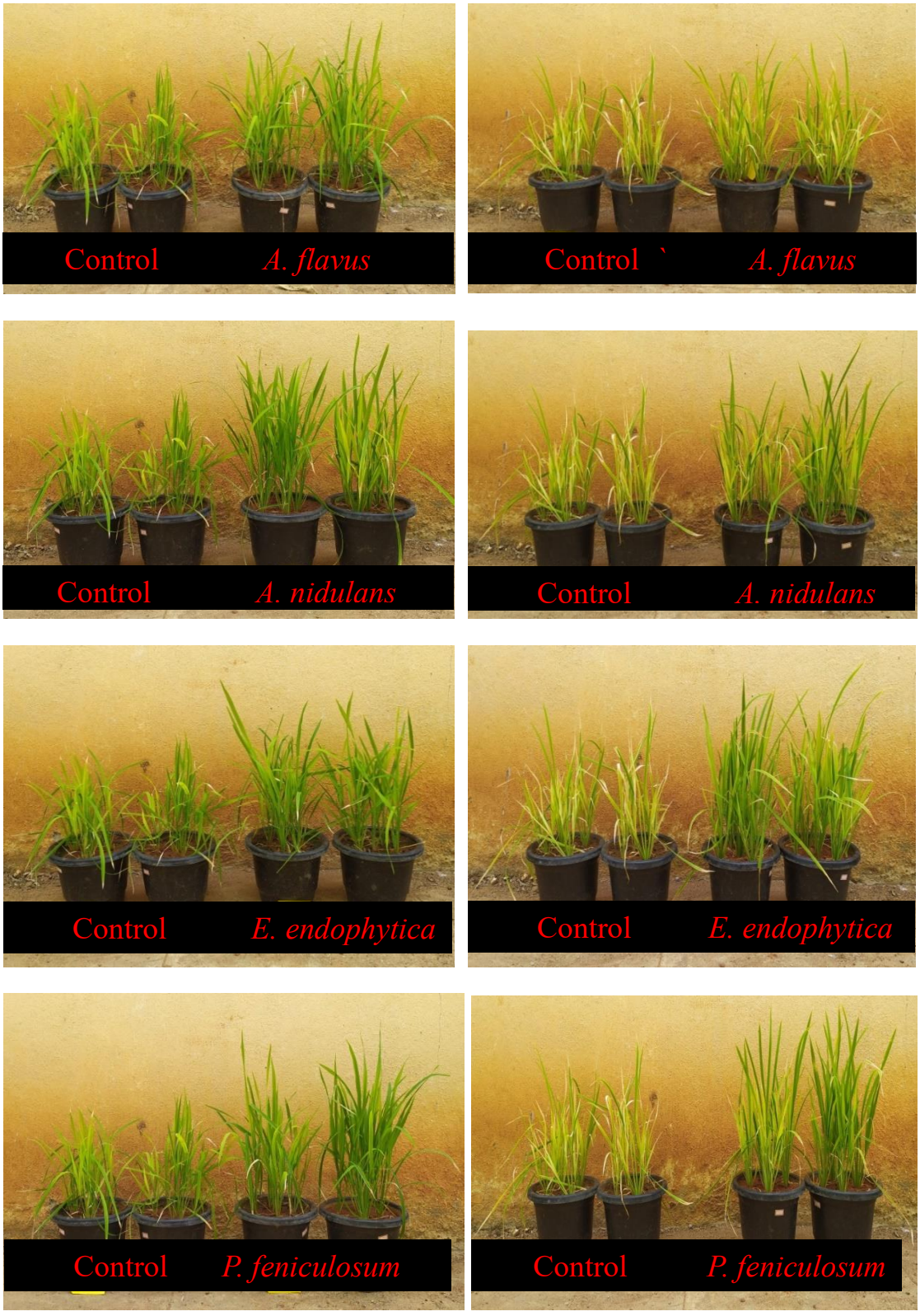


Plate 6: Influence of fungal endophytes on growth of rice under normal condition (right) as well as stress condition (left)

4.5.3 Influence of fungal endophytes on relative water content (RWC)

The results pertaining to the influence of fungal endophytes on RWC of leaf is presented in Table 7. RWC is an important indicator of water status in plants as it reflects the balance between water supply to the leaf tissue and transpiration rate. Under normal condition, there is no significant change between the treatments. But, when plants were exposed to high temperature, endophytes inoculated plants recorded higher RWC compared to un-inoculated plants. Among the four endophytes, maximum RWC was observed in the treatment inoculated with *E. endophytica* (81.33 %) followed by *P. funiculosum* (77.74 %), *A. nidulans* (76.56 %), and *A. flavus* (75.89 %). This indicated that the endophytes may involve in maintaining leaf water status by regulating transpiration rate or enhancing water uptake by increasing the root volume. Xu *et al*. (2017) reported that the *Epichloe typhina* infection played a beneficial role in tall fescue plant in maintaining higher level of leaf RWC under drought.

4.5.4 Influence of fungal endophytes on photosynthetic efficiency of rice

The results pertaining to photosynthetic efficiency of rice plant as influenced by the inoculation of fungal endophytes is presented in Table 8. Photosynthesis is the most important activity for plant growth. In the present study, photosynthetic attributes (CO_2 assimilation, stomatal conductance, intracellular CO_2 concentration and transpiration rate) did not vary significantly between endophytes inoculated plants and un-inoculated plants. But, significant variation was observed in the plants exposed to heat stress. These are in agreement with the findings of Khan *et al*. (2011) who analysed that endophytes play a role in saline tolerance of soybean plants by mutulism and significantly improve the photosynthetic attributes. Even though the photosynthetic attributes does not altered by endophytes when the plants grown in normal condition. Hence this result indicates that the endophytes respond to photosynthetic attributes under stress condition rather than normal growth condition.

Photosynthesis is one of the most sensitive physiological process to high temperature. The maximum photosynthetic activity in rice was observed at 30 ^{o}C (Yamori *et al*., 2011). The increased temperature beyond 30 ^{o}C, assimilation of CO_2 decreases significantly due to inhibition of redox reactions, rubisco activity, damage in thylakoid membrane and denaturation of chlorophyll molecules (Mathur *et al*.,

Table 8: Influence of fungal endophytes on gas exchange parameter of rice

Treatments	Photosynthetic rate (µ mol $CO_2/m^2/s$)		Stomatal Conductance (mol $H_2O/m^2/s$)		Ci (mol $CO_2/m^2/s$)		Transpiration rate (m mol $H_2O/m^2/s$)	
	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress
Control	12.30	8.64^{c}	0.17	0.11^{c}	245.59	228.09^{b}	3.82^{b}	6.01^{c}
A. flavus	16.94	9.51^{bc}	0.25	0.11^{c}	253.49	215.64^{b}	5.66^{a}	5.77^{c}
A. nidulans	16.34	10.95^{b}	0.20	0.21^{a}	234.29	293.10^{a}	5.46^{a}	8.26^{b}
E. endophytica	16.87	13.47^{a}	0.20	0.19^{b}	231.97	244.09^{b}	4.68^{ab}	9.06^{a}
P. funiculosum	15.02	13.68^{a}	0.22	0.19^{b}	257.50	228.74^{b}	5.66^{a}	8.02^{b}
C.D.@ 5%	NS	1.686	NS	0.017	NS	30.917	1.045	0.689
C.D.@ 1%	NS	2.399	NS	0.025	NS	43.975	NS	0.967
S.Em ±	1.251	0.741	0.021	0.005	14.55	9.812	0.327	0.216
C.V.	13.984	11.086	17.309	5.713	10.304	7.025	11.219	5.033

Note: Means with same superscript in a column do not differ significantly at $p \leq 0.05$ as per Duncan's Multiple Range Test (DMRT). Ci- intercellular CO2 concentration, NS- non significant

2014; Hussain *et al*., 2019). In the present study, *E. endophytica* and *P. funiculosum* inoculated plants recorded significantly higher CO_2 assimilation rate (14.35 and 13.47 µmol $CO_2/m^2/s$ respectively) which is followed by *A. nidulans* (10.95) and *A. flavus* (9.51). The un-inoculated plants showed least CO_2 assimilation (8.64 µmol $CO_2/m^2/s$) under heat stress. This suggested that the fungal endophytes improved the photosynthetic rate by protecting thylakoid membrane and chlorophyll molecules under stress conditions. Ali *et al.* (2018a) reported that the *Thermomyces* sp. treated cucumber plant maintained the PS II functional apparatus and exhibited higher photosynthesis under heat stress, 40 ^oC. The increased photosynthetic activity was reported in maize and wheat inoculated with arbuscular mycorrhizal fungus under heat stress (Zhu *et al*., 2011).

Stomatal conductance is strongly correlated with leaf photosynthesis in rice (Hirasawa *et al*., 1988). The reduction of stomatal conductance was observed in stress plants compared to normal plants except *A. nidulans* inoculated plants that increased the stomatal conductance by 17.19 per cent. In addition, *E. endophytica* and *P. funiculosum* inoculated plants significantly increased the stomatal conductance compared to un-inoculated plants (0.11 mol $H_2O/m^2/s$). The enhanced stomatal conductance could increase CO_2 uptake and improved the photosynthetic efficiency under well watered conditions (Kusumi *et al*., 2012). The intercellular CO_2 concentration in plants colonized by *A. nidulans* (293.10 mol $CO_2/m^2/s$) and *E. endophytica* (244.09 mol $CO_2/m^2/s$) was increased under heat stress. This indicates that the exchange of CO_2 and water is regulated by fungal endophytes, there by improved photosynthetic rate in heat stress.

Despite the fact that current findings revealed that decrease in stomatal conductance and increase in transpiration rate under heat stress. This could be in consequence of the variability of vapour pressure deficits of leaf and atmosphere (Leonardi *et al*., 2000; González-Rodríguez *et al*., 2005). This could also occur due to variations in water vapour exchange as a function of the degree of opening, size and stomatal pore depth and arrangement and density of stomata (Larcher, 2000; Marenco *et al*., 2005). Besides, plants avoid rise in tissue temperature by increased transpiration rate (Karwa *et al*., 2020). Among the four endophytes, the *E. endophytica* inoculated plants showed maximum transpiration rate (9.06 mmol

$H_2O/m^2/s$). This indicates that the enhanced thermal cooling capacity by endophytes for protecting leaf from scorching effect through transpiration.

4.5.5 Influence of fungal endophytes on photosynthetic pigments of rice

The results pertaining to photosynthetic pigments as influenced by inoculation of fungal endophytes is presented in Table 9. Chlorophyll status is a key index for evaluating plant photosynthetic efficiency. The chlorophyll a and b and carotenoid pigments were significantly higher in endophyte colonized plants compared to un-inoculated plants under both the conditions.

Under heat stress, reduction in photosynthetic rate was linked to decrease in chlorophyll content due to impaired biosynthesis or accelerated pigment degradation and damage of thylakoid membrane (Camejo *et al*., 2006). These effects can be reduced by endophytes (Sun *et al*., 2010, Ali *et al*., 2009). In the present study, chlorophyll a and b content were reduced under heat stress compared to normally grown plants. However, the endophyte inoculated plants showed increased chlorophyll content. The chlorophyll content is significantly higher in *A. nidulans* inoculated plants which is followed by *P. funiculosum, A. flavus* and *E. endophytica*. The least chlorophyll content was recorded in un-inoculated plants. Symbiotic microorganisms play an important role in improving chlorophyll contents of host plants. Ali *et al*. (2009) reported that the thermotolerant bacteria, *Pseudomonas* sp. strain AKM-P6 increased the total chlorophyll content in sorghum plants under heat stress. Zhu *et al.* (2011) reported that the higher chlorophyll content in mycorrhizal maize leaves as mycorrhizal symbiosis can enhance light harvesting capacity and thereby improving photosynthetic efficiency under heat stress.

High temperature caused variation in chlorophyll a/b ratio in all the treatments, which indicates that the disproportionate reduction of chlorophyll b (65.9 %) compared to chlorophyll a (49.19 %). However, an endophyte *P. funiculosum* colonized plants recorded least chlorophyll a/b ratio followed by *A. nidulans* compared to un-inoculated plants. Increased in Chl a/b ratio caused by temperature is a feature of heat-sensitive genotypes (Santarius and Müller, 1979). Hence, the un-inoculated plants are more sensitive to high temperature than endophytes colonized plants which could provide resistance to heat stress by improving the chlorophyll b content.

Table 9: Influence of fungal endophytes on photosynthetic pigments of rice

Treatments	Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Total Chlorophyll (mg/g FW)		Chlorophylls a: b ratio		Carotenoid (mg/g FW)	
	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress
Control	2.91^{e}	1.48^{e}	0.76^{e}	0.25^{c}	3.63^{e}	1.72^{e}	3.84a	5.92^{a}	0.24^{d}	0.13^{e}
A. flavus	4.38^{a}	1.68^{c}	1.34^{a}	0.30^{b}	5.79^{a}	1.96^{c}	3.27d	5.60^{a}	1.96^{a}	0.14^{c}
A. nidulans	3.34^{d}	2.20^{a}	0.94^{d}	0.43^{a}	4.26^{d}	2.60^{a}	3.55bc	5.09^{bc}	0.30^{c}	0.19^{a}
E. endophytica	3.62^{c}	1.56^{d}	1.01^{c}	0.28^{bc}	4.61^{c}	1.83^{d}	3.60b	5.50^{ab}	0.44^{b}	0.13^{d}
P. funiculosum	3.72^{b}	2.09^{b}	1.07^{b}	0.43^{a}	4.80^{b}	2.50^{b}	3.48c	4.80^{c}	0.43^{b}	0.18^{b}
C.D.@ 5%	0.046	0.036	0.029	0.035	0.062	0.066	0.088	0.478	0.032	0.005
C.D.@ 1%	0.064	0.050	0.042	0.049	0.089	0.093	0.125	0.679	0.046	0.007
S.Em ±	0.014	0.011	0.009	0.011	0.02	0.021	0.028	0.152	0.01	0.002
C.V.	0.693	1.082	1.569	5.525	0.741	1.697	1.366	4.879	2.641	1.784

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT). FW=Fresh weight.

Carotenoids act as accessory light harvesting pigments and play an essential role in photoprotection of photosynthetic apparatus (Young, 1991). It act as antioxidants and play a crucial role in plant tolerance and adaptation to abiotic stress (Sah *et al*., 2016). The carotenoid content was higher in *A. nidulans* (0.19 mg/g of FW) which is followed by other endophytes and the least was observed in un-inoculated plants (0.13 mg/g of FW). The result suggested that the endophytes might scavenge the ROS produced during heat sress and maintained carotenoid content. Arbuscular mycorrhizal fungi stabilized the lipid phase of thylakoid membrane and provided photoprotection of cellular structures and photosynthetic apparatus in maize under heat stress (Zhu *et al.,* 2011).

4.5.6 Influence of fungal endophytes on osmolytes of rice

The results pertaining to accumulation of osmolytes in rice as influenced by inoculation of fungal endophytes is presented in Table 10. During stress, cell osmotic pressure play a key role in maintaining the water potential of the plant. Osmolytes such as proline, glycine betaine, phenols, flavonoids, soluble protein, soluble sugar *etc*. are important for cellular osmotic adjustments. In the present study, osmolytes content was increased or balanced when the plants were exposed to high temperature.

There is a significant increase in proline accumulation in all the plants exposed to high temperature. However, endophytes inoculated plants showed decreased proline content compared to un-inoculated plants. The observation indicated that the endophyte colonized plants are less susceptible to elevated temperature compared to un-inoculated plants. These results are in agreement with the Bae *et al.* (2009) who reported lower accumulation of proline in cacao plants colonized by *Trichoderma hamatum* during drought stress. The accumulation of proline is a consequence of stress and does not provide tolerance (Moftah and Michel, 1987). Proline accumulation in leaf tissues of drought susceptible genotypes was comparatively higher than drought tolerant ones when subjected to mannitol induced water stress (Hien *et al*., 2003; Cha-Um *et al*., 2010).

Accumulation of glycine betaine in general is very negligible in rice plant. But, it was enhanced when plants experiences cold/heat/drought/saline stress (Shirasawa *et al*., 2006). The glycine betaine content in stressed plants is higher compared to control plants. More than 200 % betaine content was observed in

Table 10: Influence of fungal endophytes on osmolytes of rice

Treatments	Proline (µmol/g of FW)		Glycine betaine (µmol/g of FW)		Phenols (mg gallic acid equivalent/g of FW)		Flavonoid (mg rutin equivalent /g of FW)	
	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress
Control	3.36^{c}	7.34^{a}	0.46^{a}	0.67^{e}	12.19^{d}	18.19^{b}	0.35^{e}	0.32^{c}
A. flavus	3.83^{a}	5.26^{c}	0.27^{c}	1.85^{b}	24.27^{a}	17.96^{b}	0.43^{c}	0.37^{b}
A. nidulans	3.67^{b}	4.78^{d}	0.34^{b}	3.56^{a}	23.64^{a}	16.32^{c}	0.50^{b}	0.22^{d}
E. endophytica	3.77^{ab}	6.60^{b}	0.16^{d}	1.51^{c}	13.45^{c}	19.20^{a}	0.38^{d}	0.39^{b}
P. funiculosum	3.45^{c}	4.34e	0.34^{b}	1.04^{d}	19.81^{b}	15.19^{d}	0.59^{a}	0.83^{a}
C.D.@ 5%	0.159	0.274	0.014	0.092	0.922	0.776	0.022	0.025
C.D.@ 1%	0.219	0.375	0.020	0.127	1.263	1.063	0.031	0.035
S.Em ±	0.053	0.09	0.005	0.03	0.303	0.255	0.007	0.008
C.V.	2.951	3.18	3.024	3.509	3.249	2.937	3.178	3.729

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT). FW=Fresh weight.

endophytes colonized plants compared to un-inoculated plants (46.71 %). Among the four endophytes, *A. nidulans* recorded significantly higher accumulation of betaine followed by *A. flavus*, *E. endophytica* and *P. funiculosum*. These observations are in agreement with the findings of Jha *et al.* (2010) who reported increased accumulation of betain in rice leaves inoculated with *P. pseudoalcaligenes* and *B. pumilus* compared to control plants under saline stress. Betaine play a vital role in adjustment and protection of thylakoid membrane, thereby maintaining photosynthetic efficiency (Genard *et al*., 1991). Therefore, the result revealed that the endophytes colonized plants enhanced the betaine accumulation in rice.

Further, a significant variation of phenol and flavonoid content was observed in leaves of rice in between endophytes inoculated and un-inoculated plants. In endophyte inoculated plants, maximum accumulation of phenols and flavonoids were found compared to un-inoculated plants under normal condition. This indicated that the endophyte mediation can trigger the defense mechanism in plants. Similar findings were observed with Singh *et al*. (2020) who reported that *Trichoderma asperellum* and *Pseudomonas fluorescens* treated rice had higher phenol content with compared to un-inoculated plants. The high yield cultivar found higher phenol content then low yield cultivar during vegetative stage of rice and total phenol content was positively correlated to rice yield (Xuan *et al*., 2018). At high temperature, plants colonized *E. endophytica* and *P. funiculosum* recorded maximum phenols and flavonoids respectively. Other endophytes maintained higher phenol content compared to un-inoculated plants. Phenol content in plant acts as scavenger of free radicals which damage the tissue (Potapovich and Kostyuk, 2003).

Osmolyte production in symbiotic plants varies either with the type of stress, endophyte and/or plant genotype (Redman *et al*., 2011). The accumulation of high content of phenolics in *Aspergillus niger* inoculated soybean and sunflower plants under thermal stress help in mitigating the accretion of ROS and lowering of stress effects on plants (Ismail *et al*., 2020a).

4.5.7 Influence of fungal endophytes on antioxidants of rice

The results pertaining to antioxidants of rice plant as influenced by inoculation of the fungal endophytes is presented in Table 11. Plants growing under elevated temperature exhibit oxidative stress. The damaging effects of oxidative stress can be

alleviated through higher production of antioxidants (Comba *et al*., 1998). These antioxidants are super oxidase dismutase (SOD), peroxidase (POD) and catalase, which are the first line of defense against reactive oxygen species (ROS), SOD convert radical O_2^- into H_2O_2 (Bose *et al*., 2014) and then catalase decomposes H_2O_2 into O_2 and H_2O (Willekens *et al*., 1997). In the current study, endophytes inoculated plants produced higher enzymes compared to un-inoculated plants at high temperature.

The endophyte inoculated plants exposed to high temperature produced higher SOD but it was reduced in un-inoculated plants. This indicated that the endophytes enhances the activity of SOD to detoxify the O_2^- produced during heat stress. Bilal *et al.* (2021) reported that the co-inoculation of *Paecilomyces formosus* LHL10 and *Penicillium funiculosum* LHL06 increased SOD activity in soybean. Similarly, significant increase in catalase was also observed in *A. flavus, A. nidulans* and *P. funiculosum* inoculated plants. The increased catalase facilitate in scavenging H_2O_2 thereby reducing the ROS under heat stress. The antioxidants produced by endophytes during heat stress activate the resistant mechanism in plants to cope up with high temperature (Wang *et al*., 2016). Ali *et al*. (2018a) reported that the *Thermomyces* sp. confers heat tolerance in cucumber plants by stimulating SOD, POD and catalase activities.

4.5.8 Influence of fungal endophytes on phytohormones in rice

The results pertaining to phytohormone production in rice as influenced by endophytes is presented in Table 12. During heat stress, the key defense-related phytohormones (abscisic acid, salicylic acid and ethylene) increase their levels and the growth-related phytohormones (cytokinins, auxin, and gibberellins) decrease. These fluctuations ultimately causes plant senescence under high temperature (Larkindale *et al*., 2005). In the present study, increased growth as well as stress related hormones such as auxin, cytokinins, gibberellins, epibrassinolide, abscisic acid indicates the involvement of endophytes in maintaining the homeostasis of phytohormones which could provide tolerance to plants.

Table 11: Influence of fungal endophytes on antioxidant enzymes in rice

Treatments	Super oxide dismutase (U/mg of Protein)		Catalase (U/mg of Protein)	
	Without Stress	With Stress	Without Stress	With Stress
Control	6.33[c]	3.81[c]	44.12[b]	108.64[b]
A. flavus	7.77[a]	14.9[a]	64.93[a]	130.68[a]
A. nidulans	7.43[b]	15.49[a]	44.67[b]	144.32[a]
E. endophytica	0.72[d]	0.95[d]	62.34[a]	112.75[b]
P. funiculosum	0.83[d]	8.79[b]	49.15[b]	133.22[a]
C.D.@ 5%	0.245	0.544	9.430	14.814
C.D.@ 1%	0.334	0.746	13.143	21.071
S.Em ±	0.08	0.179	2.993	4.702
C.V.	3.49	4.058	9.772	6.467

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test. (DMRT)

Auxins

Indole butyric acid is (IBA) one of the auxin compound. The auxin play an important role in heat stress-induced thermomorphogenesis, including stem (hypocotyl) elongation and leaf hyponasty (Kupers *et al*., 2020). In the present study, increased IBA was observed in endophytes colonized plants compared to un-inoculated plants under heat stress (Table 12a). Enhanced IBA helps to promote the adventitious root formation which helps to improve the uptake of water and nutrient under abiotic stress (Liao *et al*., 2012; Steffens, 2014). Ludwig-Muller *et al*. (1997) reported that the influence of arbuscular mycorrhizal fungi mediated drought tolerance in maize by an increase of IBA.

Gibberellins

Gibberellins (GAs) are crucial plant hormone that is essential for plants throughout their life cycle and act as signalling molecule under abiotic stress (Abdulaziz *et al*., 2020). During high temperature, bioactive GAs (GA1, GA3, GA4

Table 12a: Influence of fungal endophytes on growth promoting phytohormones of rice

Phytohormones (ng/g of FW)	Auxin		Gibberellins				Cytokinins					
	3-Indole Butyric Acid		Gibberellic Acid 3		Gibberellic Acid 4		Benzyl aminopurine		Zeatin trans isomer		Trans zeatin Riboside	
Treatments	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress
Control	2.07^{d}	2.17^{d}	1.48^{a}	0.11^{e}	22.01^{b}	9.17^{e}	0.74^{b}	0.55^{d}	11.98^{b}	7.64^{c}	2.18^{b}	3.84^{e}
A. flavus	2.35^{b}	2.56^{b}	1.10^{b}	0.21^{d}	18.38^{c}	22.76^{b}	0.64^{c}	1.17^{a}	7.17^{c}	8.24^{b}	2.92^{a}	4.66^{d}
A. nidulans	2.19^{c}	2.93^{a}	0.56^{d}	0.54^{a}	23.09^{a}	31.71^{a}	0.64^{c}	0.65^{c}	20.63^{a}	11.88^{a}	1.48^{d}	6.33^{a}
E. endophytica	2.64^{a}	2.41^{c}	0.34^{e}	0.25^{c}	10.87^{d}	16.05^{c}	0.52^{d}	0.31^{e}	3.75^{e}	5.55^{d}	0.76^{e}	5.91^{b}
P. funiculosum	1.75^{e}	2.42^{c}	1.03^{c}	0.37^{b}	10.93^{d}	14.93^{d}	0.84^{a}	0.93^{b}	5.59^{d}	7.91^{bc}	1.98^{c}	5.25^{c}
C.D.@ 5%	0.106	0.125	0.049	0.017	0.816	1.064	0.032	0.039	0.470	0.431	0.094	0.272
C.D.@ 1%	0.145	0.174	0.068	0.023	1.128	1.472	0.044	0.054	0.650	0.595	0.128	0.377
S.Em ±	0.035	0.042	0.016	0.006	0.271	0.353	0.011	0.013	0.156	0.143	0.031	0.09
C.V.	3.169	3.334	3.594	3.79	3.174	3.733	3.117	3.585	3.177	3.466	3.302	3.477

Note: Means with same superscript in a column do not differ significantly at $p \leq 0.05$ as per Duncan's Multiple Range Test (DMRT). FW=Fresh weight.

and GA7) stay at low levels due to suppression of GA 20-oxidase genes (GA20ox1, GA20ox2, and GA20ox3) and GA 3-oxidase genes (GA3ox1 and GA3ox2) (Toh *et al*., 2008). However, in the present study endophytes colonized plants showed an increase in GA3 and GA4 at high temperature compared to normal growth temperature but reduced in un-inoculated plants (Table 12a). This indicates that endophytes might protect GA20 and GA3 oxidase genes from high temperature and helps to synthesis of higher GA3 and GA4. This increased GAs modulates the oxidative stress process and antioxidant enzyme activity, consequently suppressing the negative effect of abiotic stress (Khan *et al*., 2012). The results are in agreement with Hamayun *et al*. (2017) who reported that the synthesis of GA1 and GA4 was enhanced by 33% and 53% in *Porostereum spadiceum* AGH786 treated soybean seedlings exposed to 140 mM NaCl respectively when compared with their respective controls. Thus, by maintaining GAs under stress conditions, the endophyte is having a beneficial effect on plant for long-term survival.

Cytokinins

The most common group of cytokinins present in plants has an isoprenoid side chain and includes isopentenyladenine (iP), trans-zeatin (tZ), cis-zeatin (cZ) and benzyl aminopurine (BAP) (Mok and Mok, 2001). Heat stress reduced the content of cytokinins in rice plants and also decreases in cytokinin transportation from root to shoot *via* xylem sap and seem to be responsible for the lower number of tillers and spikelets per panicle (Wu *et al*., 2017). In the present study, increased cytokinins *viz*., tZ, cZ and BAP was observed in endophytes colonized stress plants compared to un-inoculated plants (Table 12a), which indicate that endophytes might involve in synthesis and transportation of cytokinins. The endophytes could mitigate heat stress by increasing the cytokinin level, which play important role during long-term temperature acclimation and changes in plant developmental program to recover chloroplast function and photosynthetic ability (Escandon *et al*., 2016).

Abscisic acid (ABA)

ABA serve as a thermo-priming hormone that enables plants to respond more rapidly and efficiently to heat stress It has been reported that ABA enhances heat tolerance in plants by increasing H_2O_2 levels to induce antioxidant capacity and HSPs (Islam *et al*., 2019; Li *et al*., 2021). It modulates levels of carbohydrates and energy

status through accelerated transport and enhanced metabolism of sucrose to strengthen plant thermal tolerance (Rezaul *et al*., 2019). In the present study, an increased level of ABA was observed in all the treatments when the plants exposed to high temperature (Table 12b). However, a significantly higher ABA was recorded in plants colonized with *A. nidulans* (0.85 ng/g) followed by *P. funiculosum* (0.77 ng/g), *A. flavus* (0.58 ng/g), *E. endophytica* (0.55 ng/g) and the least significant was observed in un-inoculated plants (0.29 ng/g). This indicates that endophytes mediate the thermotolerance by inducing anoxidant capacity, HSPs and sucrose metabolism. Several workers reported increased endogenous ABA level in leaves and roots during fungal associations compared to non-fungal treated plants (Allen *et al*., 1982; Esch *et al*., 1994; Herrera-Medina *et al*., 2007).

Salicylic acid (**SA**)

SA is a kind of novel phytohormones, has been suggested to promote basal thermotolerance and induce membrane thermoprotection (Kotak *et al*., 2007). A significantly increased level of SA was recorded in plants inoculated with *E. endophytica*, which is followed by *P. feniculosum*, *A. flavus* and *A. nidulans*. The least SA content was observed in un-inoculated plants (Table 12b). The enhanced SA modulates the activities of antioxidant enzymes (including catalase, superoxide dismutase, and peroxidase) and then alleviates the membrane damage caused by heat stress (Li *et al*., 2021). The SA content was significantly higher in *Phoma glomerata* associated plants in salinity stress compared to un-inoculated plants (Waqas *et al*., 2012).

Jasmonates (JAs)

JAs play a role in signal transduction in relation to stressful situation. It could confer thermotolerance through enhanced osmotic regulation by increasing the synthesis of some osmoregulators and activates the antioxidants, thereby suppressing ROS generation and reducing programmed cell death (Chen *et al*., 2020; Yang, *et al*., 2020). In the present study, endophytes inoculated plants recorded higher jasmonic acid and cis jasmone compared to un-inoculated plants (Table 12b). Among the four inoculated endophytes, significantly higher production of jasmonic acid and cis-jasmone was recorded in *A. nidulans* and *A. flavus* respectively. This finding indicate that the endophytes cope up with high temperature by activating defense system of

Table 12b: Influence of fungal endophytes on defense related phytohormones of rice

Phytohormones (ng/g of FW)	Abscisic Acid		Salicylic acid		Jasmonates				Epibrasinolide	
					Jasmonic acid		Cis-jasmone			
Treatments	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress	Without Stress	With Stress
Control	0.19^{b}	0.29^{d}	87.74^{a}	35.13^{c}	1.69^{c}	0.17^{e}	23.65^{b}	15.34^{d}	21.00^{b}	11.03^{e}
A. flavus	0.18^{c}	0.58^{c}	59.82^{b}	49.64^{b}	1.58^{c}	1.96^{c}	27.93^{a}	28.67^{a}	16.10^{e}	39.86^{a}
A. nidulans	0.43^{a}	0.85^{a}	42.19^{e}	49.52^{b}	5.92^{a}	7.27^{a}	20.88^{c}	22.08^{b}	21.92^{a}	31.50^{b}
E. endophytica	0.14^{d}	0.55^{c}	54.89^{c}	61.29^{a}	1.61^{c}	0.39^{d}	12.11^{e}	15.29^{d}	17.23^{d}	12.52^{d}
P. funiculosum	0.12^{e}	0.77^{b}	50.78^{d}	50.29^{b}	5.24^{b}	2.94^{b}	17.02^{d}	19.62^{c}	20.02^{c}	17.94^{c}
C.D.@ 5%	0.01	0.032	3.057	2.356	0.147	0.198	0.972	1.064	0.895	1.335
C.D.@ 1%	0.013	0.044	4.227	3.259	0.204	0.274	1.344	1.472	1.237	1.846
S.Em ±	0.003	0.011	1.015	0.782	0.049	0.066	0.322	0.353	0.297	0.443
C.V.	2.992	3.463	3.434	3.18	3.047	5.167	3.175	3.497	3.084	3.925

Note: Means with same superscript in a column do not differ significantly at $p \leq 0.05$ as per Duncan's Multiple Range Test (DMRT). FW=Fresh weight.

plants. Waqas *et al*. (2012) reported that the cucumber plant associated with *Phoma glomerata* had higher JA content as compared to un-inoculated plants under drought.

Epibrassinolide

Epibrassinolide is one the derivative of brassinosteroids phytohormone. Brassinosteroids have been observed to maintain redox homeostasis under heats stress by enhancing activities of enzymes involved in the ascorbate–glutathione (AsA-GSH) cycle and also improve the plant photosynthesis through improving the photosynthetic pigments, carboxylation rate of rubisco and photochemical activity of PSI (Yang *et al*., 2021). When the plants exposed to high temperature, changes in epibrassinolide was observed in between treatments. Endophyte colonized plants recorded higher amount of epibrassinolide compared to un-inoculated plants (Table 12b). This indicates that endophytes could mediate the heat tolerance by improving the photosynthesis, producing HSPs or enhancing the antioxidants through increase in host epibrassinolide. Epibrassinolide treated *Arabidopsis* and rapeseed plants mitigate the high temperature by increased HSPs synthesis and rapid resumption of protein (Kagale *et al*., 2007).

4.5.9 Confirmation of fungal endophytes present in inoculated rice plant.

The fungal endophytes were re-isolated from shoot of inoculated rice and confirmed by comparing their morphology with respective mother culture (Plate 7) and then they were further identified by the ITS region sequences and compared with mother culture sequences (Fig. 4). These sequences proved that the isolated fungi are same as mother cultures.

4.6 Plant growth promoting and stress alleviating traits of fungal endophytes

Plant growth promoting traits *viz*., siderophore production, phosphate, and potassium and zinc solubilisation of fungal endophytes (*A. flavus*, *A. niger*, *E. endophytica* and *P. funiculosum*) was analysed by appropriate techniques (Fig. 5 and 6; Plate 8).

All the endophytes have ability to produce siderophore and also solubilize phosphate, potassium and zinc. The *P. funiculosum* showed higher siderophore producing and phosphate and zinc solubilizing index compared to other endophytes.

Potassium solubilizing index was higher in *E. endophytica*. This result revealed that the endophytes are able to solubilize tri-calcium phosphate, mica and ZnO present in the media due to production of organic and inorganic acids, hydroxyl ions, carbon monoxide, exopolysaccharides or extracellular enzymes (Mehta *et al*., 2019). These endophytes have efficacy to convert elements from unavailable to available source for plant uptake by releasing their compounds to soil through root exudates, there by boost up the growth of plant. The production of siderophores in endophytic fungi associated with both marine and terrestrial habitats (Baakza *et al*., 2004).

The results pertaining to phytohormones secreated by fungal endophytes were presented in Table.13. Many fungal species secrete phytohormones such as auxin, gibberllins, cytokinins, abscisic acid (ABA) *etc*. These hormones could have a role in growth of mycelia and spore production (Gruen, 1959; Ulrich, 1960). In the present study, all the four fungal endophytes such as *A. flavus, A. niger, E. endophytica* and *P. funiculosum* were able to secret indole acetic acid (IAA), gibberellic acid (GA3), salicylic acid (SA) and abscisic acid (ABA). The secreation amount was increased in elevated temperature compared to optimum growth temperature. It might be due to adaptation to temperature by morphological variation. For example, *A. flavus* produces more spores to sustain at high temperature with maximum secretion of phytohormones. ABA is not only the stress metabolite, but also induces the synthesis of novel specific proteins, which might be important in acquired stress tolerance (Gomez *et al*., 1988). This result are in agrrement with the Marsalek *et al*. (1992) who reported that the green algae as well as cyanobacteria are able to produce extracellular ABA under salt, acid and drought stress as well as senescence.

The data pertaining to secretion of phenols, flavonoids and antioxidants by fungal endophyte is presented in Table 13. Phenols and flavonoids act as signalling molecules for plant microbe interaction (Mandal *et al*., 2010). They also act as antioxidants to reduce the oxidative stress in fungi. Maximum phenol content was observed in *A. flavus* at 40 ^{o}C and the *P. feniculosum* secreted higher amount of flavonoid at 40 ^{o}C. Secretion of phenols and flavonoids are necessary to sustain the organism at high temperature (Ismail *et al*., 2018). Under optimum growth condition, significantly higher amount of antioxidants was observed in *P. feniculosum*. This is followed by *A. flavus, A. nidulans* and *E. endophytica.* When they incubate at 40 ^{o}C, increase in antioxidants was observed in all the fungi. This indicated that the fungi

Fig 4. Phylogenetic tree of re-isolated fungal cultures with their respective sequences of mother culture. [a: *A. flavus*, b. *A. nidulans*, c. *E. endophytica* and d. *P. feniculosum*]

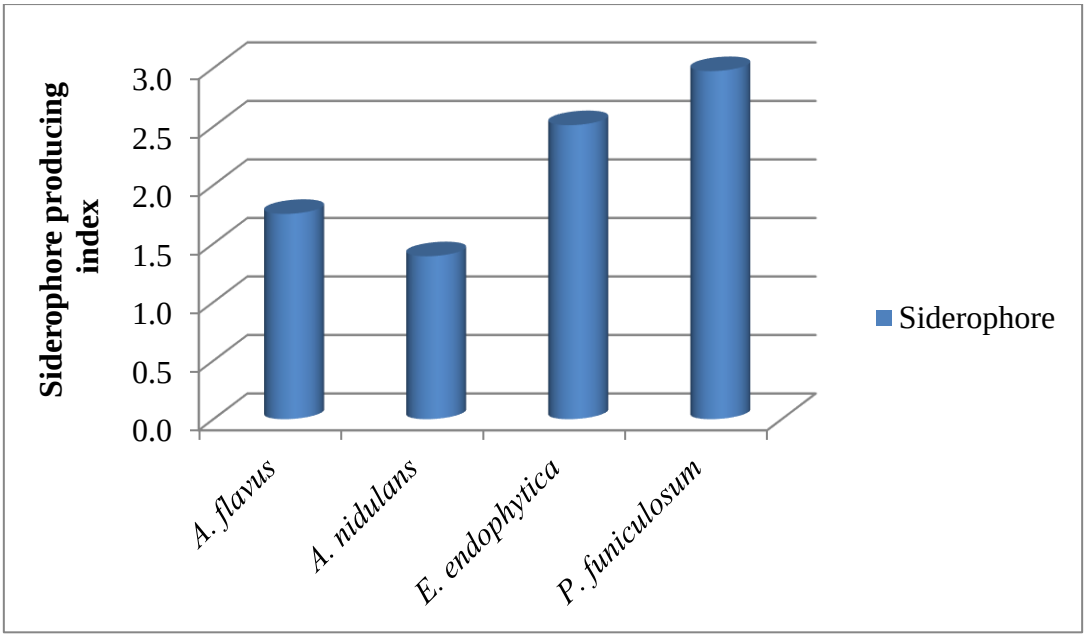


Fig 5: Siderophore production by fungal endophytes

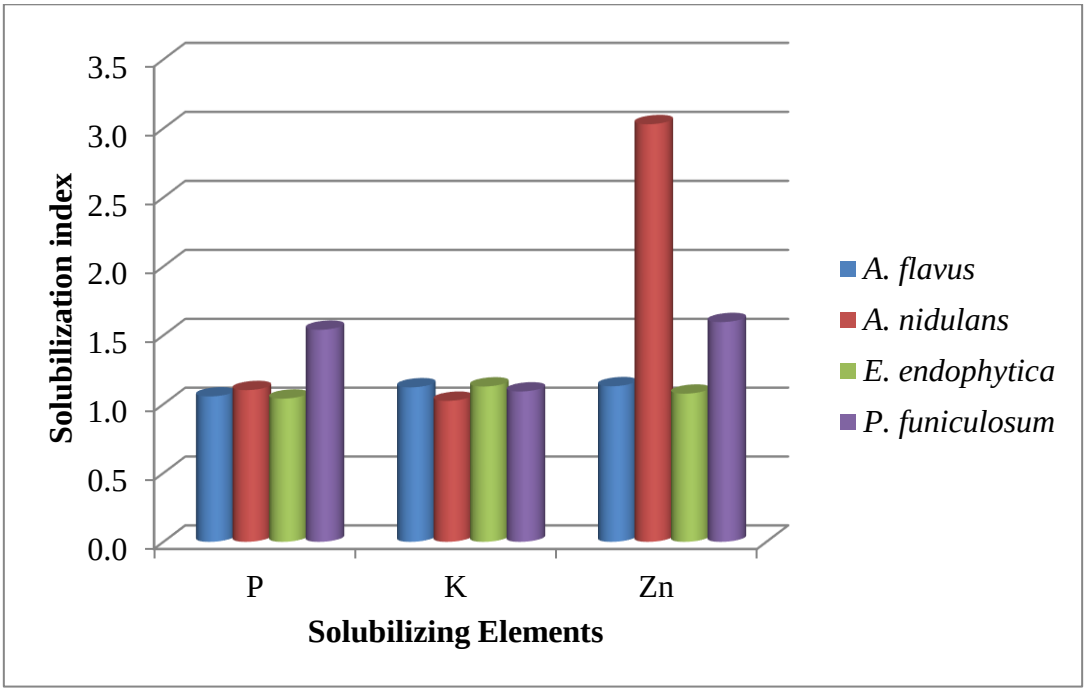


Fig. 6: Phosphate, potassium and zinc solubilisation by fungal endophytes

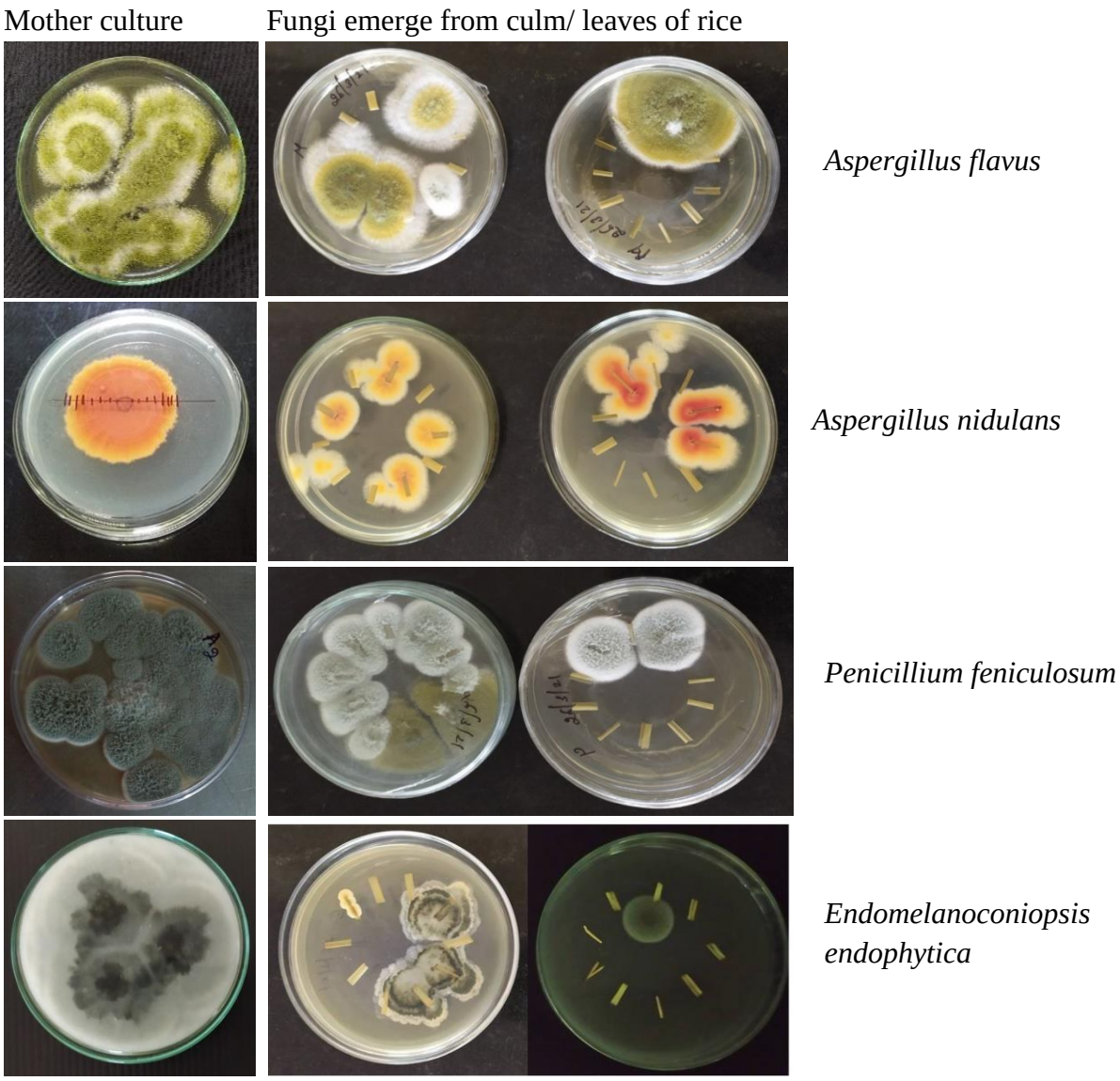
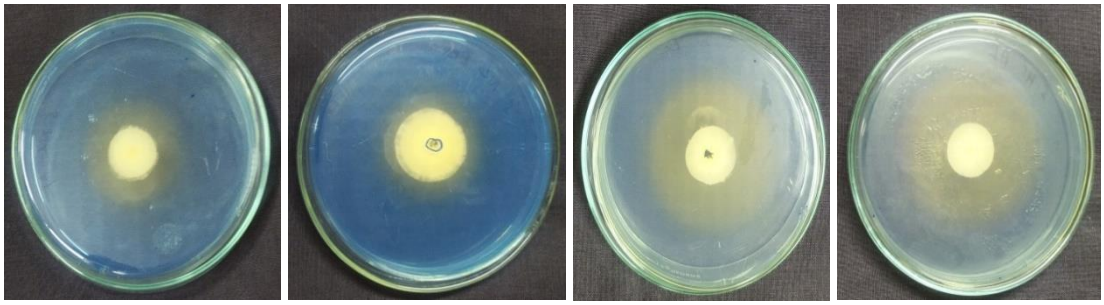
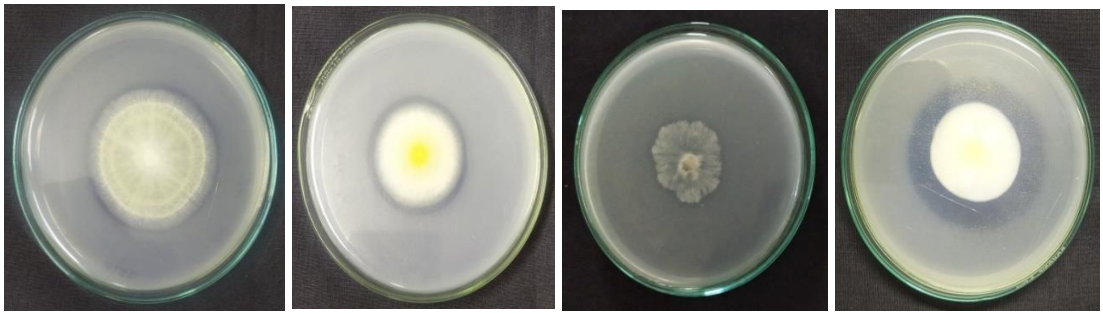


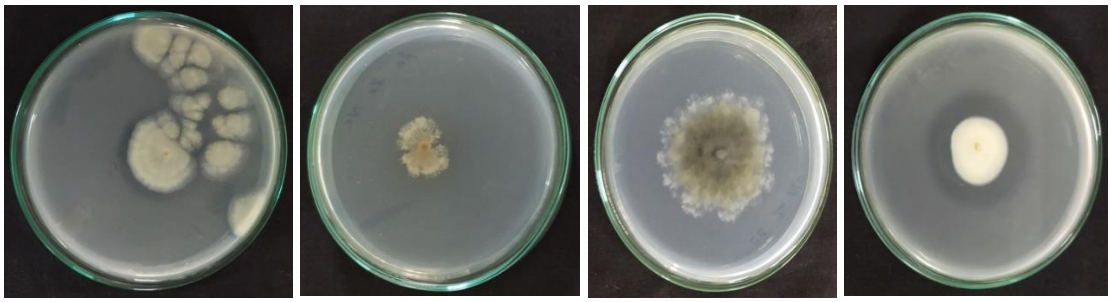
Plate 7: Comparison of re-isolated fungal endophytes with their mother culture



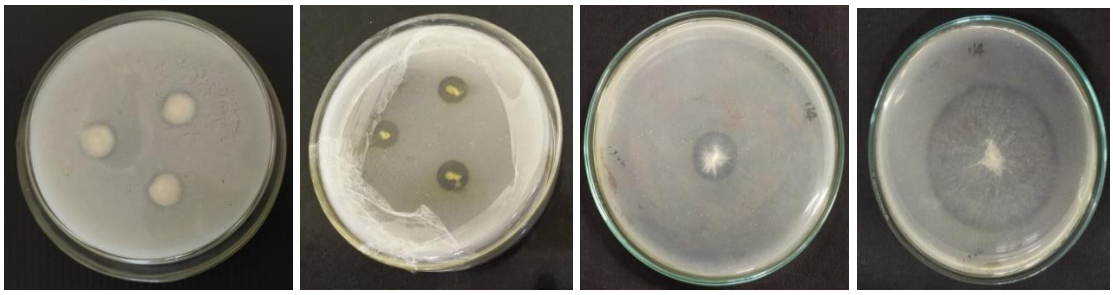
Siderophore production



Phosphate solubilization



Potassium solubilization



Zinc solubilization

Plate 8: Plant growth promoting traits of fungal endophytes [left to right *A. flavus, A. nidulans, E. endophytica* and *P. funiculosum*]

Table 13: Determination of phytohormones (µg/ml) secreted by fungal endophytes under *in-vitro*

Phytohormones		Indole acetic acid (IAA)		Gibberellic acid (GA3)		Salicylic acid (SA)		Abscisic acid (ABA)	
Incubation Temperature		30 ºC	40 ºC	30 ºC	40 ºC	30 ºC	40 ºC	30 ºC	40 ºC
Fungal endophytes	*A. flavus*	4.91^{a}	7.71^{a}	0.05^{d}	1.72^{a}	0.30^{c}	1.80^{b}	1.68^{a}	3.84^{a}
	A. nidulans	1.67^{c}	4.92^{b}	0.18^{a}	0.06^{d}	0.24^{d}	0.58^{d}	1.14^{b}	1.42^{b}
	E. endophytica	1.91^{b}	3.15^{c}	0.07^{c}	0.51^{c}	0.91^{b}	2.45^{a}	0.35^{c}	1.00^{c}
	P. funiculosum	0.21^{d}	1.18^{d}	0.14^{b}	0.88^{b}	1.30^{a}	1.65^{c}	0.07^{d}	0.59^{d}
CD @ 5%		0.127	0.226	0.008	0.046	0.034	0.08	0.04	0.081
CD @ 1%		0.175	0.312	0.011	0.063	0.047	0.11	0.055	0.111

Table 14: Determination of phenols, flavonoids and antioxidants secreted by fungal endophytes under *in-vitro*

		Phenol (µg/ml)		Flavonoid (µg /ml)		Antioxidants activity EC_{50} (%)	
Incubation Temperature		30 ºC	40 ºC	30 ºC	40 ºC	30 ºC	40 ºC
Fungal endophytes	*A. flavus*	8.66^{a}	17.87^{a}	0.14^{c}	0.19^{d}	36.43^{b}	52.58^{b}
	A. nidulans	2.10^{b}	2.56^{b}	0.47^{a}	0.58^{b}	22.61^{c}	49.82^{c}
	E. endophytica	0.96^{c}	1.27^{c}	0.22^{b}	0.49^{c}	9.21^{d}	42.02^{d}
	P. funiculosum	0.74^{d}	2.43^{b}	0.12^{c}	0.75^{a}	52.00^{a}	64.16^{a}
CD @ 5%		0.199	0.526	0.042	0.051	1.622	2.447
CD @ 1%		0.274	0.725	0.058	0.070	2.234	3.371

Note: Means with same superscript in a column do not differ significantly at $p \leq 0.05$ as per Duncan's Multiple Range Test (DMRT).

can protect themselves from oxidative stress (caused by high temperature) by enhancing the antioxidants. Hence, this experiment reveals that fungal endophytes have plant growth promoting and stress alleviated traits and could be a role in providing thermotolerance by increasing their fitness of rice plants.

4.7 Field evaluation of fungal endophytes on growth and yield of rice during summer

The fungal endophytes (*A. flavus, A. nidulans, E. endophytica and P. funiculosum*) were inoculated to rice seedlings and evaluated for growth and yield under field conditions. The data pertaining to growth and yield is presented in Table 14 and 15. All the plants inoculated with endophytes increased plant height, number of tillers, number of panicles, grain yield and straw yield compared to control. The highest growth and yield parameters were observed in *A. nidulans, P. funiculosum* and *A. flavus* which is followed by *E. endophytica* indicating that the endophyte inoculation can promote the growth and yield of rice besides imparting heat tolerance.

The results indicated that the endophytes inoculated plants improved the growth and yield of rice. It might be due to production of phytohormones. Cytokinin has pivotal role in increasing number of branches and spikelets in rice panicle for the benefit of grain yield has been corroborated more recently (Ashikari *et al*., 2005). Earlier studies are reported that fungal endophyte, *Piriformospora indica* has ability to produce cytokinin (Vadassery *et al*., 2008) which might be helpful to increase the tillers in plant system. In addition, Yuan *et al*. (2010) reported that endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus- assimilating enzymes or release of organic acids and improve the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system which in turn improve the assimilation of photosynthates and sink translocation and eventually produced larger sized and more grains of higher weight that ultimately increased the yield. The results are in confirm with Gupta *et al*. (2015) who reported that *Trichoderma longibrachiatum, Westerdykella aurantiaca, Lasiodiplodia sp.* and *Rhizopus delemar* inoculation improved the rice grain yield by increasing the yield attributes such as panicle number, spikelet number and length weight of the grain. Thus, the study, envisaged that the endophytes isolated from hot and cold desert plants can mitigate heat stress in rice.

Table 15: Influence of different endophytic fungi on growth attributes of rice at different intervals

Treatments	Plant height (cm)			Number of Tillers / hills		
	35 DAS	70 DAS	At harvest	35 DAS	70 DAS	At harvest
Control	32.02^{d}	69.20^{d}	74.85^{c}	7.86^{d}	20.05^{d}	21.05^{c}
A. flavus	36.83^{b}	76.33^{b}	79.60^{a}	12.26^{b}	24.29^{b}	25.70^{a}
A. nidulans	38.50^{a}	78.08^{a}	81.21^{a}	15.11^{a}	25.25^{a}	26.50^{a}
E. endophytica	35.32^{c}	74.32^{c}	77.83^{b}	10.46^{c}	22.49^{c}	24.02^{b}
P. funiculosum	38.32^{a}	77.55^{ab}	80.33^{a}	14.34^{a}	24.83^{ab}	26.05^{a}
C.D.@ 5 %	1.055	1.579	1.699	1.552	0.958	1.069
S.Em ±	0.339	0.507	0.545	0.498	0.308	0.343
C.V	1.871	1.35	1.385	8.302	2.631	2.784

Note: Means with same superscript in a column do not differ significantly at $p \leq 0.05$ as per Duncan's Multiple Range Test (DMRT). DAS Days After Sowing.

Table 15: Influence of different endophytic fungi on yield attributes of rice

Treatments	Panicle length (cm)	No. of panicle per hill	1000 grain weight (g)	Grain yield (t/ha)	Straw yield (t/ha)	Biological yield (t/ha)	Harvest Index
Control	15.67^{b}	15.75^{c}	19.50^{c}	3.38^{b}	9.63^{b}	13.01^{b}	0.26^{b}
A. flavus	17.91^{a}	18.52^{ab}	21.62^{ab}	4.47^{a}	11.02^{a}	15.49^{a}	0.29^{ab}
A. nidulans	18.28^{a}	18.94^{a}	22.40^{a}	4.80^{a}	10.20^{ab}	15.00^{a}	0.32^{a}
E. endophytica	17.78^{a}	17.93^{b}	20.95^{b}	4.51^{a}	10.83^{a}	15.34^{a}	0.30^{ab}
P. funiculosum	18.15^{a}	18.97^{a}	22.08^{ab}	4.72^{a}	9.81^{b}	14.53^{a}	0.32^{a}
C.D. @ 5%	0.763	0.614	1.249	0.783	1.012	1.242	0.046
S.Em ±	0.245	0.197	0.401	0.251	0.325	0.399	0.015
C.V	2.79	2.186	3.763	11.492	6.306	5.431	9.955

Note: Means with same superscript in a column do not differ significantly at p≤ 0.05 as per Duncan's Multiple Range Test (DMRT).

Plate 9: General view of the field experiment. (a) one month after sowing (b) harvesting stage.

V SUMMARY

Global temperature increases day by day due to change in climate. This raised temperature significantly affects physiological processes of agricultural crops that results in reduction in food production. Endophytes are the beneficial asymptomatic microbial partners of plants and widely used as plant growth promoters. Besides this endophytes mediate imparting abiotic stress tolerance through habitat adapted symbiosis. The present study is intended to screen and evaluate endophytic fungi for heat stress tolerance in rice.

Thirty fungal endophytes were isolated from plants growing in Thar Desert and Himalayan cold desert were screened against different temperatures (28, 30, 32, 34, 36, 38, 40, 42 and 44 ^{o} C) on potato dextrose agar *in-vitro*. Among the 30 isolates, five isolates, ACT 2, LAS 4, LAS 6, PRC 2 and SAP 3 of Thar desert and three isolates A2, A7 and X5 of Himalayan cold desert sustained the growth at higher temperature (>40 ^{o} C). The 8 endophytes were selected and identified as *Aspergillus flavus* (ACT 2), *Aspergillus nidulans* (SAP 3), *Aspergillus terrus* (PRC 2), *Chaetomium* sp. (LAS 4 and LAS 6), *Ceriporia lacerate* (A7), *Endomelanoconiopsis endophytica* (X5) and *Penicillium feniculosum* (A2) by using ITS region sequences.

The identified endophytes were evaluated for their ability to impart thermotolerance in rice by exposing to heat stress (45^{o}C for 7h/ day) for 10 days. The shoot and root growth traits such as number of tillers, leaves, fresh and dry weight, root volume, dry weight of root and biomass were recorded after 10 days of heat stress. The biomass production was reduced when the plants were exposed to high temperature due to wilting, curling and yellowing of leaves and tillers. However, the endophytes inoculated plants produced higher biomass compared to un-inoculated plants under heat stress. The plants inoculated with *A. nidulans* produced maximum biomass which is followed by *E. endophytica*, *Cheatomium* sp. L6, *A. terreus*, *P. funiculosum* and *A. flavus*. The least biomass was recorded in un-inoculated plants.

Based on stress tolerance index of growth attributes, the four best performed endophytes against heat stress *viz*., *A. flavus*, *A. nidulans*, *E. endophytica* and *P. funiculosum* were selected for analysis of physiological traits in rice. The endophyte inoculated plants showed increased relative water content, CO_2 assimilation rate,

stomatal conductance, intercellular concentration of CO_2 and transpiration rate. The endophyte inoculated plants protected chlorophyll pigments (a & b) at high temperature and maintained membrane stability by reducing lipid peroxidation. Accumulation of higher osmolytes (glycine betaine, phenol and flavonoid) and antioxidant enzymes (SOD and catalase) in rice was evidenced in endophyte inoculated plants. In addition, endophytes enhanced the production of growth hormones (auxin, gibberellins, cytokinins) and defense related phytohormones (ABA, jasmonic acid, salicyclic acid, epibrassinolide) in stressed plants in order to activate enzymatic and non-enzymatic antioxidants and HSPs. Besides, these endophytes produced plant growth promoting substances like siderophore, phosphate, potassium and Zn solubilisation, secretion of IAA, GA3, ABA and SA and defence related compounds like phenols, flavonoids and antioxidants. Field evaluation indicated the ability of these endophytes in increasing grain and straw yield of rice compared to un-inoculated plants. Thus, this study, envisaged that the endophytes isolated from hot and cold desert plants can impart tharmotolerance in rice.

Future line of work

In the present study, field trail was carried out at moderate temperature zone (central dry zone). But, the study required to evaluate these endophytes at higher temperature zones such as north eastern dry zone (Gulbarga and Raichur district) and northern dry zone (Bellary district) of Karnataka.

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APPENDIX I

Sequence data of thermotolerant fungal endophytic culture

1. *Aspergillus flavus* ACT-2

CCCCTCCCACCCGTGTTTACTGTACCTTGTTGCTTCGGCGGGGGCCGCCGGGGGCGCCCGCGCCCGCCG
GACACCATGTCTGATCTAGTGAAGTCTGAGTTTGTATCGCAATCAGTTAAAACTTTCAACAATGGATCT
CTTGGTTCCGGCATCGATGAAGAACGCAGCGAAATGCGATAACTAGTGTGAATTGCAGAATTCCGTGAA
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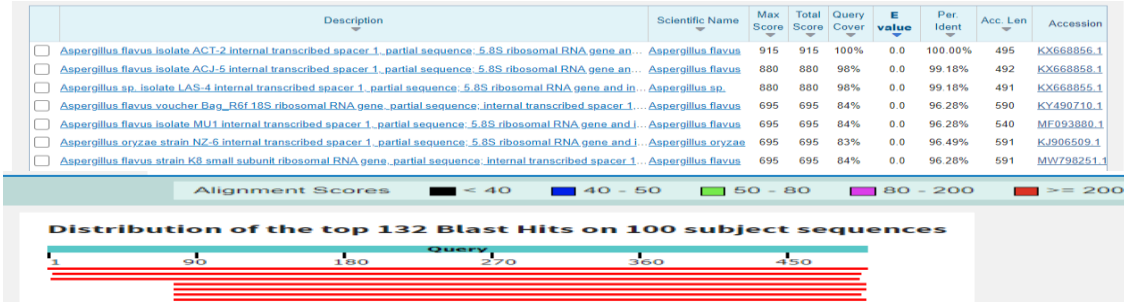

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Aspergillus flavus isolate ACT-2 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aspergillus flavus	915	915	100%	0.0	100.00%	495	KX668856.1
Aspergillus flavus isolate ACJ-5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene an...	Aspergillus flavus	880	880	98%	0.0	99.18%	492	KX668858.1
Aspergillus sp. isolate LAS-4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in...	Aspergillus sp.	880	880	98%	0.0	99.18%	491	KX668855.1
Aspergillus flavus voucher Bag_R6f 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1,...	Aspergillus flavus	695	695	84%	0.0	96.28%	590	KY490710.1
Aspergillus flavus isolate MU1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Aspergillus flavus	695	695	84%	0.0	96.28%	540	MF093880.1
Aspergillus oryzae strain NZ-6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and i...	Aspergillus oryzae	695	695	83%	0.0	96.49%	591	KJ906509.1
Aspergillus flavus strain K8 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1...	Aspergillus flavus	695	695	84%	0.0	96.28%	591	MW798251.1

2. *Aspergillus terreus* PRC2

GGTCACCTGGAAAAAACAAGTTGCAAATAAATGCGTCGGCGGGCGCCGGCCGGGCCTACGGAGCGGAAG
ACGAAGCCCCATACGCTCGAGGACCGGACGCGGTGCCGCCGCTGCCTTTCGGGCCCGTCCCCCGGGAGC
CGGGGGACGAGGGCCCAACACACAAGCCGGGCTTGAGGGCAGCAATGACGCTCGGACAGGCATGCCCCC
CGGAATACCAGGGGGCGCAATGTGCGTTCAAAGACTCGATGATTCACTGAATTCTGCAATTCACATTAG
TTATCGCATTTCGCTGCGTTCTTCATCGATGCCGGAACCAAGAGATCCATTGTTGAAAGTTTTAACTGA
TTGCAAAGAATCACACTCAGACTGCAAGCTTTCAGAACAGGGTTCATGTTGGGGTCTCCGGCGGGCACG
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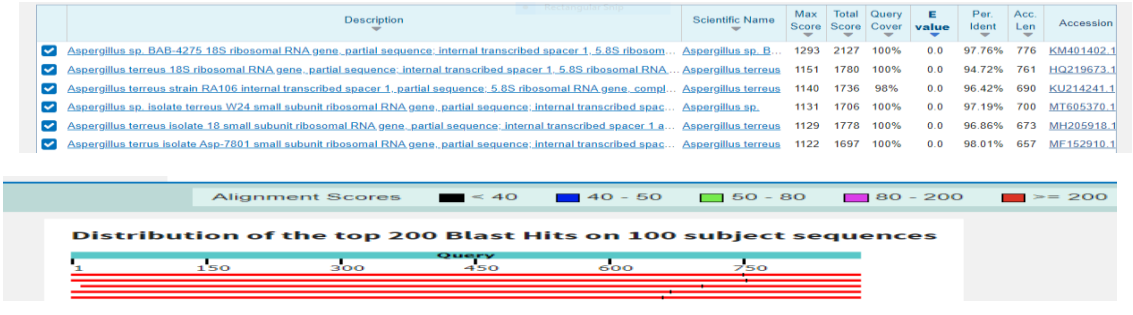

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Aspergillus sp. BAB-4275 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosom...	Aspergillus sp. B...	1293	2127	100%	0.0	97.76%	776	KM401402.1
Aspergillus terreus 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA...	Aspergillus terreus	1151	1780	100%	0.0	94.72%	761	HQ219673.1
Aspergillus terreus strain RA106 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, compl...	Aspergillus terreus	1140	1736	98%	0.0	96.42%	690	KU214241.1
Aspergillus sp. isolate terreus W24 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Aspergillus sp.	1131	1706	100%	0.0	97.19%	700	MT605370.1
Aspergillus terreus isolate 18 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 a...	Aspergillus terreus	1129	1778	100%	0.0	96.86%	673	MH205918.1
Aspergillus terrus isolate Asp-7801 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Aspergillus terreus	1122	1697	100%	0.0	98.01%	657	MF152910.1

3. *Aspergillus nidulans* SAP 3

TCCGGGCGCCCAACCTCCCACCCGTGACTACCTAACACTGTTGCTTCGGCGGGGAGCCCCCTAGGGGCG
AGCCGCCGGGGACCACTGAACTTCATGCCTGAGAGTGATGCAGTCTGAGCCTGAATACAAATCAGTCAA
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GCCTGTCCGAGCGTCATTGCTGCCCTCAAGCCCGGCTTGTGTGTTGGGTCGTCGTCCCCCCCGGGGGAC
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ATACCCGCTGAACTTAAGCATATCAATAAGGC

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Aspergillus nidulans strain SD531 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Aspergillus nidul...	952	952	100%	0.0	100.00%	555	MN901610.1
Aspergillus quadrilineatus isolate CICR1 small subunit ribosomal RNA gene, partial sequence; internal transcribe...	Aspergillus quad...	952	952	100%	0.0	100.00%	590	MW228076.1
Aspergillus sp. strain F029 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5...	Aspergillus sp.	948	948	99%	0.0	100.00%	571	MK961271.1
Aspergillus nidulans isolate 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and inter...	Aspergillus nidul...	948	948	99%	0.0	100.00%	524	MK425749.1
Aspergillus fumigatus strain Ns1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and in...	Aspergillus fumig...	948	948	99%	0.0	100.00%	537	MK879806.1
Aspergillus sp. strain Y.H. Yeh V0204 small subunit ribosomal RNA gene, partial sequence; internal transcribed s...	Aspergillus sp.	948	948	99%	0.0	100.00%	606	MH141246.1

4. *Cheatomium* sp. LAS4

GGGACTATAGCGGAGGATGCATACGTAGGTGCCCTCCCTAACTACAACCGTTGCTTCGGCGGGCGGGCG
CCCCGCGCGCCCCCCCGGCCCCCCTCGCTGGGGGCGCCCGCCGGAGGTTACCTAACTCTTGTTTACCAC
ACGGCCTCTCTGAGTCTTCTGTACTGAATAAGTCAAAACTTTCAACAACGGATCTCTTGGTTCTGGCAT
CGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAATTGCAGAATTCAGTGAATCATCGAATCTTTG
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CCGGGCCTGTGTTGGGGACCTGCGGCTGCCCGCAGGCCCCGAAAACCAGTGGCGGGCTCGCTGTCACCC
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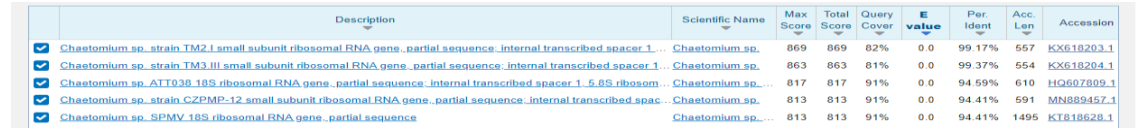

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Chaetomium sp. strain TM2.I small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Chaetomium sp.	869	869	82%	0.0	99.17%	557	KX618203.1
Chaetomium sp. strain TM3.III small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Chaetomium sp.	863	863	81%	0.0	99.37%	554	KX618204.1
Chaetomium sp. ATT038 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosom...	Chaetomium sp. ...	817	817	91%	0.0	94.59%	610	HQ607809.1
Chaetomium sp. strain CZPMP-12 small subunit ribosomal RNA gene, partial sequence; internal transcribed spac...	Chaetomium sp.	813	813	91%	0.0	94.41%	591	MN889457.1
Chaetomium sp. SPMV 18S ribosomal RNA gene, partial sequence	Chaetomium sp. ...	813	813	91%	0.0	94.41%	1495	KT818628.1

5. *Cheatomium* sp. LAS 6

CGTCTCAATACCGAAGCGATCCTAGGTCTTATTGGTGGGTGTTCTCCCGCGCCGCGAGGGGGGGGGCGA
GAGGGTTTTACGGCCGGAGCCCGCGGCACTCCCTGGGCGAGGTGTTGCTACTACGCTCGGGGTGACAGC
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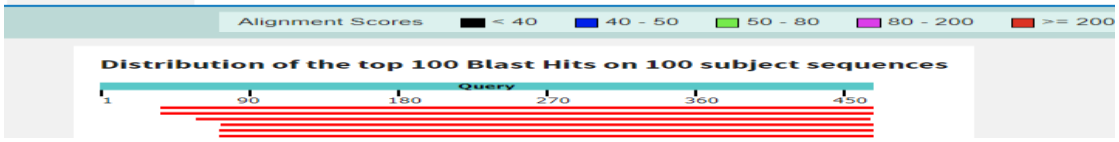
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Chaetomium sp. strain TM3.III small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Chaetomium sp.	789	789	92%	0.0	99.77%	554	KX618204.1
Chaetomium sp. strain TM2.I small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, ...	Chaetomium sp.	789	789	92%	0.0	99.77%	557	KX618203.1
Fungal sp. strain 156 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete seque...	fungal sp.	747	747	87%	0.0	99.75%	409	MG865832.1
Chaetomium sp. isolate LAS-6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, comple...	Chaetomium sp.	717	717	84%	0.0	99.49%	394	KX668854.1
Achaetomium strumarium isolate CK1129 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge...	Achaetomium str...	697	697	84%	0.0	98.49%	526	MH473825.1
Chaetomium sp. isolate Am-610 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer...	Chaetomium sp.	688	688	84%	0.0	97.99%	565	MH627288.1



6. *Ceriporia lacerate* A7

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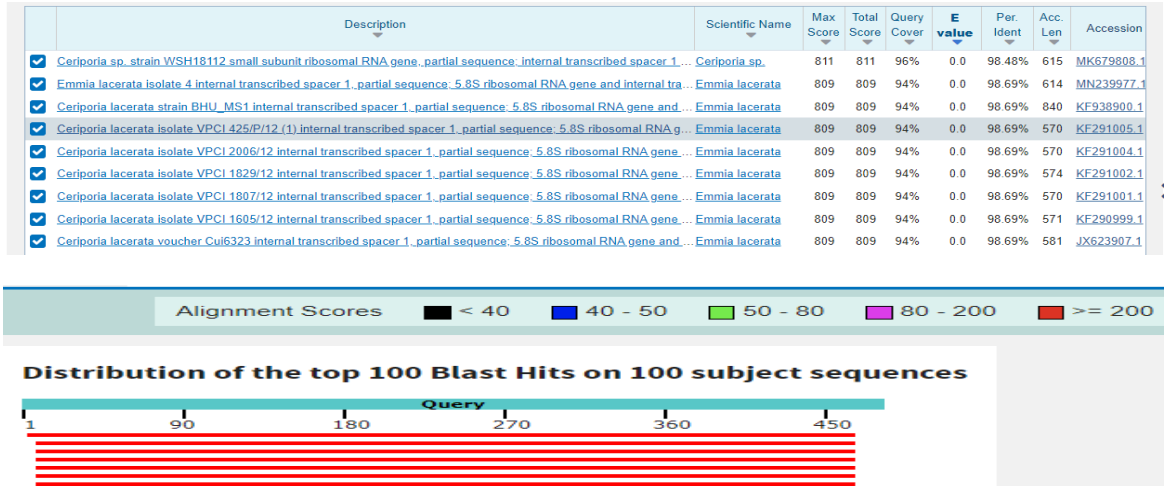

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Ceriporia sp. strain WSH18112 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1 ...	Ceriporia sp.	811	811	96%	0.0	98.48%	615	MK679808.1
Emmia lacerata isolate 4 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal tra...	Emmia lacerata	809	809	94%	0.0	98.69%	614	MN239977.1
Ceriporia lacerata strain BHU_MS1 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and ...	Emmia lacerata	809	809	94%	0.0	98.69%	840	KF938900.1
Ceriporia lacerata isolate VPCI 425/P/12 (1) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA g...	Emmia lacerata	809	809	94%	0.0	98.69%	570	KF291005.1
Ceriporia lacerata isolate VPCI 2006/12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Emmia lacerata	809	809	94%	0.0	98.69%	570	KF291004.1
Ceriporia lacerata isolate VPCI 1829/12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Emmia lacerata	809	809	94%	0.0	98.69%	574	KF291002.1
Ceriporia lacerata isolate VPCI 1807/12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Emmia lacerata	809	809	94%	0.0	98.69%	570	KF291001.1
Ceriporia lacerata isolate VPCI 1605/12 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Emmia lacerata	809	809	94%	0.0	98.69%	571	KF290999.1
Ceriporia lacerata voucher Cui6323 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and ...	Emmia lacerata	809	809	94%	0.0	98.69%	581	JX623907.1

7. *Endomelanconiopsis endophytica* X5

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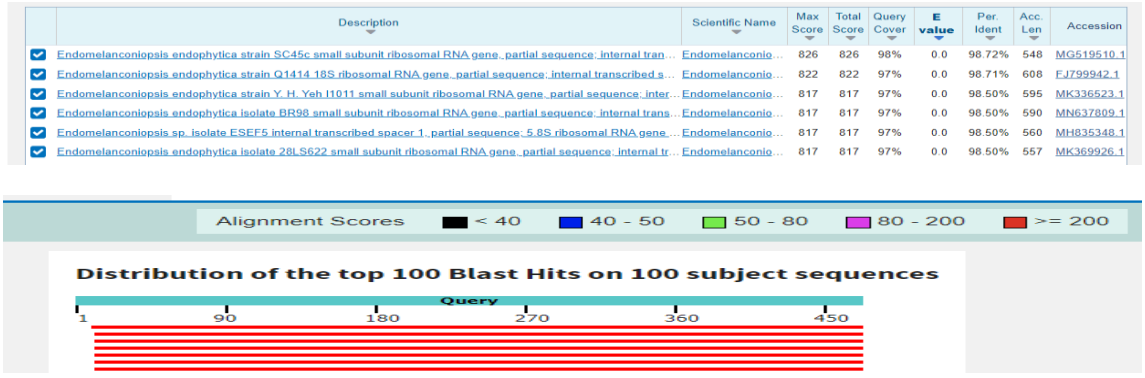

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Endomelanconiopsis endophytica strain SC45c small subunit ribosomal RNA gene, partial sequence; internal tran ...	Endomelanconio...	826	826	98%	0.0	98.72%	548	MG519510.1
Endomelanconiopsis endophytica strain Q1414 18S ribosomal RNA gene, partial sequence; internal transcribed s ...	Endomelanconio...	822	822	97%	0.0	98.71%	608	FJ799942.1
Endomelanconiopsis endophytica strain Y. H. Yeh I1011 small subunit ribosomal RNA gene, partial sequence; inter ...	Endomelanconio...	817	817	97%	0.0	98.50%	595	MK336523.1
Endomelanconiopsis endophytica isolate BR98 small subunit ribosomal RNA gene, partial sequence; internal trans ...	Endomelanconio...	817	817	97%	0.0	98.50%	590	MN637809.1
Endomelanconiopsis sp. isolate ESEF5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene ...	Endomelanconio...	817	817	97%	0.0	98.50%	560	MH835348.1
Endomelanconiopsis endophytica isolate 28LS622 small subunit ribosomal RNA gene, partial sequence; internal tr ...	Endomelanconio...	817	817	97%	0.0	98.50%	557	MK369926.1

8. *Penicilillum funiculosum* A2

AAAGAACAAGGCGGGCCCTCGTGGCCCAACCTCCCACCCTTGTCTCTCTACCCTGTTGCTTTGGCGGGC
CCACTGGGGCTCCCTGGTCGCCGGGGGACGCCTGTCCCCGGGCCCGCGCCCGCCGAAGCGCTTCGTGAA
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Sequences producing significant alignments Download New Select columns Show 100

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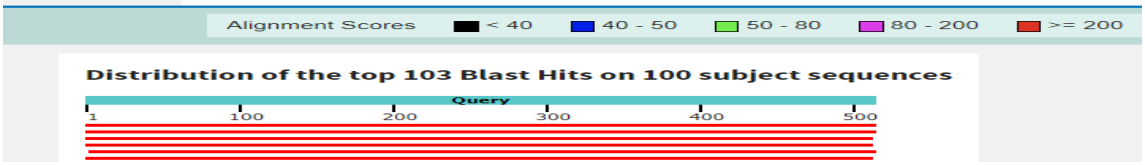
Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Talaromyces funiculosus voucher MHE 27 MC internal transcribed spacer 1, partial sequence; 5.8S ribosomal …	Talaromyces funi…	1014	1014	98%	0.0	95.22%	669	KY617055.1
Talaromyces funiculosus strain BBWT6 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA ge…	Talaromyces funi…	996	996	97%	0.0	95.01%	672	MF927596.1
Talaromyces sp. strain HBUM07199 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene…	Talaromyces sp.	992	992	97%	0.0	94.86%	851	MF662387.1
Talaromyces funiculosus strain CBS 884.70 small subunit ribosomal RNA gene, partial sequence; internal trans…	Talaromyces funi…	981	981	83%	0.0	99.09%	1452	MH859994.1
Talaromyces funiculosus isolate CR MI small subunit ribosomal RNA gene, partial sequence; internal transcribe…	Talaromyces funi…	979	979	81%	0.0	99.63%	570	MN545584.1



APPENDIX II

1. Potato Dextrose Agar

Potato	200.00 g
Dextrose	20.00 g
Agar	20.00 g
Water	1000 ml

2. Pikovskaya's Medium

Glucose	10.00 g
$Ca_3(PO_4)_2$	5.00 g
$(NH_4)_2\ SO_4$	0.50 g
$MgSO_4.\ 7H_2O$	0.10 g
$MnSO_4.\ 7H_2O$	Trace
$FeSO_4$	Trace
KCl	0.20 g
Yeast extract	0.50 g
Agar	20.00 g
Water	1000 ml
pH	6.8 to 7.0

3. Mineral salt medium

Glucose	5.00 g
$MgSO_4.7H_2O$	0.20 g
K_2HPO_4	0.10 g
KCL	0.20 g
NH_4SO_4	1.00 g
ZnO	0.1 %
Water	1000 ml
pH	7

4. Czapex Dox Agar

Sucrose	30.00 g
odium nitrate	2.00 g
Dipottassium phosphate	1.00 g
Magnesium sulphate	0.50 g
Ferrous sulphate	0.50 g
Agar	15.00 g
Water	1000 ml
pH	7.3±0.2

5. Aleksandrow Agar

Magnesium sulphate	0.50 g
Calcium carbonate	0.10 g
Mica	2.00 g
Dextrose	5.00 g
Ferric chloride	0.005 g
Calcium phosphate	2.00 g
Agar	20.00 g
Water	1000 ml
pH	7.2 ±0.2

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Influence of fungal Endophytes on growth and yield of rice (*Oryza sativa* L.)

Arpitha PS and Earanna N

Abstract
A field experiment was conducted at University of Agricultural Sciences, GKVK, Bengaluru, to study the effects of fungal endophytes on growth, yield and yield attributes of Rice (*Oryza sativa* L.). The results revealed that the plants colonized by fungal endophytes had significantly improved grain and straw yield of rice by enhancing the plant height, tiller and panicle production and also by increasing the individual grain weight. However, *Aspergillus nidulans* and *Penicillium funiculosum* colonized plants had significantly higher harvest index (0.32). Hence this study suggests that fungal endophytes could be used as a bio-stimulants for rice.

Keywords: Bio-stimulants, *Aspergillus nidulans*, *Oryza sativa* and *Penicillium funiculosum*

Introduction
Rice is the staple food of more than half of the world's population. Among the rice growing countries, India has the largest area (44 million hectares) and it is the second largest producer (131 million tonnes) of rice next to China (211 million tonnes). The rice productivity in India is 2.71 tonne per ha while the world average is 5.14 tonne per ha (IRRI, 2011). At the current population growth rate (1.5 per cent), the rice requirement of India by the year 2025 would be around 125 million tonnes. The rice production has to be enhanced to meet the food requirement of the growing population, by improving the rice growth and development. Rice growth depends on the combination of genotype, environment and management practices. At present, lots of fertilizer and pesticides are being used to raise the crop productivity which results reduction in soil fertility. To overcome these problems one of the eco-friendly approach is endophytic microbes which can be used as bio-stimulants. Endophytes have pivotal role in improving the plant growth and development, which in turn improves the sustainable yield.
Endophytes are microorganisms (fungi, bacteria, protozoa, virus or algae) those live part of their life or complete life inside living plant without causing negative effect on the host plants. The distribution of endophytes is ubiquitous and has been reported in all most all tissues of plant including leaves, stems, roots, flowers and fruits. These endophytes have intimate relation with host plant and this interaction provides benefits to both host plants and endophytic microorganisms. For host plants, endophytes can enhance plant nutrient uptake, promote host plant growth, enhance tolerance to abiotic stresses, inhibit infection by plant pathogens, and eventually increase biomass yield of the plants (Rodriguez *et al*., 2008; Mei and Flinn, 2010) [10, 9]. There has been an increase in attention to endophytic fungi in the past few years because of their beneficial properties to plants. Endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus-assimilating enzymes or release of organic acids and improves the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system. Hence, endophytes have a vital role in plant growth promotion. Even endophytes could present in rice plant such as *Chaetomium cupreum*, *Colletotrichum* spp., *Curvularia lunata*, *Fusarium solani*, *Penicillium* spp., *Fusarium oxysporum*, *Trichoderma harzianum* and *Penicillium* sp., *Aspergillus niger*, *Aspergillus flavus*, *Pythium* spp., *Rhizopus* sp (Leewijit, 2016) [6].
Few reports documented about those endophytic fungi which improved the growth of rice plant such as *Phialemonium dimorphosphorum*, *Trichoderma* sp. *Aspergillus niger* and *Fusarium* sp. (Doni *et al*., 2014; Gupta *et al*., 2015; Kandar *et al*., 2018) [2, 3, 5]. However, study on fungal interaction with rice under field condition is less explored. Hence present study was carried to evaluate the effect of fungal endophytes on growth and yield of rice crop.

Materials and Methods

Mass production of fungal endophytes

Fungal endophytes such as *A. flavus, A. nidulans*, *E. endophytica,* and *P. funiculosum* were cultured individually on PDA media, supplemented with 100 µg/L of ampicillin and grown at 30 °C (Fig. 1). After 20 days of growth, conidia were harvested from plates by adding 10 mL of sterile water and gently scraping off conidia with a sterile glass slide. The suspension was filtered through four layers of sterile cotton cheese cloth gauze. The concentration of final spore suspensions was adjusted to 10^{-5} conidia/mL with the help of haemocytometer. This suspension was used for the field experiment.

Field experiment setup

Description of location and experimental site

The field experiment was conducted at experimental plot of K-block, Department of Genetics and Plant Breeding, UAS, GKVK, Bengaluru during summer, February-June 2021. The farm is geographically situated at the altitude of 920 m above sea level 13.07 N latitude and 77.59 E longitude. The soil type here is sandy loamy soil in texture and alkaline in reaction. During crop growth period, the average rainfall was about 48.5 mm, mean relative humidity was 86 per cent and the average maximum and minimum temperature was 31.06 °C and 18.28 °C, respectively.

Experimental treatments and design

The experiment comprised of five treatments such as T1: Control, T2: *A. flavus,* T3: *A. nidulans*, T4: *E. endophytica,* and T5: *P. funiculosum* with four replications under Randomized Complete Block Design (RCBD). All the treatments received 100% RDF (Recommended Dose of fertilizer), FYM and NPK.

Cultural Practices

Prior to planting, the land was ploughed and harrowed. Later, land was levelled with plank and divided into 20 plots, each with a plot size of 2.0 m X 1.5 m. Small raised bunds were formed around each plot to mark individual treatment and irrigation channels were formed between the replications. Further, individual plots were leveled and the flat beds were converted into ridges and furrows at 30 X 10 cm distance. Rice seed was directly sown by dibbling in lines in the dry field at a depth of 3-5 cm. Immediately after sowing, light irrigation was applied through border strip method. Subsequent irrigation was given as and when needed for proper growth and development of the crop. Recommended dose of fertilizers (120 kg N, 50kg P2O5 and 50 kg K2O ha-1) was applied in all the treatments through urea, diammonium phosphate and muriate of potash. Entire dose of phosphorus and potassium and half of nitrogen were applied as basal. The remaining dose of nitrogen was top dressed equally at active tillering and panicle initiation stage.

Recording observations

Observations pertaining to the growth parameters were recorded at 35 DAS, 70 DAS and at harvest of crop. Yield attributes and yield data were collected at the time of harvest. The plant height was recorded from five hills using a meter scale from the base of the plant to the tip of the longest leaf or the panicle and expressed in centimetres. The number of tillers were counted and recorded from the five hills within the net plot. Similarly, panicle length, number of panicles were recorded from five hills of each plot. The grains were separated by threshing the crop collected from each net plot separately and were dried under sun for three days. Later winnowed and cleaned, then weight of the grains per net plot was recorded. Straw obtained from each net plot area after threshing was sun dried for four days and then weighed and expressed in q ha^{-1}. Biological yield and harvest index were calculated.

Statistical analysis

The data obtained from field experiments were statistically analyzed using and Randomized Complete Block Design (RCBD) respectively. The statistical analysis was done by using WASP: 2.0 (Web Agri Stat Package 2) statistical tool (www.icargoa.res.in/wasp2/index.php) and mean were separated by Duncan's Multiple Range Test (DMRT).

Results and Discussion

The fungal endophytes influenced the plant height and tiller numbers of rice. At 35 DAS, plant height was significantly higher in *A. nidulans* inoculated plants followed by *P. funiculosum, A. flavus* and *E. endophytica.* The least was observed in control plants. Similar significant trend was recorded during 70 DAS. Whereas during harvest, *A. nidulans, P. funiculosum* and *A. flavus* treated plants had higher plant height and showed significantly on par with each other. The results indicated that endophytes inoculated plants improve length of the plant. It might be due to production of phytohormones. Several reports documented that fungi such as *Aspergillus flavus, Aspergillus niger, Fusarium oxysporum, Penicillium corylophilum, Penicillium cyclopium, Penicillium funiculosum* and *Rhizopus stolonifer* has ability to produce gibberellin and indole actic acid (IAA) (Hasan, 2002) [4]. Mattos *et al.* (2008) [8] reported that the endophytic *Burkholderia kururiensis* promoted rice plant growth by production of the plant auxin, IAA. Our results are aligning with Kandar *et al*., (2018) [5] who also reported that *Phialemonium dimorphosphorum*, *Gaeumannomyces graminis*, and *Gaeumannomyces amomi* significantly increases the plant height of rice seedlings.

Tiller number is an important attribute for grain yield. The number of tillers were significantly maximum in plants colonized by *A. nidulans* followed by *P. funiculosum, A. flavus* and *E. endophytica* at 35 DAS and 70 DAS. During harvesting stage, these endophytes are on par with each other except *E. endophytica* which is the lower number of tillers as compared to other endophytes but significantly higher tillers compared to un-inoculated plants. Cytokinin has pivotal role in increasing number of branches and spikelets in rice panicle for the benefit of grain yield has been corroborated more recently (Ashikari *et al*., 2005) [1]. Earlier studies are reported that fungal endophyte, *Piriformospora indica* has ability to produce cytokinin (Vadassery *et al*., 2008) [11] which might be helpful to increase the tillers in plant system.

The results pertaining to yield attributes of rice plants influenced by fungal endophytes were represented in Table 2. The plants inoculated by fungal endophytes recorded higher panicle length compared to un-inoculated plants and all the endophytes showed significantly on par results with each other.

In rice plant, panicle numbers are directly proportional to tiller number. The present study support this statement.

Endophyte (*A. nidulans)* colonized plants recorded higher panicle number per hill followed by *P. funiculosum, A. flavus* and *E. endophytica.* Similar trend was observed in 1000 seed weight of rice. Improvement in yield attributes might be due to increased nutrient supply by fungal endophytes to the host plant. These results are also inline the findings of Yuan *et al.* (2010) [13] who reported that endophytes could enhance the fitness of plant by acquisition/supply of nutrients *via* siderophores, nitrogen and phosphorus- assimilating enzymes or release of organic acids and improve the absorption of water, supporting the growth of other bacteria/fungi near the rhizosphere or in plant system which in turn improve the assimilation of photosynthates and sink translocation and eventually produced larger sized and more grains of higher weight that ultimately increased the yield. Plant inoculated with plant growth promoting fungi improved the plant growth by increasing the nutrient uptake particularly nitrogen and phosphorus (Yadav *et al*., 2009) [12].

The plants colonized by fungal endophytes exhibited significantly higher grain and biological yield as compared to un-inoculated plants. *A. nidulans* and *E. endophytica* treated plants showed significantly higher straw yield followed by *A. flavus*. Our results are confirmed with Gupta *et al*. (2015) [3] who reported that *Trichoderma longibrachiatum*, *Westerdykella aurantiaca*, *Lasiodiplodia* sp. and *Rhizopus delemar* improved the rice grain yield by increasing the yield attributes such as panicle number, spikelet number and length weight of the grain. Harvest index of rice had significantly maximum in *A. nidulans* and *P. funiculosum* colonized plants which indicates that endophytes are more efficient in partitioning dry weight to seed compared to un-inoculated plants.

In conclusion, experimental results indicated that application of fungal endophytes could enhance the growth and yield attributes of rice plants.

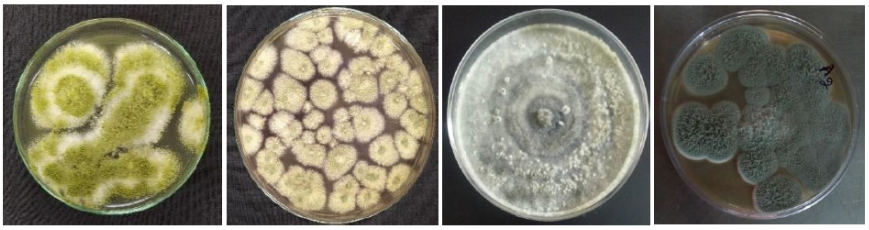

Fig 1: Growth of fungal endophytes such as *A. flavus, A. nidulans, E. endophytica* and *P. funiculosum* on PDA media (left to right)

Table 1: Influence of different endophytic fungi on growth attributes of Paddy at different intervals

	Plant height (cm)			Number of Tillers / hills		
Treatments	**35 DAS**	**70 DAS**	**At harvest**	**35 DAS**	**70 DAS**	**At harvest**
Control	32.02^{d}	69.20^{d}	74.85^{c}	7.86^{d}	20.05^{d}	21.05^{c}
A. flavus	36.83^{b}	76.33^{b}	79.60^{a}	12.26^{b}	24.29^{b}	25.70^{a}
A. nidulans	38.50^{a}	78.08^{a}	81.21^{a}	15.11^{a}	25.25^{a}	26.50^{a}
E. endophytica	35.32^{c}	74.32^{c}	77.83^{b}	10.46^{c}	22.49^{c}	24.02^{b}
P. funiculosum	38.32^{a}	77.55^{ab}	80.33^{a}	14.34^{a}	24.83^{ab}	26.05^{a}
C.D.	1.055	1.579	1.699	1.552	0.958	1.069
SE(m)	0.339	0.507	0.545	0.498	0.308	0.343
C.V.	1.871	1.35	1.385	8.302	2.631	2.784

Table 2: Influence of different endophytic fungi on yield attributes of Paddy

Treatments	**Panicle length (cm)**	**No. of panicle per hill**	**1000 grain weight (g)**	**Grain yield (t/ha)**	**Straw yield (t/ha)**	**Biological yield (t/ha)**	**Harvest Index**
Control	15.67^{b}	15.75^{c}	19.50^{c}	3.38^{b}	9.63^{b}	13.01^{b}	0.26^{b}
A. flavus	17.91^{a}	18.52^{ab}	21.62^{ab}	4.47^{a}	11.02^{a}	15.49^{a}	0.29^{ab}
A. nidulans	18.28^{a}	18.94^{a}	22.40^{a}	4.80^{a}	10.20^{ab}	15.00^{a}	0.32^{a}
E. endophytica	17.78^{a}	17.93^{b}	20.95^{b}	4.51^{a}	10.83^{a}	15.34^{a}	0.30^{ab}
P. funiculosum	18.15^{a}	18.97^{a}	22.08^{ab}	4.72^{a}	9.81^{b}	14.53^{a}	0.32^{a}
C.D.	0.763	0.614	1.249	0.783	1.012	1.242	0.046
SE(m)	0.245	0.197	0.401	0.251	0.325	0.399	0.015
C.V.	2.79	2.186	3.763	11.492	6.306	5.431	9.955

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Fungal endophytes of Himalayan Cold Desert Induces Heat tolerance in Rice (*Oryza sativa* L.)

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Abstract

The plants growing in cold desert of western Himalaya have inhabited diversified endophytes. These endophytes can provide fitness to plant under harsh environmental situations. In the current study, 22 fungal endophytes isolated from Artemisia and Xerophytic plants growing in the cold desert were screened for thermo-tolerance at different temperature ranges (28, 30 32, 34, 36, 38 and 40^{o}C) under *in vitro*. The only three isolates *viz*., A2, A7 and X5 exhibited growth up to 40^{o}C and identified as *Penicilium funiculosum* (A2), *Ceriporia lacerate* (A7) and *Endomelanconiopsis endophytica* (X5) using ITS region. These endophytes inoculated to rice seedlings and exposed to elevated temperature (45°C) for 7hr per day for 10 days to study their effect on tolerance of rice to heat stress. The results revealed that endophytes inoculated seedlings showed sustained improvement in shoot and root growth. The *E. endophytica* was chosen to be the best endophyte to impart heat stress as per Fernandez model. This study suggested that cold desert endophytes could induce heat tolerance in plants.

Keywords: Cold desert, fungal endophytes, Rice and Heat stress

Introduction

The global temperature increases day by day due to change in climate. Frequent heat waves have had serious impacts on rice production (Zhang and White, 2021). Historical data analysis envisaged that 7–8% of rice yield has been decreased due to raise in temperature to 1^{o}C (Baker *et al*., 1992). International Rice Research Institute (IRRI) demonstrated that the field trials from 1992-2003 showed 10% yield reduction of rice for every raise in one degree of minimum temperature (Peng *et al*., 2004). High temperature affects all stages of rice plant starting from germination, growth, development, reproduction and yield (Krishnan *et al*., 2011). The tiller

number decreased by 10% when temperature rise from 29/21ºC to 37/29ºC (Manalo *et al*., 1994). The synchronism between the emergence of main stem and tiller and also mobilization of nutrients among tillers were affected by high temperature resulting in decreased yield as primary tillers are directly proportional to grain yield in rice (Yoshida, 1981).

Cold deserts are found in high, flat areas, called plateaus, or mountainous areas in temperate regions of the world. Cold deserts have hot summers but extremely cold winters. The Western Himalayan cold deserts have extremes of hot and cold climate combined with excessive dryness. Soil has light grey, poor in fertility and less water holding capacity. Therefore, these desert plants develop some physiological mechanisms like CAM (Crassulacean acid metabolism), modified leaf and also take the advantage of microbial endophytes to survive in hostile environment (Zhang and White, 2021).

The endophytes can colonize the plant tissue without causing any apparent harm and provide fitness under hostile environment. Endophytes can be cultured *in-vitro* and transfer to compatible secondary plants to obtain similar benefits (Redman *et al*., 2002 and Wang *et al*., 2021). Endophytes isolated from cold deserts seems to adapt wider range of temperature as cold desert has influenced by fluctuated temperature ranges from -45ºC in winter to 40ºC in summer (Tewari and Kapoor, 2013). Therefore, we used in our study the endophytes isolated from the cold desert plants to understand induction of thermotolerance temperature sensitive rice variety IR-64.

Materials and methods

Screening for thermotolrance of endophytic isolates

The fungal endophytes isolated from Artemisia and xerophytic plants of Western Himalayan cold desert and preserved at School of Ecology and Conservation Laboratory, University of Agricultural Sciences, Bangalore-560065. The 22 isolates were procured and rejuvenated on potato dextrose agar (PDA) for the present study. The endophytic isolates were screened for temperature tolerance. Isolates were cultured in PDA plates and incubated at different temperature (28ºC, 30ºC, 32ºC, 34ºC, 36ºC, 38ºC and 40ºC) for five days. Fungal growth was measured by radial diameter of colony on fifth day of incubation.

Molecular identification of thermotolerant endophytic isolates

The endophytic isolates of genomic DNA were extracted by Cetyltrimethylammonium bromide (CTAB) method (Vainio *et al*., 1998). The internal transcribed spacer (ITS) region of genomic DNA was amplified using universal primer ITS1-F (5′ TCCGTAGGTGAACCTGCGG 3′) and ITS4-R (5′ TCCTCCGCTTATTGATATGC 3′) by polymerase chain reaction (PCR). PCR amplification was performed using Master cycler (Eppendorf, Germany) with a 20µl reaction mixture that comprised 2µl 1X taq buffer with $MgCl_2$ (1.5mM), 2µl dNTP's (10mM), 0.5µl each primer (10pmol), 0.3µl Taq DNA polymerase (3U) and 1µl template DNA (100ng). The PCR was carried out with an initial denaturation at 94°C for 4min, followed by 35 cycles at 94°C for 30s, 55°C for 1min and 72°C for 30s, and a final extension at 72°C for 12min. The PCR amplified products were sequenced by SciGenome labs, Cochin, Kerala, India. The nucleotide sequences were queried in the NCBI GenBank database using a Basic Local Alignment Search Tool (BLAST). Sequences of each fungal species and corresponding reference sequences from GenBank were subjected to ClustalW analysis. The phylogenetic tree was constructed through maximum likelihood method and Tamura- Nei model, using MEGA X. The recognized sequences were placed in GenBank with accession number.

Interaction of fungal endophytes with Rice under heat stress

Evaluation of fungal endophytes on their ability to impart heat tolerance in rice (variety IR-64) was carried out in plant growth chamber at Indian Institute of Horticulture Research (ICAR-IIHR), Hesaraghatta, Bangalore. There were two sets of experiments. 1. Heat stress (45^{o}C for 7h per day for 10 days) and 2. Without heat stress (normal temperature conditions, 30±0.5^{o}C). Each set comprised with following treatments. 1. Control (uninoculated plants) 2. *Ceriporia lacerate* 3. *Endomelanconiopsis endophytica* and 4. *Penicilium funiculosum.* Rice seeds were surface sterilized using 3 % sodium hypochlorite followed by 70 % alcohol. The surface sterilized seeds were repeatedly washed with sterile water and soaked for overnight. The pre-germinated seeds were sown in pots filled with soil and FYM (1:1w/w). Three seedlings per pot were maintained and grown for fifteen days. The thermotolerant endophytes were inoculated by stem prick method (Bhunjun *et al*., 2020) and allowed to colonize for 10 days. After colonization, set-1 seedlings were

exposed to heat (45^{o}C) for 10 days in growth chamber. Observations for plant height, number of tillers, number of leaves, root volume, fresh and dry weight of roots were recorded after 10 days of heat exposure. Similarly, observations for plants grown under normal conditions (set-2) were recorded.

Statistical Analysis

The data generated during experimentation was analyzed by one-way analysis of variance and means were separated by Duncan's Multiple Range Test (DMRT) using the software XL STAT. The 3-D plot of stress tolerance index (STI) of biomass was constructed according to Fernandez (1992) model using iPASTIC online tool kit (https://manzik.com/ipastic/).

Results

Screening and identification of thermotolerant fungal endophytes

All endophytic isolates showed good growth up to 30^{o}C, beyond that there is gradual reduction in growth. This indicated that the optimum temperature of these isolates ranges from 28 to 30^{o}C. Three isolates viz., A2, A7 and X5 recorded tolerance level up to 40^{o}C (Table 1). Hence, these isolates were selected for identification and further experiment.

Thermotolerant isolates were identified using ITS (Internal Transcribed Spacer) regions of rDNA and BLAST search. All the isolates such as A2, A7 and X5 showed 98% similarity with *Penicillium funiculosum* strain C2-20, *Ceriporia lacerate* strain BHU MS1 and *Endomelanconiopsis endophytica* strain CR3 respectively (Fig.1a-1c). The isolates A2, A7 and X5 belongs to three different genera, namely *Penicillium funiculosum, Ceriporia lacerate* and *Endomelanconiopsis endophytica* and the obtained sequences were deposited in GenBank under the accession no. OM368442, MT899187 and MT900590 respectively (Table 2). The molecular identification was reconfirmed by their macro- and micro-morphological characteristics (Fig. 2). The colony of *P. funiculosum* had greyish green with funiculose texture on PDA media and examined biverticillate condiophore with subterminal branches and ellipsoidal conidia. In case of *C. lacerate*, white fluffy colonies was observed with aseptate hyphae. Initially colourless colony was observed

in *E. endophytica* and later it become hyaline with shine black color and examined pycnidial conidiomata with ellipsoidal conidia.

Effects of endophytes isolated from cold desert on imparting thermotolerance in rice

The fungal endophytes inoculation significantly ($P < 0.01$) improved all growth attributes of rice plants except plant height under both heat stress as well as normal conditions (Table 3 and 4). An endophyte *P. funiculosum* colonized plants found superior in increasing plant height, number of tillers and leaves, root volume, fresh and dry weight of shoot and root in normal growth condition. Whereas under stress condition, the *E. endophytica* and *P. funiculosum* colonized plants showed significantly ($P < 0.01$) higher shoot and root growth parameter compared to *C. lacerate*. The un-inoculated plants produced least growth of rice.

Categories of treatments based on their performance in normal and stress conditions

The treatments were divided into four categories based on Fernandez (1992) model using stress tolerance index of biomass. The treatment *E. endophytica* inoculated plants belongs to group A that indicates the production of higher biomass under the both conditions (normal and stress). The *P. funiculosum* and *C. lacerate* fall under group B having maximum biomass only under normal growth condition. The uninoculated plants formed group D produced least biomass under both the conditions (Fig. 3).

Discussion

The numerous studies have been conducted on improvement of crop growth under heat stress using thermotolerant endophytes isolated from harsh environment or wild plants. However, the use of cold desert thermotolerant endophytes were less explored therefore we have analysed the effect of cold desert endophytes on improvement of fitness of rice under heat stress. In present study, the isolates A2, A7 and X5 were observed to be heat tolerant and grown at the range from 28^{o}C to 40 ^{o}C. This envisaged that these three isolates could sustain heat stress it might be the cold desert of Western Himalaya had extreme of hot climate (40^{o}C) during summer (Tewari and Kapoor, 2013). These endophytes were identified using ITS region of

rDNA as *P. funiculosum*, *C. lacerate* and *E. endophytica*. The ITS region of rDNA sequences is widely used to examine phylogenetic positions or relationship of a species because this region are flanked by preserved segments (18S, 5.8S and 28S genes) which provide information about the phylogeny and the taxonomic level, since their evolution is slow and they are highly similar within different taxa (Ramesh *et al*., 2017).

High temperature is one of the most important environmental stresses which severely affect the rice growth by reducing the emergence of leaves and tillers resulting in decreased biomass. In the present study, significant higher tiller number was recorded when the plants inoculated with *E. endophytica,* which might positively influenced the new tillers under heat stress by reducing the effects of heat stress on tiller bud. This is in accordance with Vila-Aiub *et al*. (2005) who reported that *Neotyphodium* sp. infected rye grass produced more tillers than uninfected plants. The endophyte *P. funiculosum* inoculated plants showed highest number of leaves compared to other endophytes which resulted in increased fresh weight of shoot. Similarly *Lolium perenne* infected with *Epichloe* endophyte had significantly higher tillers number, dry weight, leaf length and wet weight under drought condition (Jajarmi *et al*., 2015)

The root system plays a vital role in adaptation of whole plant under heat stress (Huang *et al*., 2012). Significant improved in root growth was observed in endophytes colonized plants which lead to improved absorption of nutrients and water from soil, resulting in a more vigorous plant and helps to cope of heat stress. *E. endophytica* colonized plants found better in influencing the root growth compared to others. Our results are in agreement with Waqas *et al*., (2015) who demonstrated that *Paecilomyces formosus* LWL1 improved root biomass of rice under heat stress. Higher root to shoot ratio was found in plants inoculated with *E. endophytica*, which indicate that the endophyte could protect the root system.

In conclusion, this investigation explored the possibility of using cold desert endophytes for mitigating the heat stress. The endophyte *E. endophytica* seems to be more effective in imparting heat stress tolerance in rice by improving the growth of shoot and root attributes (IR-64).

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Table 1. Effects of different temperatures on growth of the fungal colony (diameter in cm)

Endophytic fungal isolates	Temperature (ºC)						
	28	**30**	**32**	**34**	**36**	**38**	**40**
A1	6.13±0.23	5.80±0.1	5.76±0.09	2.73±0.12	1.83±0.03	0.6±0.05	-
A2	4.36±0.09	5.67±0.09	4.5±0.17	3.46±0.12	2.83±0.09	1.80±0.06	1.43±0.03
A3	6.03±0.15	5.20±0.12	4.13±0.09	2.80±0.06	3.00±0.12	0.73±0.03	-
A4	4.50±0.06	3.70±0.25	1.73±0.03	-	-	-	-
A5	5.37±0.09	4.00±0.12	2.90±0.06	2.00±0.06	1.32±0.15	-	-
A6	3.76±0.09	2.90±0.06	1.86±0.09	1.20±0.03	-	-	-
A7	5.33±0.33	4.03±0.09	4.36±0.09	3.80±0.12	2.83±0.09	2.03±0.20	1.40±0.06
A8	4.8±0.06	3.03±0.03	1.76±0.09	-	-	-	-
A9	2.30±0.06	1.90±0.06	0.63±0.09	-	-	-	-
A10	3.56±0.21	2.73±0.15	1.86±0.12	1.56±0.21	1.43±0.12	0.86±0.06	-
A11	4.03±0.12	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-
A12	1.90±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-
X1	3.50±0.17	2.93±0.09	2.26±0.09	1.36±0.09	-	-	-
X2	3.66±0.03	2.40±0.06	1.80±0.06	1.33±0.09	-	-	-
X3	5.96±0.09	5.50±0.06	5.00±0.06	4.43±0.03	1.43±0.07	-	-
X4	1.76±0.09	1.26±0.07	-	-	-	-	-
X5	8.76±0.03	8.16±0.03	8.66±0.09	8.06±0.18	3.46±0.09	1.93±0.09	1.13±0.06
X6	3.96±0.09	2.9±0.06	1.86±0.09	1.26±0.07	-	-	-
X7	4.00±0.12	2.93±0.09	2.26±0.09	1.26±0.03	-	-	-
X8	3.93±0.09	2.90±0.06	1.63±0.09	-	-	-	-
X9	1.9±0.06	2.36±0.15	3.00±0.12	3.93±0.03	1.63±0.09	0.76±0.09	-
X10	3.63±0.09	3.30±0.21	2.50±0.06	2.66±0.30	1.30±0.12	0.40±0.06	-

Data shown above are the means of three replication with ± standard error. *A- Artemisia plant X- Xerophytic plant*

Table 2. Molecular identification of thermotolerant fungal endophytes

Sl. No.	Hosts	Isolates code	Closest match	Sequence length (bp)	Query coverage (%)	Percent identity (%)	Organisms identified	NCBI Accession no.
6	*Artimisia* sp.	A2	*Penicillium funiculosum* strain C2-20	654	98	99	*Penicillium funiculosum*	OM368442
7	*Artimisia* sp.	A7	*Ceriporia lacerate* strain BHU-MS1	479	96	98	*Ceriporia lacerate*	MT899187
8	*Xerophytic plant*	X5	*Endomelanconiopsis endophytica* strain 5345	473	98	98	*Endomelanconiopsis endophytica*	MT900590

Table 3. Influence of fungal endophytes on shoot attributes of rice under stress [S] and without stress [WS]

Treatments	Plant height (cm)		No. of Tillers (/3plant)		No. of Leaves (/3plant)		Fresh wt. shoot (g/3plant)		Dry wt. shoot (g/3plant)	
	WS	S	WS	S	WS	S	WS	S	WS	S
Control	33.53±0.72^{b}	32.76±0.27^{a}	8.50±0.20^{c}	6.50±0.20^{b}	36.50±0.61^{d}	28.00±0.41^{c}	5.95±0.07^{d}	3.13±0.02^{c}	1.54±0.02^{c}	0.87±0.00^{c}
C. lacerata	35.51±1.17^{ab}	32.62±0.59^{a}	10.50±0.20^{b}	6.00±0.00^{c}	40.00±0.00^{c}	27.50±0.20^{c}	7.92±0.08^{c}	3.77±0.07^{b}	2.33±0.06^{b}	0.87±0.01^{c}
E. endophytica	36.96±0.53^{a}	32.01±0.45^{a}	11.00±0.00^{b}	7.00±0.00^{a}	45.00±0.12^{b}	37.00±0.41^{b}	8.89±0.05^{b}	3.83±0.00^{b}	2.26±0.01^{b}	1.09±0.01^{a}
P. funiculosum	36.62±0.19^{a}	32.79±0.76^{a}	14.50±0.20^{a}	6.50±0.20^{b}	55.50±0.20^{a}	39.00±0.41^{a}	10.36±0.08^{a}	4.21±0.05^{a}	2.64±0.02^{a}	1.05±0.01^{b}
($F_{3,12}$)	3.31	0.432	199.33	8.00	142.78	263.85	660.86	107.57	184.85	448.74
P	0.028	0.734	<0.0001	0.003	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at $P < 0.05$ by using Duncan's Multiple Range Test.

Table 4. Influence of fungal endophytes on root traits and total biomass of rice under stress [S] and without stress [WS]

Treatments	Root volume (cm^3/3plant)		Fresh wt. root (g/3plant)		Dry wt. root (g/3plant)		Root:Shoot		Biomass (g/3plant)	
	WS	S	WS	S	WS	S	WS	S	WS	S
Control	12.00±0.00^{b}	3.00±0.00^{c}	7.88±0.16^{c}	2.23±0.05^{d}	1.38±0.05^{bc}	0.29±0.01^{d}	0.89±0.02^{a}	0.34±0.01^{c}	2.92±0.06^{c}	1.16±0.01^{d}
C. lacerata	6.50±0.00^{d}	3.95±0.10^{b}	7.76±0.55^{c}	3.45±0.00^{c}	1.27±0.06^{c}	0.35±0.01^{c}	0.55±0.02^{d}	0.39±0.01^{b}	3.60±0.12^{b}	1.22±0.01^{c}
E. endophytica	11.50±0.20^{c}	4.25±0.02^{a}	9.88±0.15^{b}	4.03±0.06^{a}	1.46±0.02^{b}	0.47±0.02^{a}	0.64±0.01^{c}	0.44±0.02^{a}	3.72±0.03^{b}	1.56±0.01^{a}
P. funiculosum	13.90±0.04^{a}	4.00±0.00^{b}	12.73±0.07^{a}	3.64±0.00^{b}	1.98±0.04^{a}	0.39±0.01^{b}	0.75±0.01^{b}	0.36±0.01^{bc}	4.61±0.07^{a}	1.44±0.01^{b}
($F_{3,12}$)	920.23	111.39	59.85	445.68	46.17	56.97	101.74	15.35	83.62	319.94
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.000	<0.0001	<0.0001

± indicates standard error of mean (n = 4); the dissimilar letters indicate significant difference at $P < 0.05$ by using Duncan's Multiple Range Test.

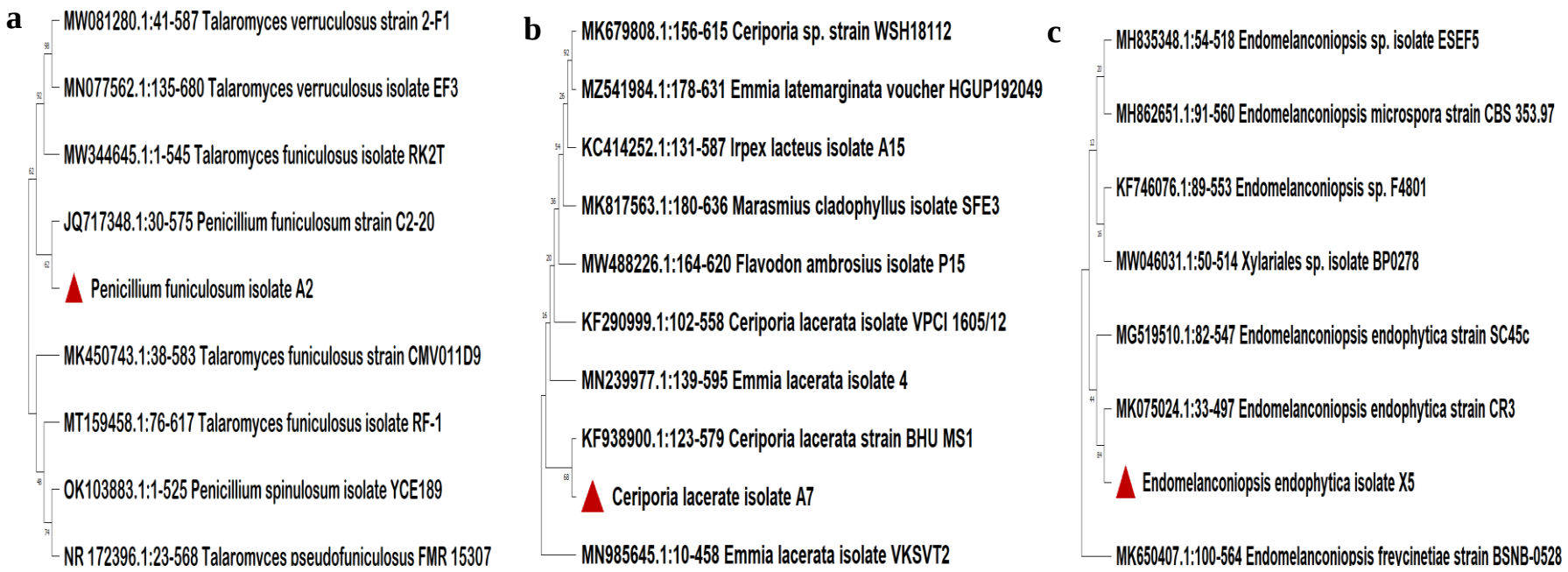


Fig. 1 MaximumLikelihood tree of the identified fungal endophytes (a) *Penicillium funiculosum* isolate A2) (b) *Ceriporia lacerate* isolate A7 and (c) *Endomelanconiopsis endophytica* isolate X5 and their closest ITS rDNA matches from the GenBank. The phylogenetic tree was constructed with bootstrap value of 500 replicates. Number at the node indicates the bootstrap value.

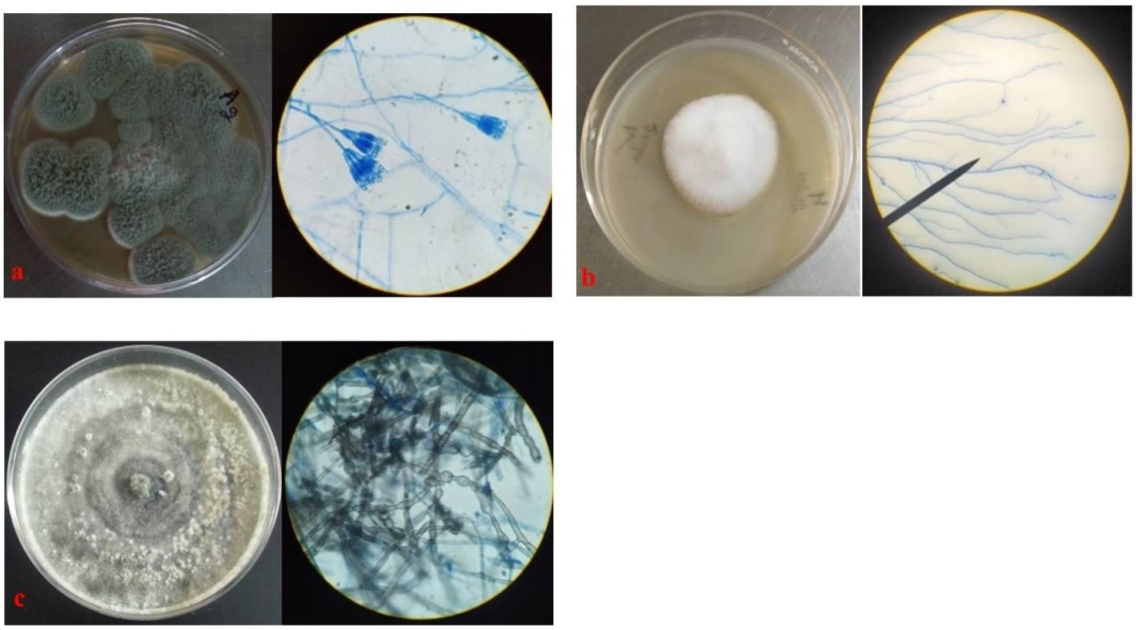
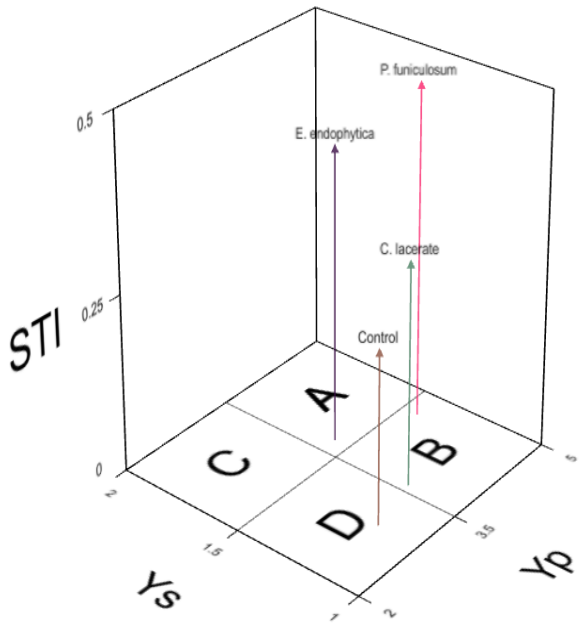


Fig. 2 Fungal colony grown on PDA medium and their fruiting body under microscope (lactophenol cotton blue stain) (a) *Penicilium funiculosum* isolate A2 colony and their conidiophore (b) *Ceriporia lacerate* isolate A7 colony and their aseptate mycelia colony and their hyphae (c) *Endomelanconiopsis endophytica* isolate X5and their mycelia.



Treatments	Yp	Ys	STI
Control	2.92	1.16	0.25
C. lacerate	3.6	1.22	0.32
E. endophytica	3.72	1.56	0.42
P. funiculosum	4.61	1.44	0.48

Fig. 3 Three dimentional plot based on Fernandez (1992) model using stress tolerance index (STI) of biomass. Yp: Biomass under normal growth condition Ys: Biomass under heat stress.