Exploration of salinity and drought stress tolerant endophytic fungi for rice cultivation

A thesis

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IN

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OF ENGINEERING & TECHNOLOGY
(Deemed to be University)

Submitted by

Gurleen Kaur Sodhi

Reg. No. 901900004

Under the supervision of

Dr. Sanjai Saxena

Professor

Department of Biotechnology

Thapar Institute of Engineering and Technology

(Deemed to be University)

Patiala – 147004, Punjab, India

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Certificate

Certified that the thesis entitled 'Exploration of salinity and drought stress tolerant endophytic fungi for rice cultivation' submitted by Gurleen Kaur Sodhi, Reg. No. 901900004 in the partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab is a record of candidate's own independent and original research work carried out by her under my supervision and guidance. The material embodied in this thesis has not been submitted in part or full to any other University or Institute for the award of any degree.

Place: Patiala
Date: 11th MARCH 2024

Dr. Sanjai Saxena
Professor
Department of Biotechnology,
Thapar Institute of Engineering and
Technology (Deemed to be University),
Patiala-147004, Punjab, India

Declaration

I, hereby declare that the work presented in the thesis entitled 'Exploration of salinity and drought stress tolerant endophytic fungi for rice cultivation' in the partial fulfilment of the requirement for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab, is an authentic record of my work carried out under the supervision and guidance of Dr. Sanjai Saxena, Professor, Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab. This report has not been submitted for the award of any degree or certificate in any other university in India or abroad.

Place: Patiala
Date: 11.03.2024

Gurleen Kaur Sodhi
Reg. No. 901900004

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"For Moses did guide them to the light,
In the darkest hour, in the blackest night."
Through challenges, one must persevere, so it helps to have someone to answer all the questions and doubts.

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List of publications

Publications

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Other publications

4. Gursharan Kaur, Rajinder Kaur, **Gurleen Kaur Sodhi** et al. (2024): Stilbenes: a journey from folklore to pharmaceutical innovation. Archives of Microbiology, 206: 1-24. **(I.F. 2.8)**

Manuscripts under preparation

5. **Gurleen Kaur Sodhi** and Sanjai Saxena (2023): Seed priming using endophytic *Nigrospora zimmermanii* mitigates abiotic stress in *Oryza sativa*.
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Book chapters

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Short courses

1. Five-day online course on Biotechnological Approaches for Bioprospecting and Waste Valorization in June, 2023, organised by Department of Biotechnology Dr. B. R. Ambedkar National Institute of Technology, Jalandhar, India.
2. Ten-day online course on Idea to Patent in June, 2022, organised by Turnip Innovations and facilitated by Dr. Rahul Kapoor.
3. Six-week online course on Industrial Biotechnology in July, 2021 authorized by University of Manchester and offered through Coursera.

Trainings and Workshops

1. One-week National e-workshop on Innovation and Intellectual Property Rights in June 2023, organised by Innovative Technology Enabling Centre (InTEC), CSIR-IMMT, Bhubaneswar, India.
2. Fifteen-day International Training cum Workshop on The future of Farming Technology and Agripreneurship Development in November, 2022, organised by Just Agriculture and National Agricultural Higher Education Project-ICAR, New Delhi, India.
3. One-day workshop on AI and Machine Learning in December, 2022, jointly organised by Thapar Institute of Engineering and Technology, Patiala and Pantech E-learning Pvt. Ltd. India.
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8. Nine-day National workshop on Taxonomy, Biodiversity, Ex-Situ Conservation and Applications of Fungi in November, 2019, organised by National Fungal Culture Collection of India, MACS' Agharkar Research Institute, Pune, India.

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1. Two-day webinar on Fermentation: Interplay of Microbes, Immunity and Nutrition February in February, 2023, organised by Department of Rural Development and Agricultural Production (RDAP) North-Eastern Hill University (NEHU), Tura Campus, Meghalaya in Association with: Biotechnology Industry Research Assistance Council (BIRAC), A Government of India Enterprise.
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4. One-day National webinar on IPR, Patents and Career Opportunities in May, 2021, organized by Federation of Asian Biotech Associations, Hyderabad, India.
5. One-day International webinar on Nanotechnological Interventions in Agri-Food Industry in October, 2020, organized by National Institute of Food Technology Entrepreneurship and Management, Sonepat, India.

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List of symbols

Symbol	Meaning
%	Percentage
~	Approximately
±	Plus, minus
°	Degree
°C	Degree Celsius
µg	Microgram
µL	Microliter
µm	Micrometre
µM	Micromolar
bp	Base pair
cm	Centimetre
g	Gram
h	Hour
L	Litre
M	Molar
mg	Milligram
min	Minute
mL	Millilitre
mm	Millimetre
mM	Millimolar
nm	Nanometre
nM	Nanomolar
psi	Pascal square inch
rpm	Revolutions per minute
s	Second
U	Unit (Activity)
v/v	Volume/volume
w/v	Weight/volume
α	Alpha
β	Beta

List of abbreviations

Abbreviation	Full Form
AAE	Ascorbic acid equivalent
ABTS	2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)
ANOVA	Analysis of variance
APX	Ascorbate peroxidase
BLAST	Basic local alignment search tool
BSA	Bovine serum albumin
CAT	Catalase
CMA	Corn meal agar
CTAB	Cetyl Trimethyl Ammonium Bromide
CZD	Czapek Dox
DNA	Deoxyribose nucleic acid
DNSA	Dinitro salicylic acid
dNTP	Deoxynucleotide triphosphates
DPPH	2,2-Diphenyl-1-picrylhydrazyl
DW	Dry weight
EDTA	Ethylenediaminetetraacetic acid
FAO	Food and Agriculture Organization of the United Nations
FRAP	Ferric ion reducing antioxidant power
FTIR	Fourier Transform Infrared
FW	Fresh weight
GAE	Gallic acid equivalent
H_2O_2	Hydrogen peroxide
HPLC	High Pressure Liquid Chromatography
IAA	Indole acetic acid
ITCC	Indian Type Culture Collection
ITS	Internal transcribed spacer
MDA	Malondialdehyde
MHA	Mueller Hinton agar
MTCC	Microbial Type Culture Collection
MTCC	Microbial type culture collection
NBT	Nitro blue tetrazolium
NCIM	National Collection of Industrial Microorganisms

OD	Optical density
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction
PDA	Potato dextrose agar
PDB	Potato dextrose broth
PEG	Polyethylene glycol
POX	Peroxidase
QE	Quercetin equivalent
RBA	Rose Bengal agar
SDA	Sabouraud agar
SNA	Synthetic nutrient agar
SOD	Superoxide dismutase
TBA	Thiobarbituric acid
TCA	Trichloro acetic acid
TE	Trolox equivalent
TE	Tris-EDTA
TW	Turgid weight
USDA	United States Department of Agriculture
WA	Water agar

Executive summary

The drastic changes in the climate have affected agriculture productivity, and it has become imperative to intensify agricultural production to meet the growing food demand globally. However, the drop in productivity of the crops can be recouped by reducing or adapting to the abiotic stresses they face via exploiting the plant-microbe interaction. Thus, Endophytic fungi play an immense role in bringing out metabolic and physiological changes within the plant to combat or adapt to stress. Hence, this study reports the isolation of fungal endophytes from various plant parts of a rain-fed (PUSA-44) and drought-resistant (Sahbhagi Dhan) rice (*Oryza sativa*) variety throughout the crop cycle. A total of 182 isolates were obtained from leaves, roots, internodes and spikes of both varieties. Of the total isolates, 120 belonged to the rain-fed and 62 to the drought-resistant rice variety. The highest isolation frequency of 11.6 and 8.8% was observed in roots of rain- fed and drought-resistant varieties, respectively, during the reproductive stage of the plant. The highest diversity of endophytic fungi was also observed at this stage, with a Shannon wiener diversity index (H') of 2.03 and 1.75 in rain-fed and drought-resistant rice. Among plant parts, the roots of rain-fed rice showed the highest diversity of endophytic fungi with an H' of 2.13. Whereas in the case of drought-resistant variety, the highest diversity was seen in the internode with H' of 1.79. Out of the 120 isolates of rain-fed variety, eight isolates (#5OSFS1a, #5OSFL6a, #6OSFL4c, #6OSFI1b, #6OSFR2d, #6OSFR2e, #7OSFS3a and #8OSFI2a) could sustain salinity and drought stress. When it comes to drought-resistant variety, three isolates (#2OSTUL6d, #2OSTUR9a and #4OSTUR1e) isolates demonstrated growth under salinity and drought stress.

On evaluating the plant growth-promoting attributes of selected isolates, #6OSFR2e from rain-fed rice and #2OSTUR9a from drought-resistant rice demonstrated the best antioxidant potential against the tested radicals. The isolates exhibited inhibition of gram-negative and gram-positive bacteria and antifungal potential. Among the rain-fed isolates, #6OSFR2e had the highest total phenolic content (241.5 ± 4.9 GAE/mg of sample), total flavonoid content (464.4 ± 4.4 QE/mg of sample), indole acetic acid (320.3 ± 5.5 µg/mL), ACC deaminase (317.4 ± 0.4 nmol α-ketobutyrate/mg protein/hour) and siderophore production (67.9 ± 0.6%). Whereas among the drought-resistant isolates, #2OSTUR9a displayed the total phenolic content (211.9 ± 0.7 GAE/mg of sample), total flavonoid content (408.4 ± 21.7 QE/mg of sample), indole acetic acid (351.0 ± 7.1 µg/mL), ACC deaminase (305.4 ± 0.8 nmol α-ketobutyrate/mg protein/hour) and siderophore production (72.6 ±

0.2%). In addition, the isolates also exhibited promising potential in mineral solubilisation, extracellular lytic enzyme and ammonia production.

Since isolates #6OSFR2e and #2OSTUR9a were predominantly the top performers, the isolates were identified using morpho-taxonomic and molecular identification tools. The isolates belonged to the same phylum, Ascomycota and were identified as *Nigrospora zimmermanii* (#6OSFR2e) and *Nigrospora oryzae* (#2OSTUR9a). Furthermore, the isolates were tested as an inoculum on the rain-fed rice PUSA-44 under a controlled and ambient environment setting. The plants were tested under different stress regimens, including salinity (150 mM NaCl), drought (10% PEG) and combined salinity and drought stress (150 mM NaCl + 10% PEG). Here, compared to the uninoculated plants, the inoculated plants had better physiochemical attributes, relative water content, pigment production, phenolic, flavonoid content, osmolyte accumulation and increased activity of ascorbate peroxidase, catalase, peroxidase and superoxide dismutase. Moreover, a decrease in lipid peroxidation and hydrogen peroxide content was also seen. To the extent of our knowledge, this is the first study demonstrating the plant growth-promoting and abiotic stress mitigation potential of endophytic *Nigrospora* sp. The findings reported in this investigation open a new realm and can be further explored to potentially replace conventionally used hazardous agrochemicals with a sustainable bioinoculant consisting of endophytic fungi.

Chapter 1

Introduction

1.1 Background

"The future is no longer about what we can prevent, but what we can mitigate."

– Dr. Katharine Hayhoe, Climate Scientist

The impact of climate change is irrefutable. The rising temperatures, water scarcity, salinization of soil, water, and depleting arable land are some of the observable effects seen because of the ongoing climate change (Bandh et al., 2021; Corwin, 2021). Scientists anticipate that this transformation will last well into the next century. The sensitivity of agriculture to these environmental predicaments presents us with a daunting challenge (Praveen and Sharma, 2020; Lesk et al., 2021). In addition, the world population is growing rampantly. Since the mid-twentieth century, humanity has witnessed a more than three-fold increase in the human population. As per the latest projections, the global population may surpass 10.4 billion by 2100 (Fróna et al., 2019). As of now, nearly 30%, i.e., 2.3 million people, suffer from world hunger, and an increase in this number is foreseen considering the population growth trends (Tripathi et al., 2019; Barrett, 2021). In order to sustain the increasing population and meet the United Nations-Sustainable Development Goal (UNSDGs) for eradicating global hunger and achieving food security and sustainable agriculture, intensification and significant changes to the current agricultural practices is crucial (UNSDG 2). This population boom will further escalate global food demand by 35-56%; taking into equation climate change, this increase in food demand could be as high as 60% (van Dijk et al., 2021; Burchfield, 2022). Of all the predicaments keeping us from achieving these goals, recent climate change and its adverse effects are the Achilles heel in the whole picture.

Due to climate variability and growing population, various factors (biotic and abiotic) arise that contribute to the overall crop yield loss. As per reports, abiotic stress is amongst the dominant causes contributing to crop yield loss by ~50% (Jeyasri et al., 2021; Zandalinas et al., 2021). Additionally, because of the stationary nature of plants, they have to endure harsh climatic conditions. Inanimate factors like extreme temperature, salinity, and drought cause abiotic stress. It has been observed that when these stress factors occur in combination, they prove to be most detrimental to the plants (Waadt et al., 2022; Zhang et al., 2022). Apart from affecting growth and yield, change the physiochemical, and molecular attributes of plants. However, extrapolation of the molecular and metabolic response of the plants to any one of the stress factors cannot elucidate the response to the combination of abiotic stress conditions (Kamarudin et al., 2018; Yang et al., 2019; Gao et al., 2019; Ishimaru et al., 2022). Under such conditions, the quality and quantity of produce generated are undesirable (Zhu, 2016; Yang et al., 2019; Razzaq et al., 2020). This effect of abiotic stress on the food crops

like rice, wheat, and maize not only impose a significant threat in sustaining the growing population but also elevates the spiralling issue of world hunger and food security. Thus, improving stress resistance is crucial for agricultural productivity and the environment.

The majestic Earth is home to diverse flora and fauna; three of the >50,000 edible plants, namely, rice, maize, and wheat, cater for 60% of the global food intake as staple crops. Rice is amongst the widely consumed staple food of the Poaceae family (Palacios-Rojas et al., 2020; Zhao et al., 2020). Because of the highly adaptable nature, rice cultivation is seen in diverse places ranging from north-eastern parts of China to central Sumatra and New South Wales in Australia (Gutaker et al., 2020). In the Indian subcontinent, nearly 5000 different rice varieties are cultivated at place below sea levels in Kerela to the high altitudes of Kashmir. The yield and water consumption are some of the factors that influence the type of rice variety being cultivated in different regions. Among these varieties, basmati and white rice are most common. The long grain basmati rice is cultivated in various northern states including Punjab (Dey, 2021; Bin Rahman and Zhang, 2023).

The 2021-2022 crop year saw 509.87 million metric tons global rice consumption (Batool et al., 2019; Yuan et al., 2021). It is consumed by people from all walks of life and forms a balanced diet alongside vegetables, pulses, meat, and poultry products. Rice is the third highest-produced agricultural commodity worldwide (FAO, 2021). India accounts for 22.5% of global production of rice, placing it in second place. Besides being a staple crop, rice plays a prominent role in various cultural festivities in India. Rice is regarded as an emblem of prosperity and auspiciousness, and various celebrations are focused on commencing from plantation to harvesting and storing paddy (Rathna et al., 2019; Schneider and Asch, 2020). In such a manner, rice has not only influenced culture, cuisine, and economy but denotes the passage of time with each cultivation stage.

In terms of nutrition, rice is currently the primary source of nutritional energy for 17 Asian and Pacific, eight African, and nine North and South American countries. In the Indian scenario, rice provides 24.1% of a person's required dietary protein supply (Kennedy et al., 2002; Sen et al., 2020; Kowsalya et al., 2022). Genetic cross-breeding practices of local rice varieties have led to 40,000 different cultivars. However, only a few of these varieties are extensively cultivated and consumed. The rice grain has been reported for its immense health benefits including reduction of cardiovascular diseases and some cancer and improvement of the gut health (Bhat et al., 2020; Carcea, 2021; Allai et al., 2022). Despite the surfeit health benefits and global consumption, rice cultivation has not prospered to meet the growing population's demand. According to Fitch Solutions, largest shortfall in the rice production is

foreseen since the last two decades. Studies suggest the vulnerability of rice to El Niño and other climate changes for the crop loss (Cherian et al., 2021). Additionally, the Agricultural Outcome report of 2022-2023 states that the demand for this staple crop is going to increase by 1.1% each year (OECD and FAO, 2022). This imbalance between the demand and supply could be primarily attributed to the changing climate and abiotic stresses.

As a kharif crop, rice uses ample amount of water for growth. In India, particularly in Punjab, an intensive rice-wheat cropping cycle, high fertilizer usage, and irrigation dependency have led to overall ecosystem imbalance. There is a decrease in the water table level, resulting in the soil salinity level also increasing (Singh et al., 2019, Pathak, 2023). These problems have taken a toll on crop productivity Abiotic stresses like salinity and drought are significant growth-restraining aspects, and rice is susceptible to the aforementioned conditions. Physiological attributes such as plant height are the key indicators of growth and development. Both salinity and drought prompts stomata closing, which causes plant height reduction (Kamarudin et al., 2018; Gao et al., 2019; Yang et al., 2019; Ishimaru et al., 2022). It further induces plant changes, affecting critical agronomic traits such as biomass, grains, yield, and harvest index (Auler et al., 2021; Liu et al., 2022). Past research has revealed as much as 70% decay in the physical traits of the rice plants under stress environment. Biochemically, rice plants undergo severe oxidative stress because of the excessive production of Reactive Oxygen Species (ROS) (Oladosu et al., 2020; Sachdev et al., 2021). A cascade of effects, including dehydration of cell membrane, reduced carbon dioxide permeability, lower water and nutrient availability, and acceleration of senescence occur, affecting the photosynthetic efficiency (Challabathula et al., 2022).

Since origin, the land plants have been exposed to a generally hostile environment. Thus, plants have developed an array of intrinsic defence mechanisms to endure biotic as well as abiotic stress during their growth cycle (in the case of crops) and life time (in the case of perennial plants) (Bhanot et al., 2021; Tuladhar et al., 2021). The defence mechanisms ranging from external structures to enzymatic and non-enzymatic antioxidants aid the plants during unfavourable conditions (He and Ding, 2022; Hossen et al., 2022). Likewise, the exogenous application of various fertilisers and growth promoters is a common practice to help with the cultivation process. Though routine consumption of such products leads to various ill health effects (Baweja et al., 2020; Rani et al., 2021). Despite the natural defences various high yield rice varieties have been lost in the face of climate change. For instance, PUSA-44, is a long duration rice variety developed by Indian Council for Agriculture Research (ICAR). Despite its extensive water consumption, it is extensively cultivated in Punjab because

of comparatively superior yield (Dwivedi et al., 2021). However, the ongoing climate change has affected the water table levels causing increased soil salinity (Kumar and Kaur, 2019). As a consequence, the cultivation of PUSA-44 is denounced.

Over the years, research has seen the development of different techniques such as utilisation of agrochemicals, cross hybridisation and genetic engineering to build stress-tolerant plant varieties (Gürsoy, 2022; Rana et al., 2022; Chen et al., 2023). Nonetheless, such techniques come with their own set of limitations involving genetic erosion, alteration of the purity of the cultivar, narrow genetic base, limitations in genetic improvement, difficulty in identification, and maintenance of accurate pedigree records (Salgotra and Chauhan, 2023). Genetically modified or GM crops are another marvel of the 21st century developed via genetic engineering. As per the International Service for the Acquisition of Agri-Biotech Applications (ISAAA), ~11.6 Mha of arable land in India is dedicated to GM crops (Rana et al., 2022). The government has approved field trials of 21 GM food crops, i.e., GM vegetables and cereals. However, the Indian State government has not allowed commercial cultivation of GM food till date (Mishra, 2020; Verma et al., 2022). Thus, the use of GM crops remains a convoluted topic.

The drastic changes in environmental conditions over time call for innovative measures that help the plants adapt to the changing environmental conditions and are sustainable as well. This brings us to a more straightforward approach to exploring the utilisation of endophytic fungi existing naturally in the biosphere. Endophytic fungi denote the mycological diversity residing in the plants without adverse effects (Yan et al., 2019; Baron and Rigobelo, 2022). Recent mycological literature has frequently used the term, although it was first described by Heinrich Friedrich Link in 1809 (Adeleke and Babalola, 2021a; Mishra et al., 2021). Fossil records of plants and various fungi dating back 400 million years ago have been recorded indicating the ancient association between the two (Krings et al., 2007). However, no precise data can pinpoint the origin owing to the intricate association.

To describe the origin of endophytes, Caiyi et al. (2004) and Li and Hu (2005) proposed endogenous and exogenous hypotheses. The former states chloroplast and mitochondria as the origin, whereas the latter states that they enter the plants from the outside. The transmission can occur vertically, with fungal hyphae penetrating the host embryo, or by horizontal transmission through sexual spores or asexual conidia (Samreen et al., 2021). The endophytic fungi and their host plant engage in a highly intimate interaction, with the fungal hyphae growing in a flattened or wedged manner against the plant cells. The fungal hyphae

and host exhibit concurrent growth, with the hyphae being attached to the host's cell wall without overpowering the plant cells (Christensen et al., 2008; Bastías et al., 2022).

Studies in the past provide evidence that plants depend on endophytic fungi for their overall development, nutrient and water uptake (Verma et al., 2021; Malicka et al., 2022). Therefore, the notion of 'Mycovitilism' coined by Vujanovic and Vujanovic (2007) following their observation of the beneficial impact of *Fusarium semitectum* on orchid seed germination, is gaining wider acceptance. This could confer an evolutionary benefit to the seeds, enabling them to encounter a ubiquitous fungal partner(s) like *Fusarium* during the crucial period of seed dispersal. Furthermore, there are extensive reports on endophytic fungi and plant growth promotion (PGP) (Baron and Rigobelo, 2022; Zhang et al., 2022). The co-evolution of endophytic fungi with the plants, coupled with the universal phenomenon of horizontal gene transfer, allows them to mimic different secondary metabolites and phytohormones produced by the host plants (Tiwari and Bae, 2020; Alam et al., 2021; Mattoo and Nonzom, 2021; Mukherjee et al., 2022). In addition, research shows that symbiotic association is a habitat-specific phenomenon unveiling endophytic fungi with unique attributes (Rodriguez et al., 2008; Bastías et al., 2022; Chaudhary and Shukla, 2023).

The changing climate over the years is among the leading cause of deteriorating plant health, affecting grain crop yield. Hence, this research work entails the isolation of fungal endophytes associated with two different rice varieties, namely, PUSA-44 and Sahbhagi Dhan. PUSA-44, is a long-duration rice variety endorsed by ICAR and is extensively cultivated in Punjab. Whereas, Sahbhagi Dhan developed by the ICAR-National Rice Research Institute is an early release rice variety, cultivated in shallow lowlands of Orissa and Jharkhand. The isolated endophytic fungi were screened against two primary abiotic stresses, salinity and drought. Furthermore, the PGP attributes of the selected endophytic fungi were evaluated. After systematized analysis, the promising isolates were tested as bio-inoculants in rice plant under stress conditions. The technology involving resilient endophytic fungi holds potential prospects to protect the rice plant from abiotic stress conditions. It may eventually reduce chemical-intensive agricultural practices into the bargain.

Chapter 2

Present approach

2.1 Hypothesis

It is widely acknowledged today that fungi live as endophytes in all plants. Although our current knowledge about the intricate cross-talk between the duo is still unfolding through numerous studies which explicitly reveal an intimate relationship (Mattoo and Nonzom, 2021). As discussed earlier in the introduction, the fungal endophytes have the ability to produce various phytochemicals otherwise produced only by the plants (Jahagirdar et al., 2019; Tiwari and Bae, 2020; Alam et al., 2021; Mukherjee et al., 2022; Chanda et al., 2023). For instance, synthesizing phytohormones is a unique characteristic of various microorganisms including fungal endophytes. These signalling molecules prompt numerous functions ranging from embryo development, stress tolerance to the overall development of plants (Cosoveanu et al., 2021). Fungal endophytes produce all five classes of phytohormones. The phytohormones play diverse roles in root formation, development of pollen tubes, seeds, cell division, and combating different stresses (Bilal et al., 2018; Zhao et al., 2021; Baron and Rigobelo, 2022; EL Sabagh et al., 2022).

In addition to phytohormones, plants take up many vital nutrients that are necessary for growth and development. But these nutrients are present as insoluble salts in soil, such as phosphorous, which is vital for growth and overall crop performance (Malhotra et al., 2018). Fungal endophytes play a crucial part in effectively solubilising such minerals (Devi et al., 2022; Iqbal et al., 2022; Chaudhary et al., 2023; Jana et al., 2023). The solubilised minerals are readily available for plants, which help stimulate root growth and regulate stomatal opening. Genera of endophytic fungi like *Penicillium*, *Trichoderma* and *Aspergillus*, have been documented for their efficient mineral solubilization properties (Adhikari and Pandey, 2019; Tandon et al., 2020; Turbat et al., 2020; Vassileva et al., 2022; Kumar and Prasher, 2023). Other minerals like potassium, play a vital role in ATP synthesis, stomatal regulation, producing proteins, starch, and cellulose, and providing general immunity against abiotic stresses (Sustr et al., 2019; Wang et al., 2021; Johnson et al., 2022). Similarly, fungal endophytes from various genera have been documented to solubilize calcium, aluminium, and zinc (Spagnoletti et al., 2017; Kaul et al., 2019; Barresi et al., 2022; Devi et al., 2022).

Another critical attribute observed in PGP microorganisms is ACC deaminase production. The enzyme produced by endophytic fungi cleaves 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ethylene, to α-ketobutyrate (Rauf et al., 2021). Consequently, a reduction in the plant ethylene levels occurs which reduces the plants' ethylene-mediated growth inhibition (Gamalero et al., 2017; Ali et al., 2019; Wang et al., 2022). Additionally, the endophytic fungi can also liberate ammonia (Mehmood et al., 2019;

Chand et al., 2020; Khalil et al., 2021). It binds the air-borne nitrogen making it accessible for the plant. Nitrogen, an essential constituent of the chlorophyll molecule, aids plants by supplying energy and enhancing crop production. Similar to the nitrogen metabolism capabilities of plants, the endophytic fungi assist the host plants in acquiring nitrogen. As a consequence, this enhances the nitrogen availability and plant growth (Ali et al., 2022; Sun et al., 2022).

Various genera of endophytic fungi have also been documented for siderophore and extracellular lytic enzyme production (Bilal et al., 2018; Chowdappa et al., 2020; Turbat et al., 2020; Khan et al., 2021). The siderophores demonstrate chelation of ferric ions found in the rhizosphere. Since iron is vital for all life forms, the siderophore molecules produced by the fungal endophytes act as growth regulators by limiting the iron availability to phytopathogens (Jana et al., 2023). Moreover, the role of endophytic siderophores in induced systemic resistance is also being investigated. Once detected by plants, a systemic response is initiated which primes the defence system of the plant and upregulates defence-related genes (Datta et al., 2022; Akram et al., 2023).

In addition, the extracellular lytic enzymes produced by endophytic fungi help them to form a symbiotic association with the host plant by allowing entry into the plant (Lu et al., 2021). Extracellular enzymes breakdown lignocellulosic materials, which both endophytic fungi and the host can assimilate. The enzymes safeguard plants from biotic stress by inhibiting different pathogens. Moreover, studies show that endophytes and plants communicate via phytochemical signals (Soni et al., 2021; Raghav et al., 2022). Endophytic fungi produce many natural products apart from putative phytochemicals of their host which belong to different chemical classes broadly belonging to alkaloids, terpenoids, flavonoids, phenolics, and tannins (Ameen et al., 2021; Chua et al., 2022; Elghaffar et al., 2022). These phytochemicals exhibit significant antimicrobial and antioxidant activities, which safeguard the plants, especially during stressful conditions. Additionally, there is a hypothesis that plant signals play a role in triggering the secondary metabolites production in endophytes (Deka and Jha, 2020).

To counteract the ROS generated during stress conditions, the production of antioxidant enzymes is a strategy employed by plants. Recent literature shows that fungal endophytes enhance the antioxidant potential of plants (Ma et al., 2019; Bianco et al., 2021; Siddiqui et al., 2022). Together the non-enzymatic and enzymatic antioxidants play a key part in regulating ionic homeostasis to combat stress. Owing to these plant growth-promoting attributes, studies have reported fungal endophytes which impart tolerance to their respective hosts against an array stressor namely salinity, drought, and high-temperature (Qiang et al.,

2019; Bilal et al., 2020; Moghaddam et al., 2021; Lubna et al., 2022; Reshna et al., 2022). The endophytes regulate the plant defence by using mechanisms ranging from signalling molecules to host gene regulations (Lu et al., 2021). Another unique characteristic of endophytic fungi is the diversity based on the type of plant and the habitat in which it is cultivated. As discussed in the introduction section, this habitat-adapted symbiosis hypothesis that the endophytes can induce promising results in stress mitigation within one growing season (Hubbard et al., 2014; Moghaddam et al., 2021; Nguyen et al., 2024). Fungal endophytes of plants thriving in the harsh conditions have been examined for their potential role in mitigating abiotic stresses (Malicka et al., 2022; Ballesteros et al., 2023).

For instance, *Fusarium* sp. isolated from a salinity-tolerant rice variety has been reported to confer salinity tolerance to a salt-sensitive rice variety (Sampangi-Ramaiah et al., 2020). Likewise, *Penicillium* and *Chaetomium* sp. associated with upland rice varieties were reported for conferring drought and salinity stress tolerance to salt-sensitive rice varieties (Pang et al., 2020). Several species of Ascomycota like *Aspergillus*, *Chaetomium*, *Penicillium*, *Piriformospora* and *Talaromyces*, from roots of rice plants have exhibited abiotic stress tolerance (Kord et al., 2019; Qin et al., 2019; Sampangi-Ramaiah et al., 2019; Siddiqui et al., 2022). On inoculation, successful colonisation of the endophytic fungi has been reported in rice. Moreover, the endophyte-inoculated plants exhibit better physiological and biochemical characteristics such as increased root and shoot growth parameters, chlorophyll content (Manasa et al., 2020; Tsai et al., 2020; Reshna et al., 2022), accumulation of osmolytes and upregulated antioxidant enzymes (Gazara et al., 2020; Gupta et al., 2023; Li et al., 2023). These positive attributes make endophytic fungi a front-runner in ameliorating plant health under stressful conditions. Hence, based on these findings, the current study was taken up to explore the answer to the following questions (a) *Can endophytic fungi tolerate more than one type of abiotic stress?* (b) *Do endophytic fungi from different rice varieties harbour different plant growth-promoting attributes?* (c) *Can endophytic fungi isolated from a particular rice variety induce abiotic stress tolerance in other rice varieties?*

To understand and answer the above research questions, the following objectives were undertaken during the course of this research work;

1. Isolation of endophytic fungi from rain-fed and drought stress varieties of rice plant
2. Screening of the endophytic fungi for salinity and drought tolerance
3. Evaluation of plant growth promoting activity of selected endophytic fungi for stress environment

Chapter 3

Review of Literature

3.1 Global Climate Change: A Catastrophe

The Earth has witnessed global climate change accompanied by variation in precipitation, extreme temperature and weather pattern. These changes are primarily brought upon by the anthropogenic activities, such as industrial operations, burning of fossil fuels, transportation, and emission of greenhouse gases into the atmosphere (Karki et al., 2020; Bandh et al., 2021; Corwin, 2021; Bhardwaj et al., 2022). By sector, energy used in industry contributes ~24 to the global greenhouse emission followed by agriculture and land use at ~18%, energy used in buildings at ~17% and transport sector at ~16% (Ritchie, 2020; FAO, 2022). Furthermore, the impact of climate change manifest in several ways within the Earth's natural systems, including but not limited to extreme heatwaves, rising sea levels, and prolonged drought conditions. These repercussions disrupt the normal functioning of human societies by amplifying the frequency and intensity of natural disasters, as well as exacerbating health risks linked to extreme weather conditions. Consequently, these challenges often necessitate the relocation of populations as a coping mechanism (Dey and Lewis, 2021; Kumar et al., 2021; Majedul, 2022) (Fig. 3.1).

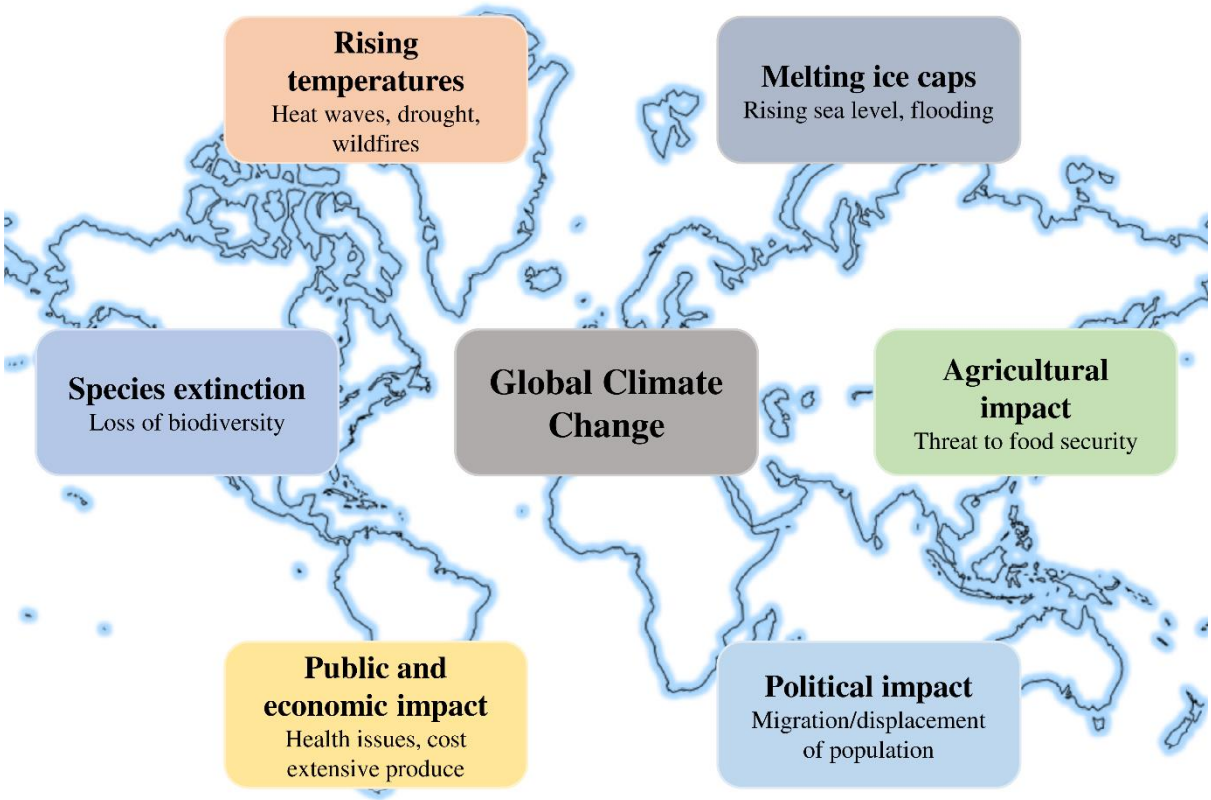

Fig. 3.1: Global climate change and its adverse effects.

The susceptibility of agriculture to climate change has led to reduced crop yields. The changes in rainfall distribution and snowmelt patterns have led to increase in drought and flood like conditions which hamper the agricultural growth and productivity. The changing

conditions also impact the soil health, which results in erosion, nutrient depletion, and decreased agricultural output (Kumari et al., 2020; Shahzad et al., 2021; Elbasiouny et al., 2022; Pathak, 2023). These changes primarily caused by the anthropogenic activities have diverse consequences on agriculture that can differ by region and the type of crop. Studies show that abiotic stress factor are the chief cause of reduction in crop yield (Khalid et al., 2019; Onyekachi et al., 2019, Jeyasri et al., 2021; Kopecká et al., 2023).

Abiotic stress describes the environmental variables such as extreme temperature, heavy metals, salinity and drought, that harm a plant's development. These stresses affect plant's physiological, biochemical and molecular functions which reduces growth and occasionally results in plant death (Shahid et al., 2020; Yadav et al., 2020, Chaudhry and Sidhu, 2022; Saini et al., 2022). For instance, drought stress reduces the chlorophyll production, water potential and wilting which adversely hinders the plant growth. Similarly, salinity stress causes a reduction in water uptake, nutrient shortages and eventually cause ionic imbalances in the plants. As a consequence, the crop yield greatly reduced (Hameed et al., 2021; Wahab et al., 2022; Zheng et al., 2023). To deal with abiotic stresses, the plants have developed a strategic coping mechanism involving the stress related proteins, regulation of gene expression and build-up of antioxidants (Singh et al., 2020; Ilyas et al., 2021, Zhang et al., 2022; Praveen et al., 2023). However, these defences are not entirely sufficient especially if there are numerous stresses present.

The reduction in crop yield and quality has a large negative economic impact on the farmers and food security. Depending upon the location, many crops may no longer be viable in specific places. In addition, because of the poor soil fertility plants may become more susceptible to pest and diseases (Nair and Nair, 2019; Usharani et al., 2019; Sade and Peleg, 2020). As a consequence, the ability to sustain the growing population is gravely hampered. According to the current trends, by 2050 the total population is predicted to exceed 9.7 billion. With over 1.3 and 4.6 billion people, Africa and Asia makeup the most populous continents (Bahar et al., 2020; Gu et al., 2021). The latest statistics show that on 2021, 9.9% of the global population i.e., ~800 million people were affected by hunger. The State of Food Security and Nutrition in the World report of 2022 says that one in every ten individuals is affected by hunger globally. Data shows that majority of the global undernourished population resides in developing countries. For instance, Asia alone houses ~418 million people affected by hunger (Chichaibelu et al., 2021; WHO, 2021, FAO et al., 2022).

The eradication of world hunger is one of the Sustainable Development Goals of the United Nations (UNSDG-2). In addition to achieving food security, the goals also emphasise the need of sustainable agriculture. It recognises the interdependence of promoting sustainable agricultural practices to achieve food security. Although this complicated subject needs a multifaceted strategy, it is a step in the right direction to achieve effective outcomes. By the support of small-scale farmers, researchers and innovators the global food production can be enhanced (Nicholls et al., 2020; Azadi et al., 2023). Recent data also shows that a sizable amount of food is wasted which can be omitted with the help of programs such as the food banks (Chen et al., 2020; Durán-Sandoval et al., 2023). For long term food security, adaptation and mitigation efforts against climate change have to be ensured. A variety of measures, such as sustainable land use practises, water management plans, and the creation of new crop varieties that are more resistant to changing climate conditions, are required to address the consequences of climate change on agriculture and sustain the growing population (Malhi et al., 2021; Abbass et al., 2022).

3.2 History of rice cultivation

The majestic Earth is home to diverse flora and fauna, out of the 50,000+ edible plants, three namely, rice, wheat and maize provide 60% of the world's food energy intake (Zhao et al., 2020; Poole et al., 2021). Rice, the seed of *Oryza* specie, is a globally consumed staple of Poaceae or the grass family (Gramineae). To understand the domestication and cultivation of rice by the human race and subsequent distribution across different parts of the globe, technological improvement that revolutionised the rice cultivation through time till date has been equally documented by historians, archaeologists as well as scientists (Fornasiero et al., 2022; Bellwood, 2023). The archaeological and linguistic data exhibits that rice was first cultivated between 13,500 to 8,200 years in China's Yangtze River basin (Normile, 1997; Vaughan et al., 2008). Following its initial cultivation, rice was widely dispersed through migration and commerce, first reaching much of east Asia, then spreading further afield, and then reaching the west because of the Columbian Exchange. African farmers independently developed the now-rare *Oryza glaberrima* rice some 3,000 years ago.

In India, domestication of wild rice species *Oryza nivar* has been reported as early as 5000 BC. However, around 2000 BC truly 'wetland' rice *Oryza sativa* var. japonica was reported (Callaway, 2014; Choi et al., 2019). Over centuries rice production in India increased due to traditional practices such as cross breeding. The existing varieties were crossed to produce strains with desirable characteristics. Consequently, the rise of commonly consumed varieties such as PUSA-basmati, Swarna and IR developed by different

organisations like Indian Agricultural Research Institute, Indian Council of Agricultural Research and International Rice Research Institute was seen (Prasanna and Rao, 2019; Samal et al., 2022). Though the breeding process aims at producing high yield better quality grains, the long-time investment to achieve these desired traits requires commitment. It also demands a forward-thinking perspective to anticipate and prepare for the agricultural scenario of the future. Othe practices such as those adopted during the green revolution and the use of genetically modified plants (GMO) have also been developed (Armanda et al., 2019; Verma et al., 2022). However, transgenic rice has not been approved in many parts of the world and this has necessitated the development of a novel intervention to sustain agriculture.

3.3 Rice production and consumption

Rice production reached ~756.7 million metric tonnes in 2020 with China and India accounting for 52% of the global total (Shahi et al., 2023). Asian farmers currently produce over 90% i.e., ~680 million metric tonnes of the world's rice (FAO-STAT, 2019) (Fig. 3.2). India ranks second in rice production with ~120 million tonnes as of 2020 (Dey, 2020; Madhu et al., 2023). Conditions like bad roads, inadequate storage, and ineffective supply chains results in significant losses in many rice producing countries. Research shows that farmers in emerging nations like India and China lose over US$89 billion year due to avoidable post-harvest farm losses. India alone could save enough food each year to sustain 70-100 million people by developing stronger infrastructure and retail networks (Bendinelli et al., 2020; Kashyap and Agarwal, 2020).

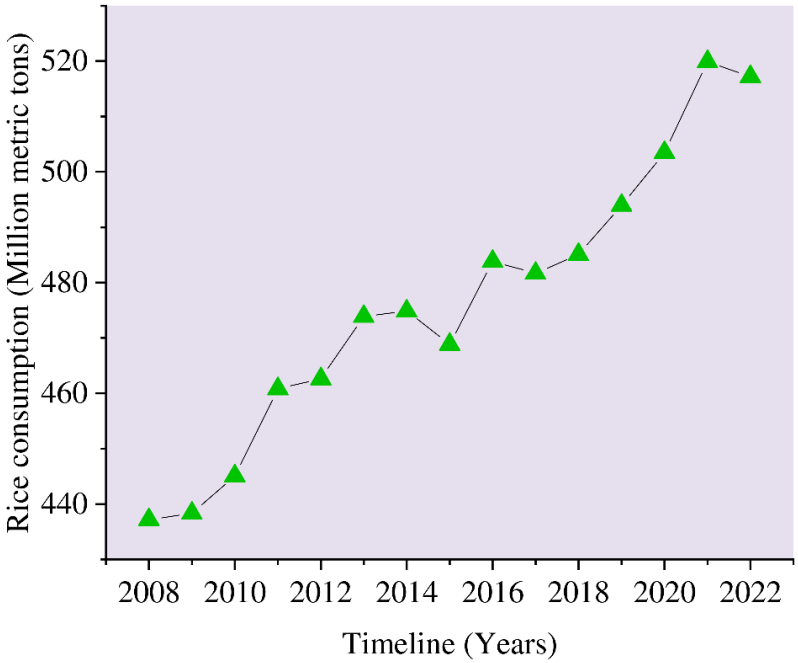

Fig. 3.2: Global rice consumption over the years.

According to research by the Korean Society of Crop Science, by 2040 the rice consumption is expected to be 590 million tonnes (Bhandari, 2019). The rice plant, *Oryza sativa* (Asian rice*), Oryza glaberrima* (African rice), *Oryza zizania*, and *Oryza porteresia* (wild rice), among others, have a wide range of variations. At one time India alone had 110,000 rice varieties. Currently, Basmati, PUSA, IR, Swarna and Sahabhagi are some of the widely consumed varieties. This trend is evident in various states of India. For instance, PUSA-44, is extensively cultivated irrigation fed rice variety of Punjab. Despite its excessive water consumption, the variety is famous among farmers due to its high yield (Dwivedi et al., 2021). Likewise, Sahabhagi Dhan is designed explicitly to combat drought in the lowlands of Orrisa and Jharkhand (Dar et al., 2020).

3.4 Health benefits of rice consumption

Being the staple food, rice is the predominant source of nutritional energy for 17 Asian and Pacific, eight African, and nine North and South American countries (FAO-STAT, 2019). A United States Department of Agriculture (USDA) nutrient data report says that 100 gram serving of rice provides 130 calories, carbohydrates, protein and negligible amount of fat (Table 3.1) (FAO-STAT, 2019; Juliano, 1993). Rice fortification with iron alone, iron combined with zinc, vitamin A, folic acid, or B-complex vitamins may help alleviate malnutrition (WHO, 2018). A thorough analysis of clinical studies on the effectiveness of iron fortification in rice revealed that the technique primarily reduced iron deficiency and raised haemoglobin levels in the blood (WHO, 2018). A key suggestion was made in the guidelines: 'In contexts where rice is a staple food, iron fortification is recommended as a public health intervention to improve the health of communities' (WHO, 2018).

The nutritional content of rice depends on various circumstances, such as strain of rice, soil health at cultivation site, polishing and manner of preparation. Several types of rice are classified based on pigments like black, brown, purple, and red rice, in addition to the white-rice varieties (Samyor et al., 2017; Chen et al., 2022). The pigment anthocyanin, which is present in significant amounts in the rice coat, is what gives the rice grains their colours (Rathna et al., 2019). In terms of nutritional value, rice is regarded as the king of cereals due to its high digestibility (Zhou et al., 2020). According to some selected studies eating whole grains can reduce heart disease, diabetes, and malignancies (Malik et al., 2019; Saleh et al., 2019; Tosh and Bordenave, 2020). The benefits of whole grains for intestinal health cannot be overstated (Anuyahong et al., 2020). Mounting evidence over the decades shows that rice has wide range of phytochemicals that fall under the category of

phenols and flavonoids, which have variety of biological functions (Rao et al., 2020; Bagchi et al., 2021; SubbuThavamurugan et al., 2023).

Table 3.1: Nutritional content in different types of rice.

Nutrients (1 cup cooked rice)	White rice	Brown rice
Energy (kcal)	205-242	216-218
Protein (g)	4.25-4.43	4.52-5.03
Total fat (g)	0.35-0.44	1.62-1.75
Carbohydrates (g)	44.51-53.44	44.77-48.84
Sugar (g)	0-0.08	0-0.68
Fiber (g)	0-0.6	~3.5
Calcium (mg)	2-16	~20
Iron (mg)	1.9-2.77	0.82-1.03
Magnesium (mg)	15-24	84-86
Phosphorous (mg)	61-69	150-162
Potassium (mg)	48-55	84-154
Sodium (mg)	0-2	2-10
Zinc (mg)	0.74-0.78	1.21-1.23
Copper (mg)	0.071-0.134	0.158-0.195
Manganese (mg)	0.664-0.746	1.765-2.139
Selenium (mcg)	11.9-14	0-19.1

Rice has polyphenolic chemicals with beneficial nutraceutical benefits for human health. These polyphenols, which include phenolic acid, anthocyanin, and proanthocyanidin, have antioxidant qualities that aid in scavenging the free radicals generated in our bodies (Verma and Srivastav, 2020; Bagchi et al., 2021; Chen et al., 2022). Moreover, it contains several bioactive substances, such as flavonoids (especially anthocyanin and proanthocyanidin), carotenoids (especially lycopene and lutein), phenolic compounds (including ferulic and caffeic acid), phytosterols (including capesterol and stigmasterol), vitamin E isoforms (including, tocotrienols) (Peanparkdee and Iwamoto, 2019; Chen et al., 2022). The bran/germ fraction, which is the outer part of the grain, contains the highest concentrations of these phytochemicals. Many cell components, including lipids, proteins, and DNA, are thought to contain bioactive chemicals that contribute to oxidative stress. It eventually results in conditions including inflammatory, cancerous, cardiovascular, and cancerous diseases (Pisoschi et al., 2021; Demirci-Çekiç et al., 2022; Kowsalya et al., 2022).

3.5 Impact of climate change on rice cultivation and production

Given the tremendous health benefits of rice consumption, it is imperative to sustainably enhance its production to keep up with the demand of population. By the year 2050, the human population may touch 9.1 billion mark but the agricultural yield is not increasing at

the same pace to meet the increasing demand (Chichaibelu et al., 2021; WHO, 2021). A rise of 70% is required in the food production to feed this growing population (Flies et al., 2018; Tamburino et al., 2020). Agricultural production has declined mainly due to abiotic and biotic factors with abiotic factors being the major contributor (Kumar et al., 2022; Kopecká et al., 2023; Radha et al., 2023). Rice is a versatile crop and can be cultivated in diverse climatic conditions including both dry and wetland environments at high and low altitudes (Batool et al., 2019; Yuan et al., 2021). However, salinity and high-water consumption are the most prevalent problem in rice growing areas, since rice is a salt sensitive crop especially in the early seedling stages, salinity not only affects the quality but productivity of rice as well (Thu et al., 2020).

Of the 130 million hectares (mha) of world rice area, ~30% area has high salt levels to allow normal rice yield. Under reasonably salt-affected soils the decrease in rice yield is anticipated to be 68% (Razzaq et al., 2020; Pathak et al., 2021). Natural events like global warming, rising water levels, excess irrigation, improper drainage and underlying rocks rich in detrimental salts are some of the reasons for increasing salt stress. As per the present scenario, 50% of the present cultivable land will be lost for agriculture by 2050 majorly due to abiotic stress factors (da Cunha et al., 2021). A soil in which the electrical conductivity (EC) exceeds 2 dS/m in the root zone area (FAO, 2021). Rice crop yield is seriously threatened by soil salt, especially in low-lying coastal locations during the dry period. The high salt concentrations affect the normal physiology, especially during the early stages of plant. Consequently, the farmers are compelled to give up these otherwise very productive sites (Nguyen and Tran, 2020; Johnson and Humphreys, 2021; Liu et al., 2022).

Salt stress affects plants in two ways: the water deficient effect, which reduces the water absorption, and the ion excess effect, which reduces growth by damaging the cells in the transpiring leaves (dos Santos et al., 2022). The most crucial stage of a plant's growth cycle is seed germination. Salinity lowers osmotic potential and induces toxicity, which affects the nucleic acid metabolism-related enzymes (Safdar et al., 2019). Salt stress also influences the photosynthetic rates. Salinity and the mineral nutrition of crops have a complicated relationship. Because of the interaction between Na^+ and NH_4^+, increased salinity results in decreased nitrogen absorption (Huang et al., 2020; Zheng et al., 2023). In areas where rice agriculture is a major source of food security, this may have a considerable effect (Schneider and Asch, 2020; Rezvi et al., 2023).

When the crop starts assimilating smaller amounts of water as compared to the evaporative demand of its surrounding atmosphere, it is considered under water stress (Kim

et al., 2020). In South and South East Asia, 19–23 million hectares of rainfed rice cultivation are frequently at risk of drought (Bhatt et al., 2021; Surendran et al., 2021). Traditional rice varieties can be severely harmed by drought conditions if there is not enough water to allow them to uptake the necessary amounts of nourishment from the soil. For instance, yield loss of ~40% have occurred in India, annually amounting to US$ 800 million (Sharma et al., 2019; Wassmann, 2019). Drought stress causes decline in photosynthesis, nutrition imbalance and yield (Iqbal et al., 2020; Dietz et al., 2021). To compete with early drought condition, large rice seeds are preferred which grow more effectively as they have a superior root mass in seedlings which facilitate the enhancement of water balance under early water stress (Raza et al., 2023). In addition, farmers use a range of management techniques to lessen the drought stress, by enhancing soil water retention, utilising drought-tolerant rice cultivars, and altering planting dates to avoid dry spells (Hussain et al., 2020; Panda et al., 2021; Zia et al., 2021).

Research has shown that besides physiological, biochemical and enzymatic attributes, salinity and drought stress also affects the rice yield (Abdallah et al., 2016; Kamarudin et al., 2018). Salinity stress reduces the number of panicles, spikes and grain weight and increases unfilled grains (Gay et al., 2010; Abdallah et al., 2016). Gay et al., 2010 witnessed an overall reduction of rice yield by 45% in some cultivars. Salt stress also impacts the photosynthetic machinery of the plants. Consequently, the plants exhibit reduced pigment production (Jamil et al., 2012; Abdallah et al., 2016). To survive the stressed environment the plants often show a rapid influx of Na^+ and reduction in K^+ and Cl^- ions (Wang et al., 2012; Thu et al., 2017). Furthermore, salt stress increases proline and sugar content indicating osmolyte accumulation (Jamil et al., 2012; Abdallah et al., 2016). Salt stress impacts the rice plants in many ways, the multifarious effects physiochemical and molecular aspects of rice plants are shown in Fig. 3.3.

Similarly, drought stress also affects the physiological, biochemical and enzymatic attributes of rice (Kamarudin et al., 2018; Ishimaru et al., 2022). Rice is primarily cultivated in irrigated areas however, even mild drought stress causes changes in the amylose and starch ratio which increases the starch digestibility of the grain and the glycaemic index (Prathap et al., 2019; Wang et al., 2022). Kumar et al. (2020) found, major variations in the studied parameters in different rice cultivars over different cultivation seasons. High glycaemic content of rice increases its digestibility; however, this leads to a sudden spike in glucose levels. Thus, consumption rice may increase the health risks in type-2 diabetic people (Jukanti et al., 2020; Ren et al., 2021). Kumar et al. (2020) also observed that rice

grains under drought stress have less amylose and resistant starch, which raises the glycaemic index. The study highlights how the effect of abiotic stresses directly impact the wellbeing of the consumers.

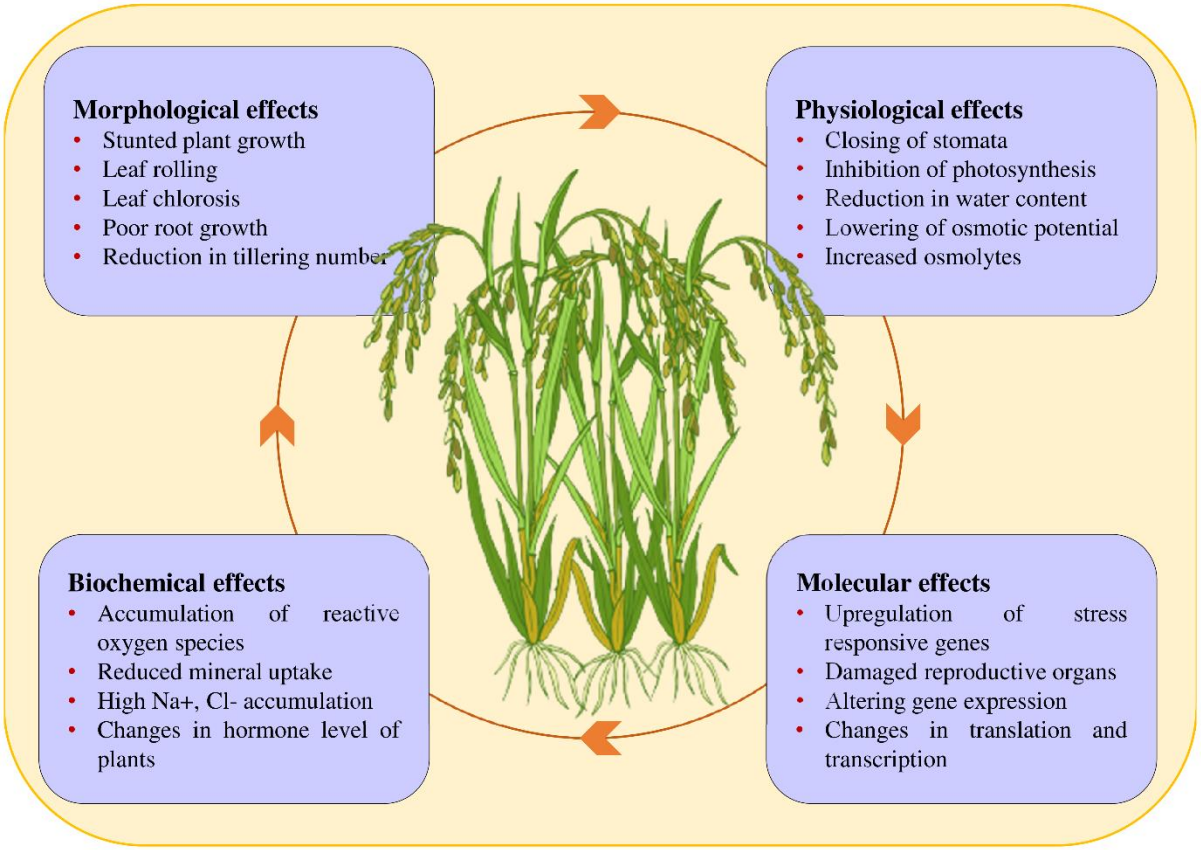

Fig. 3.3: Effects of salinity and drought stress on rice plant.

Drought stress at any development stage of rice cultivation has adverse impact (Yang et al., 2019; Ishimaru et al., 2022). Increased urbanisation and the lack of available subsurface water has led to competition for the usage of surface water, resulting in the spread of drought regions (Kookana et al., 2020; Dąbrowska et al., 2023). Rice plants exhibit low setting rate highlighting the harmful effect of the stresses on the grain (Yang et al., 2019; Zhang et al., 2022). Drought increases number of sterile spikelet's by reducing the fertility of the panicles. In addition, drought stress affected the chalky rate, length of amylopectin and intermediate chains in the rice plants. These changes in the structural integrity of rice, depreciated its sensory quality (Gao et al., 2019; Zhang et al., 2022). The stress gravely hinders the key process of photosynthesis which reduces the growth and yield. Other than the reduced yield, alterations in the quality of grain are also observed (Kamarudin et al., 2018; Yang et al., 2019). These effects range from physiological, morphological, biochemical to molecular (Gao et al., 2019; Kumar et al., 2020; Ishimaru et al., 2022). Similarly, plethora studies show the impact of abiotic stresses, especially salinity and drought on rice plants

(Table 3.2). Over the years, many techniques and methods have been employed to enumerate the impact of these stress conditions. The following section discusses the role of different resistance inducing practices and their drawbacks.

Table 3.2: Effect of abiotic stress on different rice varieties.

Rice variety	Stress	Effect	Reference
Matsumae	100 mM NaCl stress	Accumulations of Na^+, K^+, Cl^- and nitrogen in leaves. A strong effect on nitrogen metabolism in old leaves.	Wang et al., 2012
Giza 177 and Giza 178	0-60 mM NaCl stress	Reduction in photosynthetic pigments, total carbohydrate, and increase in total soluble sugars, trehalose and proline content.	Abdallah et al., 2016
Different varieties from Kyushu University Cultivated Rice Collection and World Rice Collection	12 dS/m NaCl stress	Accumulation of Na^+ in the shoots of the rice plants. Reduction in uptake of K^+ and Mg^+ ions.	Thu et al., 2017
IR64, Aeron1, MR219, MR219-4 and MR219-9	−30 kPa level	Reduction in plant growth, pigments production and yield. Enhanced proline content, enzyme activity of catalase, ascorbic acid peroxidase and guaiacol peroxidase.	Kamarudin et al., 2018
Yangliangyou 6 and Hanyou 113	−30 ± 5 kPa level	A significant reduction in physiological traits at grain filling stage. Drought stress at flowering stage has a strong influence on rice physiological traits and overall yield.	Yang et al., 2019
Rice IIYou 898	50-70% water holding capacity	Reduction in grains per panicle and the total grain number.	Gao et al., 2019
IR64 and NILs	Drought stress by termination of watering	Changes in morphological characteristics such as flower opening time, panicle length and spikelet per panicle.	Ishimaru et al., 2022

3.6 Traditional practices to combat abiotic stressors

Over the years various practices have been adopted to make plants overcome different abiotic stresses. These primarily comprise of soil amendments, mulching and different irrigation practices. The following section discusses the primary resistance inducing techniques. Although some of the approaches have yielded promising results, they have inherent set of drawbacks associated with them.

3.6.1 Agrochemicals

The food shortages across the globe saw a period of relief with intense agricultural development during the Green Revolution in 1940-1950s. Green revolution primarily focused on intensification of staple crops by improving irrigation system and employing the use of chemical fertilisers and pesticides (Gürsoy, 2022). The heavy reliance on agrochemicals improved the crop yield, however, there were negative implications such as pollution, soil degradation and biodiversity loss (Meena et al., 2020, Sellare et al., 2020; Nath et al., 2023). Additionally, studies show the ill effects of extensive chemical fertilisers and pesticides on human health. The prolonged exposure causes wide array of problems ranging from skin, respiratory, reproductive issues, increased risk of cancer and disruption of endocrine system (Elahi et al., 2019; Lee and Choi, 2020; Rani et al., 2021; Devi et al., 2022; Nath et al., 2023). Moreover, some cases of neurological issues, such as confusion, memory loss and poor coordination have also been reported (Scott and Pocock, 2021; Rezende et al., 2022; Nath et al., 2023). Over the recent years, studies have also reported carcinogenic nature of the agrochemicals. However, despite the known association, scarce studies are available as compared to the wide spread use of these agrochemicals (Burns and Juberg, 2021; Calaf, 2021; Matich et al., 2021; Pedroso et al., 2022). These major health consequences associated with agrochemicals discourages their use in intensification of agriculture.

3.6.2 Cross breeding/hybridisation

Techniques such as cross breeding or hybridisation are also employed for vigour improvement and impart tolerance to different abiotic stresses by natural acquisition of genes through recombination. Hybridisation involves cross breeding different varieties to generate a hybrid with desirable qualities (Mwangangi et al., 2019; Chen et al., 2023). Globally various hybrid rice varieties such as, Shanyou 63, IR64 and Malaysia hybrid 63 are being cultivated. Additionally, PUSA RH10, DRRH-2 and KRH-2 are some of the hybrid rice varieties cultivated in India (Taylor, 2020; Bin-Rahman and Zhang, 2023). Although the technique is time intensive and has been around for quite some time, it has its own set of

drawbacks. Cultivation of high yielding varieties reduce the genetic diversity. This makes the variety more prone to environmental perturbations and may also result in disappearance of conventional crop varieties (Begna, 2021; Singer et al., 2021; Salgotra and Chauhan, 2023).

Besides being time intensive, the hybrid seeds so obtained are cost intensive as well. The long research and development process incurs cost as it uses advanced techniques. To maintain the genetic uniformity during large scale production various quality control measures and testing are involved which adds up as production cost (Ter Steeg et al., 2022; Chakrabarty et al., 2023). Moreover, the trend of saving the seeds from one season for the next in order to reduce the cost cannot be practiced with hybrid seeds (Mueller and Flachs, 2022; Ter Steeg et al., 2022). Moreover, the hybrid varieties require extra inputs, such as fertilisers and herbicides, which is an added cost and has environmental implications (Meena et al., 2020; Pedroso et al., 2022; Nath et al., 2023). Further, if the developed hybrid has fewer nutritional attributes than the original, it could add on to the world hunger problem. Likewise, mismatching a hybrid to the diverse growing conditions around the globe could decrease the crop yield and thereby increase the problem of food shortage. Although hybridisation has been an important tool for intensification of agricultural produce, the negative connotations associated with the technique decrease its appeal.

3.6.3 Genetically modified crops (GM crops)

Another advancement of the 21st century are the genetically modified crops or GM crops. As the name suggests, plants with alterations to their genetic expressions for expression of specific traits are known are GM crops. As discussed in the earlier section, India has one of the largest acreages dedicated to GM crops, however besides BT-cotton commercial cultivation of GM crops is limited to confined trials only (Kranthi and Stone, 2020; Peshin et al., 2021; Rana et al., 2022). Research shows that the cultivation of GM crops could cause environmental disbalance such as, harmful effects on beneficial insects which are crucial for pollination. It could also have health implications like antibiotic resistance (Midtvedt, 2014; Kumar et al., 2020; Arpaia et al., 2021; Yali, 2022). Consequently, it may be daunting to treat illnesses in the population. Moreover, the development and production of GM crops is an expensive task. This could further lead to social inequality as the large agribusinesses would dominate the sector (Adlak et al., 2019; Kumar et al., 2020). The recent advances using techniques like clustered regularly interspaced short palindromic repeats (CRISPR Cas-9), transcription activator-like effector nucleases (TALENs) and zinc-finger nucleases (ZFNs) for genome editing highlight new genes and regulatory elements involved in stress tolerance. Various salinity and drought tolerance genes, such as abscisic acid (ABA),

epidermal patterning factor (EPF), salt overly sensitive (SOS1) and high-affinity K^+ transporter 1 (HKT1), have been identified for gene modifications. The advances have improved specificity, and the regulatory frameworks allow for better genetic crop safety. However, the ethical concern related to GM crops often puts one in dilemma and improving public perception of genome editing is warranted. Hence, the utilisation of GM crops remains a complex topic which needs further evaluation for a concise conclusion.

3.7 Endophytic fungi and their prospective application

Enhancing abiotic stress in crops necessitates a multifaceted strategy that is sustainable, environment friendly and the least adverse impact on human health. One such technology is exploration of the symbiotic association between microorganisms and plants. The word 'endophyte' was coined in 1809 by Johann Heinrich Friedrich Link, a German botanist (Hardoim et al., 2015). An endophyte is a microorganism residing in the host plant without causing any apparent harm (Stone et al., 2004; Sieber, 2007; Selim et al., 2012; Ali et al., 2024) (Fig. 3.4).

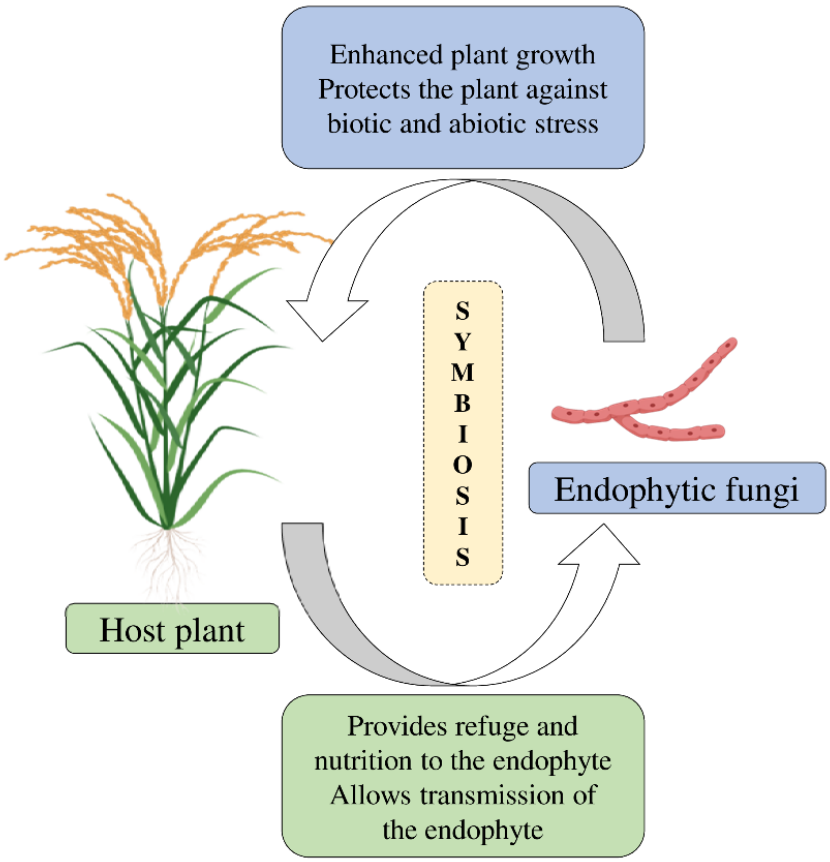

Fig. 3.4: Advantages of the symbiotic association to the host plant and fungal endophyte.

Also known as endosymbionts, endophytic fungi are ubiquitous (Verma et al., 2017; Fontana et al., 2021). The fossilised remains date back the association to 400 million years ago

(Rodriguez and Redman, 2008). Moreover, the symbiosis is believed to be the cause for plants transitioning to the land (Remy et al., 1994; Lipnicki, 2015). Different hypotheses about the origin of endophytes have been presented (Caiyi et al., 2004 and Li and Hu, 2005). Although the endophyte-plant relationship must be fully comprehended, the duo often engages in mutualism. The endophytes derive nutrition from the host, enhancing its fitness, in return (Rodriguez et al., 2009; Rho et al., 2018; Verma et al., 2021). However, the interaction is not strictly mutualistic; the endophytes may become pathogens or saprotrophs to survive under critical environment (Nelson et al., 2020; Bhunjun et al., 2023). The transmission of endophytes can occur vertically or horizontally (Rodriguez et al., 2009; Jonkers et al., 2022) (Fig. 3.5).

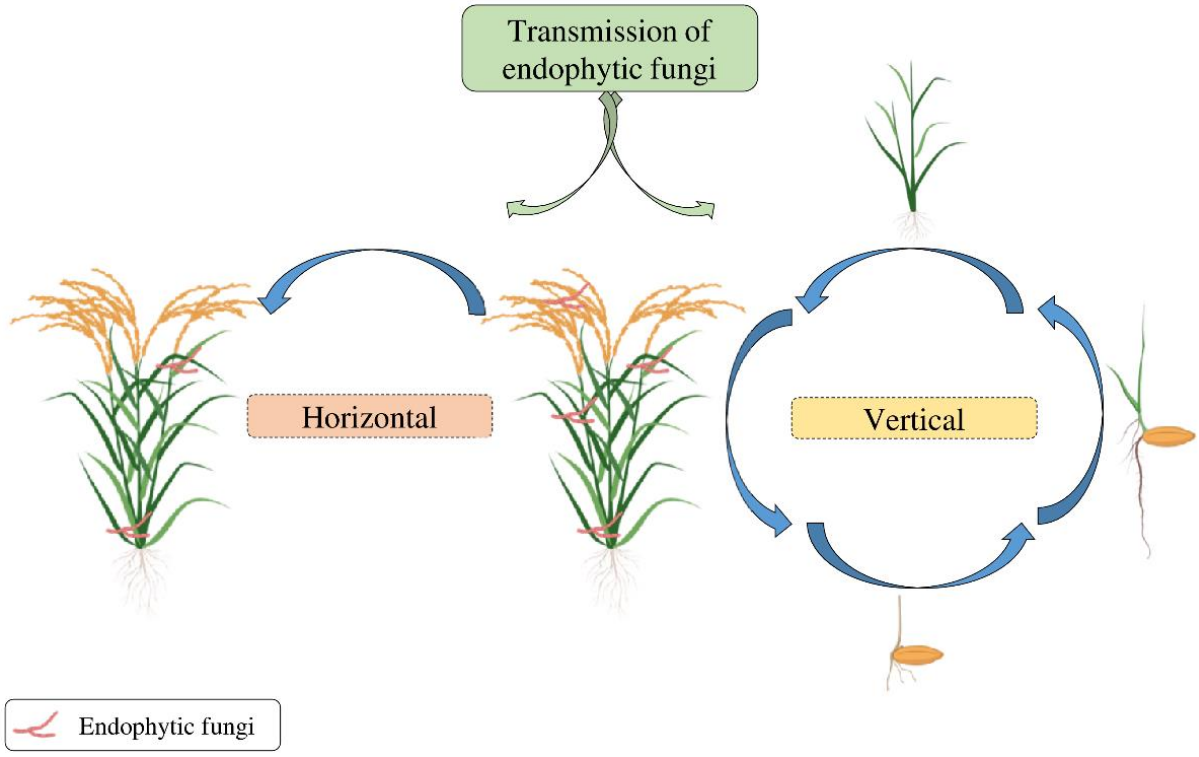

Fig. 3.5: A comparison of the horizontal and vertical transmission of endophytic fungi.

In the case of fungal endophytes, vertical transmission occurs by penetration of the fungal hyphae in the host embryo. In contrast, horizontal transmission occurs through sexual spores or asexual conidia (Samreen et al., 2021; Swamy and Sandhu, 2021; Jonkers et al., 2022). Furthermore, the endophytes can be classified by two different ways. The first method is to classify them as systemic and non-systemic. The systemic and non-systemic classification categorises the endophytes based on genetics, biology and their transmission (Wani et al., 2015). The uniqueness of systemic or true endophytes residing in a host plant does not change with changing environmental conditions. However, the diversity and number of non-systematic or transient endophytes change with changing environment.

Consequently, the non-systemic endophytes may become pathogenic under nutrient-devoid conditions (Stone et al., 2000; Rodriguez et al., 2009).

The other classification method divides the endophytic fungi into four groups (Class 1, 2, 3 and 4) based on the taxonomy, transmission mode, host range, colonisation tissue, colonisation, biodiversity and fitness benefits (Rodriguez et al., 2009; Aamir et al., 2020). The Class 1 endophytes, also known as Clavicipitaceous endophytes, are phylogenetically similar and typically found in grasses (Rashmi et al., 2019; Aamir et al., 2020). They mainly adopt a vertical transmission mode and are further segregated into Type I, II and III based on the interaction with the host. The class 2, 3 and 4 endophytes, also known as non-Clavicipitaceous endophytes, are ecologically diverse. The class 2 endophytes colonise both above and below-ground plant tissues. In comparison, endophytes of class 3 and 4 only colonise above-ground and below-ground plant parts, respectively. Overall, class 2 endophytes have been extensively studied, although the diversity and mechanisms of the interactions still need to be better understood (Bacon and White, 2000; Spence and Bais, 2015; Aamir et al., 2020)

Research has reported that the hyphae of endophytic fungi typically grow at the same rate as the host plant's tissues (Stone et al., 2000; Yan et al., 2015). Post-colonisation, different effects such as enhanced survival, uptake of nutrients, and improved physiological, biochemical and enzymatic parameters have been observed in the host plants. Past studies prove that plants depend on endophytes for their development (White et al., 2014; Strobel, 2018; White et al., 2019; White et al., 2021). Hence, the notion of 'Mycovitilism' given by Vujanovic and Vujanovic (2007) by observing the beneficial impact of *Fusarium semitectum* on orchid seed germination, is gaining wider acceptance. Recently, various scientists have successfully observed the colonisation of endophytes in seeds of different crops (Lastochkina et al., 2020; Singh et al., 2020; Maurya et al., 2021; Akter et al., 2023; Bashir et al., 2023; Chowdhury et al., 2024). This could confer an evolutionary benefit for the seeds, enabling them to encounter a fungal partner(s) during the seed dispersal period. Thus, the plants are bio prospected as they serve as a source of many undiscovered fungal species.

3.8 Plant growth-promoting attributes of fungal endophytes

Being one of the oldest habitants of earth, plants have developed an excellent defense system involving stomatal regulation, metabolic adjustments and synthesis of specific enzymes to protect against the hostile conditions (Sachdev et al., 2021; Mishra et al., 2023). Generation of reactive oxygen species (ROS) is the initial signal seen during abiotic stress. Various studies show the antioxidant potential of fungal endophytes which can aid the plant

under stress conditions (Saddique et al., 2018; Pang et al., 2020; Tsai et al., 2020; Gateta et al., 2023; Li et al., 2023). As discussed in the earlier section, the co-evolution of fungal endophytes with the host plant has enabled them to express various phytochemicals, phytohormones, and other intrinsic properties, as shown in Fig. 3.6.

Phytohormones are the plant hormones responsible for the plant's overall development (Bhatt et al., 2020; Rhaman et al., 2020; Pal et al., 2023). Initially thought to be only produced by plants, recent studies talk about phytohormone-producing endophytic fungi (Hamayun et al., 2017; Cosoveanu et al., 2021; Tian et al., 2022; Ikram et al., 2023). For instance, endophytic fungi were isolated by Khan et al. (2012) from the roots of pepper plants grown under drought stress. The isolate identified as *Chaetomium globosum* LK4 produced gibberellic (GA) and indole acetic acid (IAA). On inoculation in Waito-C rice, an increase in shoot length, biomass and chlorophyll content was observed. Chlorophyll is the chief energy source for plants utilizing which they carry out the process of photosynthesis. This increase in the chlorophyll concentrations in inoculated plants indicates the capability of endophytic fungi in growth promotion. Overall, *C. globosum* LK4 allied the growth of rice seedlings which could be attributed to the phytohormone production ability.

Similarly, Waqas et al. (2014) investigated the fungal endophytes of *Glycine max* L. (Soybean) and *Cucumis sativus* (Cucumber). Six GA-producing endophytic species of *Chrysosporium*, *Aspergillus*, *Paecilomyces*, *Penicillium*, *Phoma*, and *Paecilomyces*, were evaluated for their growth promotion ability on Waito-C and Dongjin-bye, a widely cultivated rice variety of Korea having an active GA synthesis pathway. *Paecilomyces formosus* produced the highest content of different GAs ranging from 1.1 ± 0.2 to 10.6 ± 2.6 ng/mL, as well as IAA content of 34.1 ± 3.9 µg/mL. Further, *P. formosus* inoculation enhanced the plant length, biomass and pigment content of Waito-C and Dongjin-byeo varieties.

Endophytic fungi's plant growth promotion ability on another major rice variety known for their long grains and aromatic characteristics has also been studied. Kuswinanti et al. (2015) isolated endophytic fungi associated with Pulu Mandot, a local aromatic rice cultivated in the Salukanang district of Indonesia. The isolates exhibited IAA production of 0.6 to 2.7 mg/L. Though IAA plays no apparent role in endophytic fungi, it serves many different functions in plants, from a signalling molecule to the development of plants (Ikram et al., 2018; Tian et al., 2022). In the same year, Khalmuratova et al. (2015) documented endophyte isolated from five halophytic plants, *Suaeda maritima* (Herbaceous seepweed), *Limonium tetragonum* (Sea-lavender), *Suaeda australis* (Austral Sea blite), *Phragmites australis* (The common reed), and *Suaeda glauca* (Jian peng). The application of liquid

culture of endophytic *Talaromyces pinophilus* (Su-3–4–3), exhibited the highest plant length as compared to the wild-type *Gibberella fujikuroi*. On chromatographic examination of the culture filtrate, physiologically active GAs (0.044 - 1.808 ng/mL) were detected.

GA plays a crucial part in growth and development, especially in rice, where it was first discovered. It promotes cell elongation by inducing the destruction of DELLA proteins (aspartic acid, glutamic acid, leucine, leucine, and alanine) (Ravindran and Kumar, 2019; Sabagh et al., 2021; Castro-Camba et al., 2022). Like GA, IAA is one of the significant phytohormones responsible for shoot and root development. Moreover, the exogenous application of phytohormones has exhibited a positive effect on plant growth (Duca et al., 2014; Rhaman et al., 2020; Sabagh et al., 2021; Swain et al., 2023). Hence, using fungal endophytes as a source of exogenous phytohormones could be a sustainable approach.

To explore the diversity of the fungal endophytes in different plants of extreme habitats, Khan et al. (2012) isolated endophytes from thirteen different plants, *Vitex rotundifolia* (Round-Leaved Chaste Tree), *Calystegia soldanella* (Beach morning glory), *Lathyrus littoralis* (Silky beach pea), *Polygonum convolvulus* (Wild buckwheat), *Oxalis corniculate* (Yellow wood sorrel), *Lathyrus japonica* (Beach pea), *Ixeris repenes* (Hama nigana), *Glehnia littoralis* (American silvertop), and *Salsola komarovi* (Okahijiki), growing in dunes around Pohang beach, Korea. The culture filtrates of the isolates were tested for growth promotion on Waito-C rice. Here, 82.7% fungal isolates enhanced plant height and shoot length. Considering the promising attributes exhibited by the majority of the isolates, it can be speculated that some of these isolates may exhibit growth promotion under stress conditions. Various other studies have reported similar findings (Al-Hosni et al., 2018; Bhatt et al., 2020; Yustisia et al., 2020; Castro-Camba et al., 2022; Ikram et al., 2023).

Besides phytohormones, endophytic fungi exhibit various other PGP properties. For instance, production of 1-aminocyclopropane-1-carboxylate deaminase (ACC deaminase). The endophytic fungi stimulate growth during stress conditions by sequestering the ACC produced by plants. Consequently, ethylene production by the plant is reduced. Depending on the concentration, ethylene, a multidimensional stress hormone, promotes or inhibits plant growth. Over the years, various studies have reported ACC deaminase production by fungal endophytes (Zhang et al., 2019; Rehman et al., 2022; Wang et al., 2022; Ikram et al., 2023). Iron is another vital mineral that plays a vital function in plants. In addition to photosynthesis and chlorophyll synthesis, iron is the central component of many enzymes and co-factors. Siderophores are small organic molecules, synthesised during iron-deficient conditions (Bilal et al., 2018; Ikram et al., 2023). During stressful conditions, siderophores

play a vital role by binding to toxic traces and heavy metals. This complex formation restrains the movement of heavy metals through the roots, reducing the accumulation of metals in plants. On the contrary, they provide essential nutrients to the associated microorganisms (Albelda-Berenguer et al., 2019). The documentation of siderophore production by endophytic fungi is consistent throughout the literature (Chowdappa et al., 2020; Turbat et al., 2020; Poveda et al., 2021; Ikram et al., 2023).

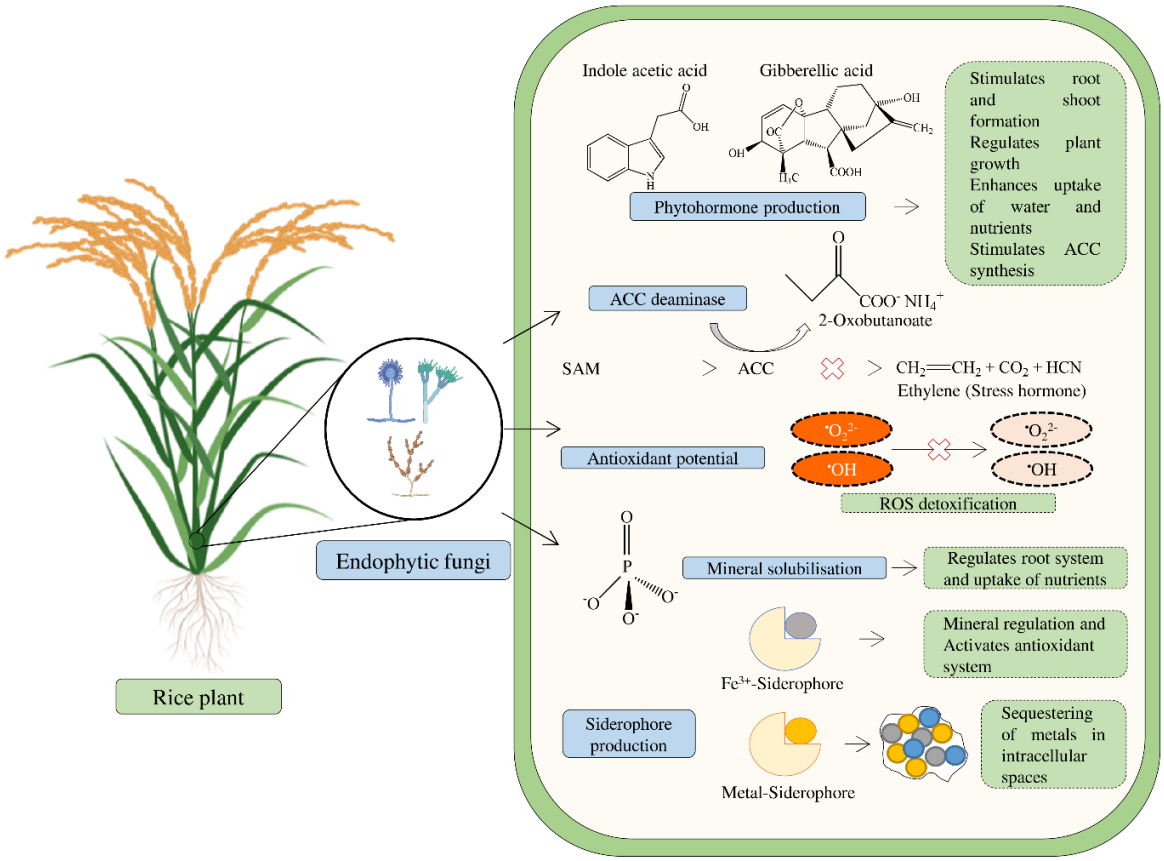

Fig. 3.6: Various plant-growth promoting attributes of fungal endophytes.

Another unique property of endophytic fungi is mineral solubilization, which enables plants to efficiently uptake minerals (Kaul et al., 2019; Chand et al., 2020; Tandon et al., 2020; Ratul et al., 2023). In a report, Potshangbam et al. (2017) isolated fungal endophytes from Moirangphou, an indigenous rice variety in Manipur, India. On biochemical analysis, isolate ENF-49 exhibited siderophore production and the highest phosphate solubilisation index of 1.62 ± 0.012. Phosphate plays a vital role in plants in terms of energy transfer and regulation of protein synthesis (Chand et al., 2020; Khan et al., 2023). During stressful conditions, it aids the plant by enhancing its root system for better uptake of water and nutrients, but the insoluble phosphate hinders the plants to utilize it effectively (Rawat et al., 2021; Khan et al., 2023). Hence, the phosphate-solubilizing endophytic fungi help especially during stress conditions.

In a similar study, endophytic *Aspergillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 from roots of *Oxalis corniculate* (Creeping woodsorrel) were isolated, and their PGP potential was evaluated on Waito-C variety of rice (Bilal et al., 2018). Isolate BRL1 exhibited phosphate solubilisation potential; additionally, both the isolates exhibited siderophore and IAA production potential. On inoculation, the isolates increased plant length, FW, DW, and chlorophyll content. IAA affects plant growth by regulating embryonic development, apical dominance, and the transition to blooming (Al-Hosni et al., 2018; Hu et al., 2018; Li et al., 2023). Thus, this increase in growth parameters of rice on inoculation of endophytes could be attributed to the production of phytostimulatory hormones, which aid the plant under non-stress conditions.

It is hypothesized that the extracellular enzymes produced by the fungal endophytes protects the host plant against abiotic stress as enzymes help in creating a symbiotic association with plants (Yadav, 2018; Verma et al., 2022). Positive results for the production of lytic enzymes such as amylase, cellulase by the endophytic fungi have been observed (Sunitha et al., 2013; Atugala and Deshappriya, 2015; Khan et al., 2016; Sornakili et al., 2020; Ratul et al., 2023). Similar studies of rice fungal endophytes exhibiting PGP attributes are shown in Table 3.2. Nitrogen is another vital chemical that is essential for plants. It is the critical chlorophyll component responsible for photosynthesis (Ali, 2020; Mengesha, 2021). In a study, Sun et al. (2019) evaluated the symbiotic effect of endophytic *Phomopsis liquidambari* on Wuyunjing 23, a Japonica rice variety widely cultivated in China. On inoculation, enhanced accumulation of NH_4^+-N and NO_3^-N at the tillering and the maturing stage was also observed. Despite low nitrogen levels, the endophyte-inoculated plants exhibited a remarkable increase in shoot length, biomass and grain weight which could be attributed to the biological nitrogen-fixing capability of the endophytic fungi (Adeleke and Babalola, 2021b).

The group further evaluated the symbiotic association of endophytic, *Diaporthe liquidambaris*, on the Wuyunjing 23 rice variety. The endophyte was pre-labelled with a green fluorescent protein (GFP) through the vector plasmid pCT74. On inoculation, an increase in chlorophyll concentration was observed, whereas the rubisco content increased by 57.6%. RuBisCo (Ribulose-1,5-bisphosphate carboxylaseoxygenase) is responsible for carbon fixation; it helps plants to convert atmospheric carbon into energy-rich molecules. Endophyte inoculation also enhanced the water-soluble carbohydrates (WSC) by ~35–45% (Sun et al., 2019). The WSC accumulates around and about the stems in the plants as

energy reservoirs for further aiding the growth and development of grains which could prove beneficial under stress environment (Afzal et al., 2021; Saddhe et al., 2021).

Studies have also documented the growth-promoting traits of dark septate endophytes (DSEs) in rice varieties. Vergara et al. (2019) evaluated the effect of DSEs on Piauí rice, a low nitrogen-utilizing variety native to Maranhao-Brazil. The endophytes A101 and A103 successfully colonized the roots of the rice plant and enhanced the biomass, tiller, and leaf number. The isolates also enhanced the uptake of nitrogen (N), phosphorous (P), potassium (K) and other minerals. Components like K and manganese (Mn) ensure optimum plant growth. Likewise, P forms the vital component of the energy unit (ATP), whereas Mg forms the core of the chlorophyll molecule. Zn plays a vital function in driving metabolic reactions and calcium plays the structural role of forming cell walls and membranes and as an intracellular messenger (Shrestha et al., 2020; Kaur et al., 2023). An increase in the activity of proton pump H^+-ATPase, responsible for transporting H^+ ions for electron transport chain and plant respiration, was also observed. The upregulation was confirmed by the gene expression of inoculated and uninoculated plants.

Though many studies have reported the effect of fungal endophytes on the rice plant's physiochemical attributes, our knowledge of the molecular mechanism behind these positive effects is limited (Sharma et al., 2024). For better understanding, transcriptome analysis comprising the differential expression of *Trichoderma asperellum* SL2 inoculated and uninoculated rice seedlings were compared using next-generation sequence technology by Doni et al. (2019). The gene ontology (GO) analysis exhibited that 150 genes involved in photosynthesis were upregulated in *T. asperellum* SL2 inoculated seedlings. The GO analysis highlights the difference in biological processes, cellular locations, and molecular functions of inoculated and uninoculated samples. Moreover, several genes associated with chlorophyll biosynthesis, stomatal, and root development were upregulated in inoculated seedlings (Nivedita et al., 2020; Jiang et al., 2021). Talking about molecular functioning and metabolism, evident results were observed with the upregulation of 84 catalytic activity and 39 ion-binding genes. Since rice is a C3 plant, it relies on the catalytic activity of Rubisco, and the upregulation in inoculated seedlings indicates the potential of endophytic fungi to regulate molecular signalling and enhance plant growth (Zhongming et al., 2020).

Further, the study observed upregulation of cellular components, such as 238 genes of thylakoid membranes, 192 genes of chloroplast, and 76 genes of cytosol, was observed. Thylakoid is the site of oxygenic photosynthesis and photochemical reactions in plants. The chloroplast and cytosol are responsible for capturing light energy and protein production,

sorting, and transport, respectively (New et al., 2018). A noteworthy response to *T. asperellum* SL2 inoculation was the upregulation of defense response genes associated with systemic acquired resistance (SAR). SAR is an analogue of the innate immune response and is triggered in the whole plant. The immune system utilizes the pathogen recognition receptors (PRRs) to locate the pathogen via their pathogen-associated molecular patterns (PAMPs) or pathogen effectors. Upon recognition, the immune system induces an effector-triggered immunity (ETI) or effector-triggered susceptibility (ETS) or PAMP-triggered immunity (PTI). This locally triggered response is signalled throughout the whole plant and is thus known as SAR (Klessig et al., 2018). Similarly, other studies demonstrating the PGP potential of endophytic fungi on rice are shown in Table 3.3.

Table 3.3: Plant growth-promoting attributes of fungal endophytes and their effects.

Endophyte	Host plant	Rice variety	Response	References
Fusarium oxysporum, Emericella nidulans	*Ipomoea batatas* (Sweet potato)	IR-64	Enhanced plant height.	Hipol et al., 2012
Paraconiothyrium sp.	*Capsicum annuum* (Bell pepper)	*Waito*-C and Dongjin-beyo	Enhanced shoot length, shoot FW and seedling biomass.	Khan et al., 2012
Aspergillus caespitosus LK12 and *Phoma sp.* LK13	*Moringa peregrina* (Moringa)	*Waito*-C and Dongjin-beyo	The isolates exhibited GA production in various quantities and significantly increased the shoot length.	Khan et al., 2014
Absidia sp. and *Cylindrocladium* sp.	*Oryza sativa* L. Suwandel and Kaluheenati (Rice)	Suwandel and Kaluheenati	Dual inoculation significantly increased plant height, FW and DW.	Atugala et al., 2015
Phomopsis liquidambari	*Bischofia polycarpa* (Chinese bishopwood)	Wuyunjing 7	Increase in the available nitrate and ammonium contents, increased the potential nitrification rates, affected the abundance and community structure under low N conditions.	Yang et al., 2015
Aspergillus flavus Y2H001	*Perilla frutescens* (Beefsteak)	*Waito*-C	The isolate exhibited GA production and enhanced root and shoot length.	You et al., 2015
Unidentified	Mangrove tree	*Oryza sativa* L. Cempo Ireng	Promoted germination of seeds, stem height and root height.	Tumangger et al., 2018
Preussia sp. BSL-10	*Boswellia sacra* (Frankincense tree)	*Waito*-C rice and Jin so mi rice	The isolate produced nitric oxide, GA, and IAA and enhanced plant growth.	Al-Hosni et al., 2018
Phomopsis liquidambari	-	Wuyunjing 23	Promoted aerenchyma formation in root, root porosity and radial oxygen loss, enhancement of oxidation-reduction potential, IAA and ethylene levels.	Hu et al., 2018
Phomopsis liquidambari	*Bischofia polycarpa* (Chinese bishopwood)	Wuyunjing 23	*P. liquidambari* decreased N and P loss by 24.59% and 17.46% per pot, respectively and activated soil functional genes to accelerate nutrient turnover in the rice rhizosphere. It also influenced the patterns of microbiota led to an optimized microbial with a higher level of available nutrient supplies.	Tang et al., 2019
Piriformospora indica	-	Wild type rice and over-expressing the vacuolar H^+-PPase (AVT rice)	Enhanced plant height, fresh and dry matter of shoot, photosynthetic rate, stomatal conductance, intrinsic water use efficiency, carboxylation efficiency and nutrient uptake in both shoots and roots.	Bertolazi et al., 2019
Unidentified	Sinjai local red rice	Sinjai local red rice	The isolate produced 0.015 mg/mL IAA after 48 hours of incubation.	Yustisia et al., 2020
Phomopsis liquidambaris	*Bischofia polycarpa* (Chinese bishopwood)	Wuyunjing 21	CRISPR/Cas9 gene disruption *ku70* or *ku80* gene, *PmkkA* gene as a result higher chitinase and glucanase activity.	Huang et al., 2020

3.9 Abiotic stress alleviation in rice using fungal endophytes

The damage plants bear because of non-living factors, such as extreme temperatures, drought, and salinity, is termed abiotic stress (Zhang et al., 2022). The vast economic losses caused by these abiotic stressors make them a key focus of agricultural research (Oshunsanya et al., 2019; Pathak et al., 2021). Although depending on the location or area, the effect may be either beneficial or detrimental, but pertaining to farming, the effects are predominantly detrimental (Talukder et al., 2021; Chaudhry and Sidhu, 2022).

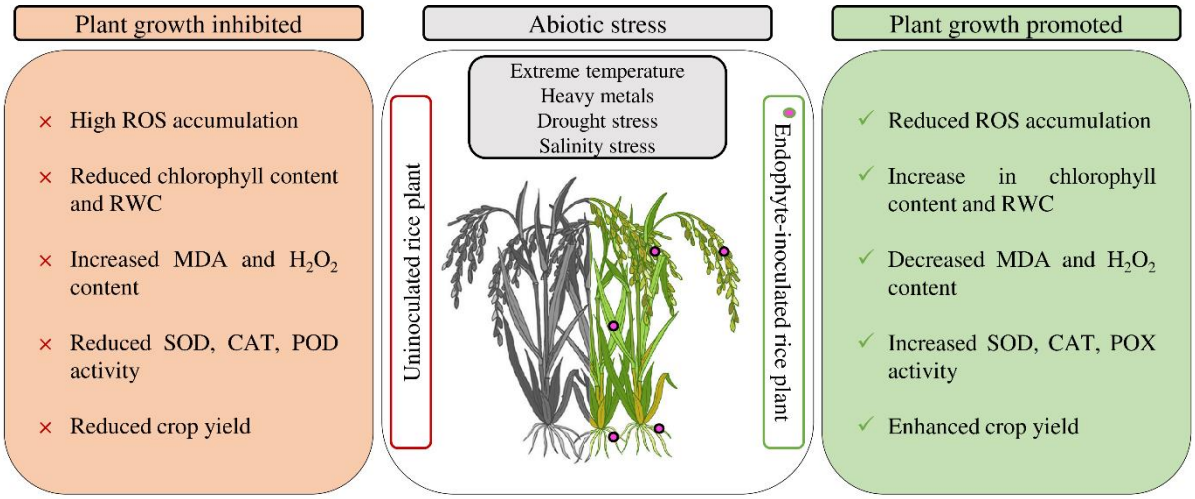

Fig. 3.7: Effect of fungal endophyte inoculation in plants under abiotic stress (Where ROS reactive oxygen species; RWC relative water content; MDA malonaldehyde; H_2O_2 hydrogen peroxide; SOD superoxide dismutase; CAT catalase; POX peroxidase).

3.9.1 Effect of fungal endophytes in rice plant under drought stress

Water deficit is a principal abiotic stress that affects crop yield. Since rice is a kharif crop, a high amount of water is required for its cultivation, and water scarcity is a major consequence of the ongoing climate change (Singh et al., 2021; Muthuvel and Amai, 2022; Singh et al., 2022). To ensure the viability of endophytes in such irrigation systems, the notion of mycovitilism or seed soaking should be used as the preferable application technique. Drought stress reduces the water potential, RWC, and turgor pressure of the plants. This is followed by a reduction in photosynthetic pigments, ROS generation, and plant death in severe cases (Fig. 3.7). Some plant species have developed methods to withstand such conditions; nonetheless, rice plants do not have the ability to withstand drought stress. Various scientists have reported drought-tolerant endophytic fungi from different plants (Shukla et al., 2012; Idhan et al., 2018; Tandon et al., 2020; Xu et al., 2022; Singh et al., 2023).

To combat the issue, a study pursued the effect of *P. indica* on miR159 and miR396 of rice plants under drought (Mohsenifard et al., 2017). Physical attributes like shoot FW,

DW, and relative water content (RWC) were higher as compared to the uninoculated plants. Similarly, Saddique et al. (2018) observed enhanced FW, DW of the roots and shoots in *P. indica* inoculated rice genotypes, WC-297 (drought tolerant), Caawa (moderately drought tolerant), and IR-64 (drought susceptible) under 15% PEG-6000 drought stress. In another study, Pang et al. (2020) observed enhanced physiological attributes in rice plants inoculated with endophytic *Talaromyces cellulolyticus* FN4 under 10% PEG-6000 stress. The PGP effect of the endophyte isolated from upland rice insinuates the possibility of a different diversity of microbes because of habitat adopted symbiosis. Tsai et al. (2020) evaluated the outcome of *P. indica* on grain yield and growth of Tainung 67 (a japonica variety of rice). An increase panicles per plant, filled grains per plant, grain weight per plant and seedling survival rate was observed. Li et al. (2021) evaluated the effect of endophytic strain EF0801, identified as *Sordariomycetes* sp., on Liaoxing1 variety of rice exposed to 5-20% PEG-6000 induced drought stress. Enhanced plant height, shoot DW, and chlorophyll content was seen in inoculated plants.

Saddique et al. (2018) also observed an increase in chlorophyll and proline content which could be attributed to the enhanced mineral acquisition because of *P. indica* inoculation. The results are concurrent as observed by Pang et al. (2020). *T. cellulolyticus* FN4 inoculation enhanced osmolyte content under drought. Tsai et al. (2020) studied the photosynthetic machinery of endophyte associated rice plants under drought. The Fv/Fm values of uninoculated plants decreased to 0.60 after exposure to stress conditions compared to the inoculated seedlings with 0.76 Fv/Fm values. Improved photosynthetic efficiency in the inoculated plants was observed, which could be attributed to reduced damage to the photosystem prompted by *P. indica*. In the case of stomatal closing, 41.1% and 50.2% of stomata were completely closed in uninoculated and inoculated plants, respectively. The surface temperature of inoculated plants was 0.5 °C higher than the uninoculated plants. Various studies have reported similar findings where inoculation of endophytic fungi has resulted in enhanced physiological and biochemical attributes under drought stress (Shukla et al., 2012; Santos et al., 2017; Idhan et al., 2018; Tandon et al., 2020; Xu et al., 2022).

Additionally, the studies also documented enhanced antioxidant enzyme system. Pang et al. (2020) saw a significant increase in catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) activity as well as critical biomarkers, closely involved in protecting the plant during drought stress. Likewise, Behera et al. (2018) observed an increase in ascorbate peroxidase (APX), SOD, POX, cell membrane stability index (%),

proline, and phenol content of rice plant under drought stress on inoculation of endophytic *Trichoderma* sp. Further, Qin et al. (2019) evaluated the effect of endophytic *Aspergillus fumigatus* SG-17 on the Nipponbare variety of rice under drought stress. Inoculation of SG-17 increased the proline content, relative membrane permeability, and RWC and decreased the malondialdehyde content (MDA) under stress conditions. Tsai et al. (2020) also observed lower levels of MDA in inoculated plants, along with an increase in glutathione/glutathione disulfide (GSSG) ratio, CAT, and glutathione reductase levels.

Mohsenifard et al. (2017) analysed the expression pattern of *P. indica* inoculated rice plants under drought. The two miRNAs were more than five times up-regulated on inoculation of *P. indica* under stress conditions. miR159 is a GAMYB gene encoding microRNA present in the majority of land plants. The GAMYB genes are directly responsible for transducing the gibberellin signal. In comparison, miR396 regulates plant agronomically essential traits (Millar et al., 2019). Similarly, Qin et al. (2019) observed an enhanced expression of heat shock protein (HSP) 70 in rice plants under drought stress inoculated with a compound from endophytic *Aspergillus fumigatus* SG-17. The 70 kD HSP is involved in the metabolism of ROS. The positive effect of compound (Z)-N-(4-hydroxystyryl) formamide (NFA) on the rice plant suggests the efficacy of NFA in combating the impact of drought by regulating the oxidative pathway of the plant.

Li et al. (2021) noted an increase in the content of various mineral nutrients such as K, Ca, Mg, P, and Mn increased in leaves and roots on inoculation of *Sordariomycetes* sp. EF0801. Furthermore, lactate, fumarate, acetate, and succinate content also increased in leaves and roots on inoculation of *Sordariomycetes* sp. EF0801 under stress conditions. Lactate is responsible for the maintenance of cellular homeostasis, whereas fumarate provides an alternative carbon source. Likewise, acetate helps maintain metabolic flexibility, and succinate aids oxidative metabolism of plants (Araújo et al., 2011; Fu et al., 2020; Jain et al., 2020). Table 3.4 shows similar studies which have reported PGP traits of fungal endophytes under drought stress. In nature, the prolonged periods of droughts, low precipitation, and impending water uptake by the plant causes the accumulation of salts in the topsoil, thereby increasing the salinity levels (Uddin et al., 2016). Hence, determining the impact of endophytic fungi on plants under salinity stress is also imperative.

Table 3.4: Effect of fungal endophytes on different rice varieties under drought stress.

Endophyte	Host plant	Rice variety	Response	Stress	References
Trichoderma harzianum	-	Kalanamak 313	Reduced wilting, delayed changes in stomatal conductance, net photosynthesis and leaf greenness. Increase in stress induced metabolites and phenolics content, decrease in proline, MDA and H_2O_2 contents.	Drought stress induced by termination of watering	Shukla et al., 2012
Dark septate endophyte	*Oryza glumaepatula* (Wild rice)	Nipponbare and Piauí	Promoted rice plant growth, decreased oxidative stress.	0-264.246 g/L PEG-6000 induced drought stress	Santos et al., 2017
Unidentified	-	IMPARI 20	Enhanced chlorophyll-a, chlorophyll-b concentration, total chlorophyll content.	Drought stress	Idhan et al., 2018
Unidentified	Local aromatic rice variety of Indonesia	-	Plant height, tiller number, root length, root weight, number of filled grains and production per hectare were higher.	Drought stress	Syamsia et al., 2020
Trichoderma koningiopsis (NBRI-PR5)	Rice plant	-	Production of organic acids for solubilizing insoluble tri-calcium phosphate at high pH stress was observed whereas, in drought conditions accumulation of poly-phosphate in mycelia and alkaline phosphatase enzyme was reported.	pH 7-10, 2.5-30% (w/v) PEG and glycerol induced drought stress	Tandon et al., 2020
Aspergillus fumigatus and *Chaetomium globosum*	*Myricaria laxiflora* (Tamarisk)	Nipponbare	Two natural antioxidants, Z-N-4 hydroxystyryl formamide and chaetoglobosin A, can reverse the decline trend of oxidative parameters caused by long-term flooding, such as MDA, SOD, ethanol dehydrogenase, and NADPH oxidase.	Flooding stress induced by fully submerging the seedlings for eight days	Xue et al., 2021
Phomopsis liquidambaris	*Bischofia polycarpa* (Chinese bishopwood)	Wuyunjing 23	Enhanced chlorophyll and soluble sugar contents, ATP, and activities of critical aerobic and anaerobic respiration enzymes.	Hypoxia caused by waterlogging	Hu et al., 2021
Piriformospora indica	-	Nipponbare, Zhonghua11, Zhonghan3, OsPIN2 transgenic lines-Hei-Jing2 and OsPIN2 mutant	Enhanced rhizosheath formation.	60-80% field capacity	Xu et al., 2022

3.10 Effect of fungal endophytes in rice plant under salinity stress

Accumulation of excessive salt content is known as salt stress and is a detrimental condition for plants. Soils with electrical conductivity of 2 dS/m or above, i.e., ~20 mM sodium chloride (NaCl) concentrations, are termed saline (Paz et al., 2020; FAO, 2021). As per the latest data, by the year 2050, salt stress will affect 50% of the global arable land. Since rice is a salt-sensitive crop, it is gravely affected (Acosta-Motos et al., 2017; Zheng et al., 2023). A plausible cause of enhanced salt tolerance of plants inoculated with endophytic could be attributed to the improved nutrient uptake owing to the mineral solubilization capability of endophytic fungi, protection of photosynthetic machinery, regulation of water use efficiency, upregulation of the antioxidant enzyme system and osmolytes (Pang et al., 2020; Ghorbani et al., 2021; Zin et al., 2021; Ghosh et al., 2022). However, the exact mode of action of endophytic fungi is yet to be determined. As discussed in the earlier sections, horizontal gene transfer between the fungal endophytes and plants allows the endophytic fungi to express various host plant characteristics. Thus, depending upon the habitat of the plant from which the endophytic fungi have been isolated, their stress tolerance capabilities can vary. Studies have shown that fungal endophytes isolated from plants growing in extreme environments such as saline soil exhibit resistance to high salinity (Khan et al., 2012; Waqas et al., 2015; Sangamesh et al., 2018; Pang et al., 2020).

To study fungal endophytes under salinity, Khan et al. (2012) isolated *Paecilomyces formosus* LHL10. On inoculation in Waito-C and Dongjin-byeo, *P. formosus* LHL10 enhanced the shoot length, FW, and pigment content of rice seedlings. Similarly, Qin et al. (2017) isolated endophytes from salt-tolerant plants. The isolates could tolerate 2–4% of NaCl, 2% KCl stress, varying pH ranges (6– 11) and high temperatures of up to 32 °C. On inoculation in rice plant, the tested isolates enhanced the biomass production, plant height and significantly promoted seedling growth. Likewise, Siddiqui et al. (2021) evaluated endophytic fungi from the roots of a halophyte grass for salt tolerance. Under 50-150 mM NaCl stress endophyte *Aspergillus terreus* enhanced the plant height, FW, DW, and RWC of inoculated rice. Sampangi-Ramaiah et al. (2020) studied *Fusarium* sp. inoculation under salt stress in IR-64 rice variety. The endophyte exhibited 77% growth at 1.5 M NaCl concentration compared to the control. On inoculation under salt stress, the tiller number/plant and total biomass increased.

The improved root architecture of the inoculated plants enables them to effectively utilize nutrients from the soil, thereby increasing the plant's stress tolerance. Siddiqui et al. (2021) also observed enhanced chlorophyll, APX, CAT, SOD content and Na^+/K^+ ratio in *A.*

terreus inoculated rice plants. On the contrary, a decrease in H_2O_2 and MDA content was seen. Similarly, Sampangi-Ramaiah et al. (2020) documented an increase in the total chlorophyll content, CO_2 assimilation rate, stomatal conductance, and transpiration rate in IR-64 rice seedlings on inoculation *Fusarium* sp. under salt stress. The increased chlorophyll synthesis in inoculated plants suggests the activation of biosynthetic enzymes and reduced damage to the PSII system. The lower H_2O_2 and MDA content of the inoculated plants indicates increased activity of the antioxidant system. Various studies have documented similar findings where inoculation of endophytic fungi has enhanced physiochemical and enzymatic attributes of rice plant under salinity stress (Jogawat et al., 2013; Soares et al., 2016; Hamayun et al., 2017; Kord et al., 2019; Reshna et al., 2022).

To study the in-depth mechanism of stress tolerance by the endophyte Jogawat et al. (2016) isolated the *piHOG1* gene was isolated from the genome of the endophytic fungus *Piriformospora indica*. A yeast homolog of MAPK p38, HOG1, is involved in stress responses. The effect of *PiHOG1* on rice plants was evaluated by creating a knockdown transformant KD-*PiHOG1* using RNA interference. On inoculating the rice plants with both variants, a substantial decrease in physiochemical parameters was seen in the case of KD-*PiHOG1*. Moreover, genes *PiHOG1*, *PiHSP78*, *PiENA1* and *PiGRE2* were upregulated in plants inoculated with *PiHOG1*. Furthermore, the downregulation of *STL1*, *GPD*, and *PFK26* genes related to glycerol accumulation in the KD-*PiHOG1* transformant was observed. Since glycerol maintains osmotic homeostasis, accumulation of glycerol is necessary for the endophyte to colonize in the host plant. In osmotic stress, the influx of Na^+/K^+ occurs, which is regulated by the *ENA1*, an ATPase pump. Downregulation of *PiENA1* was observed in KD-*PiHOG1*, implying a strong influx of Na^+ causing toxicity in the host plant. Finally, upregulation of salinity tolerant genes *PiSLC9M* and *PiCP450* in *PiHOG1* inoculated plant was observed. The study sheds light on the response of *PiHOG1* in mitigating salinity stress in rice and presents *P. indica* as a novel bio-stimulant.

In response to salt stress, alternative splicing of transcripts is a common phenomenon observed in plants. It allows the plants to produce multiple isoforms of proteins from a single gene and adds a level of regulation to the expression of genes. Sampangi-Ramaiah et al. (2019) investigated the impact of a salt-tolerant endophytic *Fusarium* sp. on alternative splicing of IR-64 variety of rice under 150 mM salt stress. As a result, 44,403 splicing events were observed in endophyte-inoculated plants. Furthermore, 51,944 novel alternative splicing events were identified, which were reduced to 46, 707 in the inoculated plants. Of the total alternative splicing events, 3634 were unique to

inoculated plants. Moreover, the majority of the genes in alternative splicing events cater to the cellular and biological processes of the plant, whereas a few are related to molecular functioning. The results indicate the importance of splicing events for plants to adapt to stress conditions and how inoculation of endophyte relieves the plant of some of the stress. It can be hypothesized that the endophytic fungi prohibit the stress-induced alternate splicing events under salt stress.

In another study, Sampangi-Ramaiah et al. (2020) observed upregulation of 1348 genes were, and downregulation of 1078 genes in rice plants inoculated with *Fusarium* sp. under salt stress. The upregulated genes were related to oxidoreductases, dehydrogenases, and transcription factors involved in salt tolerance. Along with the genes, upregulation of differential gene expression (DGEs) was also observed; 122 DGEs were upregulated in inoculated plants. The upregulated DGEs are involved in signaling, reception kinases, and external signal perception. However, their specificity needs to be elucidated for developing real-world applications. Similarly, over years various studies have highlighted the stress mitigation by fungal endophytes (Jogawat et al., 2013; Soares et al., 2016; Hamayun et al., 2017; Kord et al., 2019; Reshna et al., 2022) (Table 3.5). An overview of the studies has suggested the immense possibility of endophytes in PGP and mitigation of various abiotic stresses. It can be said that fungal endophytes help in improving the physiological, biochemical and enzymatic levels of inoculated rice which enables the host plants to combat abiotic stresses.

Table 3.5: Effect of fungal endophytes on different rice varieties under salinity stress.

Endophyte	Host plant	Rice variety	Response	Stress	References
Sebacina vermifera	-	Tarom Hashemi	Enhanced growth, yield and nutrition of rice.	NaCl concentration of 3, 6 and 9 dS/m^2	Pirdashti et al., 2012
Piriformospora indica	-	Pusa basmati-1, IARI	Enhanced root and shoot lengths, FW and DW, chlorophyll a, b, and carotenoids.	200-300 mM NaCl	Jogawat et al., 2013
Penicillium janthinellum LK5	*Solanum lycopersicum* L. (Tomato)	*Waito*-C and Dongjin-beyo rice seedlings	Enhanced GA content, CAT, POD, GSH activity. Decreased MDA and JA content.	140 mM NaCl	Khan at al., 2013
Multiple	*Phragmites australis* (The common reed)	*Oryza sativa* L.	Exhibited propensity for growth promotion, enhanced root and shoot length.	25-175 mg NaCl/kg of soil	Soares et al., 2016
Trichoderma spp.	-	PD-11	A decrease in MDA and H_2O_2 contents, increase in proline and phenolics content. Gene expression profiling for *SOD*, *APX*, phenyl ammonia lyase (*PAL*), and pyrroline-5-carboxylate synthase (*P5CS*) revealed induced changes in gene expression pattern were significantly in treated seedlings.	0-240 mM NaCl	Rawat et al., 2016
Porostereum spadiceum AGH786	*Glycine max* (Soybean)	*Waito*-C rice	Enhanced GA, isoflavones content, and reduced ABA and JA.	70-140 mM NaCl	Hamayun et al., 2017
Trichoderma harzianum	-	Kernel	Increase in RWC and stomatal conductance, better photosynthetic performance, higher pigment concentrations, CAT, SOD activities. Decrease in H_2O_2 content.	25-200 mM NaCl	Yasmeen et al., 2017
Piriformospora indica	-	Hashemi	Affected the differential abundance of miRNAs regulated genes and transcription factors linked to import of K ions into the root cells, the export of Na ions.	0-100 mM NaCl	Kord et al., 2019
Phomopsis liquidambaris	*Bischofia polycarpa* (Chinese bishopwood)	Wuyunjing 7	Enhanced root-shoot length, FW and DW, and overall growth rice seedlings. Increase in ACC accumulation, ACC synthase and ACC oxidase activities also observed.	100-200 mM/L NaCl	Siddikee et al., 2021
P. indica	-	VTL-6 and Manu Ratna	Enhanced shoot and root lengths, FW and DW, seedling vigor, root volume and root shoot ratio. Low Na^+/K^+ ratio.	100-500 mM NaCl level	Reshna et al., 2022
Serendipita indica	-	Tarom, Amol2, IR66946 and Neda	Enhanced shoot/root FW and changes in several PSII parameters.	60 mM NaCl level	Rezaei et al., 2022

Chapter 4

Materials and Methods

4.1 Collection of plant samples

Healthy, asymptomatic parts (leaves, roots, internode and spikes) of *Oryza sativa* var. PUSA-44, a rain-fed rice variety, were collected from Fatehgarh Sahib, Patiala, Punjab-India (30.6174° N, 76.3888° E) during the crop season (June-October, 2020). The plant parts were collected at intervals of 15 days from the stage of plantation till harvesting of the crop. In total, 8 samples were obtained in 120 days.

Additionally, the seeds of *Oryza sativa* var. Sahbhagi Dhan, a drought resistant rice variety, were procured from Indian Council of Agricultural Research-National Rice Research Institute (ICAR-NRRI), Cuttack, Odisha. The seeds were sown in a selected area in Patiala, Punjab-India (30.3574° N, 76.3666° E). Healthy, asymptomatic plant parts (leaves, roots, internode and spikes) of *Oryza sativa* var. Sahbhagi Dhan were collected during June-October, 2021 crop season. In total, 7 samples were obtained in 105 days after every 15 days from the stage of plantation till harvesting of the crop. All the plant samples were carefully placed in aseptic zip-lock bags, and kept at 4 °C till further processing.

Table 4.1: Geographical co-ordinates of the plant sample collection.

Location	*Oryza sativa* variety	Sampling site	State	Geographical co-ordinates
1	PUSA-44	Fatehgarh Sahib	Punjab	30.6174° N, 76.3888° E
2	Sahbhagi Dhan	Thapar Institute of Engineering and Technology	Punjab	30.3574° N, 76.3666° E

4.2 Analysis of physiochemical characteristics of soil from sampling site

The soil sample from the site of endophyte isolation was collected to access its physiochemical characteristics. The collected plant samples were carefully placed in sterile zip-lock bags, for further processing.

4.2.1 Determination of pH

In 10 mL of deionised water, soil sample (10 g) was introduced. Post stirring the pH was recorded with a pH meter [Cole-Parmer P200] (McLean, 1983).

4.2.2 Estimation of organic carbon and organic matter

The Walkley-Black method was adopted. Briefly, in 10 mL of potassium dichromate ($K_2Cr_2O_7$) (1N), 1 g soil was dispensed. Subsequently, 20 mL H_2SO_4 was added. Post filtration, 10 mL 95% (w/v) H_3PO_4 and 1 mL diphenylamine was added. The sample was titrated against 0.5 N $(NH_4)_2Fe(SO_4)_2 \cdot 6H_2O$ till brilliant green colour is achieved (Heanes, 1984). The process was repeated without the soil sample. The organic carbon content was assessed as follows;

Organic carbon (%) = $\frac{(T1-T2) \times N \times 0.003 \times 100}{W} \times C$

Where, T1 and T2 = titre value of blank and soil sample; N = normality of acid; W = weight of soil sample (g); C = correction factor i.e., 1.33

Furthermore, the organic matter was estimated using the following formula;

Organic matter (%) = Total organic carbon × 1.72

4.2.3 Estimation of total and available phosphorus

Briefly, to 1 g of soil sample, 72% $HClO_4$ was added for digestion. Post digestion, the mixture was filtered using a Whatman filter paper no. 04. Subsequently, to 2 mL of the supernatant, 2-3 drops of p-nitrophenol was added and titrated against 4 N NaOH. Further, the total phosphorous content was analysed as per Murphy and Riley (1962). For estimation of available phosphorous, to 1 g of soil sample, a pinch of activated charcoal and 20 mL of 0.5 N sodium bicarbonate ($NaHCO_3$) were added. It was kept at 120 rpm for 30 mins. Subsequently 5 mL of the filtrate from this mixture was taken in a fresh Erlenmeyer flask and ammonium molybdate ($(NH_4)_6Mo_7O_{24}$) solution was added. Subsequently, addition of dilute stannous chloride ($SnCl_2$) was done and followed by absorbance recording at 660 nm using a microplate reader [Powerwave 34, Biotek, USA]. The same process was repeated without the soil sample. For quantification, a standard curve of different concentrations of phosphorous was prepared (Olsen, 1954).

4.2.4 Estimation of total nitrogen content

To 10 g of moist soil sample, 30 mL concentrated H_2SO_4 was added in a Kjeldhal flask. Post mixing, 10 g of Hibberd's mix [K_2SO_4, $FeSO_4$ and $CuSO_4$ in ratio 10:1:0.5], 1 g salicylic acid ($C_7H_6O_3$) and 5 g sodium thiosulphate ($Na_2S_2O_3$) were introduced. The contents were heated until the appearance of green-yellow colour. Subsequently, post filtration 10 mL of filtrate was added to a distillation flask followed by 40% NaOH. Whereas, 0.1 N H_2SO_4 with few drops of methyl red as indicator was placed in the delivery tube. Post distillation, the distillate was titrated using 0.1 N NaOH (Kirk, 1950).

Nitrogen content (%) = $\frac{14 \times (A-B) \times N}{1000}$

Where, A = volume of acid (sample); B = volume of acid (blank); N = acid normality.

4.3 Isolation of endophytic fungi

For isolation, the protocol by Schulz et al. (1993) was adopted with minor modifications. The collected plant samples were washed to eliminate any dirt and debris and allowed to air dry before further processing.

For surface sterilisation, the plant parts were dipped in 1% (v/v) sodium hypochlorite (NaClO) for 5 mins, 70% (v/v) ethanol for 2 mins and 30% (v/v) ethanol for 30 seconds. The surface sterilised samples were then rinsed with distilled water and aseptically kept on sterile blotting sheet for drying. Post drying, they were aseptically cut into a segment of 2-3 mm and inoculated onto half strength potato dextrose agar (PDA) and water agar (WA) plates augmented with streptomycin (100 mg/L). The inoculated plates were kept at 26 ± 2 °C for 12 days with a 12-hour light/dark photoperiod. To check the efficiency of surface sterilisation, imprints of the sterilised plant parts were taken on half strength PDA and WA plates and observed for any growth. The inoculated plates were monitored every day for emergence of any fungal hyphae or growth. To obtain pure colonies the advancing hyphae were picked form an end of the colony and inoculated onto fresh PDA. Each isolate was given a specific code as shown in Fig.4.1.

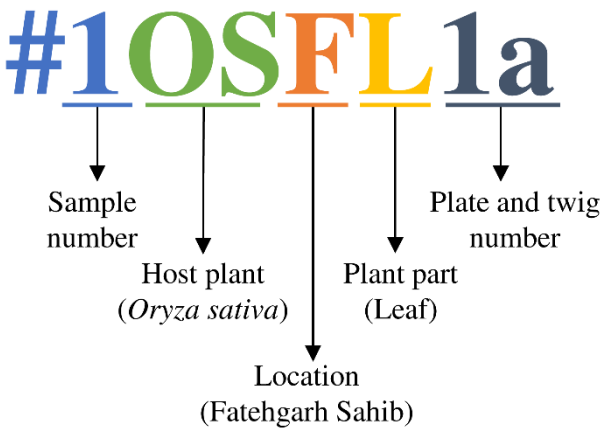

Fig 4.1: Coding outline for endophytic fungal isolates.

4.4 Maintenance and preservation of endophytic fungi

PDA slants prepared by adding 39 g PDA [Hi-media-GMH096] to 1000 mL distilled water pH 5.3 ± 2, supplemented with 10% (v/v) glycerol. The slants were autoclaved for 15 mins at 121 °C and 15 psi. Actively growing cultures were aseptically point inoculated onto the slants and incubated at 26 ± 2 °C for 12 days with a 12-hour light/dark photoperiod till growth was observed. The slants were then transferred to 4 °C till further use (Paul et al., 2015).

4.5 Morpho-taxonomic studies of endophytic fungi isolated from rain-fed and drought-resistant rice variety

The endophytic fungal isolates were tentatively identified using classical morpho-taxonomic method. The isolates were grown over Corn Meal agar (CMA), Czapek Dox agar (CZD), Pine Leaf agar (PLA), Rose Bengal agar (RBA), Synthetic Nutrient Deficient agar (SNA), Sabouraud Dextrose agar (SDA), and Water agar (WA) for 15-20 days at 26 ± 2 °C with a 12-hour light/dark photoperiod. Morphological features, such as basic shape of the fungal colony, diameter of the colony, colour, elevation, margin, opacity and colony growth rate were recorded. Additionally, for observing the microscopic features mycelial mass of the actively growing culture was placed over a glass slide along with a water droplet. Using a fine needle, the mycelia was teased in order to separate the filaments. For staining Lacto-phenol cotton

blue was used and the slide was observed under different magnification (10-100X). The microscopic characteristics such as hyphae, conidia, and other cellular bodies were observed [Nikon E200, Tokyo, Japan]. At least 30 micrometric observations per structure were analysed using Image J software [National Institutes of Health, USA] (Ellis and Ellis, 1985; Barnett and Hunter, 1972).

4.6 Distribution of endophytic fungi of rain-fed and drought-resistant rice variety

Based on the tentative identification, studies on distribution of endophytic fungi throughout the crop cycle and among different plant parts were carried out.

4.7 Diversity studies of endophytic fungi of rain-fed and drought-resistant rice variety

To study the diversity of fungal endophytes, isolation frequency, and various diversity indexes were accessed. The indexes were also calculated throughout the crop cycle and among different plant parts.

4.7.1 Calculation of isolation frequency

The isolation frequency (IF) was assessed using the following formula (Ikram et al., 2023);

$$\text{IF } \% = \frac{\text{No.of individual fungi recorded}}{\text{Total no.of segments}} \times 100$$

4.7.2 Shannon Wiener diversity index

It was assessed to measure the diversity of species as follows (Shannon, 1948);

$$H = \sum_{i=1}^{s} Pi \ln Pi$$

Where, H = diversity index, s = no. of species, Pi = the ratio of individuals belonging to i^{th} species to the total no. of individuals across all species.

4.7.3 Simpson's diversity index

To measure the diversity and relative abundance of each species the following formula was used (Simpson, 1951);

$$D = 1\left(\frac{\sum n\,(n-1)}{N\,(N-1)}\right)$$

Where, n = total number of organisms of a species, N = total number of organisms of all species.

4.7.4 Sorenson's Index of Similarity

To access the overlap between the two populations from different rice varieties the following formula was used (Osono and Mori, 2004);

$$QS = \frac{2a}{(2a+b+c)}$$

Where, a = number of common species in both populations; b and c = number of species specific to each population.

4.7.5 Species evenness

To measure the distribution of the species the following formula was used (Poole, 1974);

$$E = \frac{H'}{\ln S}$$

Where, H' = Shannon-Wiener Index; S = total number of species.

4.7.6 Species richness

To measure the total number of species in the community the following formula was used (Whittaker, 1977)

$$Richness = \frac{S}{\sqrt{N}}$$

Where, S = total number of species; N = total number of isolates of all species.

4.8 Preliminary screening of endophytic fungi for salinity and drought stress tolerance using plate assay

The isolated endophytic fungi were screened for salinity and drought stress using *in vitro* plate assay. To induce salinity stress, 0.5-2.0 M (w/v) NaCl was augmented to PDA plates and the media was autoclaved for 15 mins at 121 °C and 15 psi. An active 5–7-day old fungal culture was point inoculated onto the media and incubated at 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod. Mean diameter was recorded every day for 10 days and compared against a control having no NaCl. The test was done in triplicates and the growth was assessed as follows;

$$Growth\ \% = \frac{Diameter\ of\ test\ (mm)}{Diameter\ of\ control\ (mm)} \times 100$$

Similarly, to induce drought stress conditions, 5-20 % poly-ethylene glycol-6000 (PEG-6000) amended plates were prepared by overlaying PEG-6000 solutions on top of the agar plates. Likewise, an active 5–7-day old fungal culture was point inoculated onto the media and incubated at 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod. Mean diameter was recorded every day for 10 days and compared against a control having no PEG-6000. The test was performed in triplicates and the growth was determined as mentioned above (Van der Weele et al., 2000; Verslues and Bray, 2004; Ripa et al., 2019; Sampangi-Ramaiah et al., 2020).

4.9 Secondary screening of selected endophytic fungi for salinity and drought stress tolerance using broth assay

The endophytic isolates that exhibited the least reduction in growth under the highest tested stress concentration as compared to the respective control were then subjected to salinity as well as drought screening in submerged culture conditions as described in section 3.6. To a Erlenmeyer flask, 25 mL potato dextrose broth (PDB) was added which was supplemented with 0.5-2 M NaCl (w/v) to induce salinity stress whereas PDB with no NaCl served as the control. The flasks were autoclaved for 15 mins at 121 °C and 15 psi. Subsequently a 5 mm mycelial plug of the actively growing 5–7-day old culture was aseptically added and the flasks were incubated 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod with 120 revolutions per minute (rpm). Post the culmination, the mycelial mass was harvested with a pre-weighed Whatman filter paper no. 04 [Merck, Millipore, USA]. The wet weight of the mycelial mass was recorded followed by drying at 80 °C for 2 days. After drying the weight of the mycelial mass was recorded and the growth was calculated as follows;

$$\text{Growth \%} = \frac{Wet\ weight - Dry\ weight\ (test)}{Wet\ weight - Dry\ weight\ (control)} \times 100$$

Similarly, for drought stress evaluation, 25 mL pre-sterilised PDB was supplemented with 5-20% of PEG-6000 whereas PDB flasks containing no PEG-6000 served as control. This pre-sterilized medium was inoculated with a 5 mm mycelial plug of the actively growing 5–7-day old culture was aseptically and incubated at 120 rpm, 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod. Post incubation the same protocol was adopted as described earlier in this section and the growth was determined as mentioned above (Van der Weele et al., 2000; Verslues and Bray 2004; Ripa et al., 2019; Sampangi-Ramaiah et al., 2020).

4.10 Correlation analysis between plate and broth assay

To explore the quantitative relationship between plate and broth assay for salinity and drought stress tolerance, a correlation analysis was carried out (IBM SPSS Statistics, ver. 28.0.1.1 (15)). After launching SPSS, variables were selected from the dataset. The correlation coefficient was set to Pearson and scatter plots were generated for interpretation of the results.

4.11 Correlation analysis between salinity and drought tolerance

To explore the quantitative relationship between different endophytic fungi for salinity and drought stress tolerance, a correlation analysis was performed as discussed earlier (4.10).

4.12 Evaluation of plant growth promoting (PGP) attributes

4.12.1 Production of culture filtrate

Briefly, a 5 mm mycelial plug of 5-7-days old actively growing culture were cultivated in a Erlenmeyer flask with 60 mL pre-sterilised PDB and kept at 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod. The mycelial mass was harvested using Whatman filter paper no. 4 and centrifuged for 10 mins at 10,000 rpm. The supernatant was moved through a 0.22 µm nitrocellulose filter to obtain cell free extract [Merck, Millipore, USA] (Dwibedi and Saxena, 2020).

4.12.2 Solvent extraction

Liquid-liquid extraction was done using solvent ethyl acetate. The solvent and culture filtrate were taken in the ratio 3:1 and shaken vigorously. This step was repeated three times. For dehydration of organic layer anhydrous sodium sulphate was used and evaporated using rotatory evaporator [DLAB RE100-Pro, China]. Post drying, the crude fraction was dissolved in methanol and used for estimation of antioxidant and antimicrobial potential (Gautam et al., 2022).

4.12.3 Estimation of Total Phenolic Content (TPC)

Folin-Ciocalteu (FC) method was adopted. In brief, 100 µL FC reagent mixed with 100 µL fungal extract (concentration 1 mg/mL) was kept at room temperature for 10 mins. Post incubation, 200 µL of 6% Na_2CO_3 was added and kept at room temperature for 60 mins. Using a microplate reader [Powerwave 34, Biotek, USA] the absorbance was observed at 760 nm with gallic acid (concentration range 100-500 µg/mL) as standard. The TPC was denoted as µg of Gallic acid equivalent (GAE) per mg of extract (Ainsworth and Gillespie, 2007).

4.12.4 Estimation of Total Flavonoid Content (TFC)

It was estimated as per the protocol by Hossain et al. (2011) with some changes. In brief, 800 µL of deionised water and 60 µL 5% w/v of $NaNO_2$ was added to 200 µL of fungal extract (concentration 1 mg/mL) and kept at room temperature for 5 mins. Post incubation, 60 µL of 10% w/v $AlCl_3$ was introduced and kept for 1 minute at room temperature. Then, 400 µL of 1N NaOH was mixed and the result was read at 510 nm using a microplate reader [Powerwave 34, Biotek, USA]. Quercetin (concentration range 50-500 µg/mL) served as a standard. The total phenolic content was denoted as µg of Quercetin equivalent (QE) per mg of extract.

4.12.5 *In-vitro* antioxidant potential

The free radical scavenging potential of selected isolates was assessed by different assays.

4.12.5.1 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging assay

It was performed as per the protocol of Dhayanithy et al. (2019) with minor modifications. The free radical DPPH when dissolved in methanol produces a violet/ purple colour which becomes yellow in the presence of anti-oxidants. This colour change is recorded spectrophotometrically at 517 nm. Briefly this test comprises of using 20 µl of the fungal extract (concentration range of 200-1000 µg/mL) to which was added 230 µl of DPPH solution prepared in methanol. This was kept at room temperature in dark for 30 min and then result was assessed at 517 nm using a microplate reader [Powerwave 34, Biotek, USA]. Quercetin (concentration range 200-1000 µg/mL) served as a standard and working DPPH as the control. The DPPH radical scavenging capability was denoted as QE equivalents per milligram of extract. The percentage free radical scavenging (%FRS) was calculated as:

$$\% \text{ FRS} = \frac{Absorbance\ (Control) - Absorbance\ (Sample)}{Absorbance\ (Control)} \times 100$$

The linear regression between concentrations and corresponding % FRS was used for IC_{50} value calculation.

4.12.5.2 Trolox equivalent antioxidant capacity (TEAC)

It was assessed as per the protocol given by Re et al. (1999) with minor changes. The method employs utilisation of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$). $ABTS^+$ stock was generated by mixing 2.45 mM potassium persulphate ($K_2S_2O_8$) and 7 mM ABTS (prepared in 100 mM phosphate buffer saline /pH 7.4) in equal volume. Post incubation at room temperature for 16 hours the stock solution of ABTS radical was diluted to an absorbance of 0.9-1.0 at 734 nm using phosphate buffer saline. The assay was performed by adding 1 mL of ABTS radical to 10 µL of the fungal extract (concentration range 200-1000 µg/mL). The mixture was kept at room temperature for 6 mins and result was recorded at 734 nm using a microplate reader [Powerwave 34, Biotek, USA]. Trolox (concentration range 200-1000 µg/mL) served as a standard. The TEAC was denoted as microgram of Trolox equivalent (TE) per milligram of extract. The %FRS and IC_{50} was calculated using the formula mentioned in the section above.

4.12.5.3 Hydrogen-peroxide radical scavenging assay (H_2O_2)

It was performed as per the protocol of Ruch et al. (1989) with minor changes. In brief, 600 µL of 43 mM H_2O_2 (prepared in 1 M phosphate buffer /pH 7.4) was added to fungal extract (concentration range 200-1000 µg/mL) and incubated at room temperature for 10 mins. The

result was recorded using a UV-Vis spectrophotometer [UV-1900, Shimadzu Corp., Japan] at 230 nm Ascorbic acid (concentration range 200-1000 µg/mL) served as a standard. The H_2O_2 scavenging activity was denoted as microgram of Ascorbic Acid equivalent (AAE) per milligram of extract. The %FRS and IC_{50} was calculated using the formula mentioned in the section above.

4.12.5.4 Ferric ion reducing antioxidant power (FRAP)

It was performed as per the protocol of Benzie and Strain (1996) with minor modifications. To prepare FRAP reagent, a mixture of 20 mM iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), 300 mM sodium acetate buffer and 10 mM 2,4,6-tripyridyl-s-triazine ($C_{18}H_{12}N_6$) was added in the ratio 1:10:1. Briefly, 1 mL FRAP reagent and 10 µL of 1mg/mL fungal extract were mixed and kept for 20 mins at room temperature. The result was assessed at 595 nm using a microplate reader [Powerwave 34, Biotek, USA]. Ascorbic acid (concentration range 10-100 µg/mL) served as a standard. The FRAP activity was expressed as µg of AAE per milligram of extract.

4.12.6 Evaluation of antimicrobial potential

4.12.6.1 Anti-bacterial assay

The antibacterial efficiency of selected isolates was assessed against both gram-negative (*Escherichia coli* MTCC 739 and *Enterobacter aerogenes* NCIM 5139) and gram-positive (*Bacillus subtilis* MTCC 736 and *Staphylococcus aureus* MTCC 96) bacteria using agar well diffusion assay. The selected isolates were subjected to production of filtrates and subjected to liquid-liquid extraction as mentioned in section 4.12.1 and 4.12.2 respectively. Muller Hinton (MH) Agar plates were prepared and swabbed with 0.5 McFarland adjusted cultures under sterile conditions. Furthermore, 5 mm wells were punched in the plates to which 30 µL of 1 mg/mL fungal extract was added. The extract was kept undisturbed for 20 mins following which the plates were incubated at 37 °C for the night. Streptomycin [Hi-Media, India] was the positive control whereas an uninoculated well served as a negative control. The antibacterial potential was measured by recording the inhibition zone formed around the well (Rios et al., 1988).

4.12.6.2 Anti-fungal assay

The antifungal potential of selected endophytic fungi was assessed against *Alternaria alternata* (ITCC 6129), *Botrytis cinerea* (ITCC 6011), *Colletotrichum gloeosporioides* (ITCC 3801) and *Fusarium moniliforme* (ITCC 6240) using dual culture method. Briefly, 5 mm plugs of actively growing 5-7-day old culture and pathogen were inoculated 5 cm apart on petri plate containing Saboraud dextrose agar (SDA) media and kept at 26 ± 2 °C for 7 days. The growth of the

isolates and the pathogen was recorded each day till 7 days. The percentage inhibition was assessed as follows (Kumar et al., 2023)

$$\% \text{ Inhibition} = \frac{R1-R2}{R1} \times 100$$

Where, R1 = Radial growth of pathogen; R2 = Radial growth of pathogen in presence of fungal endophyte.

4.12.7 Production of phytohormones

Indole acetic acid (IAA) and gibberellic acid (GA3) production in the culture filtrates was assessed by qualitative and quantitative methods. Briefly, a 5mm mycelial plugs of 5-7-days old actively growing culture were transferred to an Erlenmeyer flask having 60 mL pre-sterilised CZD broth. In case of IAA, the media was supplemented with L-tryptophan. This was kept at 26 ± 2 °C for 10 days with a 12-hour light/dark photoperiod. Subsequently, a cell free culture filtrate was obtained as described in section 4.12.1.

4.12.7.1 Qualitative detection of phytohormones using UV-Visible spectrophotometer

The UV/Vis spectra of the culture filtrate were accessed in 100-700 nm range using a 1 cm quartz cuvette [UV-1900, Shimadzu Corp., Japan] (Kamnev et al., 2001; El-Sayed et al., 2019). The absorption maxima of the culture filtrates were compared with standard IAA [Hi-media PCT0803] and GA3 [Hi-media, PCT0830].

4.12.7.2 Quantification of phytohormones using High-performance liquid chromatography (HPLC)

For IAA quantification, the pH of culture filtrate was acidified to 3.0 using glacial acetic acid. For extraction, ethyl acetate was used in the ratio 3:1 and shaken vigorously. This step was repeated three times. For dehydration of organic layer anhydrous sodium sulphate was used and evaporated using rotatory evaporator [DLAB RE100-Pro, China]. Post drying, the crude fraction was dissolved in methanol and passed through 0.22 µm nitrocellulose membrane [Merck, Millipore, USA] (Qiang et al., 2019).

For estimation of GA3, the pH of culture filtrate was acidified to 2.2 using glacial acetic acid. For extraction, ethyl acetate was used in the ratio 3:1 and shaken vigorously. This step was repeated three times. For dehydration of organic layer anhydrous sodium sulphate was used and evaporated using rotatory evaporator [DLAB RE100-Pro, China]. Post drying, the crude fraction was dissolved in 60% (v/v) methanol and the pH was set to 8.0. The extract was passed through 0.22 µm nitrocellulose membrane [Merck, Millipore, USA] (Ben Rhouma et al., 2020).

4.12.7.3 Indole acetic acid quantification (IAA)

For quantification of IAA, isocratic elution was followed using a mobile phase comprising of deionized water, acetonitrile and acetic acid in the ratio 40:60:1 (Wary et al., 2022). Operational conditions were, flow rate 1 mL/min, oven temperature 40 °C and injection volume 10 µL. Different concentrations (100-500 µg/mL) of standard IAA [Hi-media, PCT0803] were prepared in HPLC-grade methanol. The retention times of the analyte and standard were compared and quantified using the peak area.

4.12.7.4 Gibberellic acid quantification (GA3)

For quantification of GA3, isocratic elution was followed using a mobile phase comprising of 80% (v/v) methanol and 20% (v/v) deionized water (Ben Rhouma et al., 2020). Operational conditions were, flow rate 1 mL/min, oven temperature 35 °C and injection volume 10 µL. To determine the concentration of GA3, different concentrations (10-50 µg/mL) of standard GA3 [Hi-media, PCT0830] were prepared in HPLC-grade methanol. The retention times of the analyte and standard were compared and quantified using the peak area.

4.12.8 Estimation of protein content

The protein content of the culture broth of selected endophytic fungi was estimated using Bradford's reagent (Bradford, 1976). To 1 mL of the cell free supernatant, 3 mL 1X Bradford's reagent [composition: Coomassie brilliant blue G-250 prepared in 95% (v/v) ethanol and 85% (w/v) phosphoric acid] and kept at 37 °C for 30 mins. Post incubation, the sample was analysed at 595 nm using a microplate reader [Powerwave 34, Biotek, USA]. Bovine serum albumin (concentration range 1-5 mg/mL) was used as a standard.

4.12.9 Production of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC deaminase)

For evaluation of ACC deaminase production, the selected isolates were cultivated in DF media [composition (g/L): 6 g Na_2HPO_4, 4 g KH_2PO_4, 2 g glucose, 2 g gluconic acid, 2 g citric acid, 0.2 g $MgSO_4.7H_2O$ and trace elements 124.6 mg $ZnSO_4.7H_2O$, 78.22 mg $CuSO_4.5H_2O$, 11.9 mg $MnSO_4.H_2O$, 10 mg H_3BO_3, 10 mg MoO_3 and 1 mg $FeSO_4.7H_2O$] supplemented with different concentrations (1-5 mM) of ACC for 10 days at 28 ± 2 °C, 120 rpm (Dworkin and Foster, 1958). Followed by centrifugation at 10,000 rpm for 10 mins. The precipitate was dissolved in 0.1 M Tris HCl buffer pH 7.6 and centrifuged at 16,000 rpm. The same step was repeated using 0.1 M Tris HCl buffer pH 8.5. Further, 1 mL toluene was mixed, followed by addition of 0.56 M HCl and 2,4-dinitrophenylhydrazine (DNPH). The cells were kept at 30 °C for 30 mins. Post incubation, 2 M NaOH was introduced and the result was recorded at 540 nm using a microplate reader [Powerwave 34, Biotek, USA]. Standard curve of α-ketobutyrate

(concentration range 0.1-0.5 µM) was plotted (Zhang et al., 2019). Furthermore, the verification of α-ketobutyrate produced in ACC supplemented media was done using a Fourier-transform infrared spectroscopy (FTIR) in an IR Tracer-100 [Shimadzu Corp., Japan].

4.12.10 Production of siderophores

The selected endophytic fungi were accessed for siderophore production potential as per the universal Chrome Azurol S (CAS) method by Schwyn and Neilands (1987) with slight modifications. The CAS reagent was prepared by mixing CAS dye, $FeCl_3.6H_2O$ and hexadecyltrimethylammonium bromide in the ratio 5:1:4. The endophytic fungi were grown in iron-deficient succinate medium [composition (g/L): 6 g K_2HPO_4, 4 g succinic acid, 3 g KH_2PO_4, 1 g $(NH_4)_2SO_4$, 0.2 g $MgSO_4.7H_20$] and incubated at 26 ± 2 °C for a period of 7 days. Followed by centrifugation at 8,000 rpm for 10 mins and filtration through Whatman filter paper no. 04. To 1 mL of the cell free filtrate, 1 mL of CAS dye was added and kept for 20 mins at room temperature. The result was analysed at 630 nm using a microplate reader [Powerwave 34, Biotek, USA]. The uninoculated culture broth served as a reference and the siderophore production was estimated using the following formula:

Siderophore production (psu) = $\frac{Absorbance\ (Reference) - Absorbance\ (Sample)}{Absorbance\ (Reference)} \times 100$

4.12.11 Mineral solubilisation

The selected isolates were evaluated for the capacity to solubilise minerals present in the soils.

4.12.11.1 Phosphate

The phosphate solubilization ability was accessed using Pikovskaya's Agar medium [composition (g/L): 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.02 g NaCl, 0.02 g KCl, 0.003 g $FeSO_4 \cdot 7H_2O$, 0.003 g $MnSO_4 \cdot H_2O$, 5 g $Ca_3\ (PO_4)_2$, 10 g glucose, 0.5 g yeast extract, 15 g agar, and 1000 mL distilled water) was supplement with bromophenol blue]. Subsequently, a 7-day old, 5 mm mycelial plugs of selected isolates were inoculated and kept at 26 ± 2 °C for a period of 7 days. Control set comprised of sterile agar plugs with no culture. Three replicates were tested for each fungal isolate. A yellow-coloured halo along the growth indicated phosphate solubilizing activity on 7th day (Pikovskaya, 1948). The solubilization index was assessed as follows (Premono et al., 1996);

SI= $\frac{Colony\ diameter\ (mm) + Halo\ diaeter\ (mm)}{Colony\ diameter\ (mm)}$

4.12.11.2 Zinc

The zinc solubilization ability was accessed using zinc oxide agar media [composition (g/L): 1 g $(NH_4)_2SO_4$, 1 g ZnO, 0.2 g KCl, 0.2 g K_2HPO_4, 0.1 g $MgSO_4$, 15 g agar, and 1000 mL distilled water] (Fasim et al., 2002). Subsequently, a 7-day old, 5 mm mycelial plugs of selected

isolates were inoculated on the agar media and kept at 26 ± 2 °C for 7 days. Control set comprised of sterile agar plugs with no culture. A clear halo along the colony indicated zinc solubilizing activity on 7th day. The zinc solubilization index was calculated by the formula mentioned in previous section.

4.12.12 Production of extracellular lytic enzymes

The ability to produce extracellular enzymes of the selected isolates was defined by the Enzymatic Index (EI) calculated as follows (Florencio et al., 2012);

$$EI = \frac{Colony\ diameter\ (mm) + Halo\ diameter\ (mm)}{Colony\ diameter\ (mm)}$$

4.12.12.1 Amylase production assay

To assess amylase production Glucose Yeast Peptone Agar (GYP) supplemented with 0.2%(w/v) of starch [composition (g/L): 1 g glucose, 0.1 g yeast extract and 0.5 g peptone, 15 g agar] was used as a substrate. Briefly 5 mm mycelial plug was aseptically inoculated and kept at 26 ± 2 °C for 7 days. The control comprised of uninoculated 5 mm PDA plug. After 7 days the plates were immersed 1% (w/v) iodine. The production of amylase was indicated by the formation of yellow zones on blue-black background (Lee et al., 2014). The enzyme activity was ascertained by calculating the EI.

14.12.12.2 Cellulase production assay

GYP medium was augmented with 1% (w/v) Carboxymethyl Cellulose as a substrate for cellulase production. Briefly 5 mm mycelial plug of the selected endophytic fungus was aseptically inoculated and kept at 26 ± 2 °C for 7 days. The control comprised of uninoculated 5 mm PDA plug. After 7 days of incubation, the plates were immersed in 0.5% Congo red solution for 20 mins. These were subsequently de-stained for 15 mins using 1 M NaCl, subsequently the de-staining solution was decanted. The production of cellulase was seen as a yellow halo (Lee et al., 2014). The enzyme activity was ascertained by calculating the EI.

14.12.12.3 Laccase production assay

For assessing the laccase production, 0.005% 1- naphthol supplemented GYP medium was prepared. Briefly a 7-day old, 5 mm mycelial plug of selected endophytic fungi were seeded in the centre of the GYP+1-napthol plates and allowed to incubate at 26 ± 2 °C for a period of 7 days. The control comprised of uninoculated PDA plug only. The change of colour from colourless to purple/violet coloured halo around the colony was formed due to laccase activity (Lee et al., 2014). The enzyme activity was ascertained by calculating the EI.

14.12.12.4 Pectinase production assay

For accessing the pectinase production, pectin agar media [composition (g/L): 5 g pectin, 1 g yeast extract, 15 g agar, pH 5.0] was used. Briefly 5 mm mycelial plug was aseptically inoculated and kept at 26 ± 2 °C for 7 days. The control comprised of uninoculated 5 mm PDA plug. After 7 days of incubation, the plates were immersed in aqueous hexadecyltrimethylammonium bromide ($C_{19}H_{42}BrN$), formation of clear zones on translucent background indicated pectinase activity (Sunitha et al., 2013). The enzyme activity was ascertained by calculating the EI.

4.12.13 Production of ammonia

Ammonia production was assessed using Nessler's reagent (Chand et al., 2020). The fungal endophytes were cultivated in Czapek' Dox broth at 26 ± 2 °C for a period of 7 days. Followed by centrifugation at 8,000 rpm for 10 mins and filtration through Whatman filter paper no. 04. To 5 mL of the cell free supernatant, 1 mL Nessler's reagent was added. The change in colour from pale yellow to brownish indicated the intensity of ammonia production with respect to the uninoculated culture broth which served as a reference.

4.12.14 Production of hydrogen cyanide (HCN)

The selected endophytic fungi were also accessed for production of hydrogen cyanide using PDA media supplemented with glycine (Ripa et al., 2019). A 5 mm mycelial plugs of 7 days old culture were inoculated on PDA. The petri plates were sealed using a parafilm along with Whatman filter paper no. 01, soaked in 0.05% (w/v) picric acid positioned on the lid of the plate and incubated at 26 ± 2 °C for a period of 7 days. The colour change of filter paper from yellow to reddish-brown indicated HCN production. An uninoculated plate served as the control where no colour change was observed.

4.13 Isolation of genomic DNA

Molecular identification involved the isolation of genomic DNA of the selected endophytic fungal isolates by Cetyl trimethylammonium bromide (CTAB) method (van Burik et al., 1998) with minor modifications. Briefly, 0.5 g of fungal mycelia was scrapped off from 4 to 5-day old culture of the selected endophytes and crushed using liquid nitrogen using a mortar and pestle. Further cell lysis was performed by addition of 1 mL extraction buffer which was composed of 1% (w/v) CTAB, 1M Tris HCl, 0.5 M EDTA, 5 M NaCl and 2% cetrimide. This reaction mixture was incubated at 60 °C for 30 mins. Thereafter 1.5 µL of RNase solution was added and the reaction mixture was incubated at 37 °C for 15 mins. The extraction of lysate was carried out using phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuged at 12,000 rpm for 10 mins. The precipitation of genomic DNA from the aqueous layer was done using

chilled isopropanol. The resulting pellet was washed using 80% (v/v) ethanol, air dried, dissolved in TE buffer and stored at -20 °C. The quality of the isolated genomic DNA was assessed by recording the absorbance at 260 and 280 nm. Whereas estimation of concentration of the genomic DNA was done as follows:

Concentration (µg/mL) = Absorbance $_{260\ nm}$ × 50 (µg/mL) × dilution factor

4.14 PCR amplification of genomic DNA

The amplification of the Internal Transcriber Spacer (ITS) region 1, 5.8S, ITS 4, β-tubulin (TUB2) and Translation elongation factor 1-α (TEF) of the genomic DNA was done. For ITS, primers ITS 1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'TCCTCCGCTTA TTGATATGC-3') were used whereas for TUB2, BT-2a (5'-GGTAACCAAATCGGTGCTGCTTTC-3') and BT-2b (5'-ACCCTCAGTGTAGTGACCCTTGGC-3') were used. Similarly, for TEF primers TEF1-728F (CATCGAGAAGTTCGAGAAGG) and EF-2 (GGA(G/A)GTACCAGT(G/C)ATCATGTT) were used. Briefly, a 25 µL of reaction mixture containing 25 ng of extracted genomic DNA, 0.8 µM of each primer, 2.5 mM of dNTP (Bangalore GeNei), 1.5 mM $MgCl_2$ [GeNei, Bangalore], 1.5 U of Taq DNA Polymerase [GeNei, Bangalore] in 10 X Taq buffer [GeNei, Bangalore] was prepared. For ITS region, the conditions for thermal cycler consisted of initial denaturation at 96 °C for 5 min followed by 39 cycles at 95 °C for 1 min, 58 °C for 1.30 min, 72 °C for 1 min and final extension at 72 °C for 5 min (White et al., 1990). For TUB2 region, initial denaturation at 95 °C for 4 mins followed by 34 cycles at 94 °C for 1 min, 56 °C for 0.5 min, 72 °C for 1 min and final extension at 72 °C for 10 min (Glass and Donaldson, 1995). For TEF region, the same conditions of ITS region were opted with annealing at 52 °C (Carbone and Kohn, 1999). The PCR amplicons were examined using gel electrophoresis in a 1.5% agarose gel at 40V, gel imaging was performed under UV light in Bio-Rad Gel documentation system. The purified amplicons were sent for sequencing to Biokart, Bangalore-India and Agharkar Research Institute-NFCCI, Pune-India.

4.15 Phylogenetic tree construction

Post sequencing, the sequences were analysed using Sequencher ver. 5.4.6 (www.genecodes.com) for their purity (>90%), aligned and then submitted to GenBank. nBLAST algorithm software was used to undertake similarity search of final ITS, TUB2 and TEF sequences. MEGA 11 was used to align the reference sequences obtained from nBLAST analysis with the ITS, TUB2 and TEF sequences. The alignment file comprised of 10 sequences, 7 type species and the sequences under study. Maximum likelihood method based on Tamura-Nei model with 1000 bootstrap was used for the phylogenetic tree construction.

4.16 Preparation of inoculum

Oryza sativa var. PUSA-44 was used as a model for evaluating the selected endophytic fungi. A spore suspension of selected endophytic fungi E1 = #6OSFR2e (isolated from rain-fed rice) and E2 = #2OSTUR9a (drought-resistant rice) was prepared, the number of spores were determined under Nikon microscope (Nikon E200, Tokyo, Japan) using a haemocytometer (1×10^6 no. of spores).

4.17 Effect of abiotic stress on seed germination

A total of 100 surface sterilised (using 1% sodium hypochlorite) seeds were placed from germination under the different sets. The designations used are E = Endophyte, SS = salinity stress, DS = drought stress, CS = combine stress.

Different treatments used are:

E^-S^-: Uninoculated control

E^-SS^+: Uninoculated + 150 mM NaCl (salinity stress)

E^-DS^+: Uninoculated + 10% PEG 6000 (drought stress)

E^-CS^+: Uninoculated + 150 mM NaCl + 10% PEG 6000 (combined stress)

$E1^+S^-$: Endophyte 1 inoculated control

$E1^+SS^+$: Endophyte 1 + 150 mM NaCl (salinity stress)

$E1^+DS^+$: Endophyte 1 + 10% PEG 6000 (drought stress)

$E1^+CS^+$: Endophyte 1 + 150 mM NaCl + 10% PEG 6000 (combined stress)

$E2^+S^-$: Endophyte 2 inoculated control

$E2^+SS^+$: Endophyte 2 + 150 mM NaCl (salinity stress)

$E2^+DS^+$: Endophyte 2 + 10% PEG 6000 (drought stress)

$E2^+CS^+$: Endophyte 2 + 150 mM NaCl + 10% PEG 6000 (combined stress)

The inoculated seeds were treated with the respective inoculum, whereas the control set was treated with deionised water. The seeds were kept in a moistened muslin cloth at 25 °C for 3 days. A spray bottle was used to maintain the moisture in the seeds by spraying the respective solutions (deionised water or inoculum) every day for 3 days. The number of germinated seeds were counted and the total germination was calculated as follows (Hossen et al., 2022);

Total germination (%) = $\frac{No.of\ germinated\ seeds}{Total\ no.of\ seeds} \times 100$

4.18 Pot trials under controlled environment-growth chamber

The plant growth promotion features of the selected endophytic isolates viz., #6OSFR2e and #2OSTUR9a under salinity, drought and combined stress was assessed via pot trials under controlled environment. The endophyte treated and untreated seedlings were planted in pots (7 × 4 × 7.5 cm; top diameter × base diameter × height) containing sterile and moistened soil in a randomised block design (n=3) for each of the sets as mentioned in section 4.17. Seven days after planting, the uninoculated seedlings were watered using ½ strength Hoagland solution, whereas ½ strength Hoagland solution supplemented with 150 mM NaCl, 10% PEG-6000 and 150 mM NaCl + 10% PEG-6000 was used for inducing salinity, drought and combined stress, respectively (Hoagland and Arnon, 1950). This regimen of stress induction was followed for the next three days. The pots were grown under photoperiod of 12 hours with an average temperature of 30 ± 2 °C.

4.19 Pot trials under ambient environmental conditions

The plant growth promotion features of #6OSFR2e and #2OSTUR9a under salinity, drought and combined stress was assessed via pot trials under ambient environment as well. The endophyte treated and untreated seedlings were planted in pots (14 × 11 × 11.5 cm; top diameter × base diameter × height) containing sterile and moistened soil in a randomised block design (n=3) for each of the following sets as mentioned in section 4.17. Fourteen days after planting, the uninoculated seedlings were watered using ½ strength Hoagland solution, whereas ½ strength Hoagland solution supplemented with 150 mM NaCl, 10% PEG-6000 and 150 mM NaCl + 10% PEG-6000 was used for inducing salinity, drought and combine stress, respectively (Hoagland and Arnon, 1950). This regimen of stress induction was followed for the next seven days. The pots were grown under photoperiod of 12 hours with an average temperature of 30 °C/25 °C (Day/Night).

4.20 Estimation of physiological parameters of the rice plant

Physical parameters of the plants such as root length, shoot length, root and shoot fresh weight, and shoot dry weight were recorded to assess the changes under stress (Reshna et al., 2022).

4.21 Estimation of biochemical parameters of rice plant

4.21.1 Relative water content (RWC)

For RWC, the plant samples were harvested and washed using distilled water to remove any dust or debris. The fresh weight was recorded and the samples were then immersed in distilled water till they attained constant turgid weight following which they were dried in the oven and RWC was calculated using the following formula (Smart and Bingham, 1974);

$$\text{RWC} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Fresh weight} - \text{Turgid weight}} \times 100$$

4.21.2 Total phenolic content (TPC)

Folin-Coicalteu's method as per González-Teuber et al. (2022) was adopted with minor modifications. Briefly, 1N FC (Folin-Coicalteu's)-reagent was added to the test sample containing the harvested plant followed by 6% (w/v) sodium carbonate (Na_2CO_3). It was incubated at room temperature for 1 hour. Post incubation, the absorbance was recorded at 760 nm using a microplate reader [Powerwave, Biotek, USA]. Gallic acid (concentration range 100-2000 µg/mL) was used as the standard. The TPC was expressed as GAE per gram of sample.

4.21.3 Total flavonoid content (TFC)

Aluminium chloride ($AlCl_3$) colorimetric method by Ali et al. (2021) was adopted with minor modifications. Briefly, 5% (w/v) sodium nitrite was added to the test sample followed by addition of 10% (w/v) aluminium chloride ($AlCl_3$) and 1N sodium hydroxide (NaOH). The reaction mixture was incubated at room temperature for 10 mins. Post incubation, the absorbance was read at 510 nm using a microplate reader [Powerwave 34, Biotek, USA]. Quercetin (concentration range 100-2000 µg/mL) was used as the standard. The TFC was expressed as QE per gram of sample.

4.21.4 Chlorophyll and carotenoid content

The leaf samples were harvested and washed using distilled water. The samples were then crushed in 80% of chilled acetone to get a homogenous mixture. This mixture was centrifuged at 8000 rpm for 10 mins. Absorbance of the resultant supernatant was recorded at 480, 650 and 663 nm using a microplate reader [Powerwave 34, Biotek, USA]. The chlorophyll and carotenoid content were estimated using the following equations (Arnon, 1949);

Chlorophyll a (µg/g FW) = $12.2 \times A_{663} - 2.81 \times A_{645}$

Chlorophyll b (µg/g FW) = $20.12 \times A_{645} - 5.03 \times A_{663}$

Total chlorophyll content (µg/g FW) = Chlorophyll a + Chlorophyll b

Carotenoid content (µg/g FW) = $\frac{1000 \times \text{A480} - 3.27 \times \text{Chl a} - 104 \times \text{Chl b}}{229}$

4.21.5 Total sugar content

Phenol-sulfuric acid method by Dubois et al. (1956) was adopted. The plant samples were washed and oven dried and suspended in 80% ethanol. The sample was then centrifuged at 8000 rpm for 10 mins to obtain the supernatant was used for further testing. To the supernatant, 100 µL of phenol reagent and 5 mL of concentrated sulfuric acid was added. This was then incubated at room temperature for 30 mins. Post incubation, the result was read at

485 nm using a microplate reader [Powerwave 34, Biotek, USA]. Glucose (concentration range 10-100 µg/mL) was used as the standard.

4.21.6 Reducing sugar

For estimation of reducing sugar content, dinitrosalicylic acid (DNSA) reagent was used (Sumner, 1925). The plant samples were washed and oven dried and suspended in 80% ethanol. The sample was then centrifuged at 8000 rpm for 10 mins and the obtained supernatant was used for further testing. Briefly, to 1 mL of supernatant, 2 mL DNSA reagent [composition: 43 mM DNSA, 0.4 M sodium hydroxide (NaOH) and 1.55 M sodium tartrate ($C_4H_4Na_2O_6$)] was added. The reaction mixture was incubated at 90 °C for 10 mins in a water bath (NSW, India) and allowed to cool. The result was recorded at 560 nm using a microplate reader [Powerwave 34, Biotek, USA] and expressed as mg/g DW with D-glucose as standard.

4.21.7 Proline content

The harvested leaf sample was crushed using liquid nitrogen and suspended in 3% (w/v) sulphosalicylic acid ($C_7H_6O_6S$). It was then centrifuged at 8000 rpm for 10 mins and the supernatant was incubated along with acid-ninhydrin reagent in boiling water bath [NSW, India]. Post incubation toluene was added and the absorbance was recorded at 520 nm using a microplate reader [Powerwave 34, Biotek, USA]. For quantification, different concentrations of the standard L-proline [Hi-media, RM061] were prepared and concentration was expressed as µmol/g FW (Bates et al., 1973).

4.21.8 Malondialdehyde content (MDA)

To access the lipid peroxidation, formation of MDA was evaluated as described by Heath and Packer (1968) with minor modifications. In brief, 0.5 gram of leaf sample were ground to a fine powder and suspended in 0.1 % (w/v) trichloroacetic acid and centrifuged at 8000 rpm for 10 mins. Subsequently, 0.5% (w/v) thiobarbituric acid was added and incubated for 30 mins in a boiling water bath [NSW, India]. Post incubation, the reaction mixture was allowed to cool following which the absorbance was recorded at 532 nm using a microplate reader [Powerwave 34, Biotek, USA] and quantified using the extinction coefficient of 155 $mM.cm^{-1}$. The MDA content was expressed as nmol/FW.

4.21.9 Hydrogen peroxide content

To access the damage caused by H_2O_2 formation, the leaf samples were crushed to a fine powder and suspended in 0.1% (w/v) trichloroacetic acid (Velikova et al., 2000). It was then centrifuged at 8000 rpm for 10 mins. To the resultant supernatant, 10 mM potassium phosphate (K_3PO_4) buffer pH 7 and 1 M potassium iodide (KI) were added and incubated at room temperature in dark for 60 mins. Post incubation, the result was recorded at 390 nm

using a microplate reader [Powerwave 34, Biotek, USA]. H_2O_2 was used to prepare the standard curve and expressed as µmol/g FW.

4.22 Estimation of enzymatic parameters of rice plant

4.22.1 Total protein content and enzyme assays

For estimation of enzyme assays, the leaf samples were crushed using 0.1 M phosphate buffer pH 7. The resultant homogenate was centrifuged at 8000 rpm for 10 mins and the supernatant designated as the crude enzyme extract was used for further analysis. The total protein content was estimated using Bradford assay, briefly 1X Bradford reagent (prepared by dissolving Coomassie Brilliant Blue G-250 in 95% (v/v) ethanol and 85% (w/v) phosphoric acid) was added to the crude enzyme extract. The absorbance was assessed at 595 nm using a microplate reader [Powerwave 34, Biotek, USA] and quantified using a calibration curve of bovine serum albumin (BSA) (Bradford, 1976).

4.22.2 Ascorbate peroxidase (APX) assay

A reaction mixture comprising of 50 mM phosphate buffer having pH 7, 30% (v/v) H_2O_2, 10 mM EDTA, 100 mM ascorbate and crude enzyme extract was prepared (Nakano and Asada, 1981). The decrease in absorbance was recorded at 290 nm using a UV-Vis spectrophotometer [UV-1900, Shimadzu Corp., Japan] for 3 mins, where reaction mixture without the crude enzyme extract served as a blank.

$$\text{Enzyme activity} = \frac{\frac{\Delta A}{\Delta t} \times T}{\xi \times V \times P}$$

Where, $\Delta A/\Delta t$ = change in absorbance w.r.t time, T = total volume of reaction mixture (mL), ξ = molar extinction coefficient ($mM^{-1}cm^{-1}$), V = volume of crude enzyme extract (mL), P = protein content (mg/mL)

The results were expressed as U/mg protein, where one unit equals the amount of enzyme required to breakdown 1 µM substrate per minute.

4.22.3 Catalase (CAT) assay

The catalase activity was assessed using a reaction mixture of 50 mM phosphate buffer having pH 7, 30% (v/v) hydrogen peroxide and crude enzyme extract (Aebi, 1984). Absorbance was recorded at 240 nm using a UV-Vis spectrophotometer [UV-1900, Shimadzu Corp., Japan], where reaction mixture without the crude enzyme solution served as a blank.

4.22.4 Peroxidase (POX) assay

The peroxidase activity was assessed using a reaction mixture of 50 mM phosphate buffer having pH 7, 1% (w/v) o-Dianisidine ($C_{14}H_{16}N_2O_2$) solution, 1% hydrogen peroxide and crude enzyme solution (McEwen, 1971). The absorbance was recorded at 470 nm using a microplate

reader [Powerwave 34, Biotek, USA], where reaction mixture without the crude enzyme extract served as a blank.

4.22.5 Superoxide dismutase (SOD) assay

The superoxide dismutase activity was accessed using a reaction mixture of 50 mM phosphate buffer having a pH 7.8, 1 M methionine, 1 mM NBT (Nitroblue tetrazolium salt), 3 mM EDTA-disodium and 0.2 mM riboflavin and the crude enzyme extract. It was then incubated for 20 mins under fluorescent lights at room temperature. Similarly, the reaction mixture without the crude enzyme solution served as a control. Post incubation the result was read at 560 nm using a microplate reader [Powerwave 34, Biotek, USA] (Beauchamp and Fridovich, 1971).

$$\text{SOD activity} = \frac{\frac{Absorbance\,(Control) - Absorbance(Sample)}{Absorbance\,(Control)}}{50\% \times V \times P} \times T$$

Where, V = volume of sample, P = protein content (mg/mL), T = total volume (mL)

4.23 Root colonisation assay

For confirmation of colonisation by endophytic fungi, root staining method by Phillips and Hayman (1970) was employed. Freshly harvested roots were treated with 10% KOH, acidified using 1% HCl. The roots were stained using 0.5% trypan blue (w/v) and observed under a Nikon microscope (E200, Tokyo, Japan). The colonisation frequency was calculated as follows:

$$\text{Root colonisation (\%)} = \frac{No.of\ roots\ colonised}{Total\ no.of\ roots\ examined} \times 100$$

4.24 Statistical analysis

All the tests were performed in triplicate and the data is represented as mean ± SD. One-way ANOVA analysis followed by Tukey's post hoc was performed using GraphPad Prism to analyse the variation and significant difference. Two-way ANOVA analysis was also performed using GraphPad Prism to assess the interaction between different factors.

Chapter 5

Results (In-vitro)

5.1 Physiochemical characteristics of the soil from sampling site

The soil from plant collection site, location 1 at Fatehgarh Sahib had the following physiochemical attributes: largely neutral pH 7.3 ± 0.1, organic carbon content of 0.05 ± 0.0%, organic matter of 0.9 ± 0.0%, total nitrogen content of 0.1 ± 0.0%, total phosphorous content of 987.0 ± 4.6 mg/kg, and available phosphorous content of 94.7 ± 2.9 mg/kg. Whereas the soil from plant collection site, location 2 at the Thapar Institute of Engineering and Technology had the following physiochemical attributes: a pH of 7.8 ± 0.1, organic carbon content of 0.6 ± 0.0%, organic matter of 1.1 ± 0.0%, total nitrogen content of 0.2 ± 0.0%, total phosphorous content of 885.3 ± 5.9 mg/kg, and available phosphorous content 105.0 ± 2.0 mg/kg.

5.2 Isolation of endophytic fungi from rain-fed and drought tolerant rice variety

In this study, endophytic fungi were isolated from rain-fed (PUSA-44) and drought-resistant (Sahbhagi Dhan) *Oryza sativa* cultivars (Fig. 5.1).

Fig. 5.1: a. Plant parts of PUSA-44 (Bar: 10 mm); b. Seeds of Sahbhagi Dhan; c. Cultivated plant of Sahbhagi Dhan for endophyte isolation.

Fig. 5.2 shows the emerging endophytic colonies from the plant segments, which were purified, assigned a specific code and preserved till further use. From the rain-fed rice variety, PUSA-44, 120 isolates existing as endophytes were recovered during the entire crop cycle. Of the 120 isolates, 41, 31, 25 and 23 were from leaves, roots, internode and spikes, respectively (Table A1-Appendix). Likewise, 62 isolates were procured from the entire crop cycle of drought-resistant rice variety, Sahbhagi Dhan. Of the 62 isolates, 19, 22, 12 and 9 were from leaves, roots, internode and spikes, respectively (Fig. 5.3) (Table A2-Appendix). Some of the purified isolates obtained during the work are shown in Fig. 5.4.

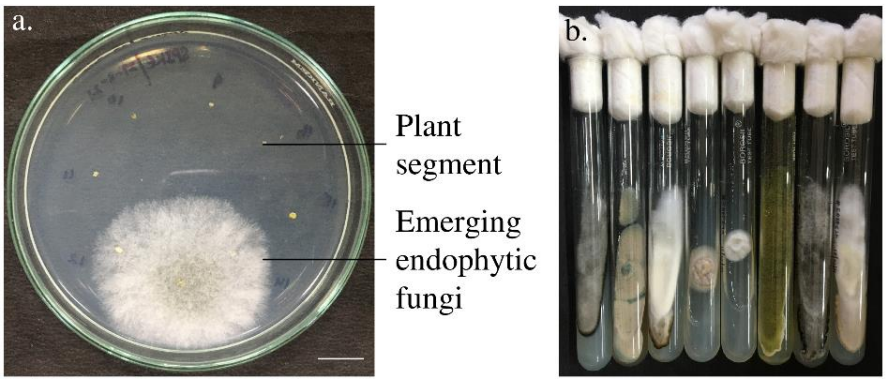

Fig. 5.2: a. Potato dextrose agar plate showing plant segments and emerging endophytic isolate (Bar: 10 mm); b. Pure isolates preserved in slants containing glycerol.

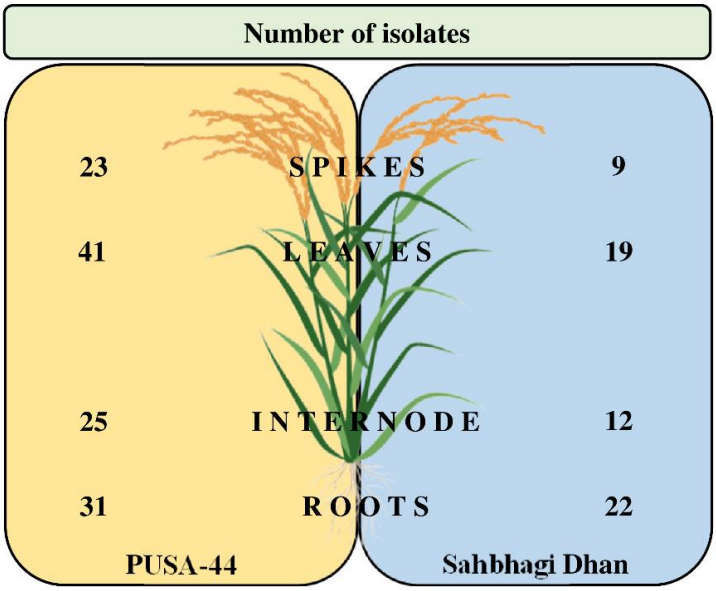

Fig. 5.3: The number of endophytic fungal isolates from different plant parts of rain-fed (PUSA-44) and drought resistant (Sahbhagi Dhan) rice during the crop cycle.

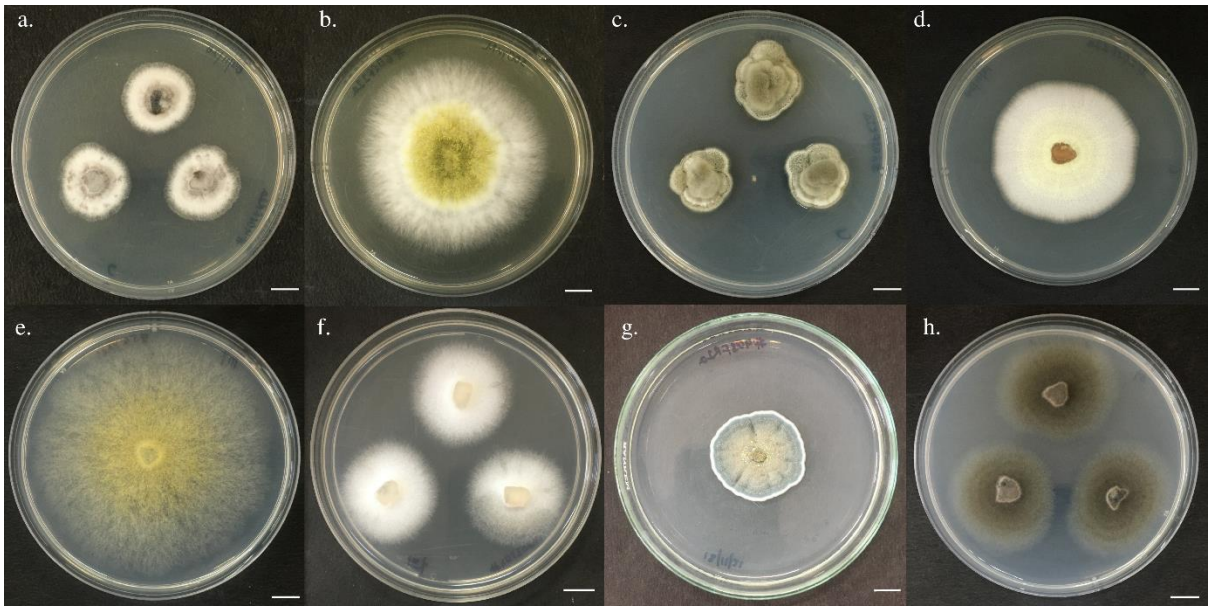

Fig. 5.4: a. *Nigrospora* sp. b. *Aspergillus* sp. c. non-sporulating d. *Paecilomyces* sp. e. *Rhizopus* sp. f. *Fusarium* sp. g. *Penicillium* sp. h. unidentified (Bar: 10 mm).

5.3 Morpho-taxonomic studies of the isolated endophytic fungi

The isolated endophytic fungi were tentatively identified based on the plate morphology, colony morphology and microscopic characteristics. Of the identified isolates from rain-fed rice variety, 45, 16.7, 13.3 and 2.5% belonged to Sordariomycetes, Dothideomycetes, Eurotiomycetes and Zygomycetes class, respectively (Fig. 5.5).

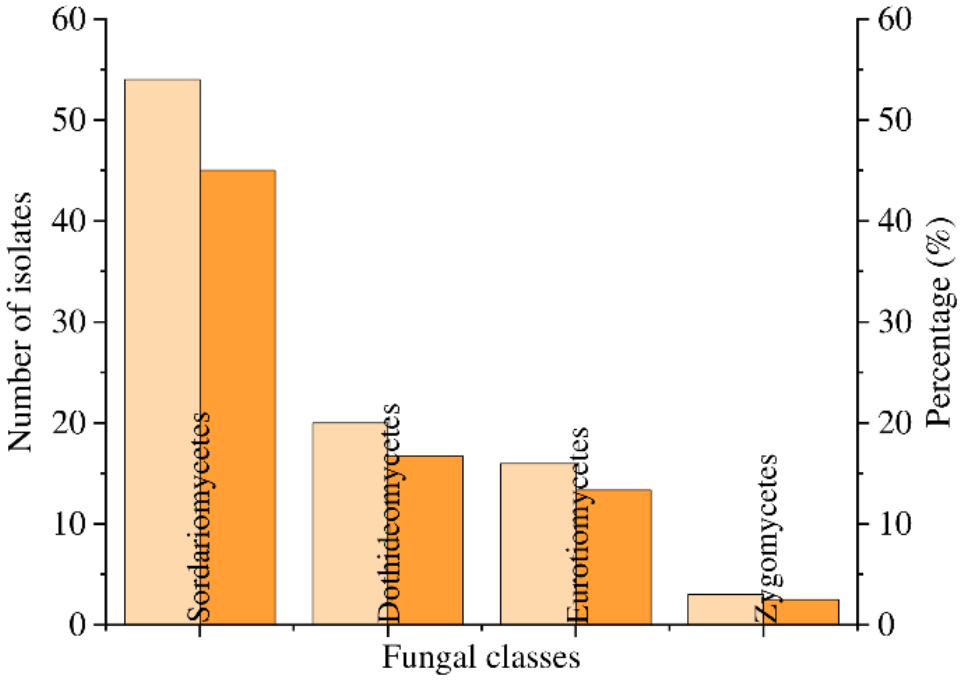

Fig. 5.5: Fungal endophytes isolated from rain-fed rice variety-PUSA-44.

Furthermore, the 120 isolates represented 11 different genera, namely, *Alternaria*, *Aspergillus, Cladosporium, Colletotrichum, Curvularia, Penicillium*, *Fusarium*, *Nigrospora*, *Paecilomyces*, *Pestalotiopsis* and *Rhizopus*. Among the mentioned genera, 25, 15.9, 13.3, 7.5 and 5% isolates, belonged to *Nigrospora*, *Fusarium*, *Alternaria*, *Penicillium* and *Aspergillus*, respectively. Furthermore, less than 3% were from *Curvularia*, *Rhizopus*, *Colletotrichum*, *Paecilomyces*, *Cladosporium* and *Pestalotiopsis* each (Fig. 5.6a). Similarly, of the identified isolates from drought resistant rice variety, 53.2% belonged to Sordariomycetes, whereas 12.9% belonged to Dothideomycetes, and less than 5% of isolates belonged to Eurotiomycetes and Zygomycetes (Fig. 5.7).

The 62 isolates represented eight genera: *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Nigrospora*, *Paecilomyces* and *Rhizopus*. Notably, 29, 21 and 9.7% isolates were from *Fusarium*, *Nigrospora* and *Cladosporium* species. Moreover, less than 5% belonged to *Alternaria*, *Aspergillus*, *Colletotrichum*, *Paecilomyces* and *Rhizopus* (Fig. 5.6b). Microscopic attributes of some of the isolated endophytes are shown in Fig. 5.8.

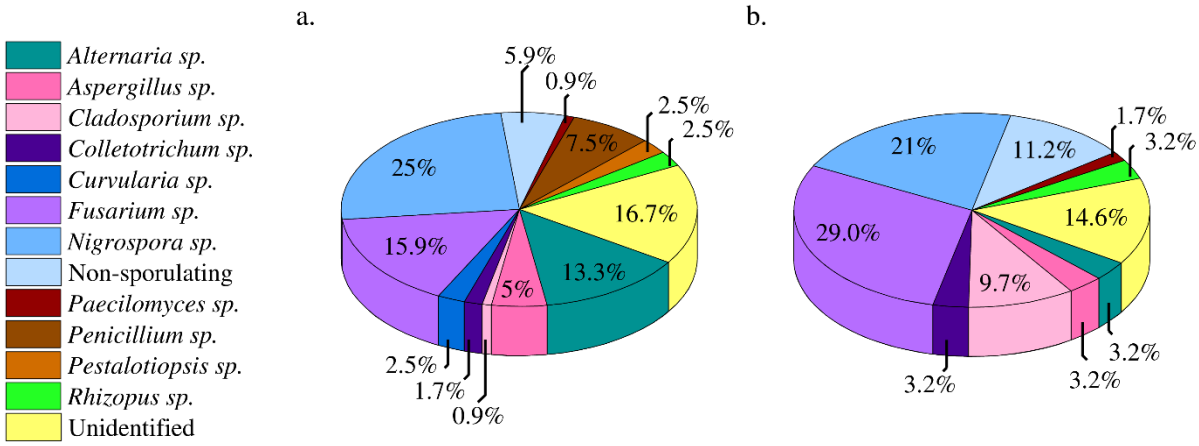

Fig. 5.6: Distribution of endophytic fungi isolated from a. rain-fed; b. drought resistant rice variety.

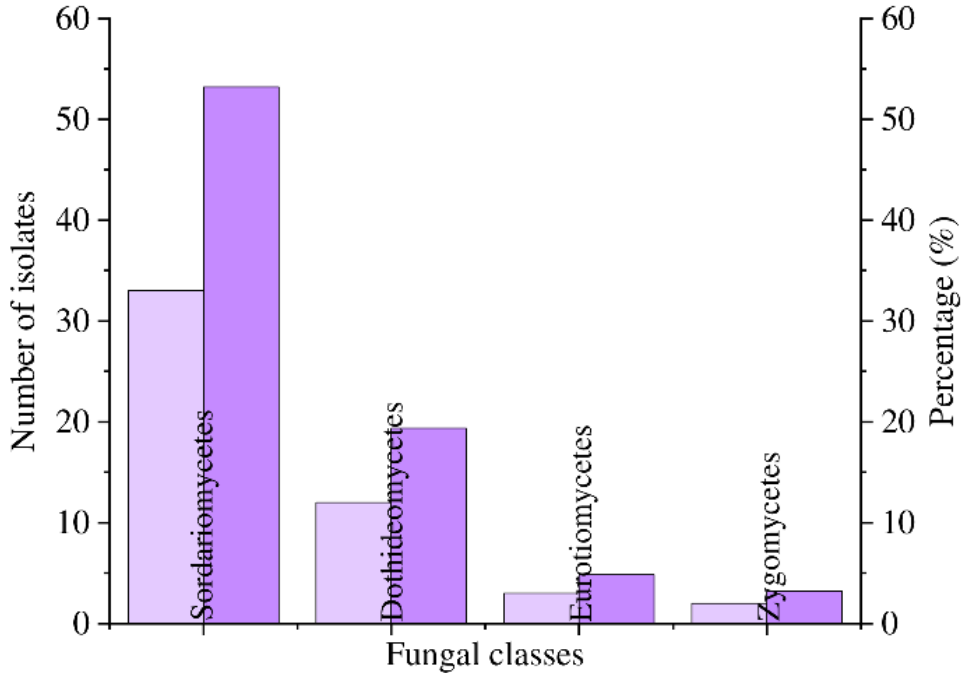

Fig. 5.7: Fungal endophytes isolated from drought resistant rice variety Sahbhagi Dhan.

Altogether, endophytic isolates belonging to eight genera were commonly isolated from the two rice varieties, i.e., PUSA-44 and Sahbhagi Dhan. The eight genera included *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Nigrospora*, *Paecilomyces* and *Rhizopus*. Whereas genera *Curvularia*, *Penicillium* and *Pestalotiopsis* were isolated from rain-fed rice variety only.

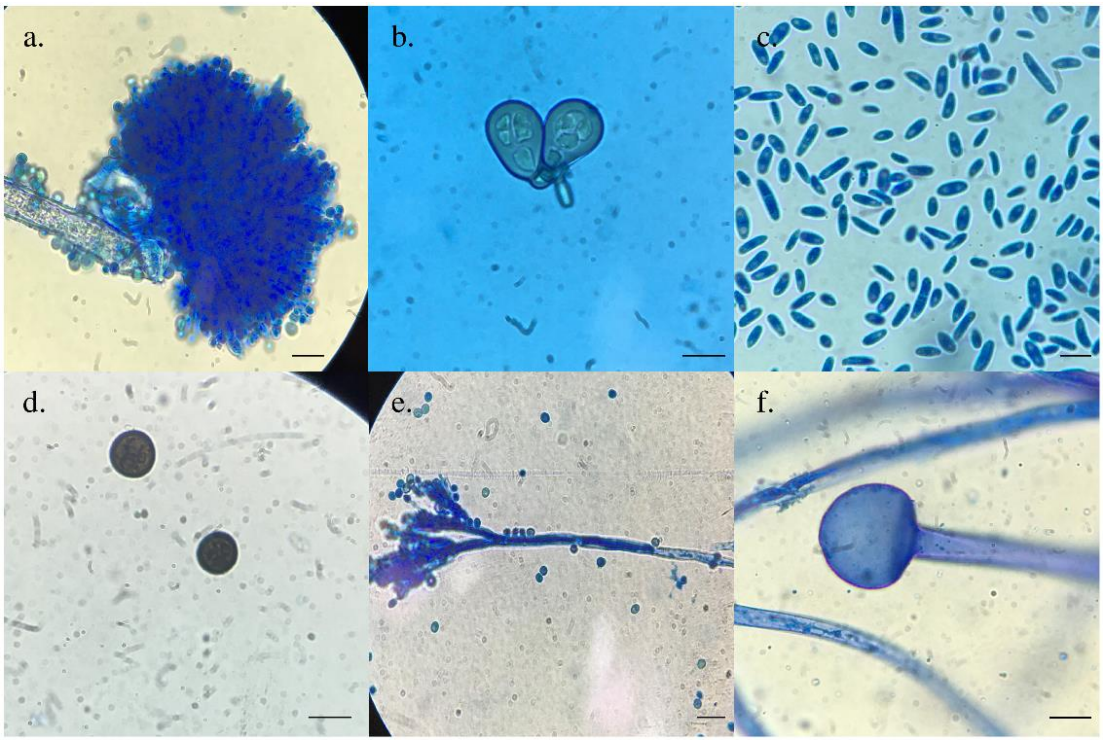

Fig. 5.8: a. *Aspergillus* sp.; b. *Curvularia* sp.; c. *Fusarium* sp.; d. *Nigrospora* sp.; e. *Penicillium* sp.; f. *Rhizopus* sp. (Bar: 10 µm).

5.4 Distribution of endophytic fungi of rain-fed and drought-resistant rice variety throughout the crop cycle

Tentative identification revealed that in the case of rain-fed rice, *Nigrospora* was the dominant genus isolated throughout the crop cycle (Fig. 5.9). During the first isolation cycle (15 days post-sowing), 33.3% of the isolated endophytic fungi were identified as *Nigrospora* sp. Notably, 16.7% of the isolated endophytic fungi were identified as *Colletotrichum* sp. The isolates from this genus were not observed again throughout the crop cycle (Fig. 5.9a). Similarly, during the second isolation cycle (30 days post-sowing), 33.3% of the isolates were identified as *Nigrospora* and *Alternaria* sp. (Fig. 5.9b). In the third isolation cycle which was carried out after 45 days post sowing, 25% of the isolated endophytic fungi were identified as *Fusarium* sp., whereas the frequency of *Alternaria* sp. dipped to 12.5%. Interestingly, no *Nigrospora* sp. were isolated during this time. Here, endophytic fungi of genus, *Cladosporium*, appeared with 12.5% isolates. Furthermore, genus *Colletotrichum*, *Cladosporium* were not observed again in the crop cycle (Fig. 5.9c).

During the reproductive phase of the plant from fourth to sixth isolation cycle (60-90 days post-sowing), although still the highest, *Nigrospora* sp. saw a declining trend. Of the isolated endophytic fungi, 42.9, 27.8 and 23.3% were identified as *Nigrospora* sp. (Fig. 5.9d-f). Isolates from the genus *Penicillium*, *Pestalotiopsis* and *Rhizopus* were also observed from the fourth to seventh isolation (60-105 days post-sowing). During the seventh isolation cycle

(105 days post-sowing), 27.8% of isolated endophytic fungi were identified as *Alternaria* sp. Closely followed by 22.2% isolates of *Nigrospora* sp. (Fig. 5.9g). During the eighth isolation (120 days post-sowing), 30% of the isolated endophytic fungi were identified as *Fusarium* sp., closely followed by *Alternaria* and *Nigrospora* sp. with 25% (Fig. 5.9h).

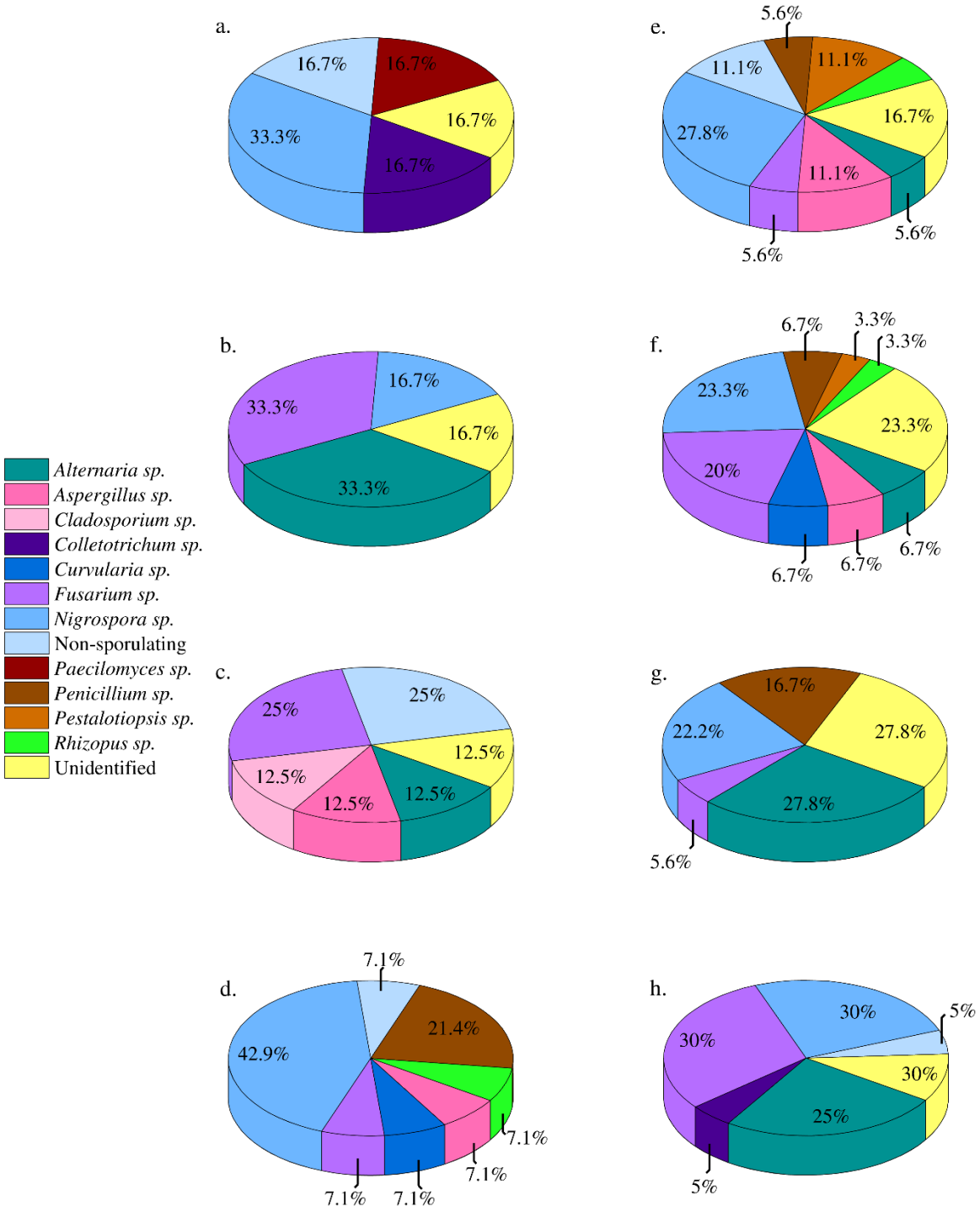

Fig. 5.9: Distribution of endophytic fungi isolated from different phases of PUSA-44 growth cycle a. First isolation-15th day; b. Second isolation-30th day; c. Third isolation-45th day; d. Fourth isolation-60th day; e. Fifth isolation-75th day; f. Sixth isolation-90th day; g. Seventh isolation-105th day; h. Eighth isolation-120th day.

In the case of drought resistant rice, *Fusarium* and *Nigrospora* were the dominant genera isolated throughout the crop cycle (Fig. 5.10). During the first isolation (15 days post-sowing), 60% of the isolated endophytic fungi were identified as *Fusarium* sp. In addition, 20% of isolates of *the Cladosporium* genus were also observed (Fig. 5.10a).

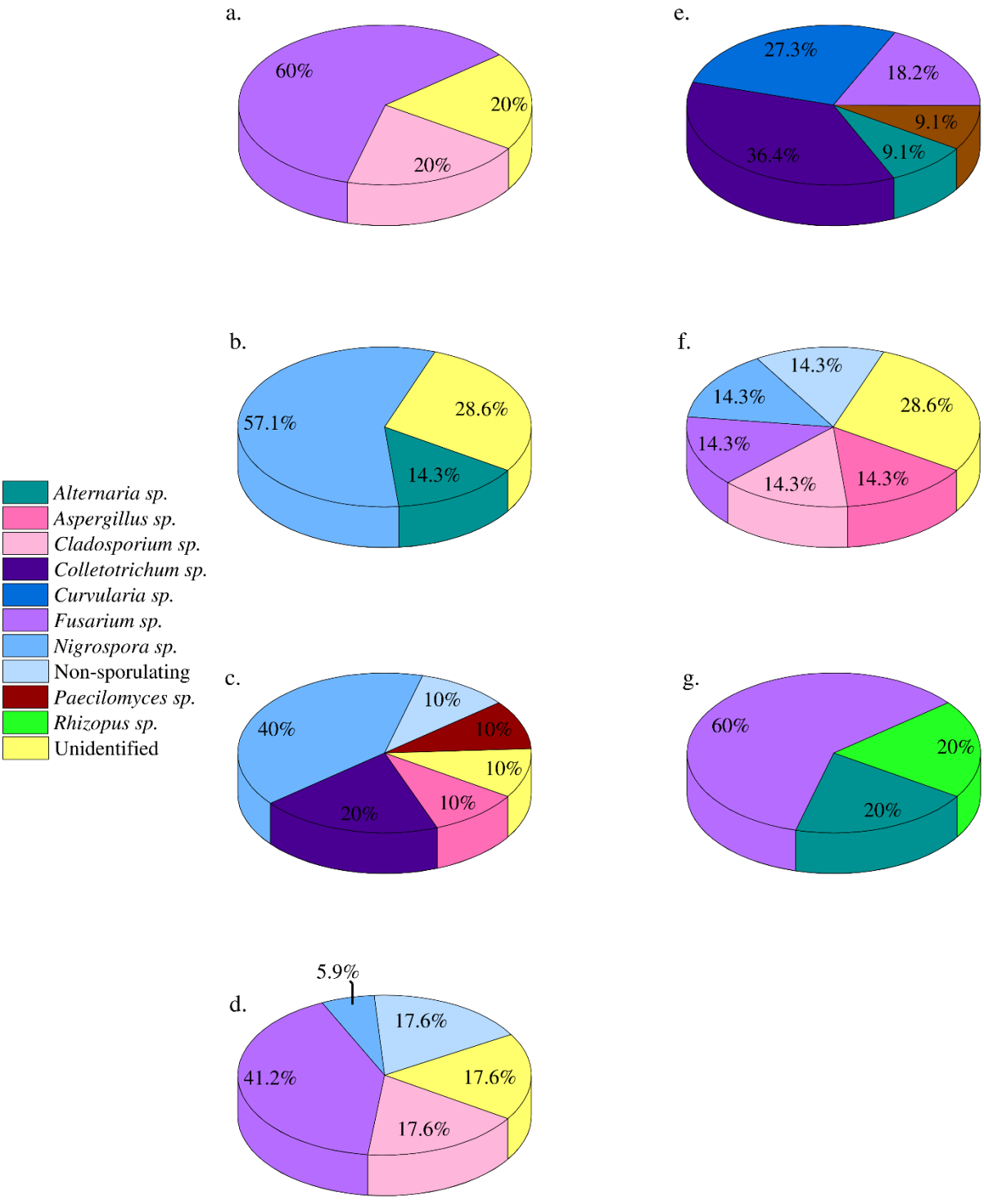

Fig. 5.10: Distribution of endophytic fungi isolated from different phases of Sahbhagi Dhan growth cycle a. First isolation-15th day; b. Second isolation-30th day; c. Third isolation-45th day; d. Fourth isolation-60th day; e. Fifth isolation-75th day; f. Sixth isolation-90th day; g. Seventh isolation-105th day.

During the second isolation (30 days post-sowing), 57.1% of the isolated endophytic fungi were identified as *Nigrospora* sp. At the same time, 14.3% of isolates of *the Alternaria* genus were seen (Fig. 5.10b). During the third isolation (45 days post-sowing), *Nigrospora* was again the dominant genera with 40% isolates. Notably, no *Fusarium* sp. was isolated during these stages, whereas isolates belonging to *Aspergillus* sp. were observed for the first time. In addition, 20% isolated endophytic fungi of the *Colletotrichum* genus and 10% isolates of *the Paecilomyces* genus were observed for the only time during the crop cycle (Fig. 5.10c).

During the fourth isolation (60 days post-sowing), isolates of genus *Fusarium* made a reappearance contributing 41.2% to the total isolated endophytic fungi (Fig. 5.10d). The trend followed through to the fifth isolation (75 days post-sowing) with 36.4% isolates of *the Fusarium* genus, closely followed by 27.3% isolates of *Nigrospora*. Here, 9.1% isolated endophytic fungi of *the Rhizopus* genus were observed for the first time in the crop cycle (Fig. 5.10e). During the sixth isolation (90 days post sowing), isolates from different genera, such as *Aspergillus*, *Cladosporium*, *Fusarium* and *Nigrospora*, were seen in equal proportions (Fig. 5.10f). At last, during the seventh isolation (105 days post sowing), 60% of the isolated endophytic fungi belonged to *Fusarium* sp. (Fig. 5.10g).

5.5 Distribution pattern of endophytic fungi among the plant parts of the rain-fed and drought resistant rice varieties

Based on the morphological data, the preponderance of different genera of fungal endophytes in different plant parts of the two varieties of rice, namely PUSA-44 and Sahbhagi Dhan. In the case of leaf isolates of rain-fed rice, 32.5 and 20% of the isolated endophytic fungi belonged to *Nigrospora* and *Fusarium* (Fig. 5.11a). Interestingly, 5% of isolates of the *Colletotrichum* genus were isolated from leaves. Similarly, out of the 24 internode isolates, 30% belonged to *Alternaria* sp. whereas 24% belonged to *Nigrospora* sp. (Fig. 5.11b). *Paecilomyces* genus was reported from the internode only with 4% isolation frequency. Among the root isolates, *Nigrospora* and *Fusarium* were the dominant genera, with 15.6% isolation rate (Fig. 5.11c). Notably, 3.1% of isolates of the genus *Cladosporium* were also reported from the root and was not reported from any other plant part. Likewise, in the case of spikes, *Nigrospora* was the dominant genus with 26.1% isolates, followed by *Fusarium* and *Alternaria* with 17.4% isolates (Fig. 5.11d). Additionally, isolates of *Rhizopus* genes were isolated from leaves, roots and spikes, whereas genera *Curvularia* and *Pestalotiopsis* were only isolated from roots and spikes. Similarly, isolates of *the Aspergillus* genus were only isolated from leaves and internodes of rain-fed rice variety.

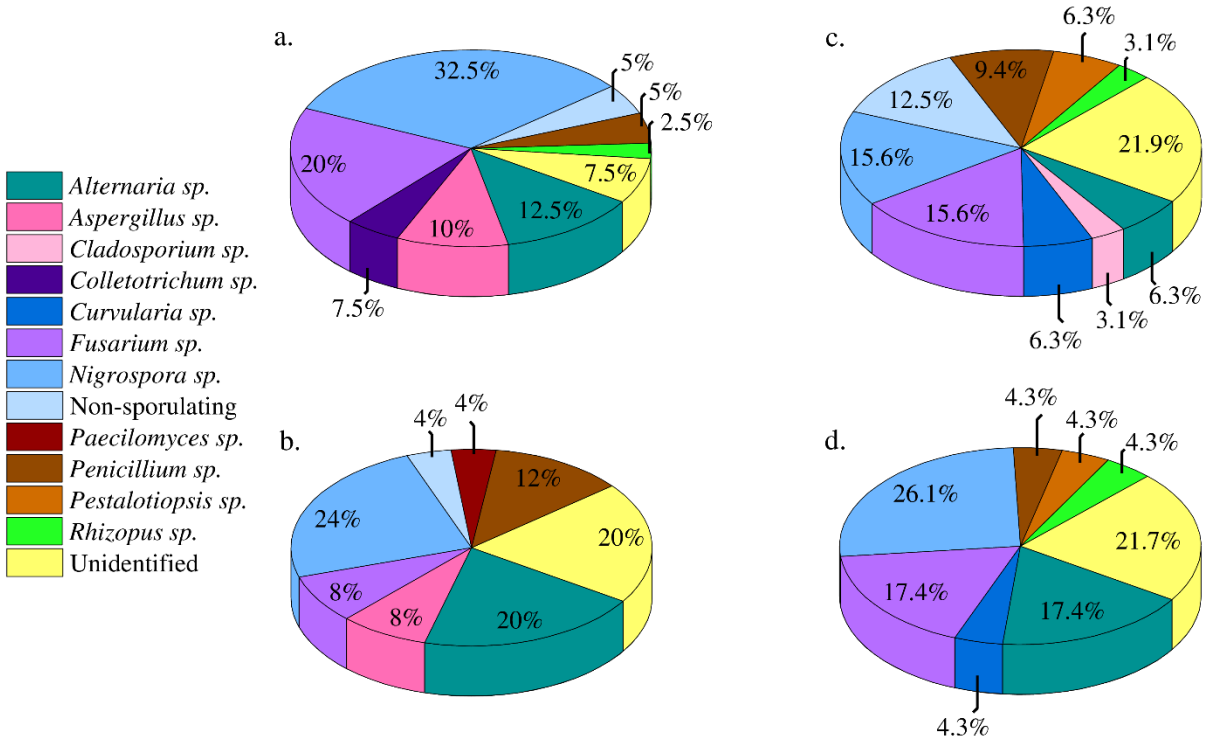

Fig. 5.11: Distribution of endophytic fungi isolated from different plant parts of rain-fed rice a. Leaf; b. Internode; c. Root; d. Spike.

In the case of drought resistant rice, among the leaf isolates, 36.8% belonged to *Nigrospora*, followed by *Fusarium*. In addition, *Alternaria* was the only other genus identified among the isolated endophytic fungi from leaves (Fig. 5.12a).

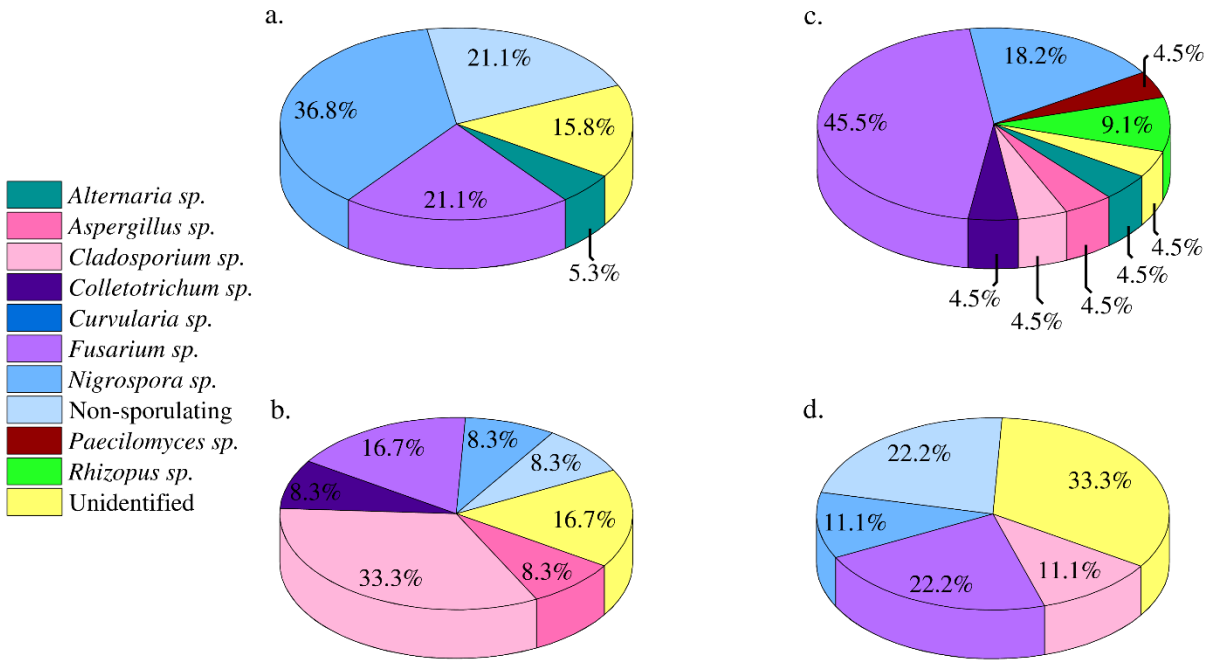

Fig. 5.12: Distribution of endophytic fungi isolated from different plant parts of drought resistant rice a. Leaf; b. Internode; c. Root; d. Spike.

In the internode, *Cladosporium* was the dominant genera with 33.33% isolates, followed by 16.7% isolates of *Fusarium* (Fig. 5.12b). Likewise, *Fusarium* was also the dominant genera among root and spike isolates at 45.5 and 22.2% isolation rate, respectively (Fig. 12c-d). Notably, 9.1 and 4.5% isolates of *Rhizopus* and *Paecilomyces* were also observed from roots. These genera were not isolated from any other plant part. Similarly, the genus *Alternaria* was only isolated from the leaves and roots. Whereas isolates of genus *Aspergillus*, *Cladosporium* and *Colletotrichum* were only isolated from the internode and roots.

5.6 Isolation frequency of endophytic fungi of rain-fed and drought resistant rice variety

In the case of the rain-fed rice variety, PUSA-44, same isolation frequency of fungal endophytes was seen leaves and roots i.e., 2.5% (first isolation) and 3.8% (2^{nd} to 3^{rd} isolation) during the vegetative stage of the plant. In contrast, comparatively lesser isolates (1.3-2.5%) were recovered from the internode. Interestingly, at the start of reproductive stage (5^{th} isolation) there were no fungal isolates from the internode (Fig. 5.13).

On the other hand, 5% isolation frequency was recorded from the spike. During the reproductive stage 11.2 and 10% isolation frequency was recorded from roots and leaves, respectively. However, the isolation frequency from roots declined to 6.2 and 1.3% at 105^{th} day and at the end of the growth cycle, respectively. The isolation frequency from spike was 7.5 and 8.8% around the 105^{th} day and at the end of the growth cycle, respectively (Fig. 5.13a).

On the contrary, in the drought-resistant rice variety, Sahbhagi Dhan, the isolation frequency of fungal endophytes from the internode was same throughout the vegetative stage. Comparatively lower numbers of isolates were obtained from the roots during the same duration. However, the isolation frequency of endophytes from roots increased during 4^{th} isolation (Fig. 5.13b). The highest isolation frequency of 8.8% was observed at this stage from the roots. In addition, a 5% isolation frequency was observed from the spikes. As observed in the other variety the isolation frequency from roots declined to 2.5% near the end of the growth cycle. Similarly, a decreasing pattern towards the end of the growth cycle was observed in leaves, internodes and spikes.

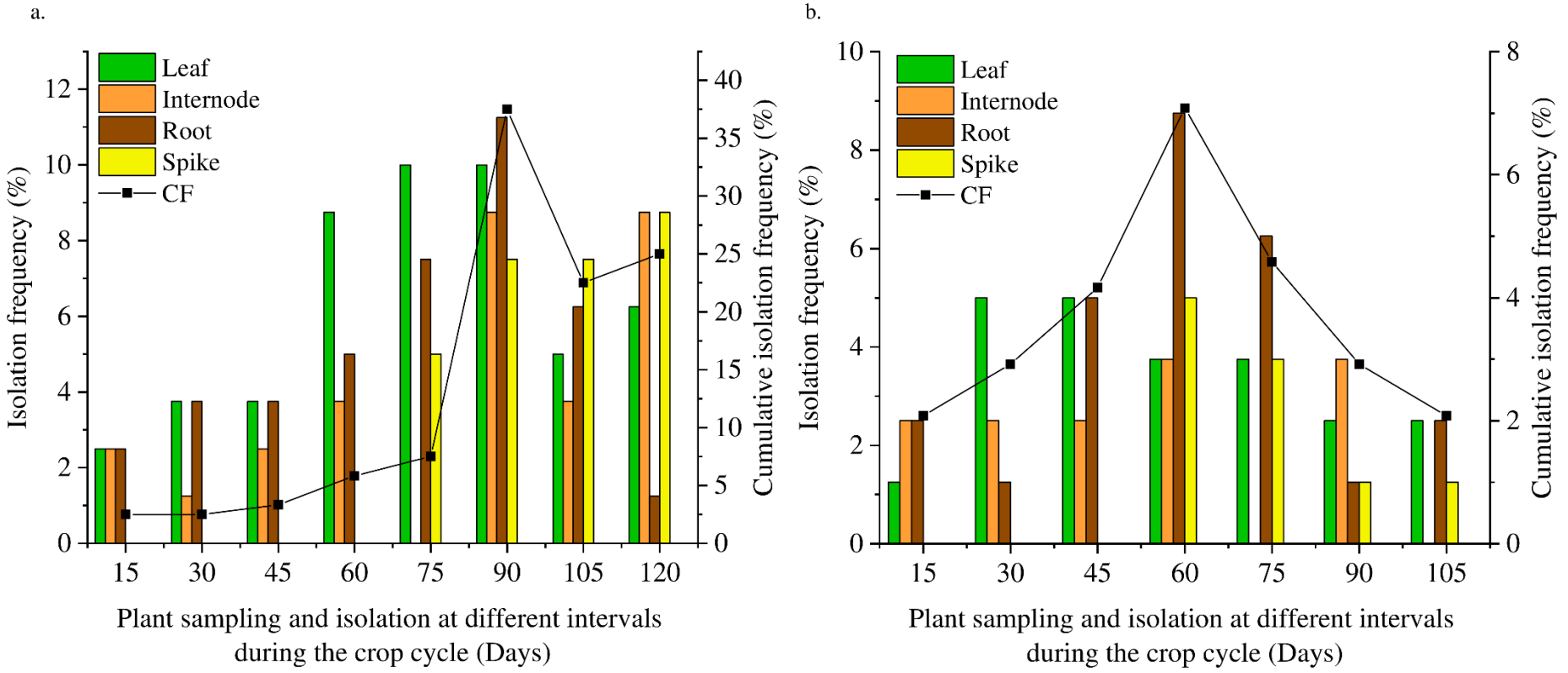

Fig. 5.13: Isolation and cumulative isolation frequency of fungal endophytes of a. Rain-fed rice variety-PUSA-44; b. Drought resistant rice variety-Sahbhagi Dhan.

5.7 Diversity studies of endophytic fungi of rain-fed and drought-resistant rice variety

Further, high values of diversity indices were seen in the endophytic populations from both rice varieties. Simpson's index (D) of 0.14 and 0.16 was observed in rain-fed and drought resistant rice, respectively. Likewise, the Shannon wiener diversity index of 2.14 and 1.95 was seen in rain-fed and drought resistant rice, respectively (Table 5.1).

Table 5.1: Diversity indexes for rain-fed and drought resistant rice.

Indexes	Rice variety	
	Rain-fed (PUSA-44)	Drought resistant (Sahbhagi Dhan)
Simpson's index (D)	0.14	0.16
Simpson's diversity index (1-D)	0.86	0.84
Simpson's reciprocal index (1/D)	7.15	6.36
Shannon wiener diversity index (H')	2.14	1.95
Evenness	0.83	0.85
Richness	1.00	1.01
Total number of isolates	120	62

Overall, the rain-fed rice possessed a higher diversity of endophytes than drought resistant. Both the varieties had similar species evenness and richness indexes. An evenness index of 0.83 and 0.85 was seen in rain-fed and drought resistant rice, respectively. A richness index of 1.00 and 1.01 was observed in the said varieties, respectively (Table 5.1). Furthermore, Sorenson's index assessed the similarity between the fungal endophytes isolated from rain-fed and drought resistant. A high index of 0.84 was observed, indicating 84.21% similarity between the populations isolated from both varieties.

5.8 Diversity studies of endophytic fungi of rain-fed and drought-resistant rice variety throughout the crop cycle

The diversity of fungal endophytes was also assessed throughout the crop cycle. An increase in the Simpson's index was observed throughout the crop cycle of rain-fed rice. Here, the lowest index of 0.07 was observed during the vegetative phase (15-45th day) of the crop cycle. In contrast, the highest index of 0.2 was seen around the initiation of the reproductive phase (60th day). On the contrary, in drought resistant rice, the Simpson's index increased during the reproductive phase and decreased during maturation. The lowest index of 0.05 was observed around the maturation phase (90th day), whereas the highest index of 0.33 was seen during the vegetative stage 15-30th day).

On analysing the Simpson's diversity index, the highest diversity with an index of 0.93 was observed during the vegetative phase of rain-fed rice, after which a decrease was seen.

On the other hand, the highest diversity with an index of 0.95 was seen during the maturation phase of drought resistant rice (Fig. 5.14a). In the case of the Shannon Wiener diversity index, an asymmetrical bell shape curve was observed in both varieties. The highest diversity with an index of 2.03 and 1.75 was observed in rain-fed and drought resistant rice respectively, during the reproduction to maturation phase of the cycle (Fig. 5.14b). In addition, the species' evenness declined with a value of 0.84 in rain-fed rice around the initial reproductive phase (60^{th} day). In contrast, a sharp increase with a value of 0.97 was seen in drought resistant rice around the maturation phase (90^{th} day) (Fig. 5.15a). The species richness declined, with a value of 1.17 close to the end of the crop cycle in rain-fed rice. In drought-resistant rice, there was a sharp increase with a value of 2.26 close to the end of the crop cycle (Fig. 5.15b).

5.9 Diversity studies of endophytic fungi of rain-fed and drought-resistant rice variety among plant parts

The diversity of fungal endophytes was also assessed among different plant parts of the rice plant. The highest diversity among different plant parts of rain-fed rice was observed in the roots with Simpson's index (D) of 0.11 (Table 5.2). This was closely followed by internode and spikes whereas the lowest diversity was seen in leaves with Simpson's index (D) of 0.16. Likewise, the highest Shannon wiener diversity index of 2.13 was also observed in roots of rain-fed rice. The different plant parts exhibited an evenness index ranging from 0.87-0.93 whereas the highest Richness index of 10 was observed in the roots.

Interestingly, in the case of drought resistant rice, the highest diversity among different plant parts was observed in the internode with Simpson's index (D) of 0.12 (Table 5.3). This was closely followed by spikes. Whereas the leaves and roots had lowest diversity. The highest Shannon Wiener diversity index of 1.79 was also observed in internode of drought resistant rice. This was closely followed by roots with Shannon wiener diversity index of 1.73. The evenness index ranged from 0.79-0.95 whereas the highest Richness index of 9 was observed in the roots.

On analysing the different plant parts of rain-fed rice, highest similarity with Sorenson's index of 0.88 i.e., 88.88% was seen between the isolates of root and spike (Fig. 5.16a). This was closely followed by leaf-internode with Sorenson's index of 0.82. On the contrary, the least similarity was seen among isolates of internode and spike with Sorenson's index of 0.62 (Fig. 5.16a). Similarly, in the case of drought resistant rice, the highest similarity with Sorenson's index of 0.83 was observed in isolates of internode and spike. In comparison, the least similarity was seen among isolates of leaf-root and root-spike with Sorenson's index of 0.57 (Fig. 5.16b).

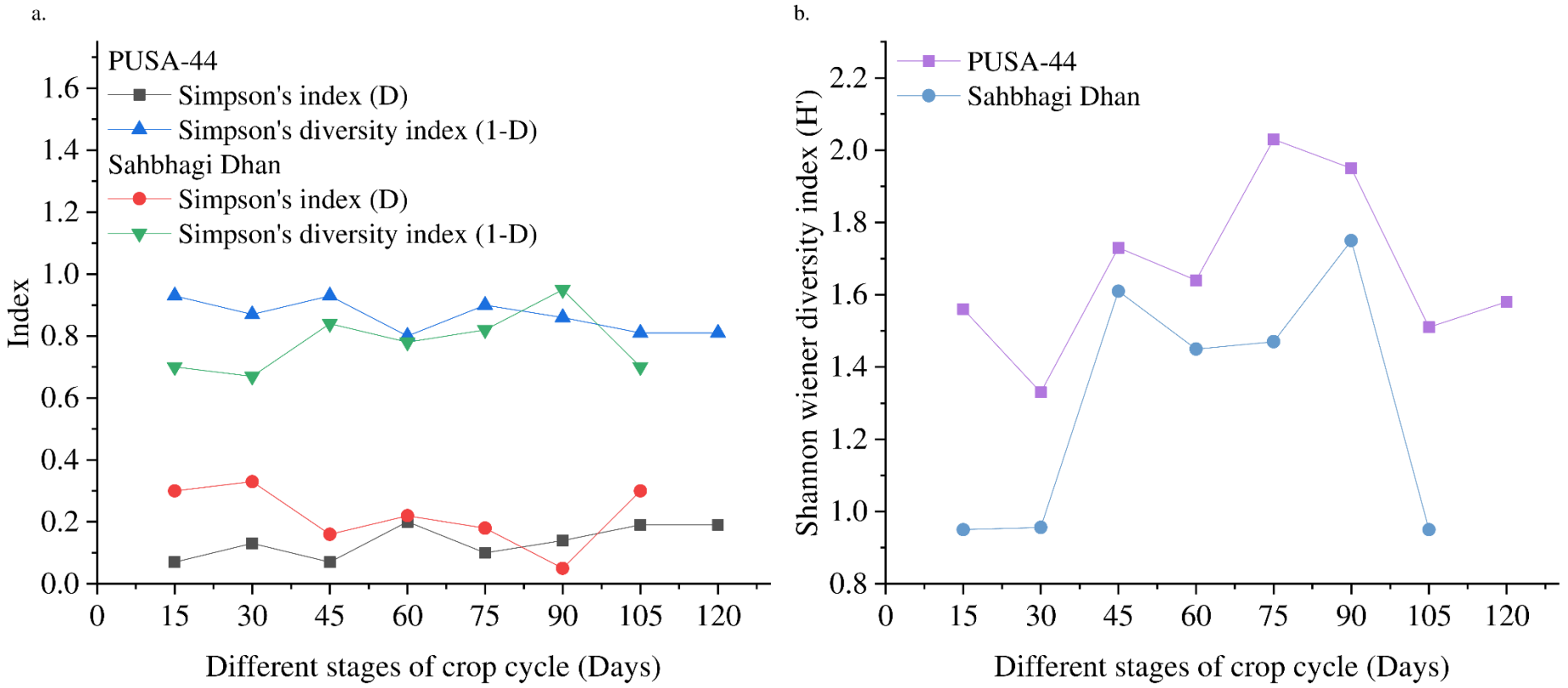

Fig. 5.14: Diversity analysis of endophytic fungi isolated from rain-fed and drought resistant rice during different stages of crop cycle a. Simpson's index and Simpson's diversity index; b. Shannon wiener diversity index.

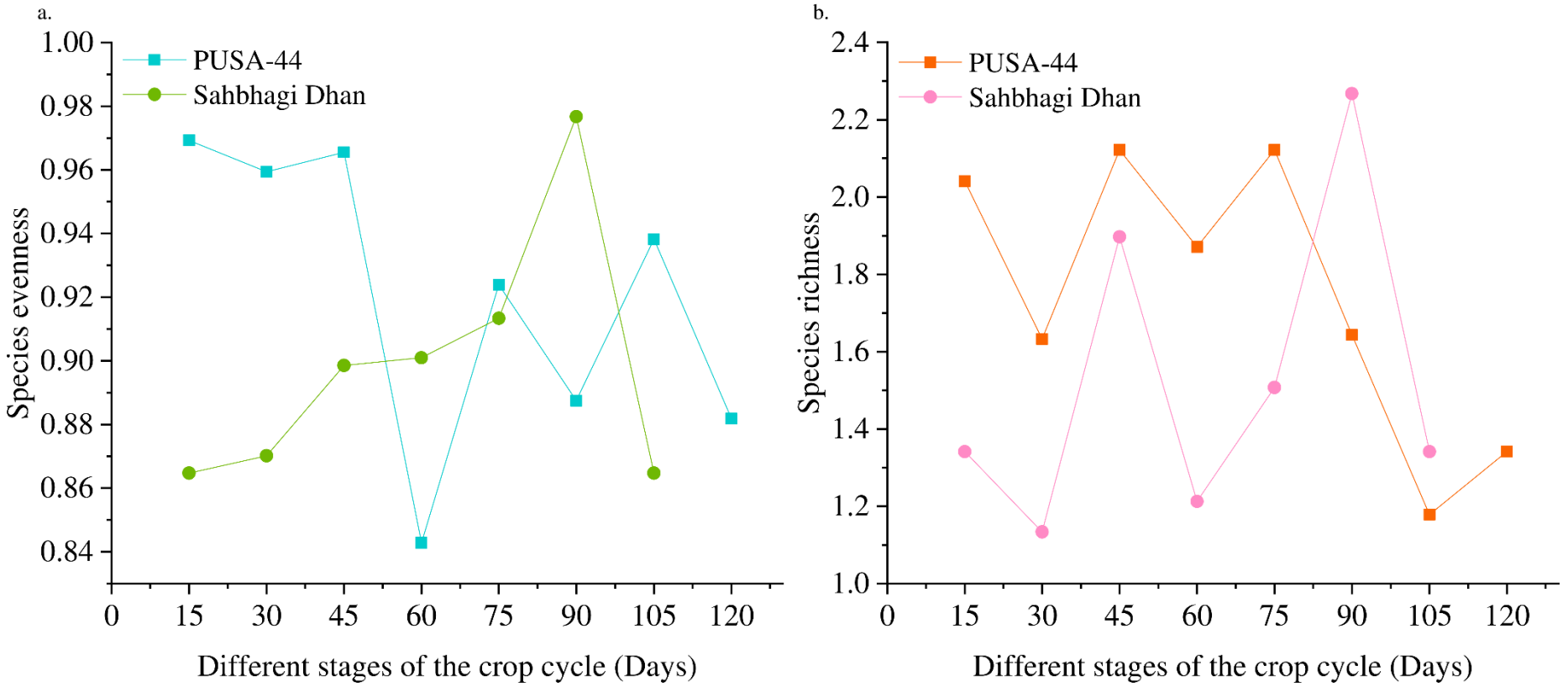

Fig. 5.15: Diversity analysis of endophytic fungi isolated from rain-fed and drought resistant rice during different stages of crop cycle a. Species evenness; b. Species richness.

Table 5.2: Diversity indexes for different plant parts of rain-fed rice.

Indexes	Rain-fed rice (PUSA-44)			
	Leaf	Root	Internode	Spike
Simpson's index (D)	0.16	0.11	0.13	0.15
Simpson's diversity index (1-D)	0.84	0.89	0.87	0.85
Simpson's reciprocal index (1/D)	6.09	9.36	7.5	6.84
Shannon wiener diversity index (H')	1.91	2.13	1.9	1.84
Evenness	0.87	0.93	0.92	0.88
Richness	9	10	8	8
Total number of isolates	40	32	25	23

Table 5.3: Diversity indexes for different plant parts of drought resistant rice.

Indexes	Drought resistant rice (Sahbhagi Dhan)			
	Leaf	Root	Internode	Spike
Simpson's index (D)	0.21	0.23	0.12	0.14
Simpson's diversity index (1-D)	0.79	0.77	0.88	0.86
Simpson's reciprocal index (1/D)	4.75	4.44	8.25	7.2
Shannon wiener diversity index (H')	1.47	1.73	1.79	1.52
Evenness	0.91	0.79	0.92	0.95
Richness	5	9	7	5
Total number of isolates	19	22	12	9

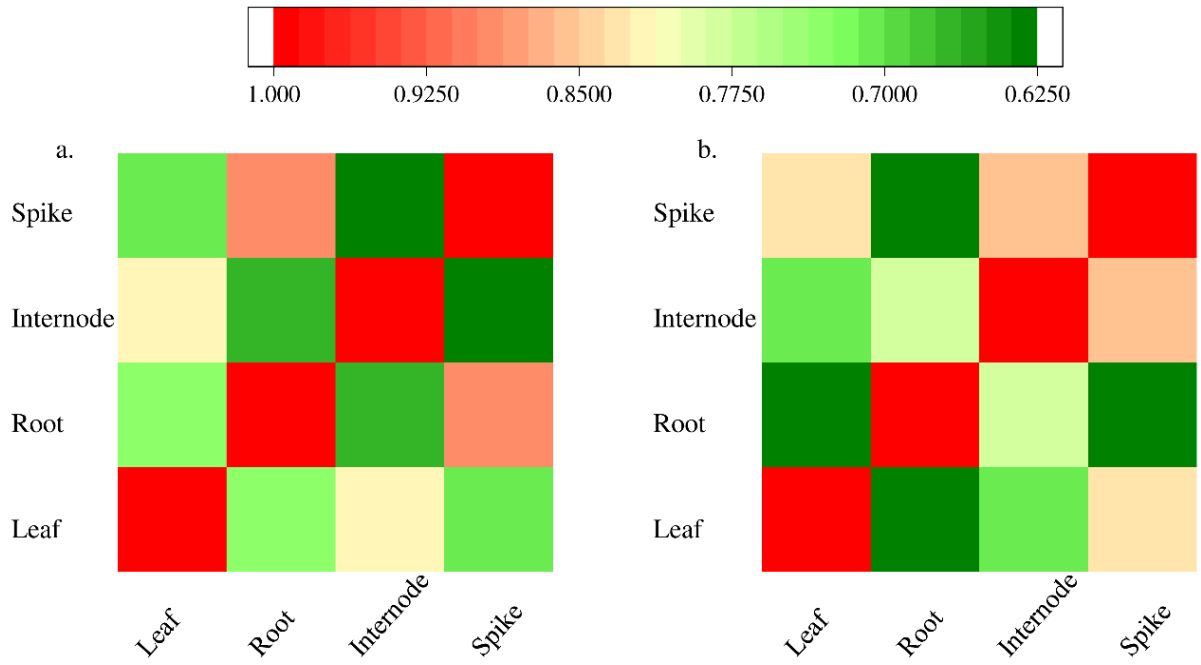

Fig. 5.16: Heatmaps showing Sorenson's index of endophytic fungi isolated from different plant parts of a. rain-fed rice; b. drought resistant rice variety.

5.10 Screening of endophytic fungi for abiotic stress tolerance

5.10.1 Salinity stress tolerance using plate and broth assay

On screening the endophytic isolates of rain-fed rice for salinity stress using plate assay, an overall reduction in growth was observed with an increase in NaCl concentration (Fig. 5.17). One-way ANOVA analysis revealed a significant difference among tolerance level of isolates (Table A3, A4-Appendix). It was observed that only 18 out of 25 internode isolates exhibited over 70% colony growth in a plate assay when 0.5 M NaCl salinity stress was induced. However, when the salinity stress was enhanced to 1 M and 1.5 M NaCl, the number of isolates surviving the stress and exhibiting over 70% colony growth reduced to 10 and 5, respectively. Further enhancement in the salinity stress to 2 M NaCl reduced the isolates to 2. The isolates, #6OSFI1b and #8OSFI2a exhibited 74.8 ± 2.9 and 73.5 ± 1.3% colony growth compared to the respective controls at 2 M NaCl (Fig. 5.17a).

Of the 32 endophytic isolates of the root, 26, 12 and 5 isolates exhibited over 70% colony growth under 0.5, 1.0 and 1.5M NaCl stress, respectively. Only two isolates, #6OSFR2e and #6OSFR2d could sustain further salinity stress using 2 M NaCl exhibiting 83.3 ± 0.0 and 76.7 ± 2.9% colony growth, respectively (Fig. 5.17b). In the case of leaves, of the 40 endophytic isolates, 27, 23 and 9 isolates exhibited over 70% colony growth in the plate assay under 0.5, 1.0 and 1.5 M NaCl induced salinity stress. When salinity stress using 2M NaCl was induced only two isolates, #6OSFL4c and #5OSFL6a exhibited 74.8 ± 2.2 and 70.1 ± 1.1% colony growth (Fig. 5.17c). In the case of 23 spike isolates, 14, 9 and 5 isolates exhibited more than 70% plate growth under salinity stress induced at a concentration of 0.5, 1.0 and 1.5 M NaCl. However, at salinity stress induced at 2M NaCl only two isolates, #5OSFS1a and #7OSFS3a with 80.5 ± 3.3 and 70.9 ± 1.1% growth as compared to the controls (Fig. 5.17d).

A similar trend was observed under broth assay, where a decrease in biomass was observed on increasing the NaCl concentration (Fig. 5.18). Out of the 11 tested internode isolates, #6OSFI1b and #8OSFI2a exhibited the highest growth of 74.8 ± 0.0 and 73.5 ± 0.0% under salinity stress induced using 2 M NaCl (Fig. 5.18a). Similarly, among the 12 root isolates, #6OSFR2e and #6OSFR2d exhibited 83.3 ± 0.0, and 76.7 ± 0.0% growth, respectively (Fig. 5.18b). Whereas out of the 20 endophytic leaf isolates, #6OSFL4c, #5OSFL6a exhibited 74.8 ± 0.0, 70.0 ± 0.0 % growth, respectively (Fig. 5.18c). Among the 9 spike isolates, #5OSFS1a and #7OSFS3a exhibited 80.9 ± 0.0, and 70.9 ± 0.0% growth under 2 M NaCl stress, respectively (Fig. 5.18d). Here, the isolates demonstrating more than 70% growth compared to their respective controls were selected for further analysis. Some of the tested isolates are shown in Fig. 5.19.

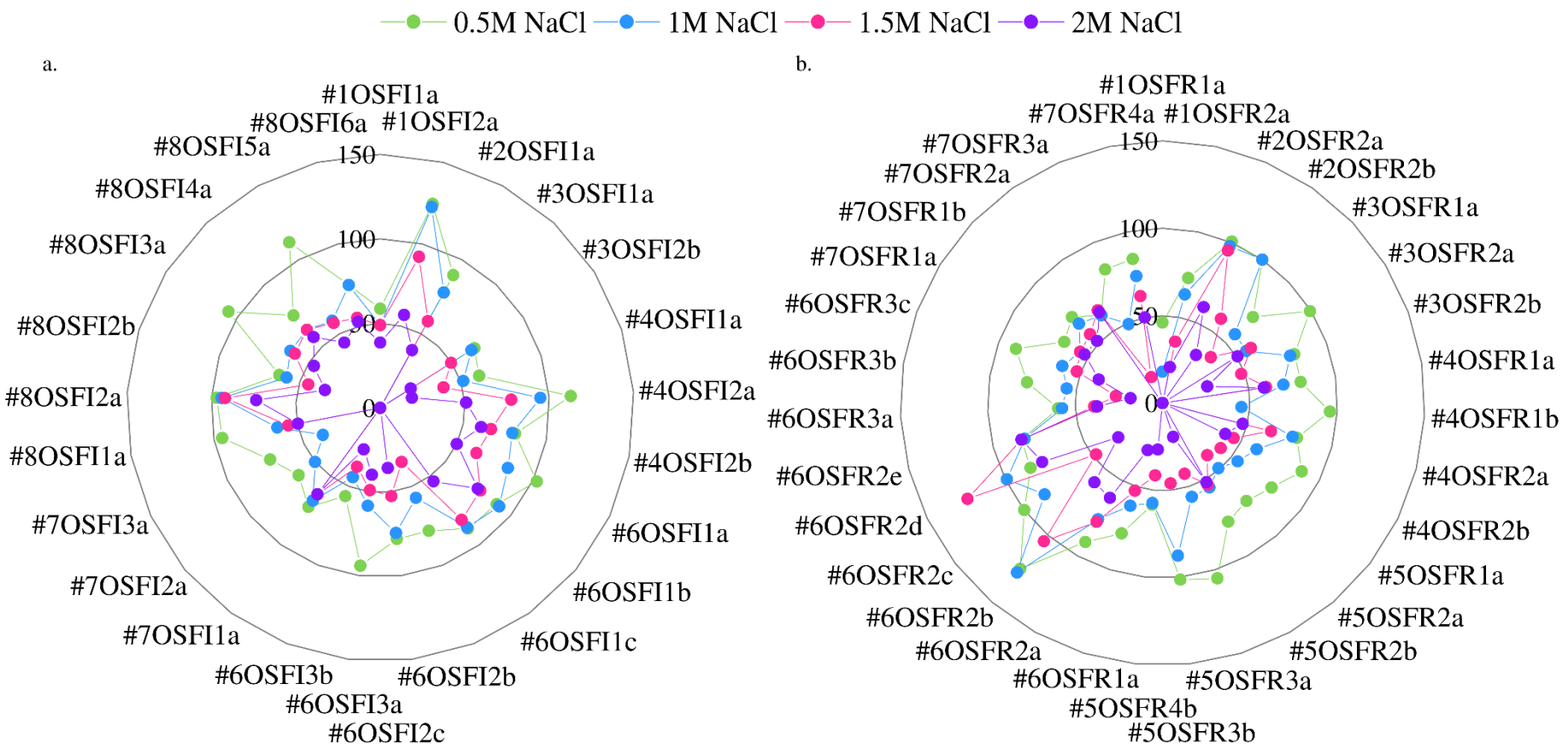

Fig. 5.17: Isolates from a. Internode; b. Root of rain-fed rice exhibiting growth under different levels of (w/v) NaCl in plate assay. The data represents mean ± SD, n=3.

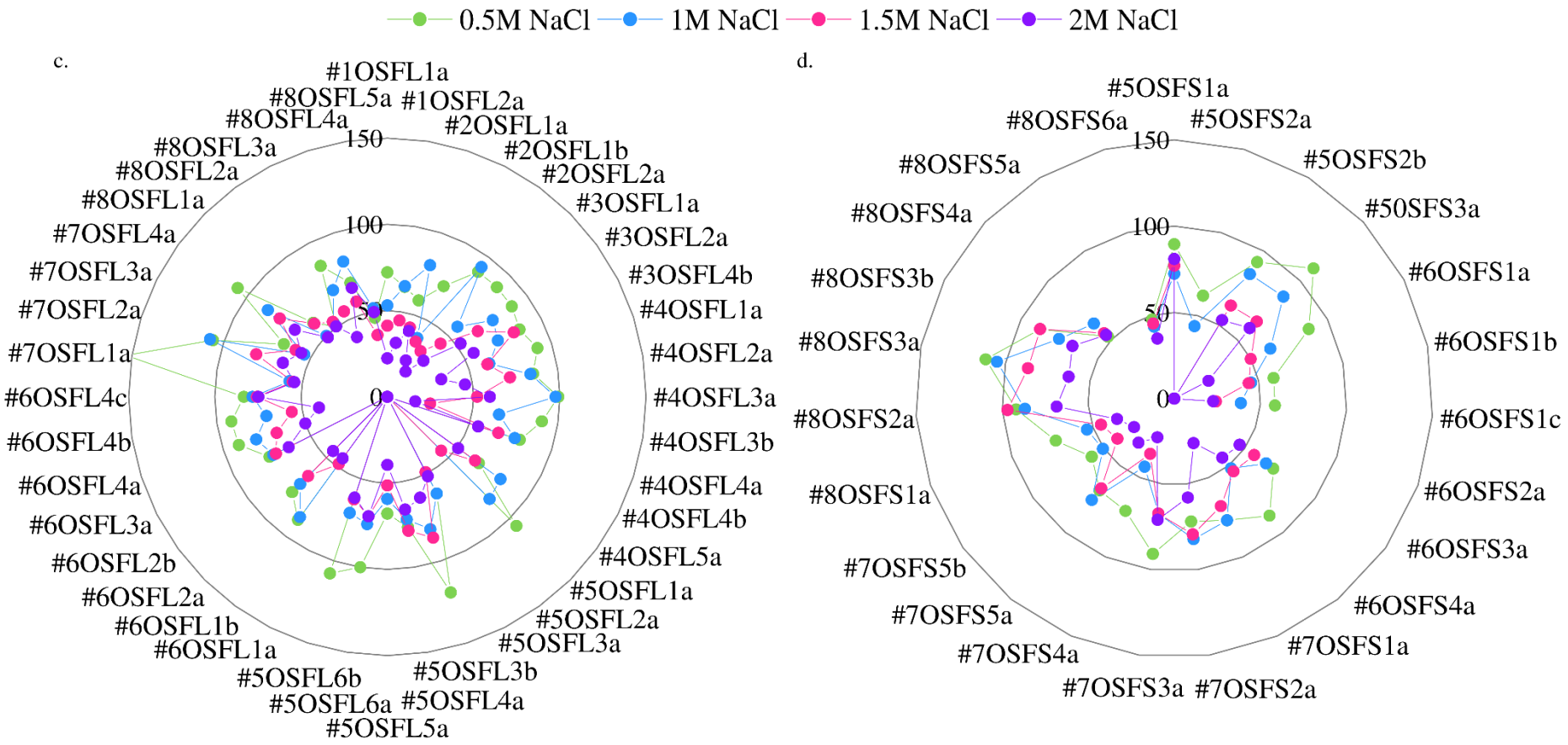

Fig. 5.17: Isolates from c. Leaves; d. Spikes of rain-fed rice exhibiting growth under different levels of (w/v) NaCl in plate assay. The data represents mean ± SD, n=3.

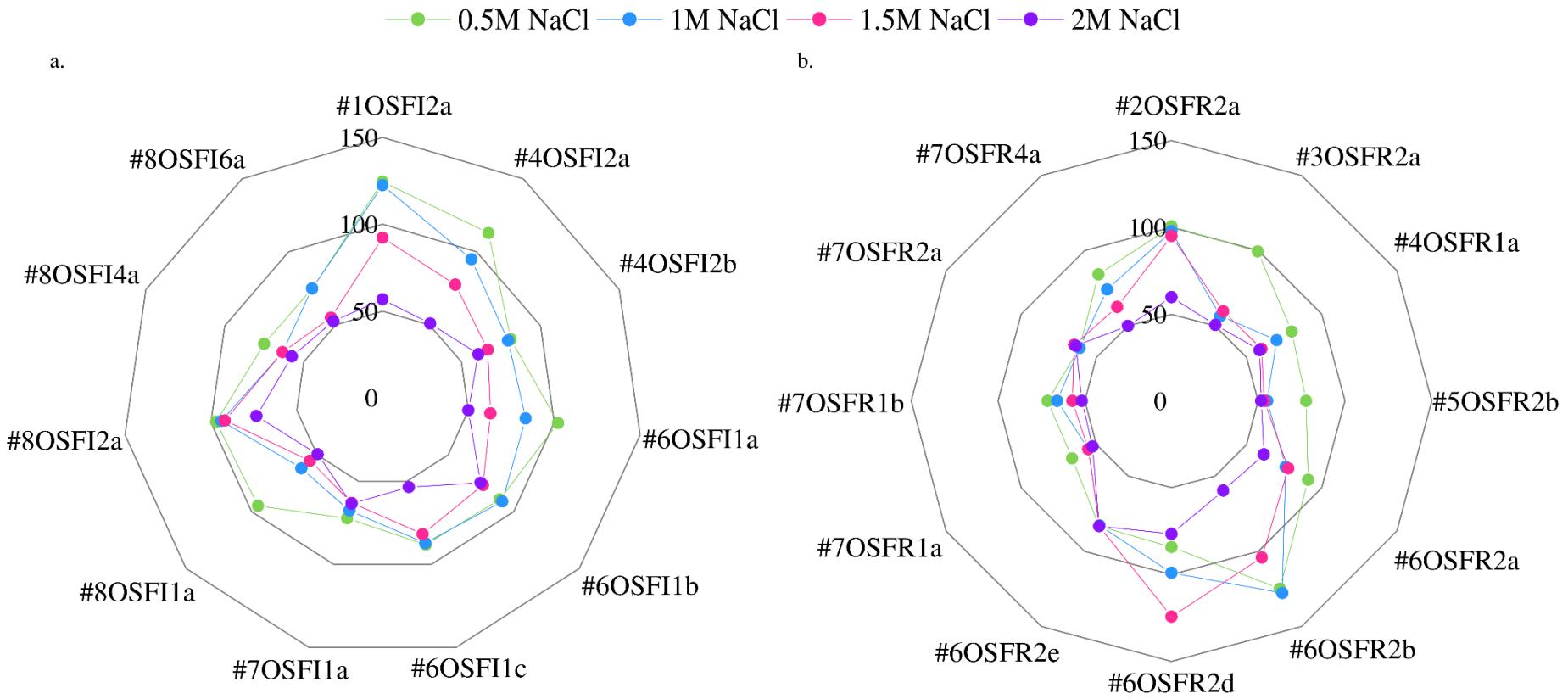

Fig. 5.18: Isolates from a. Internode; b. Root of rain-fed rice exhibiting growth under different levels of (w/v) NaCl in broth assay. The data represents mean ± SD, n=3.

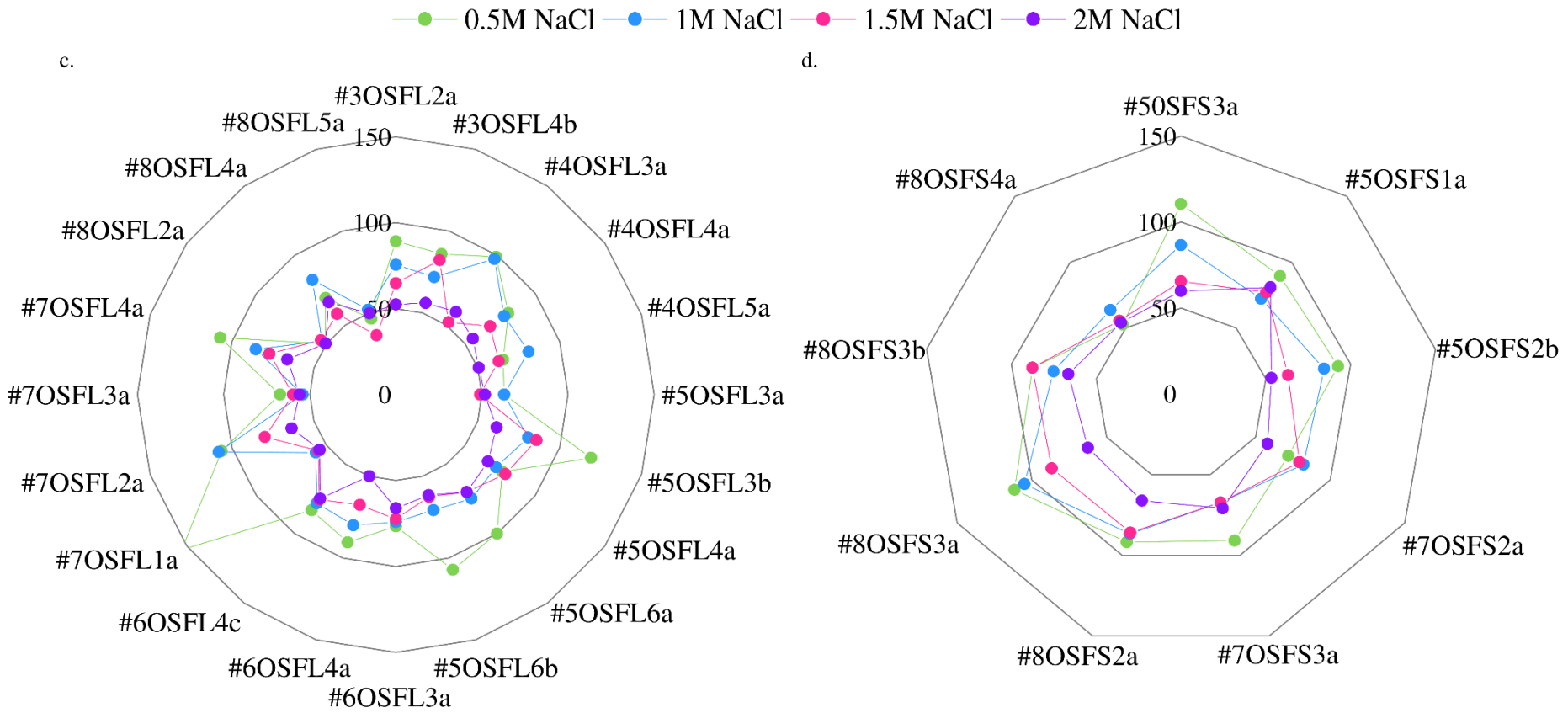

Fig. 5.18: Isolates from c. Leaves; d. Spikes of rain-fed rice exhibiting growth under different levels of (w/v) NaCl in broth assay. The data represents mean ± SD, n=3.

On screening the endophytic isolates of drought resistant rice for salinity stress using plate assay, a reduction in growth was observed in most isolates with an increase in NaCl concentration (Fig. 5.20). It was seen that only 4 out of 12 internode isolates displayed over 70% growth under 0.5 and 1 M NaCl. However, none of the isolates exhibited more than 70% growth when 1.5 M NaCl salinity stress was induced (Fig. 5.20a). Of the 22 endophytic isolates of the root, 9 and 7 exhibited more than 70% growth under 0.5 and 1 M NaCl, respectively. Only two isolates, #4OSTUR1e and #2OSTUR9a exhibited 79.7 ± 1.9 and 73.3 ± 0.8% colony growth, respectively, when compared to the control (Fig. 5.20b-c). In the case 19 leaf isolates, 13 and 11 exhibited more than 70% growth at 0.5 and 1 M NaCl stress. On increasing the salinity stress to 1.5 M NaCl, isolate #2OSTUL6d exhibited the colony growth of 73.9 ± 1.4%. However, on further increasing the salinity stress to 2 M NaCl, none of these isolates exhibited more than 70% growth as compared to the control. On the contrary, none of the 9 spike isolates exhibited more than 70% growth, even at the lowest NaCl concertation of 0.5 M (Fig. 5.20d). Some of the tested isolates are shown in Fig. 5.19.

A similar trend was recorded under broth assay, where a decrease in biomass was evident with increasing NaCl concentration. The three tested internode isolates exhibited more than 70% growth at 0.5 and 1 M NaCl. However, none of the isolates could survive on increasing the stress to 1.5 M NaCl (Fig. 5.21a). In the case of 9 root isolates, #4OSTUR1e, #2OSTUR9a exhibited 78.9 ± 0.1, 73.1 ± 0.2% growth, respectively, for 1.5 M NaCl stress. Similarly, out of the 14 leaf isolates, #2OSTUL6d exhibited 73.1 ± 0.0% growth when subjected to 1.5 M NaCl stress (Fig. 5.21b-c). Notably, none of the spike isolates exhibited more than 70% growth even at the lowest NaCl concertation of 0.5 M (Fig. 5.21d).

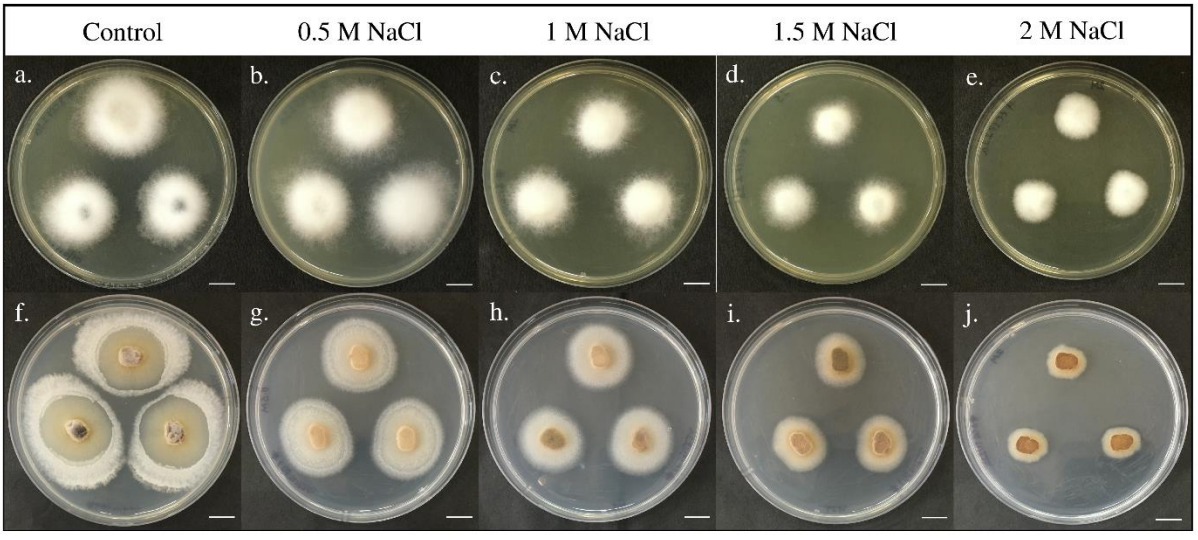

Fig. 5.19: Endophytic fungal isolates of a-e. rain-fed rice; f-j. drought resistant rice under different concentrations of NaCl (Bar: 10 mm).

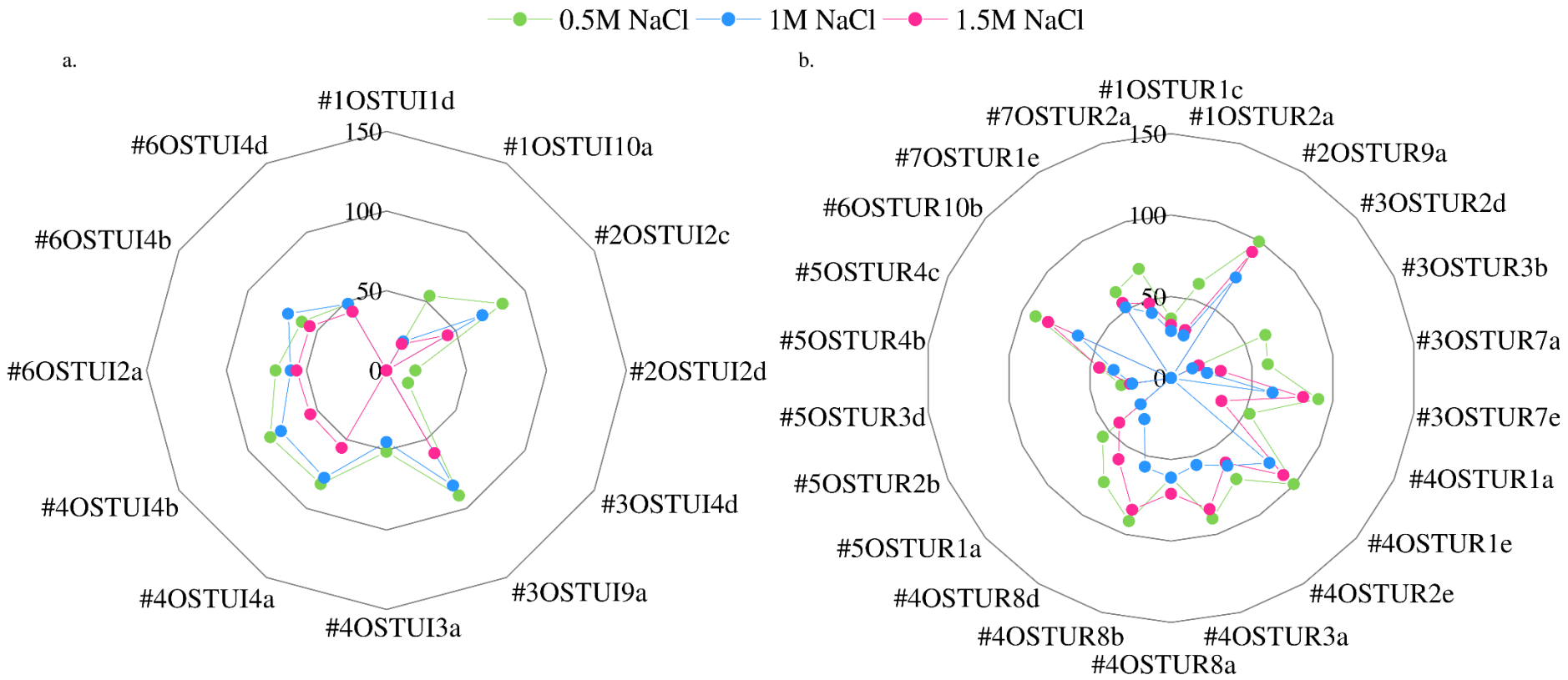

Fig. 5.20: Isolates from a. Internode; b. Root Spikes of drought resistant rice exhibiting growth under different levels of (w/v) NaCl in plate assay. The data represents mean ± SD, n=3.

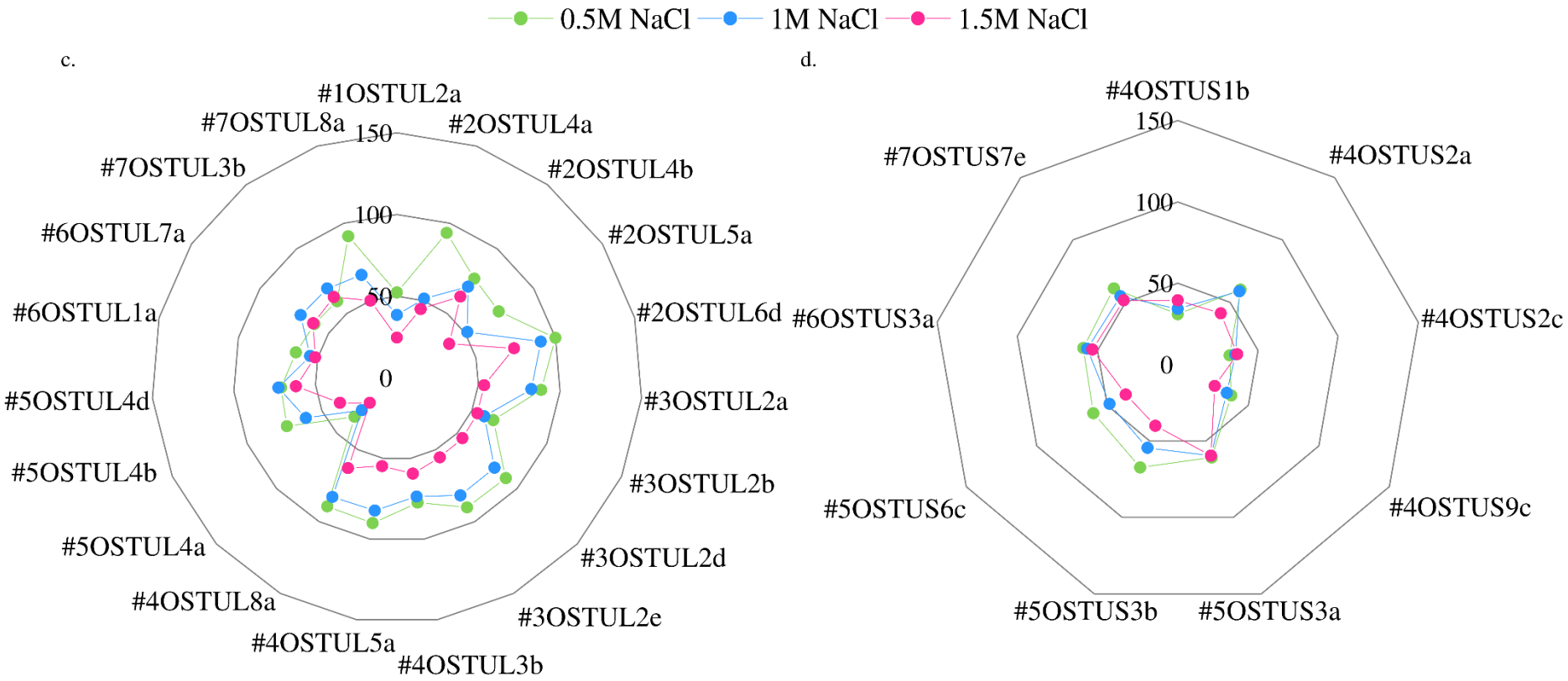

Fig. 5.20: Isolates from c. Leaves; d. Spikes of drought resistant rice exhibiting growth under different levels of (w/v) NaCl in plate assay. The data represents mean ± SD, n=3.

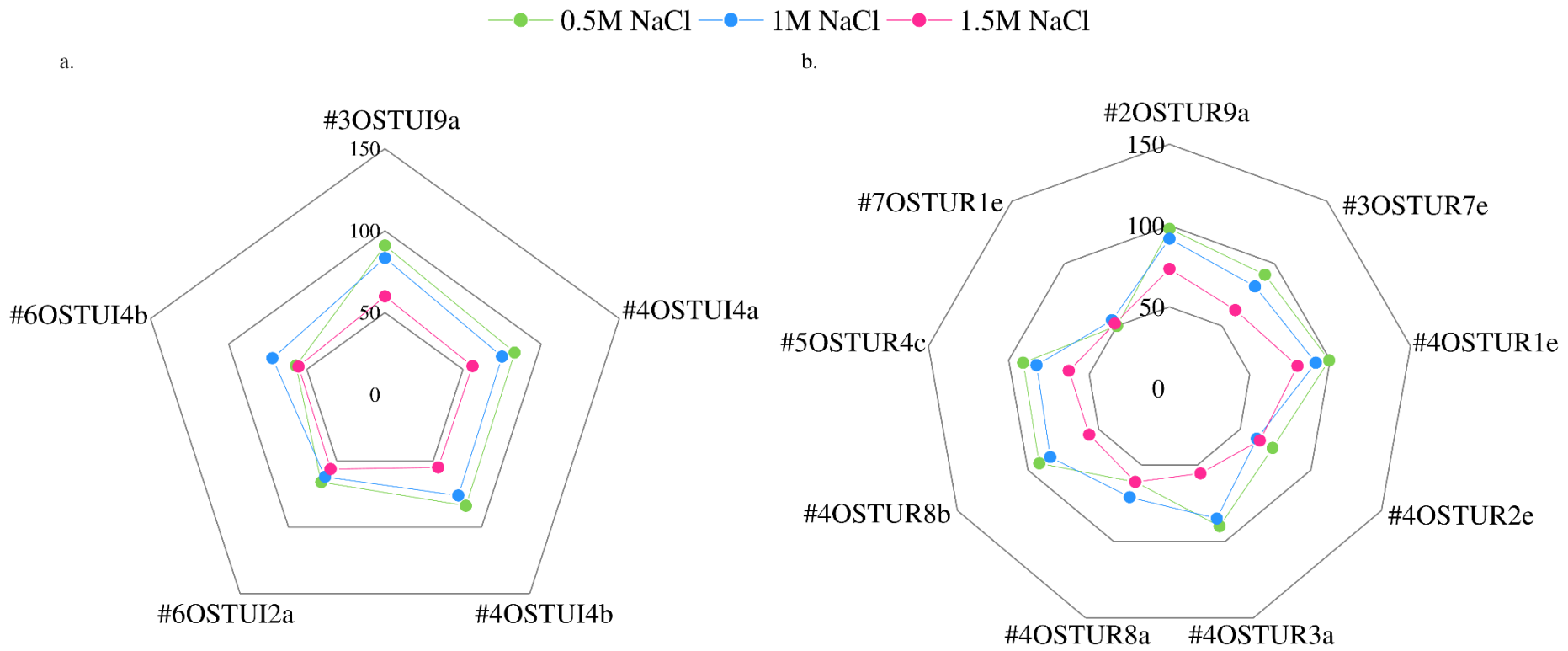

Fig. 5.21: Isolates from a. Internode; b. Root of drought resistant rice exhibiting growth under different levels of (w/v) NaCl in broth assay. The data represents mean ± SD, n=3.

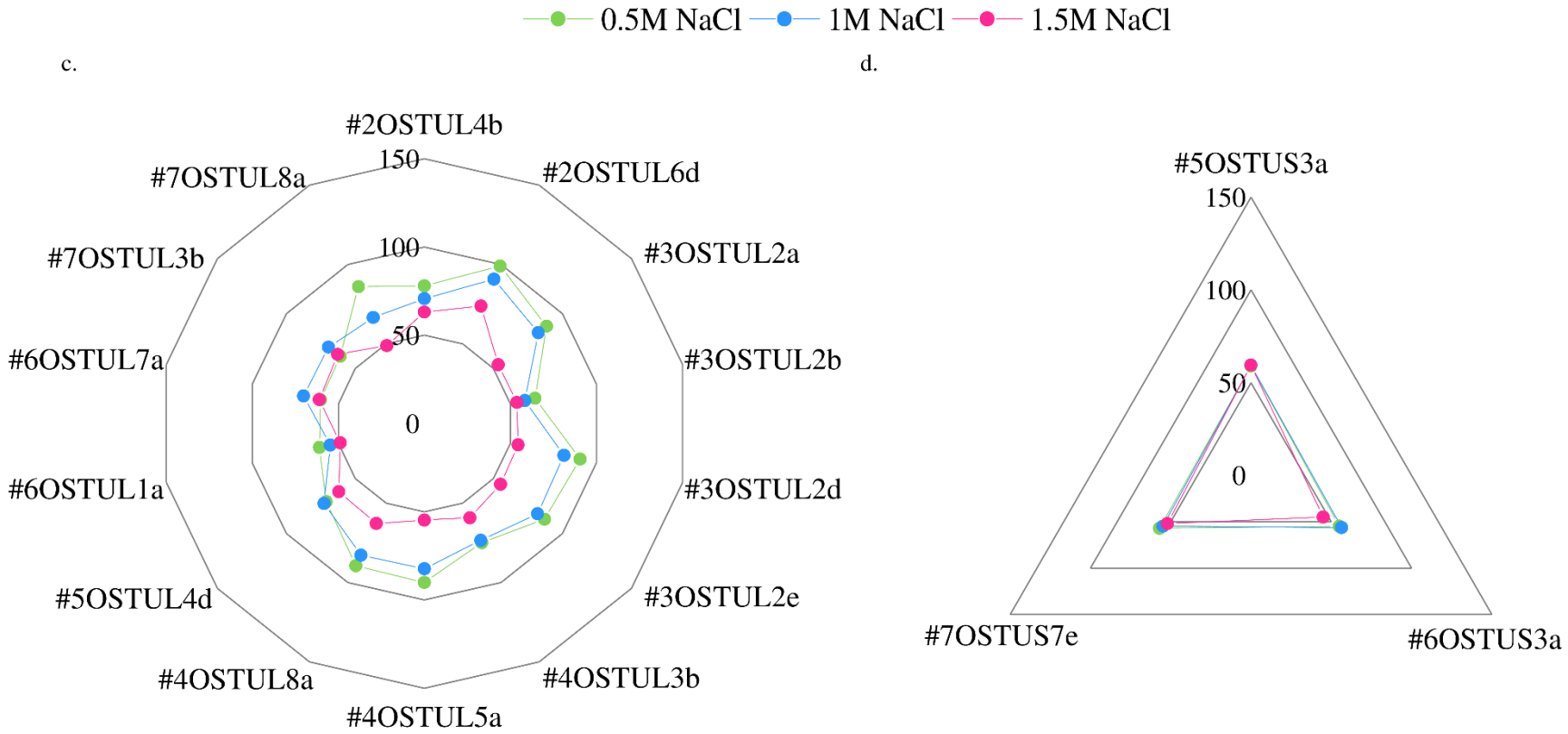

Fig. 5.21: Isolates from c. Leaves; d. Spikes of drought resistant rice exhibiting growth under different levels of (w/v) NaCl in broth assay. The data represents mean ± SD, n=3.

5.10.2 Drought stress tolerance using plate and broth assay

On screening the isolates of rain-fed rice for drought tolerance, an overall reduction in growth was observed with an increase in PEG concentration (Fig. 5.22). One-way ANOVA analysis revealed a significant difference among tolerance level of isolates (Table A3, A4-Appendix). On testing the 25 internode isolates, 21, 12 and 7 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. Further increasing the concentration to 20% PEG only one isolate, #8OSFI2a exhibited 76.0 ± 0.4% colony growth (Fig. 5.22a). Of the 32 endophytic isolates of root 26, 16 and 5 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. Only two isolates, #6OSFR2e and #6OSFR2d could sustain further drought stress with 20% PEG exhibiting 84.8 ± 0.9 and 78.5 ± 1.3% colony growth (Fig. 5.22b). In the case of 40 leaf isolates, 30, 23 and 10 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. However, under 20% PEG stress, only one isolate, #6OSFL4c exhibited 80.7 ± 1.2% growth (Fig. 5.22c). A similar trend was observed while testing endophytic isolates from spike. Among the 23 spike isolates, 14, 11 and 7 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. Whereas under 20% PEG stress only two isolates, #5OSFS1a and #7OSFS3a exhibited more than 70% growth with 88.7 ± 2.7 and 75.3 ± 2.4% growth (Fig. 5.22d).

Furthermore, in broth assay, a reduction in biomass was observed on increasing the PEG concentration (Fig. 5.23). The 11 internode isolates tested exhibited more than 70% growth under 5% PEG stress. However, this number reduced to 8 and 5 isolates when 10 and 15% PEG was used to induce drought stress. Under 20% PEG stress only one isolate, #8OSFI2a exhibited 73.5 ± 0.0% growth (Fig. 5.23a). Among the 12 root isolates, 10, 7 and 5 exhibited more than 70% growth under 5, 10 and 15% PEG stress. Under 20% PEG stress, isolates #6OSFR2e and #6OSFR2d exhibited 85 ± 0.0 and 78 ± 0.0% growth (Fig. 5.23b). In the case of the 21 leaf isolates, 15 exhibited more than 70% growth under 5% PEG stress. The number remained constant for 10% PEG stress. However, only eight isolates exhibited more than 70% growth under 15% PEG stress. Isolates #6OSFL4c exhibited 83.3 ±1.2% growth under 20% PEG stress (Fig. 5.23c). Among the tested nine spike isolates, 6 exhibited more than 70% growth under 5 and 10% PEG stress. This number reduced to 5 isolates under 15% PEG stress, whereas only two isolates #5OSFS1a and #7OSFS3a exhibited 80.9 ± 0.0 and 71.9 ± 1.1% growth under 20% PEG stress (Fig. 5.23d). Some of the tested isolates are shown in Fig. 5.24.

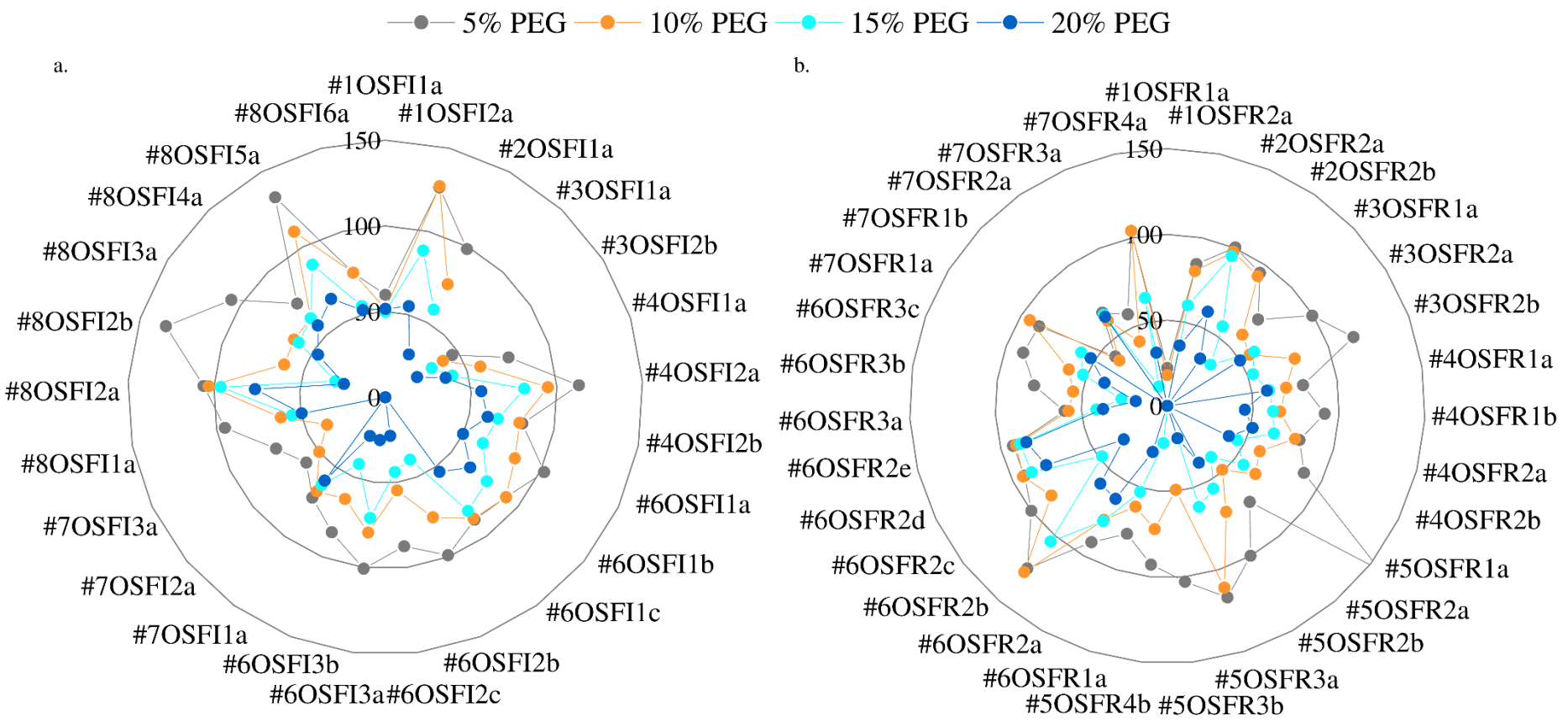

Fig. 5.22: Isolates from a. Internode; b. Root of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in plate assay. The data represents mean ± SD, n=3.

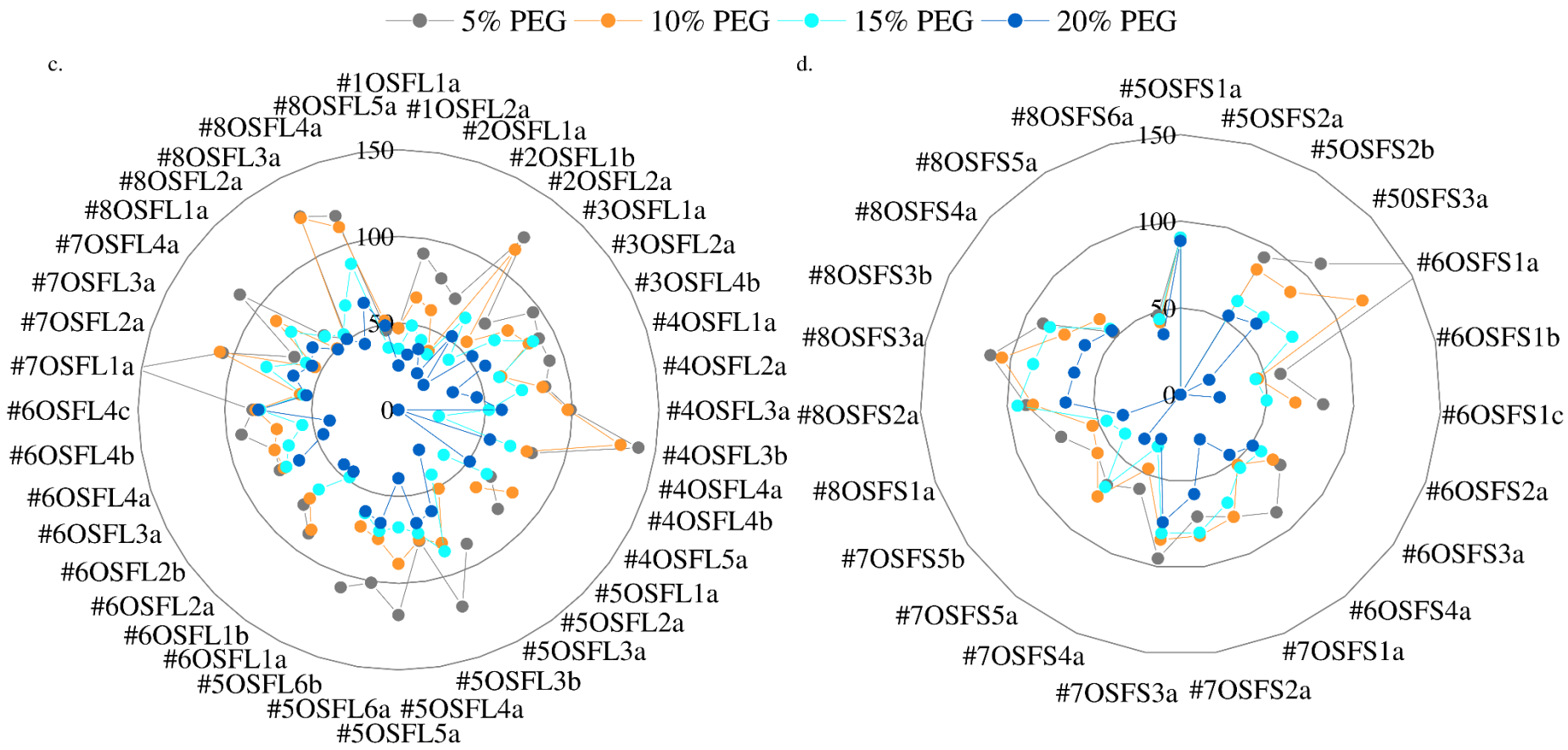

Fig. 5.22: Isolates from c. Leaves; d. Spikes of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in plate assay. The data represents mean ± SD, n=3.

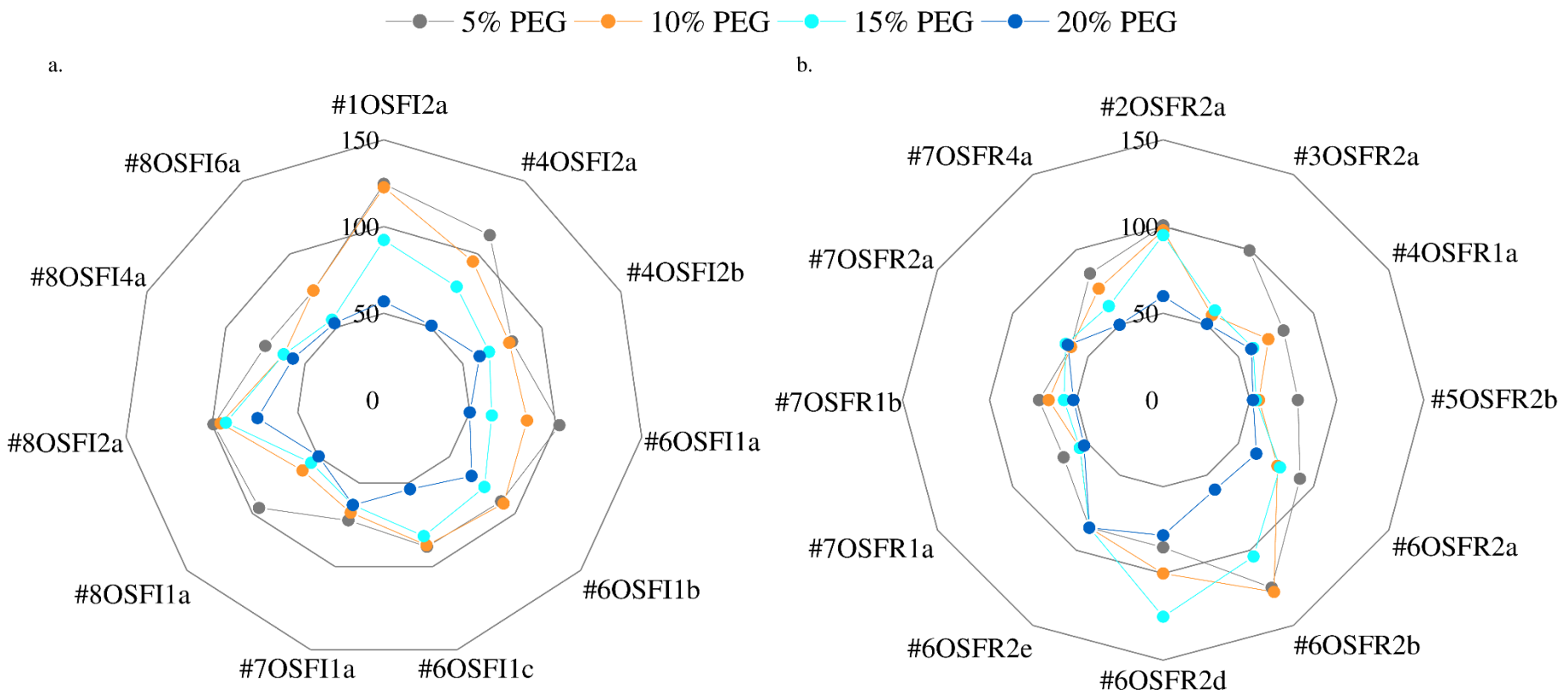

Fig. 5.23: Isolates from a. Internode; b. Root of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in broth assay. The data represents mean ± SD, n=3.

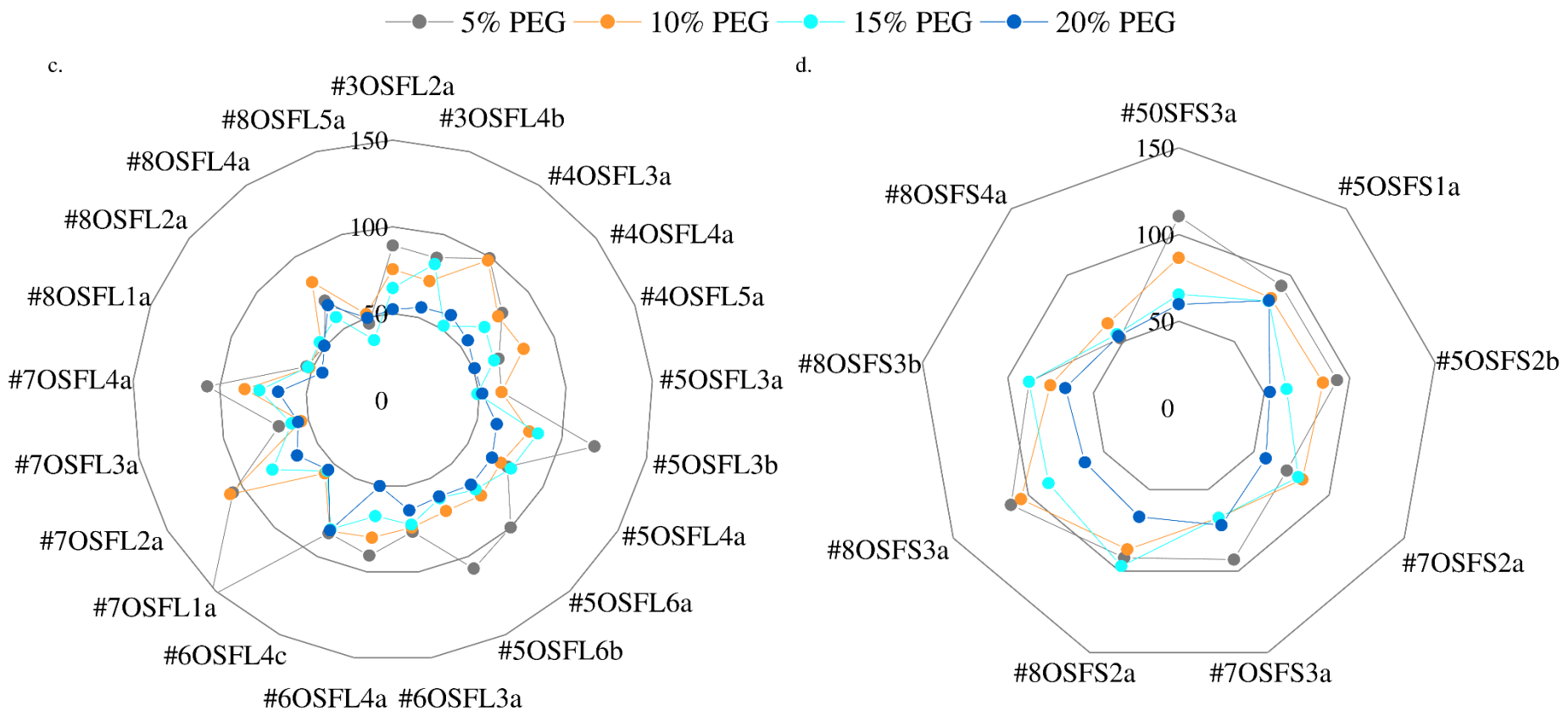

Fig. 5.23: Isolates from c. Leaves; d. Spikes of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in broth assay. The data represents mean ± SD, n=3.

On screening the isolates of drought resistant rice for drought tolerance, an overall reduction in growth was observed with an increase in PEG concentration (Fig. 5.25). A total of 6 out of 12 internode isolates, exhibited more than 70% growth under 5 and 10% PEG stress. However, no isolate exhibited growth beyond 10% PEG stress (Fig. 5.25a). Among the 22 isolates of root, 15, 6 and 4 exhibited more than 70% growth under 5, 10 and 15% PEG stress, respectively. Under 20% PEG stress isolates, #4OSTUR1e and #2OSTUR9a exhibited 77.8 ± 0.9 and 77.6 ± 0.8% colony growth (Fig. 5.25b). Among the 19 leaf isolates, 15, 10 and 6 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. Only one isolate, #2OSTUL6d, exhibited 71.2 ± 0.4% growth under 20% PEG stress (Fig. 5.25c). Among the 9 spike isolates, 5 and 3 exhibited more than 70% growth under 5 and 10% PEG stress, whereas no isolate exhibited growth beyond this concentration (Fig. 5.25d).

Further screening of the isolates under broth assay revealed a similar trend where the biomass decreased with increase in PEG concentration (Fig. 5.26). All the 5 tested internode isolates exhibited more than 70% growth under 5 and 10% PEG stress (Fig. 5.26a). However, no isolate exhibited growth beyond 10% PEG stress. In the case of 9 root isolates, 8, 6 and 4 exhibited more than 70% growth under 5, 10 and 15% PEG stress. Two isolates, #4OSTUR1e and #2OSTUR9a exhibited growth of 78 ± 0.0 and 76 ± 0.0% under 20% PEG stress (Fig. 5.26b). Among the tested 16 leaf isolates, 14, 11 and 6 isolates exhibited more than 70% growth under 5, 10 and 15% PEG stress. However, under 20% PEG stress, only #2OSTUL6d exhibited 72.0 ± 0.0% growth (Fig. 5.26c). The four tested spike isolates exhibited more than 70% growth under 5% PEG stress. This number reduced to 3 isolates at 10% PEG stress. However, none of the isolates exhibited growth beyond 10% PEG stress (Fig. 5.26d).

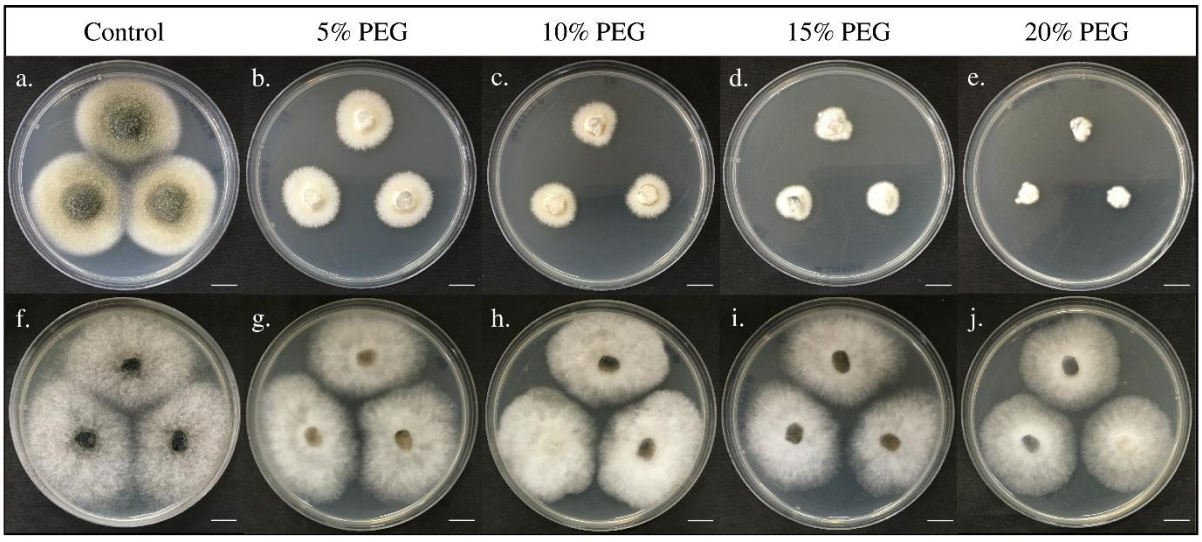

Fig. 5.24: Endophytic fungal isolates of a-e. rain-fed rice; f-j. drought resistant rice under different concentrations of PEG-6000 (Bar: 10 mm).

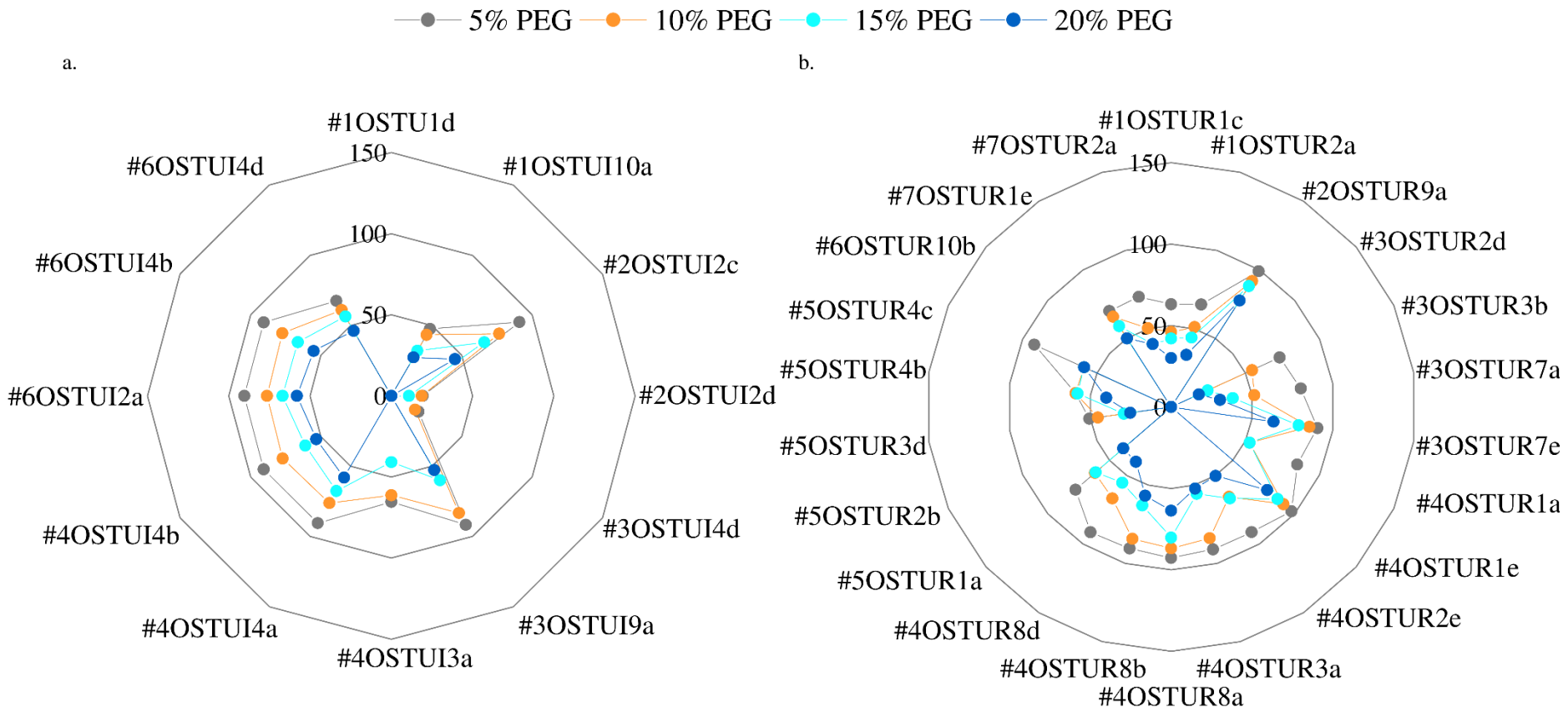

Fig. 5.25: Isolates from a. Internode; b. Root of drought resistant rice exhibiting growth under different levels of (w/v) PEG-6000 in plate assay. The data represents mean ± SD, n=3.

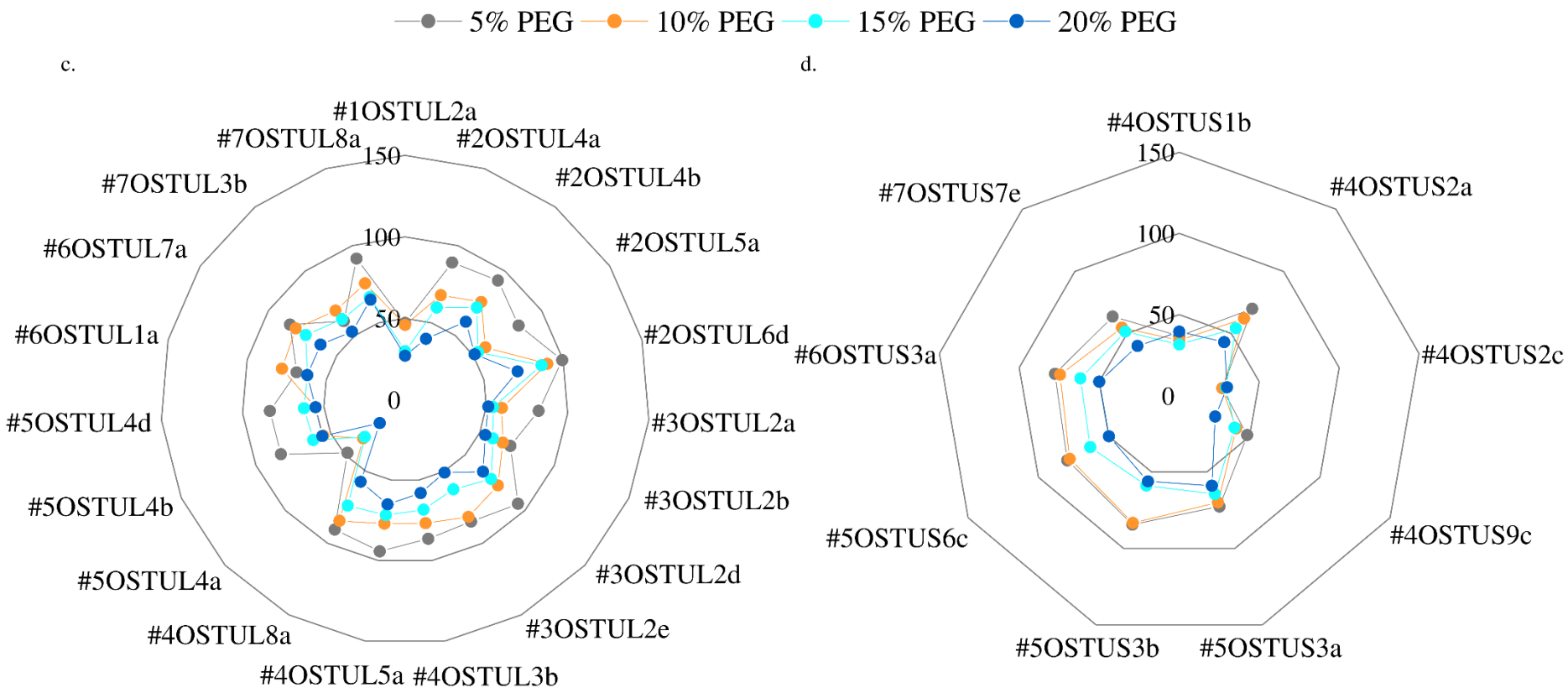

Fig. 5.25: Isolates from c. Leaves; d. Spikes of drought resistant rice exhibiting growth under different levels of (w/v) PEG-6000 in plate assay. The data represents mean ± SD, n=3.

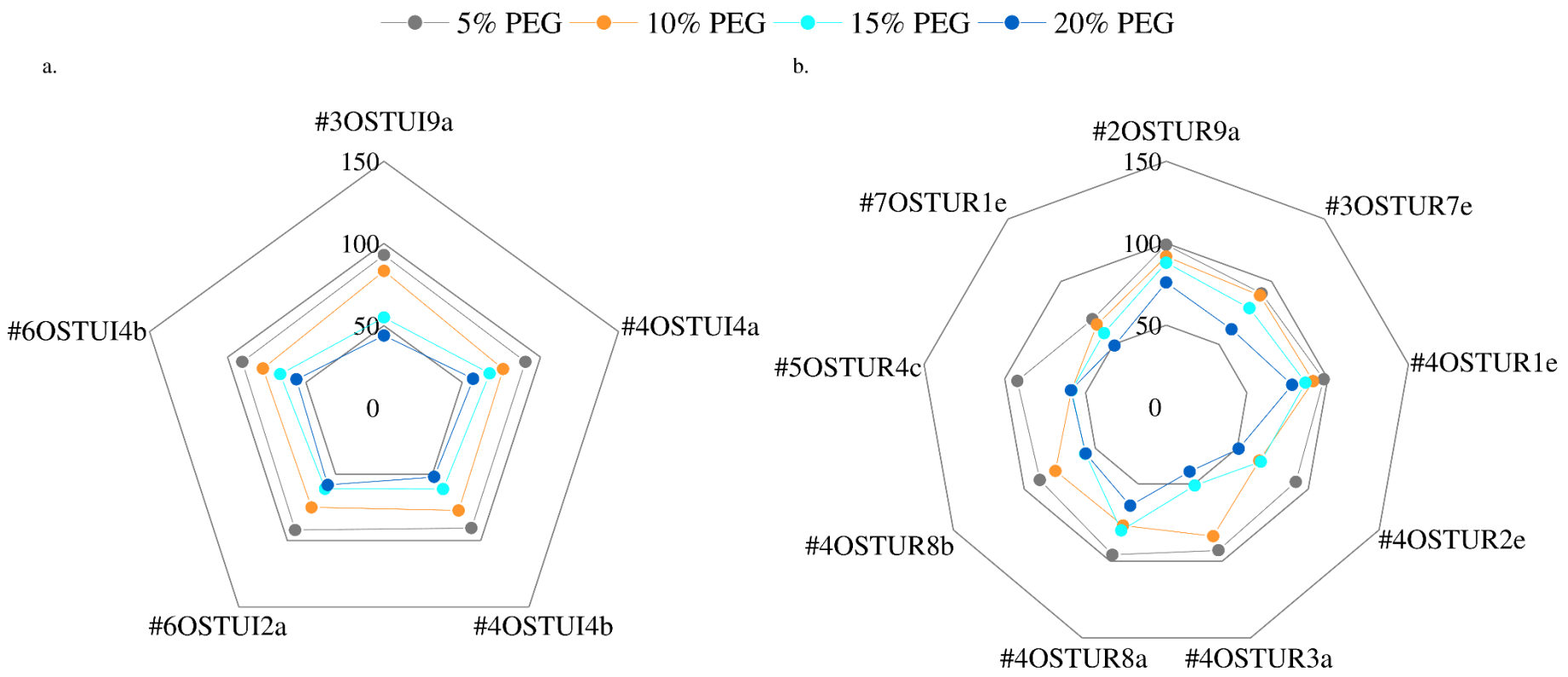

Fig. 5.26: Isolates from a. Internode; b. Root of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in broth assay. The data represents mean ± SD, n=3.

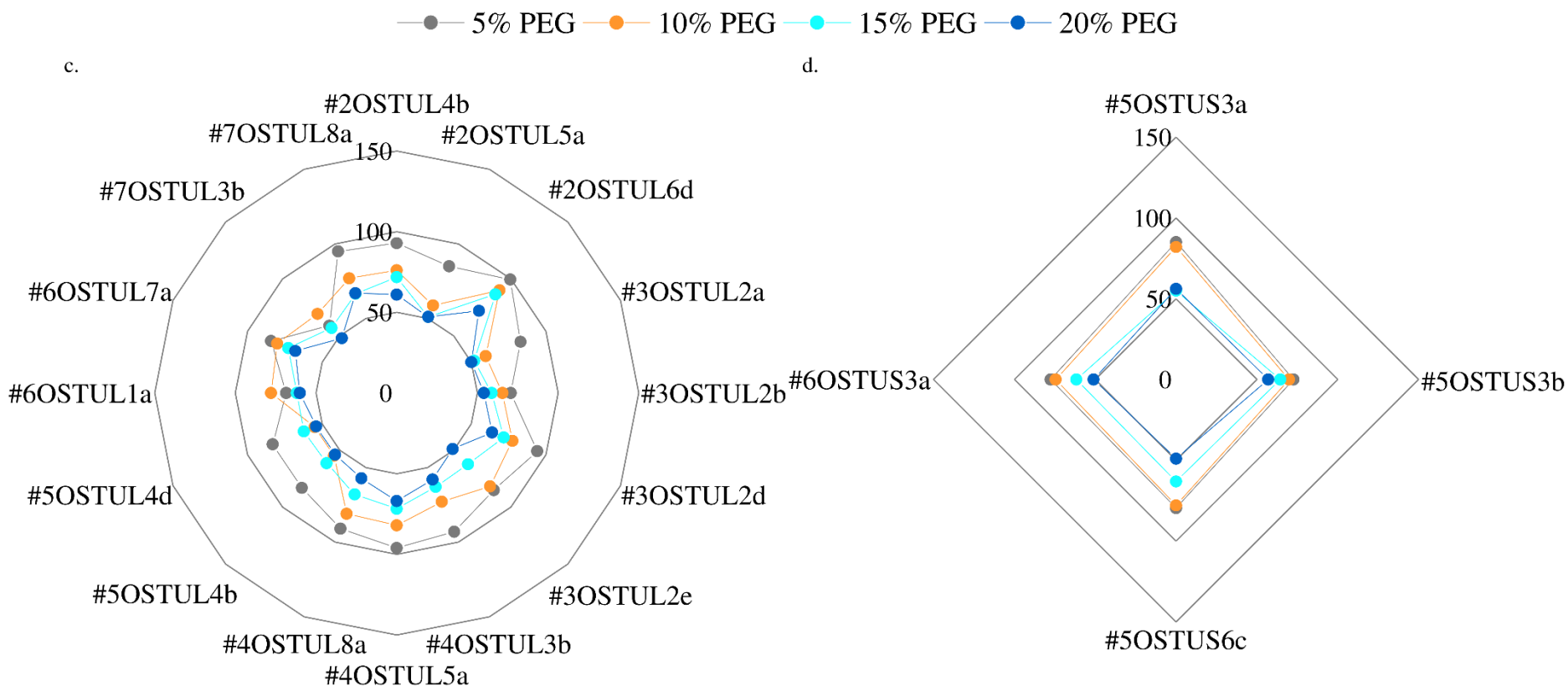

Fig. 5.26: Isolates from c. Leaves; d. Spikes of rain-fed rice exhibiting growth under different levels of (w/v) PEG-6000 in broth assay. The data represents mean ± SD, n=3.

5.11 Correlation analysis between plate and broth assay

A positive correlation was detected between plate and broth assay at different salinity and drought stress levels ($p < 0.001$). For isolates of rain-fed variety, at varying NaCl concentrations (0.5 to 2 M), Pearson's correlation coefficient (PCC) of 0.989, 0.939, 0.988, 0.998 was obtained (Fig. 5.27a). Whereas for the varying drought stress levels (PEG concentration 5 to 20%), PCC of 0.884, 0.861, 0.796, 0.795 was observed (Fig. 5.27b). Similarly, a PCC of 0.985, 0.998, 0.978 and 0.984 for drought-resistant rice isolates was observed at increasing NaCl concentration (Fig. 5.27c). Whereas, a PCC of 0.988, 0.970, 0.973 and 0.948 was observed at increasing PEG concentration (Fig. 5.27d).

5.12 Correlation analysis between drought and salinity stress

The growth of endophytic fungi under varying levels of salinity and drought exhibited a positive Pearson correlation coefficient in both rice varieties ($p < 0.001$). In the case of rain-fed rice, the endophytic fungi exhibited a similar growth pattern under increasing salinity and drought stress. Under plate assay, the most significant correlation of PCC 0.913 was observed between the growth of endophytic fungi at 2 M NaCl and 20% PEG. In contrast, the growth at 2 M NaCl and 5% PEG exhibited the lowest PCC value of 0.424 (Fig. 5.28a). Under broth assay, the most significant correlation of PCC 0.999 was observed between the growth of endophytic fungi at 0.5 M NaCl and 5% PEG. This was closely followed by 0.995 and 0.993 between growth at 1 M and 10% PEG and 1.5 M NaCl and 15% PEG. The lowest PCC value of 0.270 was observed between growth at 1 M NaCl and 20% PEG (Fig. 5.28b).

On the contrary, in the case of drought-resistant rice, the endophytic fungi did not exhibit a similar growth pattern under increasing salinity and drought stress. In the plate assay, the most significant correlation of PCC 0.953 was observed between the growth of endophytic fungi at 1.5 M NaCl and 20% PEG. The lowest PCC value of 0.601 was seen between growth at 2 M NaCl and 20% PEG (Fig. 5.28c). In the broth assay, the most significant correlation of PCC 0.942 was observed between the growth of endophytic fungi at 1.5 M NaCl and 20% PEG. In comparison, the growth at 2 M NaCl and 10% PEG exhibited the lowest PCC value of 0.294 (Fig. 5.28d).

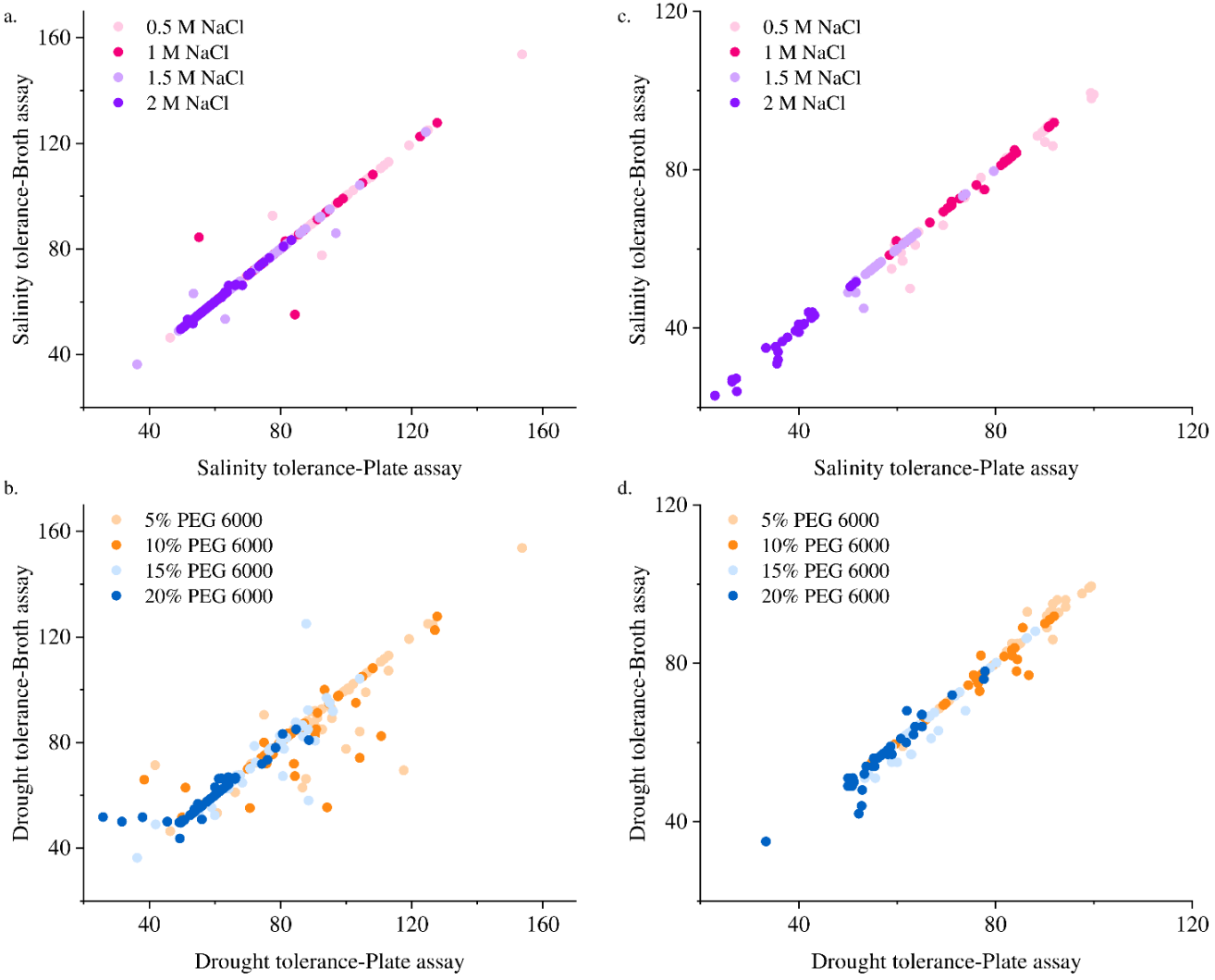

Fig. 5.27: Correlation analysis between plate and broth screening assay at different concentrations of NaCl and PEG 6000 a. Salinity tolerance of rain-fed rice isolates; b. Drought tolerance of rain-fed rice isolates; c. Salinity tolerance of drought resistant rice isolates; d. Drought tolerance of drought resistant rice isolates. The values represent mean ± SD, n=3.

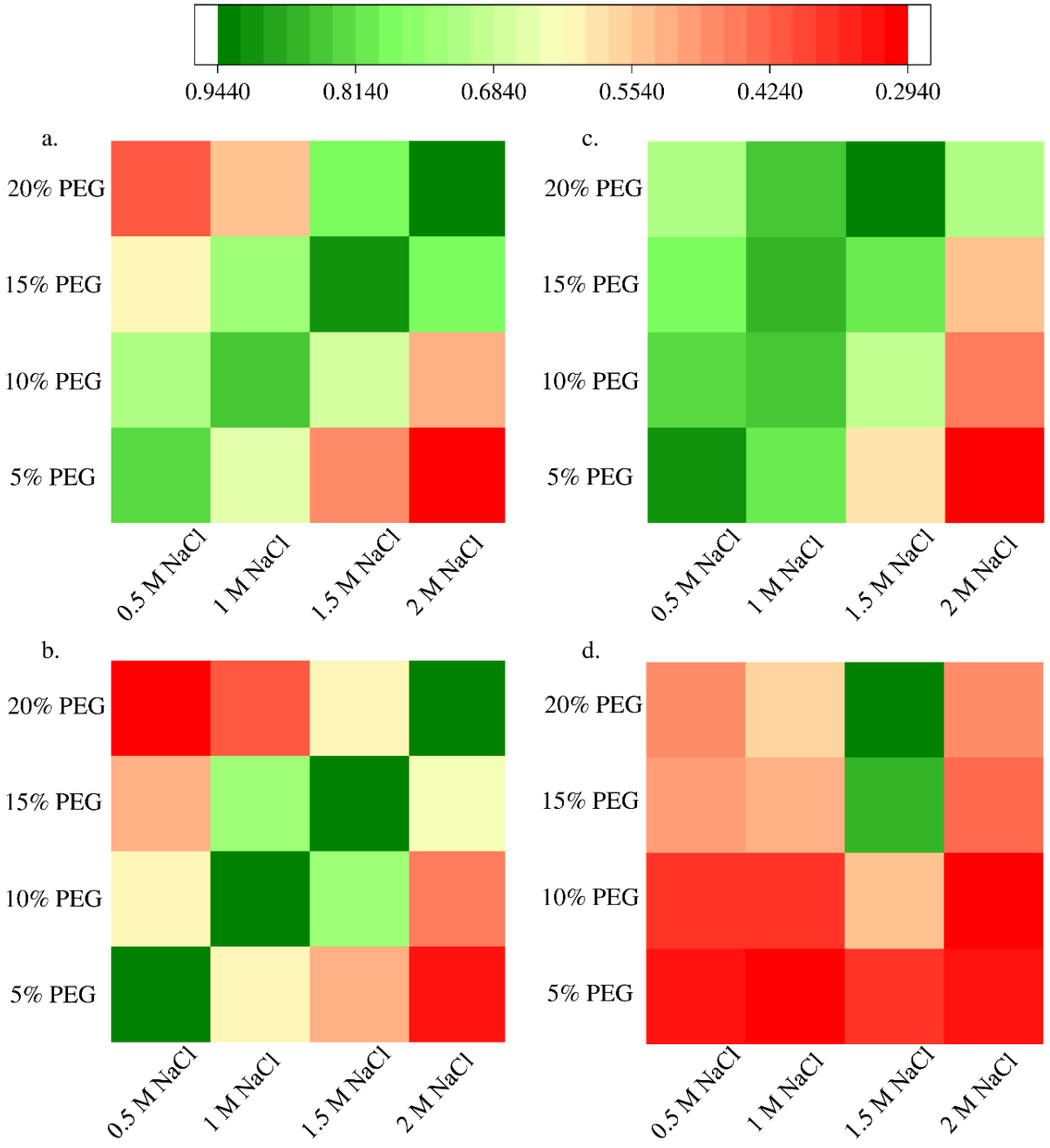

Fig. 5.28: Heatmaps showing Pearson correlation coefficient between growth of endophytic fungi under different concentrations of NaCl and PEG a. Rain-fed rice isolates under plate assay; b. Rain-fed rice isolates under broth assay; c. Drought resistant rice isolates under plate assay; d. Drought resistant rice isolates under broth assay.

5.13 Plant growth promoting attributes of selected endophytic fungi

5.13.1 *In-vitro* assay for Total Phenolic and Total Flavonoid Content

Among the tested isolates of rain fed variety, #6OSFR2e exhibited the highest TPC of 241.5 ± 4.9 GAE/mg of sample, followed by #7OSFS3a with 187.2 ± 2.3 GAE/mg of sample (Fig. 5.29a). Similarly, the highest TFC of 464.4 ± 4.4 QE/mg of sample was also exhibited by isolate #6OSFSR2e. This was closely followed by isolate #7OSFS3a at 444.1 ± 21.9 QE/mg of sample. In the case of drought-resistant rice, isolate #2OSTUR9a exhibited the highest TPC of 211.7 ± 0.3 GAE/mg of sample. The highest TFC of 408.3 ± 21.7 QE/mg of sample was also observed in isolate #2OSTUR9a. Followed by isolate #4OSTUR1e with TFC production of 342.7 ± 18.0 QE/mg of sample (Fig. 5.29b) (Table A5-Appendix).

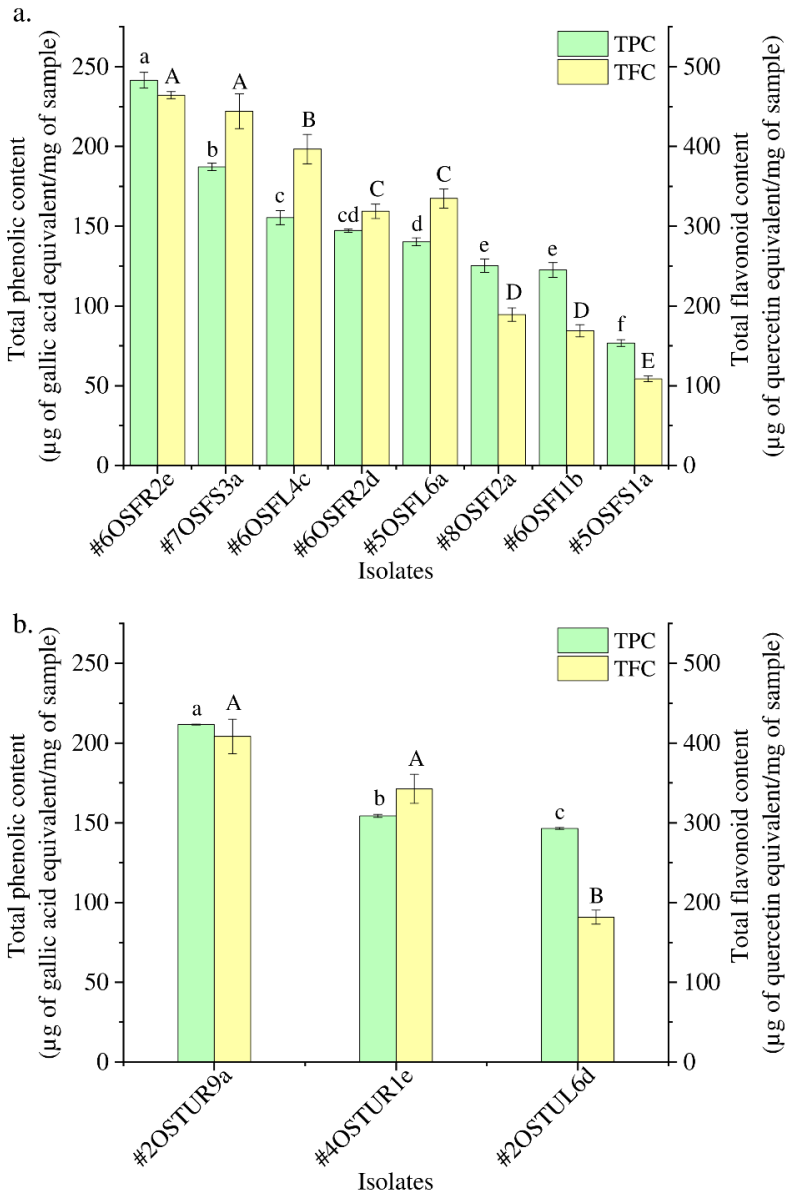

Fig. 5.29: Total phenolic and flavonoid content of selected endophytic fungal isolates of a. rain-fed rice; b. drought resistant rice variety. The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

5.13.2 *In-vitro* antioxidant potential

The tested isolates of both rice varieties also exhibited scavenging of different free radicals under *in vitro* conditions (Fig. 5.30). In the case of DPPH, the standard Quercetin exhibited a % FRS of 94.8 ± 0.6%. Among the rain-fed rice isolates, #6OSFR2e and #7OSFS3a exhibited the highest % FRS against DPPH radical with 88.8 ± 3.1 and 79.7 ± 1.0%, whereas isolate #5OSFS1a had the lowest % FRS of 50.0 ± 0.6% (Fig. 5.30a). Furthermore, a linear regression was used to calculate the IC_{50} value for each fungal extract. Here, isolates #7OSFS3a and #6OSFR2e had the best IC_{50} value of 345.4 ± 21.7 and 391.6 ± 20.7 µg/mL (Table 5.4). Among the drought-resistant rice isolates, #2OSTUR9a exhibited the highest % FRS of 82.4 ± 0.3% and the best IC_{50} value of 365.5 ± 10.0 µg/mL (Fig. 5.30d).

In the case of ABTS radical, Trolox exhibited the highest % FRS of 87.7 ± 0.2 %. Among the rain-fed rice isolates, #6OSFR2e, #6OSFL4c and #7OSFS3a demonstrated the maximum % FRS of 86.0 ± 0.20, 84.8 ± 0.2 and 81.0 ± 0.8% against ABTS radical (Fig. 5.30b). The isolate #6OSFR2e exhibited the best IC_{50} value of 366.0 ± 5.9 µg/mL. Among the drought-resistant rice isolates, #2OSTUR9a exhibited the highest % FRS value of 85.6 ± 0.3%, whereas the isolates #2OSTUL6d and #4OSTUR1e had a comparable % FRS of 79.6 ± 0.4 and 79.4 ± 0.3%, respectively (Fig. 5.30e). The isolate #2OSTUR9a also had the best IC_{50} value of 357.5 ± 7.1 µg/mL (Table 5.4).

In the case of H_2O_2 radical, standard ascorbic acid had the highest % FRS of 86.3 ± 0.1%. Among the rain-fed rice isolates, #6OSFR2e exhibited a comparable % FRS of 85.3 ± 0.3%, followed by isolate #6OSFR2d with % FRS of 82.2 ± 0.7%. In comparison, isolate #5OSFS1a had the lowest % FRS of 41.3 ± 1.2% against H_2O_2 radical (Fig. 5.30c). In addition, isolate #6OSFR2e had the best IC_{50} value of 227.8 ± 3.8 µg/mL, followed by isolate #7OSFS3a with IC_{50} of 284.9 ± 8.0 µg/mL (Table 5.4). Among drought-resistant rice isolates, #2OSTUR9a exhibited the highest % FRS of 82.15 ± 0.4%, closely followed by isolate #4OSTUR1e with % FRS of 80.5 ± 0.4% (Fig. 5.30f). Likewise, isolate #2OSTUR9a had the best IC_{50} value of 231.5 ± 7.7 µg/mL (Table 5.4).

Furthermore, among the rain-fed rice isolates, #6OSFR2e, #7OSFS3a and #6OSFL4c exhibited the highest FRAP activity of 78.9 ± 0.5, 76.8 ± 0.4 and 76.0 ± 0.3 Fe (II) equivalent/mg of extract. The lowest FRAP activity of 50.3 ± 0.4 Fe (II) equivalent/mg of extract was observed in isolate #6OSFI1b. Among drought-resistant rice isolates, #2OSTUR9a had the highest FRAP activity of 75.0 ± 0.5 Fe (II) equivalent/mg of extract. In contrast, isolate #4OSTUR1e demonstrated the lowest FRAP activity of 60.8 ± 2.0 Fe (II) equivalent/mg of extract (Table 5.4; Table A5-Appendix).

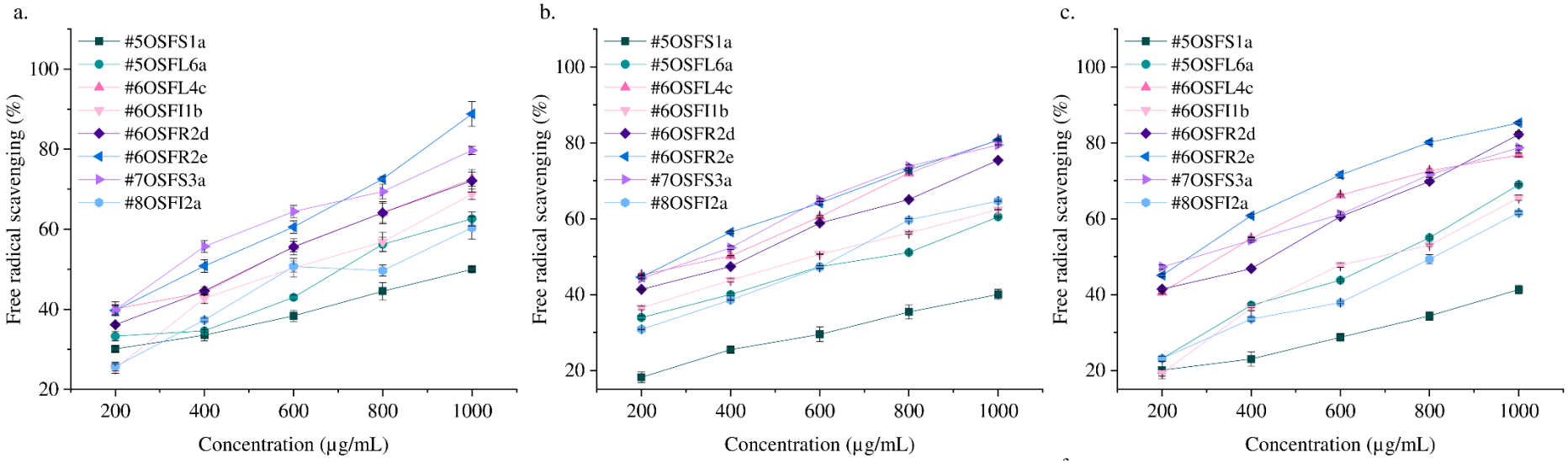

Fig. 5.30: Free radical scavenging activity of rain-fed rice isolates against a. DPPH radical; b. ABTS radical; c. H_2O_2 radical. The values represent mean ± SD, n=3.

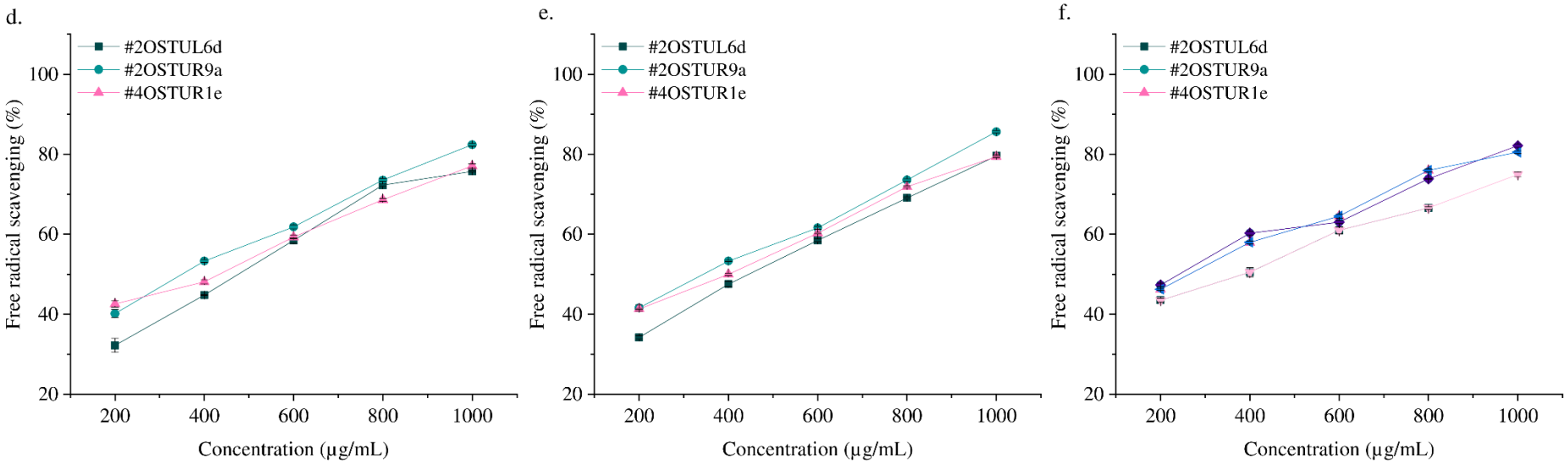

Fig. 5.30: Free radical scavenging activity of drought resistant rice isolates against d. DPPH radical; e. ABTS radical; H_2O_2 radical. The values represent mean ± SD, n=3.

Table 5.4: IC_{50} values and Ferric ion reducing antioxidant power activity of selected isolates from rain-fed and drought resistant rice.

Variety/ Isolates	IC_{50} (µg/mL)			Ferric ion reducing antioxidant power (µg Fe (II) equivalent/mg of extract)
	DPPH scavenging	Trolox equivalent antioxidant capacity	Hydrogen peroxide scavenging	
Standard	340.7±3.2^{e}	353.5±7.2^{f}	209.4±6.8^{h}	-
Rain-fed				
#5OSFS1a	1024.0±46.2^{a}	939.1±41.9^{a}	898.2±27.5^{a}	70.0±0.5^{d}
#5OSFL6a	701.0±1.9^{b}	706.6±7.1^{b}	680.5±5.9^{c}	68.9±0.6^{de}
#6OSFL4c	474.5±11.8^{d}	348.1±4.7^{ef}	329.7±6.1^{e}	76.0±0.3^{b}
#6OSFI1b	624.3±30.6^{c}	602.9±4.3^{c}	702.9±4.3^{c}	50.3±0.4^{g}
#6OSFR2d	501.8±12.2^{d}	422.4±3.1^{de}	404.9±5.6^{d}	73.6±0.3^{c}
#6OSFR2e	391.6±20.7^{e}	366.0±5.9^{f}	227.8±3.8^{gh}	78.9±0.5^{a}
#7OSFS3a	345.4±21.7^{e}	362.5±6.6^{f}	284.9±8.0^{f}	76.8±0.4^{ab}
#8OSFI2a	728.9±8.1^{b}	640.9±5.0^{c}	793.2±12.2^{b}	51.4±0.4^{g}
Drought-resistant				
#2OSTUL6d	482.6±11.6^{d}	461.3±4.9^{d}	364.2±16.2^{e}	67.1±0.2^{e}
#2OSTUR9a	365.5±10.0^{e}	357.5±7.1^{f}	231.5±7.7^{gh}	75.0±0.5^{bc}
#4OSTUR1e	392.7±15.4^{e}	384.4±1.8^{ef}	252.0±5.9^{fg}	60.8±2.0^{f}

The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

5.13.3 *In-vitro* antimicrobial potential

All the tested isolates of rain-fed and drought-resistant rice varieties exhibited antimicrobial potential (Table A5-Appendix). The zone diameter of inhibition (ZDI) of some of the tested isolates is shown in Fig. 5.31. The positive control exhibited a ZDI of 20.3 ± 0.6, 18 ± 1, 18.3 ± 0.6 and 16.3 ± 0.6 mm against *Escherichia coli* (MTCC 739), *Enterobacter aerogenes* (NCIM 5139), *Bacillus subtilis* (MTCC 736) and *Staphylococcus aureus* (MTCC 96), respectively. The endophytic isolates of both the rice varieties exhibited inhibition of gram-positive and gram-negative bacteria.

In the case of rain-fed rice, isolates #6OSFR2e and #7OSFS3a showed the largest ZDI of 18.3 ± 0.6 and 15.7 ± 0.6 mm against *E. coli*. Likewise, isolates #6OSFR2e, #7OSFS3a and #6OSFL4c also exhibited 15.3 ± 1.2, 13.00 ± 1.00 and 12.3 ± 0.6 mm ZDI against *Enterobacter aerogenes.* In addition, the isolates #6OSFR2e and #7OSFS3a exhibited 15.7 ± 0.6 and 12.7 ± 0.6 mm ZDI against *Bacillus subtilis*. In contrast, 13.7 ± 0.6 and 14.3 ± 0.6 mm ZDI was observed against *Staphylococcus aureus* (Table 5.5). Similarly, in the case of drought-resistant rice, the isolate #2OSTUR9a exhibited the highest ZDI of 17.3 ± 1.2 and

13.3 ± 0.6 mm against *E. coli* and *Enterobacter aerogenes*. And, a ZDI of 16.3 ± 0.6 and 14.3 ± 0.6 mm was observed against *Bacillus subtilis* and *Staphylococcus aureus* (Table 5.5).

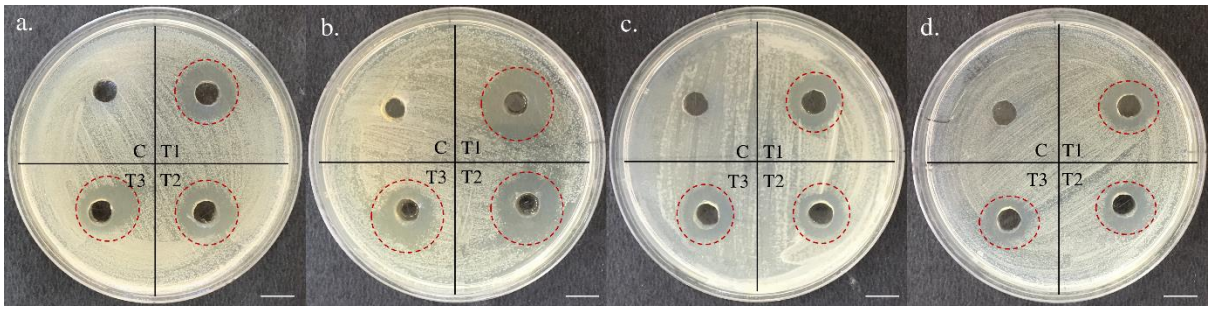

Fig. 5.31: *In-vitro* antibacterial activity of selected endophytic fungi of rain-fed and drought resistant rice varieties against a. *Escherichia coli* (MTCC 739); b. *Enterobacter aerogenes* (NCIM 5139); c. *Bacillus subtilis* (MTCC 736); d. *Staphylococcus aureus* (MTCC 96) (where C = Control; Tn = Test. Bar: 10 mm).

Table 5.5: Antibacterial activity of selected endophytic fungal isolates.

Variety/ Isolate	Zone of inhibition (mm)			
	***Escherichia coli* (MTCC 739)**	***Enterobacter aerogenes* (NCIM 5139)**	***Bacillus subtilis* (MTCC 736)**	***Staphylococcus aureus* (MTCC 96)**
Streptomycin	20.3±0.6^{a}	18.0±1.0^{a}	18.3±0.6^{a}	16.3±0.6^{a}
Rain-fed				
#5OSFS1a	3.7±0.6^{e}	3.3±0.6^{e}	5.7±0.6^{de}	4.3±0.6^{c}
#5OSFL6a	3.0±1.0^{e}	4.7±1.2^{de}	3.0±1.0^{e}	6.7±1.2^{c}
#6OSFL4c	11.3±0.6^{c}	12.3±0.6^{b}	9.0±1.0^{c}	11.0±1.0^{b}
#6OSFI1b	7.0±1.0^{d}	7.3±0.6^{cd}	9.3±1.2^{c}	6.7±1.2^{c}
#6OSFR2d	9.7±0.6^{c}	8.7±1.2^{c}	8.3±0.6^{cd}	7.3±1.2^{c}
#6OSFR2e	18.3±0.6^{a}	15.3±1.2^{ab}	15.7±0.6^{a}	13.7±0.6^{ab}
#7OSFS3a	15.7±0.6^{b}	13.0±1.0^{b}	12.7±0.6^{b}	14.3±0.6^{a}
#8OSFI2a	4.7±1.2^{de}	3.7±0.6^{e}	7.0±1.0^{cd}	6.7±1.2^{c}
Drought-resistant				
#2OSTUL6d	7.0±1.0^{b}	6.7±1.2^{c}	4.7±0.6^{b}	2.0±0.0^{d}
#2OSTUR9a	17.3±1.2^{a}	13.3±0.6^{b}	16.3±0.6^{a}	14.3±0.6^{b}
#4OSTUR1e	9.0±1.0^{b}	7.3±1.2^{c}	6.7±0.6^{b}	6.3±0.6^{c}

The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

Furthermore, the tested isolates of rain-fed and drought-resistant rice varieties also exhibited antifungal potential (Fig. 5.32). The isolates #6OSFR2e and #7OSFS3a displayed the utmost inhibition, 42.5 ± 0.8 and 33.7 ± 0.9% against *Alternaria alternata* (ITCC 6129). At the same time, isolates #6OSFR2e, #7OSFS3a and #6OSFL4c exhibited 45.0 ± 1.7 and 34.4 ± 1.0% inhibition against *Botrytis cinerea* (ITCC 6011). The isolates also demonstrated inhibition against *Colletotrichum* and *Fusarium*. The highest inhibition of 49.6 ± 1.9 %

against *Colletotrichum gloeosoriodes* (ITCC 3801) was seen by isolate #6OSFR2e, closely followed by isolate #7OSFS3a at 39.0 ± 3.9%. The isolates #6OSFR2e, #7OSFS3a and #6OSFL4c also exhibited 35.2 ± 2.3, 32.9 ± 1.3 and 31.5 ± 0.5% inhibition against *Fusarium moliniforme* (ITCC 6240) (Table 5.6). Similarly, in the case of drought-resistant rice, isolate #2OSTUR9a exhibited the highest inhibition of 29.1 ± 1.6, 61.1± 1.0, 42.2 ± 2.7 and 30.0 ± 0.5% against *Alternaria alternata*, *Botrytis cinerea*, *Colletotrichum gloeosporioides* and *Fusarium moniliforme*, respectively (Table 5.6).

Table 5.6: Antifungal activity of selected endophytic fungal isolates.

Variety/ Isolate	Inhibition (%)			
	***Alternaria alternata* (ITCC 6129)**	***Botrytis cinerea* (ITCC 6011)**	***Colletotrichum gloeosporioides* (ITCC 3801)**	***Fusarium moniliforme* (ITCC 6240)**
Rain-fed				
#5OSFS1a	6.6±2.0^{e}	21.7±0.0^{c}	9.7±2.2^{d}	11.3±0.2^{d}
#5OSFL6a	5.4±0.1^{e}	12.8±1.0^{d}	25.2±2.3^{c}	14.1±0.1^{cd}
#6OSFL4c	19.3±1.0^{d}	34.0±1.0^{b}	40.6±2.0^{b}	31.5±0.5^{ab}
#6OSFI1b	6.0±1.0^{e}	22.8±1.0^{c}	30.9±2.2^{c}	15.9±1.9^{c}
#6OSFR2d	23.5±0.2^{c}	32.2±1.0^{b}	25.2±0.9^{c}	30.0±1.3^{b}
#6OSFR2e	42.5±0.8^{a}	45.0±1.7^{a}	49.6±1.9^{a}	35.2±2.3^{a}
#7OSFS3a	33.7±0.9^{b}	34.3±1.0^{b}	39.0±3.9^{b}	32.9±1.3^{ab}
#8OSFI2a	6.0±1.0^{e}	21.7±0.0^{c}	27.6±2.3^{c}	6.6±0.8^{e}
Drought-resistant				
#2OSTUL6d	10.8±0.1^{b}	5.6±1.0^{b}	21.1±3.2^{b}	11.3±0.2^{b}
#2OSTUR9a	29.1±1.6^{a}	61.1±1.0^{a}	42.2±2.7^{a}	30.0±0.5^{a}
#4OSTUR1e	6.6±2.0^{b}	8.3±1.7^{b}	22.8±1.0^{b}	12.2±1.5^{b}

The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

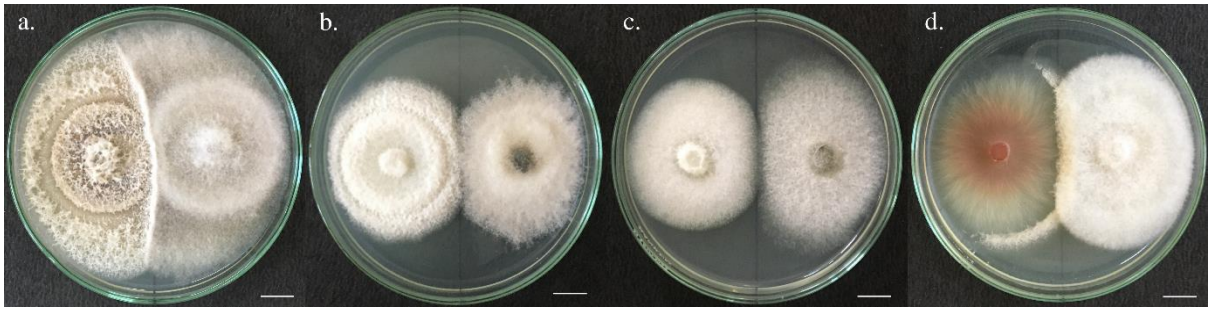

Fig. 5.32: *In-vitro* antifungal activity of selected endophytic fungi of rain-fed and drought resistant rice varieties against a. *Alternaria alternata* (ITCC 6129); b. *Botrytis cinerea* (ITCC 6011); c. *Colletotrichum gloeosoriodes* (ITCC 3801); d. *Fusarium moniliforme* (ITCC 6240) (Bar: 10 mm).

5.13.4 Qualitative and quantitative estimation of phytohormones

The crude extract of selected endophytic fungi of rain-fed and drought-resistant rice exhibited indole acetic acid (IAA) and gibberellic acid (GA3) production capabilities. The standard IAA and crude extract exhibited absorption maxima at 280 nm (Fig. 5.33a). An absorption maximum of 254 nm was observed for standard GA3 and crude extract. (Fig. 5.33b).

The retention time of the standard indole acetic acid (IAA) and crude extract was found to be 9.9 minutes from the HPLC analysis (Fig. 5.34a). In comparison, standard GA3 and the crude extract had the retention time of 2.2 minutes (Fig. 5.34b). On quantification, among the rain-fed rice isolates, #6OSFR2e exhibited the highest IAA production of 212.8 ± 3.8 µg/mL, followed by isolates #7OSFS3a and #6OSFL4c at 167.1 ± 1.1 and 135.0 ± 3.4 µg/mL. All the isolates displayed an increase in IAA production on supplementation of tryptophan. The IAA production of #6OSFR2e increased to 320.3 ± 5.5 µg/mL with the addition of tryptophan. Likewise, isolate #7OSFS3a exhibited IAA production of 306.4 ± 5.2 µg/mL with the addition of tryptophan (Fig. 5.35a).

Interestingly, isolate #8OSFI2a did not exhibit any significant increase in IAA production on addition of tryptophan (Fig. 5.35a). Similarly, auxin production of 109.8 ± 2.6 µg/mL among drought-resistant rice isolation was observed in isolate #2OSTUR9a. Which increased to 351.0 ± 7.1 µg/mL on tryptophan supplementation (Fig. 5.35c). Production of another phytohormone i.e., gibberellic acid was also assessed by the selected isolates. Among the rain-fed rice isolates, the highest GA production of 23.4 ± 1.1 and 19.2 ± 0.1 µg/mL was observed by isolates #7OSFS3a and #6OSFR2e, respectively (Fig. 5.35b). Likewise, the maximum GA production of 26.8 ± 0.9 µg/mL among drought-resistant rice isolates was detected in isolate #2OSTUR9a (Fig. 5.35d) (Table A5-Appendix).

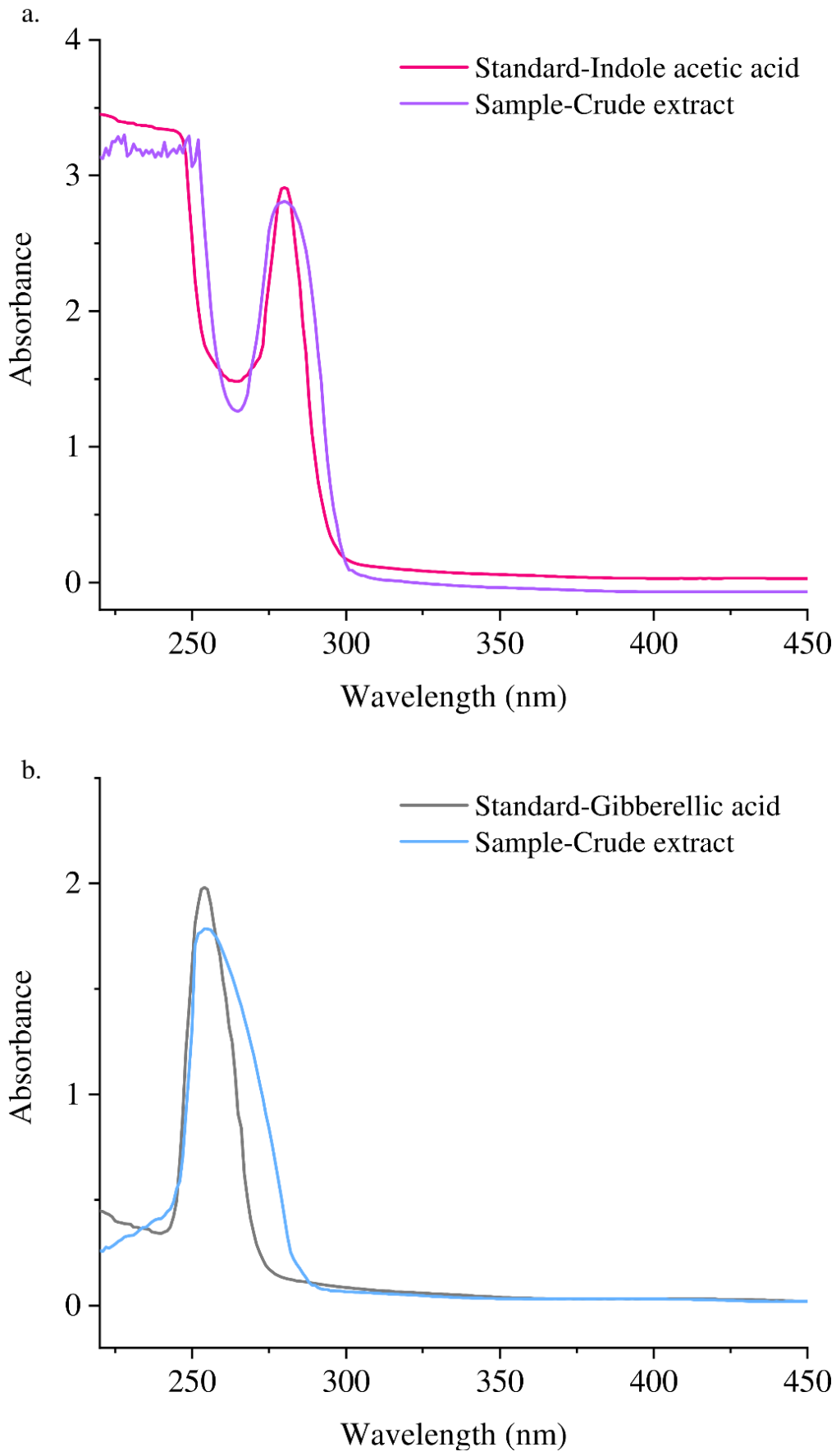

Fig. 5.33: UV-visible spectra of a. Indole acetic acid (IAA); b. Gibberellic acid (GA3) and crude extract of selected endophytic fungal isolates.

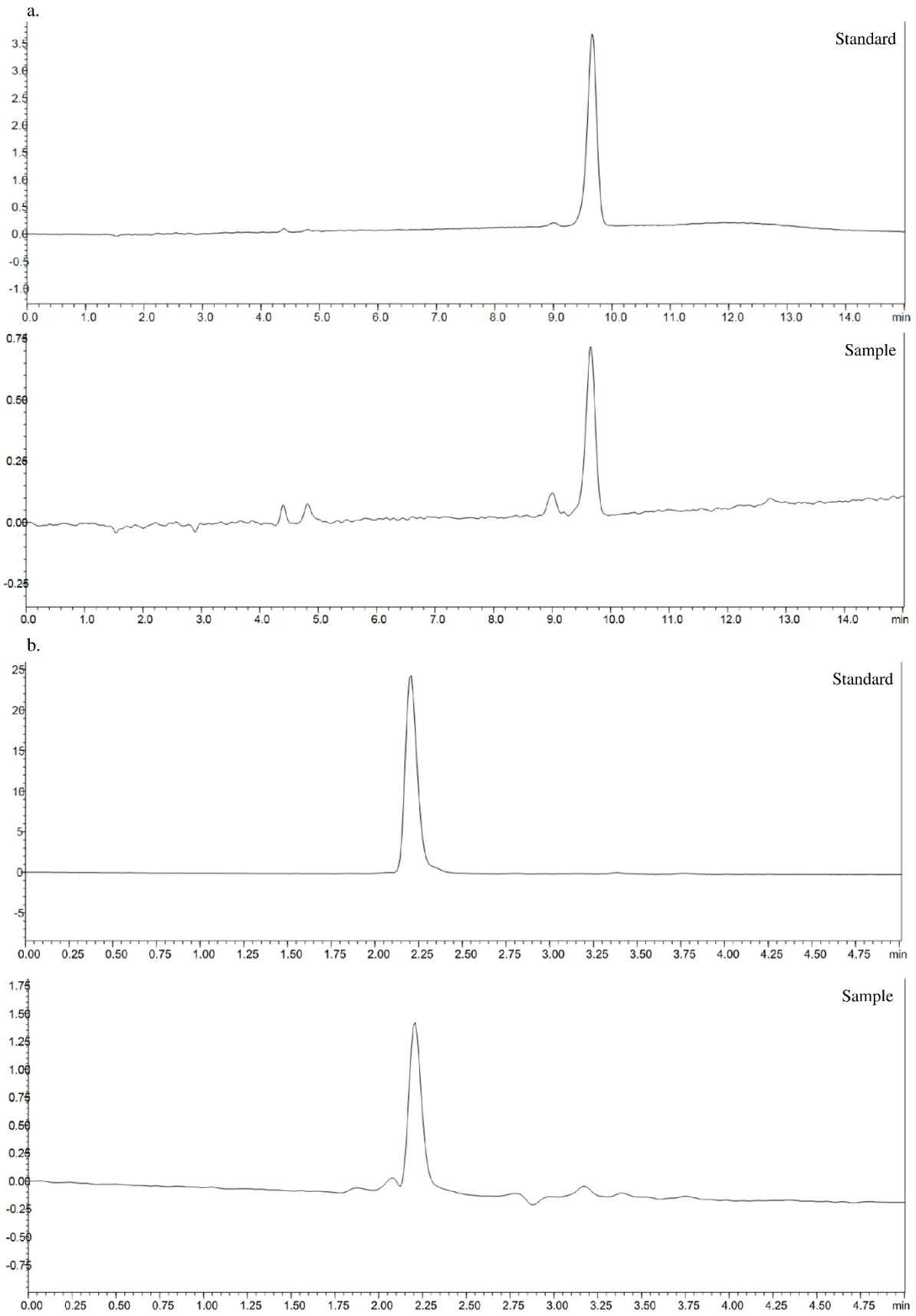

Fig. 5.34: HPLC chromatogram of a. Indole acetic acid (IAA); b. Gibberellic acid (GA3).
(IAA: 40:60:1 deionised water, acetonitrile, acetic acid; flow rate 1 mL/min at column oven temperature of 40 °C; GA3: 4:1 deionised water, methanol; flow rate 1 mL/min at 35 °C).

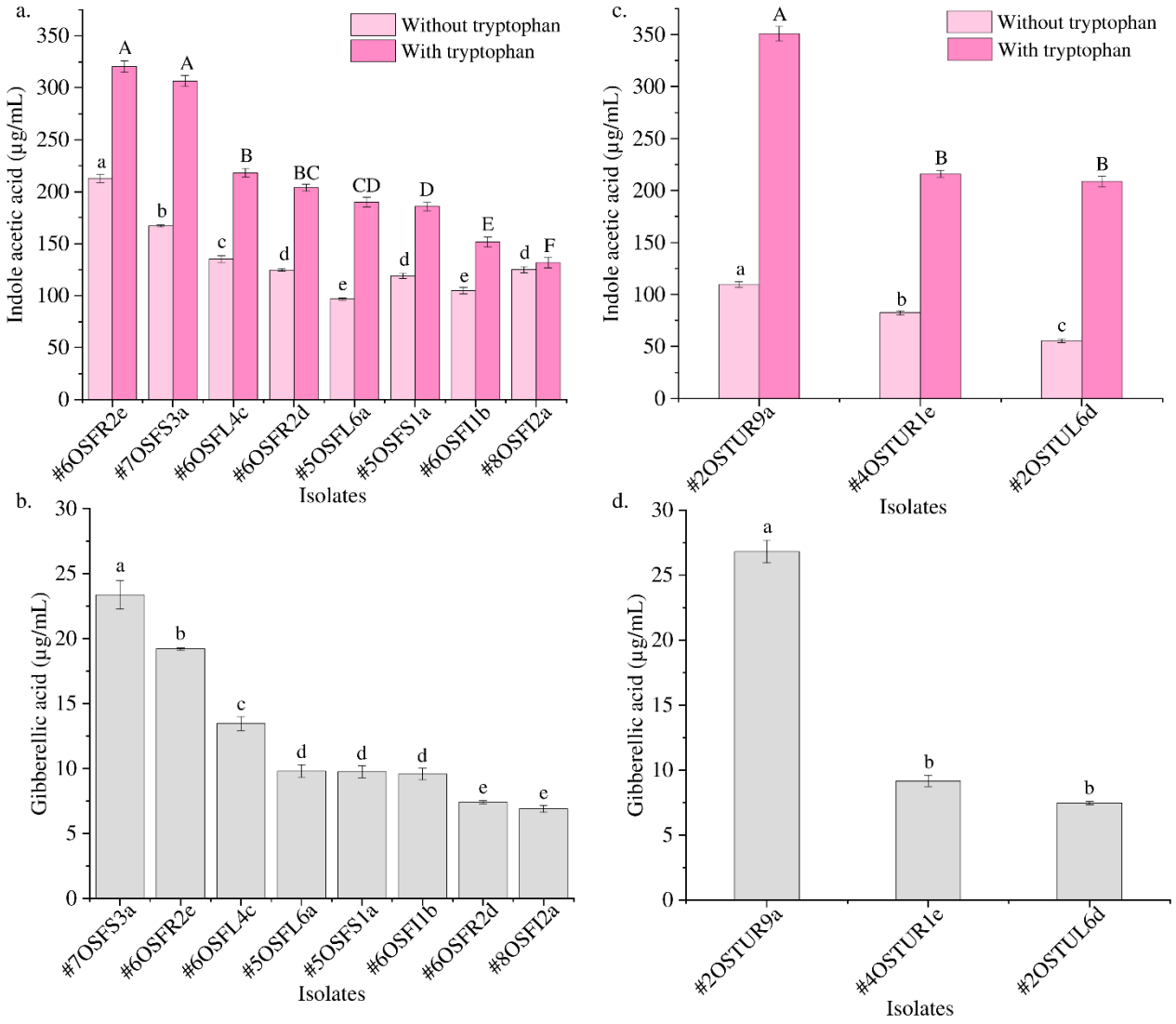

Fig. 5.35: Indole acetic acid (IAA) and gibberellic acid (GA3) production of selected endophytic fungal isolates of a, b rain-fed rice; c, d. drought resistant rice. The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

5.13.5 Production of ACC deaminase

The selected isolates exhibited ACC deaminase activity ranging from 47.6 ± 2.1 to 317.4 ± 0.4 nmol α-ketobutyrate/mg protein/hour. In addition, the isolates exhibited an increase in ACC deaminase production with an increase in concentration of ACC. Among the rain-fed rice isolates, #6OSFR2e had the highest ACC deaminase production of 317.4 ± 0.4 nmol α-ketobutyrate/mg protein/hour at 5 mM ACC concentration. It was followed by #7OSFS3a and #6OSFL4c with 263.5 ± 0.7 and 220.3 ± 0.8 nmol α-ketobutyrate/mg protein/hour at 5 mM ACC concentration (Fig. 5.36a). Interestingly, the increase in ACC deaminase activity beyond 3 mM ACC concentration was non-significant (Table A5-Appendix).

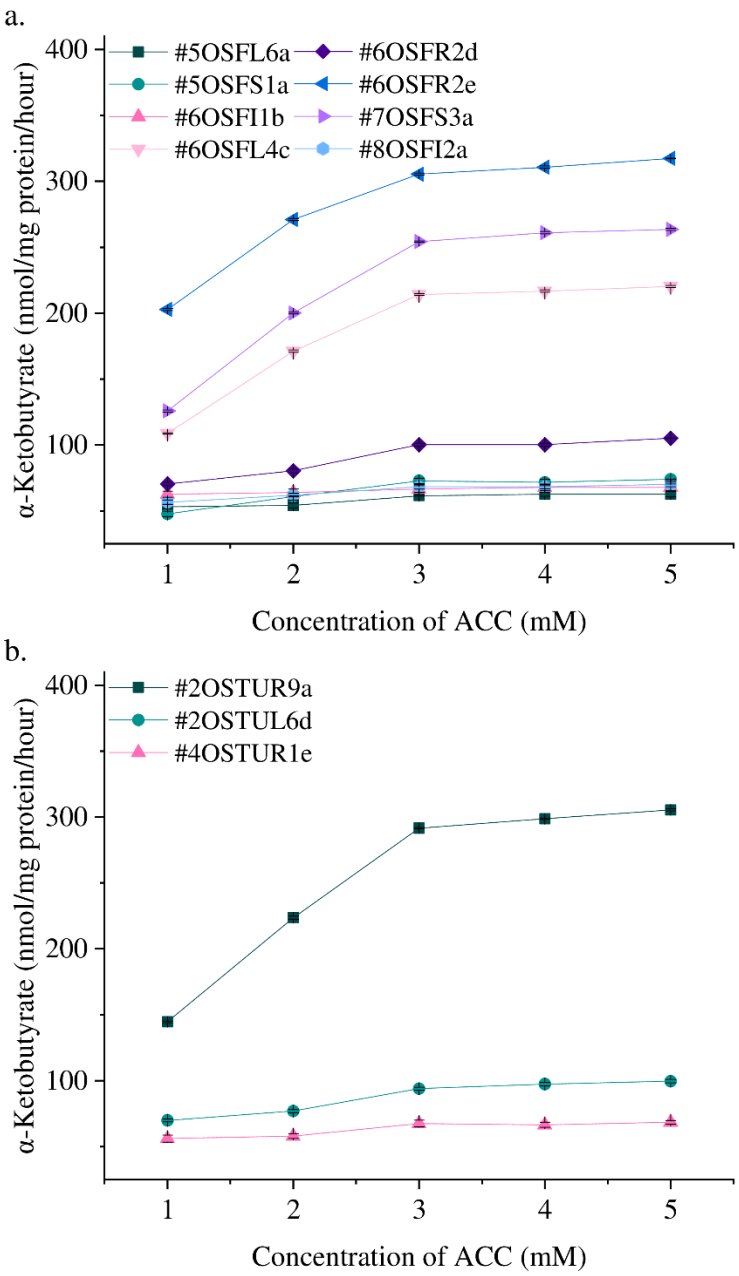

Fig. 5.36: Production of ACC deaminase by selected endophytic fungal isolates of a. rain-fed rice; b. drought resistant rice. The values represent mean ± SD, n=3.

Among the drought-resistant rice isolates, #2OSTUR9a exhibited the highest ACC deaminase activity of 305.4 ± 0.80 nmol α-ketobutyrate/mg protein/hour at 5 mM ACC concentration. This was followed by isolate #2OSTUL6d with an activity of 99.5 ± 1.3 α-ketobutyrate/mg protein/hour. In contrast, isolate #4OSTUR1e exhibited the lowest ACC deaminase activity (Fig. 5.36b).

Furthermore, Fourier transform infrared (FTIR) analysis was carried out to confirm α-ketobutyrate liberation. The crude extract of selected endophytic fungi peaked at 3291 and 1629 cm^{-1}, indicating the presence of amino and ketonic functional groups. The same corresponding peaks were observed on analysing the standard α-ketobutyrate (Fig. 5.37).

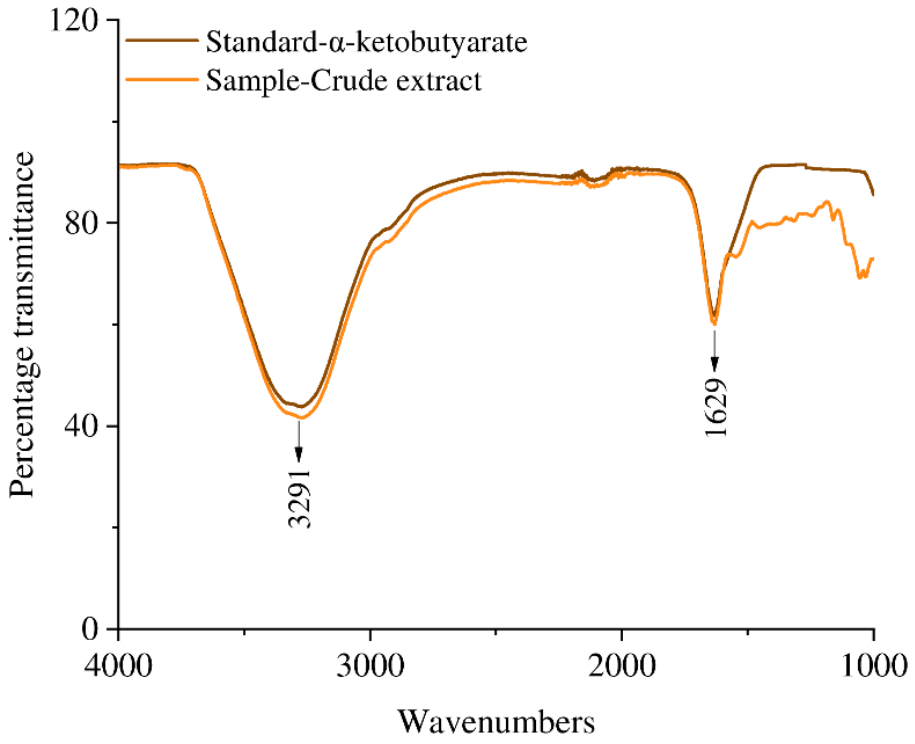

Fig. 5.37: FTIR spectra of standard α-ketobutyrate and crude extract of endophytic fungal isolates.

5.13.6 Production of siderophores

The selected endophytic fungi also exhibited varying degrees of siderophore production. Fig. 5.38a shows a change in colour of CAS dye from blue to yellow with the addition of culture filtrate indicating siderophore production. Among the rain-fed rice isolates, #6OSFR2e exhibited the highest siderophore production of 67.9 ± 0.6%, closely followed by #7OSFS3a with 67.0 ± 0.2%. The lowest siderophore production of 8.0 ± 0.4% was observed in isolate #5OSFS1a (Fig. 5.38b). Among drought-resistant rice isolates, #2OSTUR9a exhibited the highest siderophore production of 72.6 ± 0.2%. In contrast, the lowest production was observed in isolate #4OSTUR1e with 15.0 ± 0.3% (Fig. 5.38c) (Table A5-Appendix).

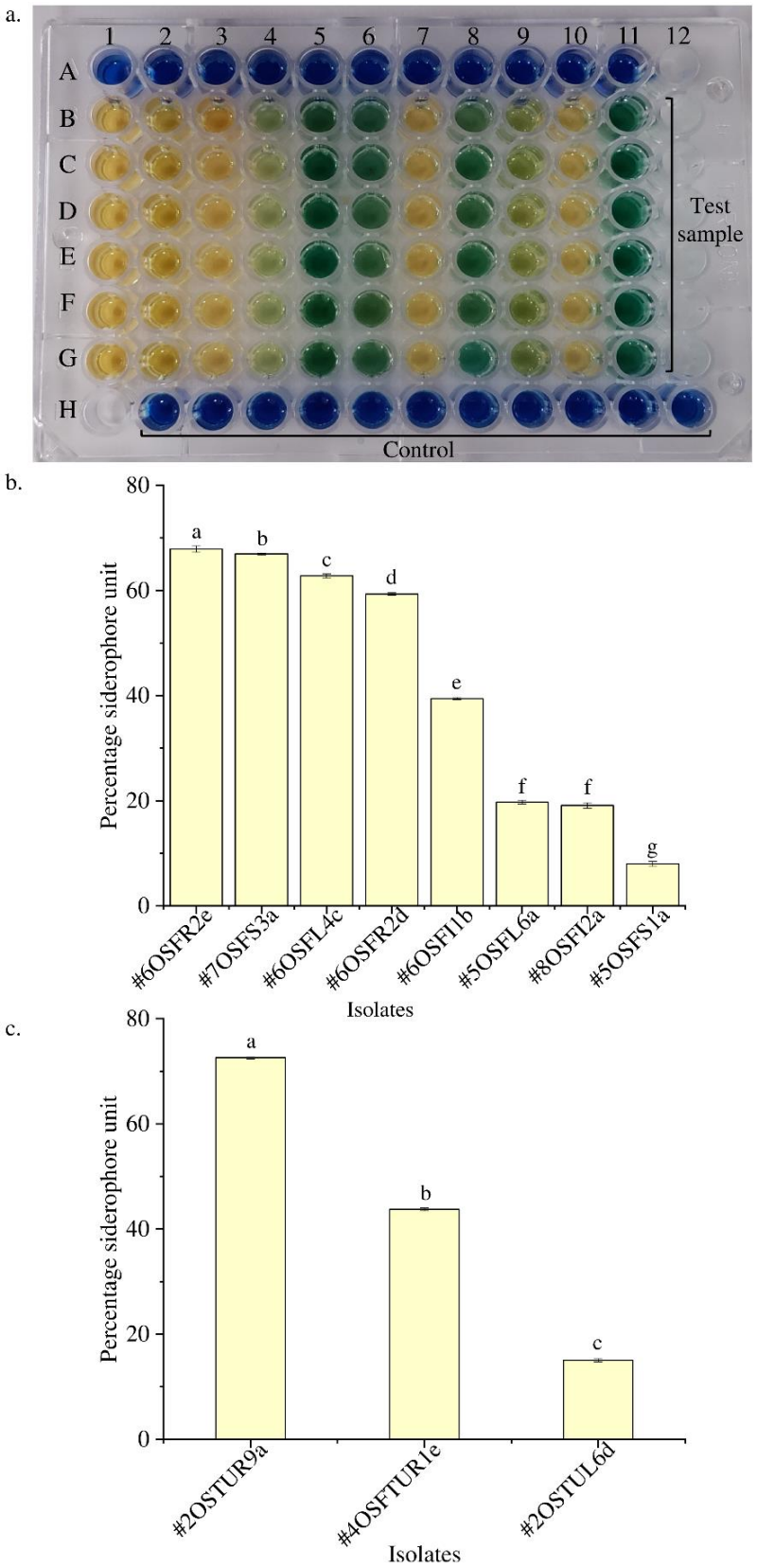

Fig. 5.38: a. A 96-well plate showing chrome azurol S (CAS) assay for detection of siderophore by selected endophytic fungal isolates (where control = CAS dye; test sample = CAS dye + culture filtrate); Siderophore production potential of selected endophytic fungal isolates of b. rain-fed rice isolates; c. drought resistant rice isolates. The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

5.13.7 Mineral solubilisation

Most of the selected isolates exhibited the ability to solubilise more than one type of mineral. Fig. 5.39 shows the phosphate and zinc solubilisation potential by selected endophytic fungi. In the case of rain-fed rice isolates, phosphate solubilisation potential with a PI of 1.1 ± 0.0, 1.1 ± 0.00 and 1.0 ± 0.0 was observed in isolate #6OSFR2d, #6OSFR2e and #6OSFL4c, respectively. Notably, isolates #5OSFL6a and #6OSFI1b exhibited no phosphate solubilisation potential. Furthermore, #6OSFR2d displayed the greatest zinc solubilisation potential with a ZSI of 2.9 ± 0.2, followed by #6OSFR2e with a ZSI of 2.3 ± 0.2 (Fig. 5.40a). In drought-resistant rice, PI value of 1.1 ± 0.0, 1.1 ± 0.0 and 1.0 ± 0.0 was observed in case of isolate #2OSTUR9a, #2OSTUL6d and #4OSTUR1e, respectively. Notably, none of the selected isolates of drought-resistant rice variety exhibited zinc solubilisation potential (Fig. 5.40b) (Table A5-Appendix).

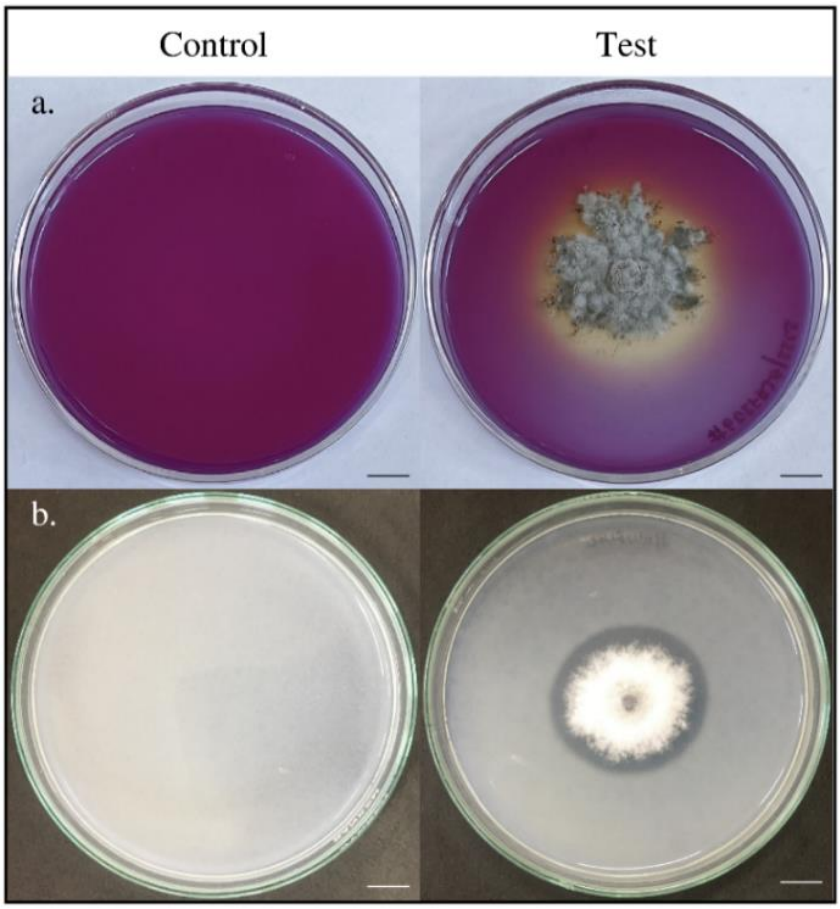

Fig. 5.39: The mineral solubilisation ability of selected endophytic fungal isolates denoted by the zone formation around the growing colonies a. Phosphate; b. Zinc (Bar: 10 mm).

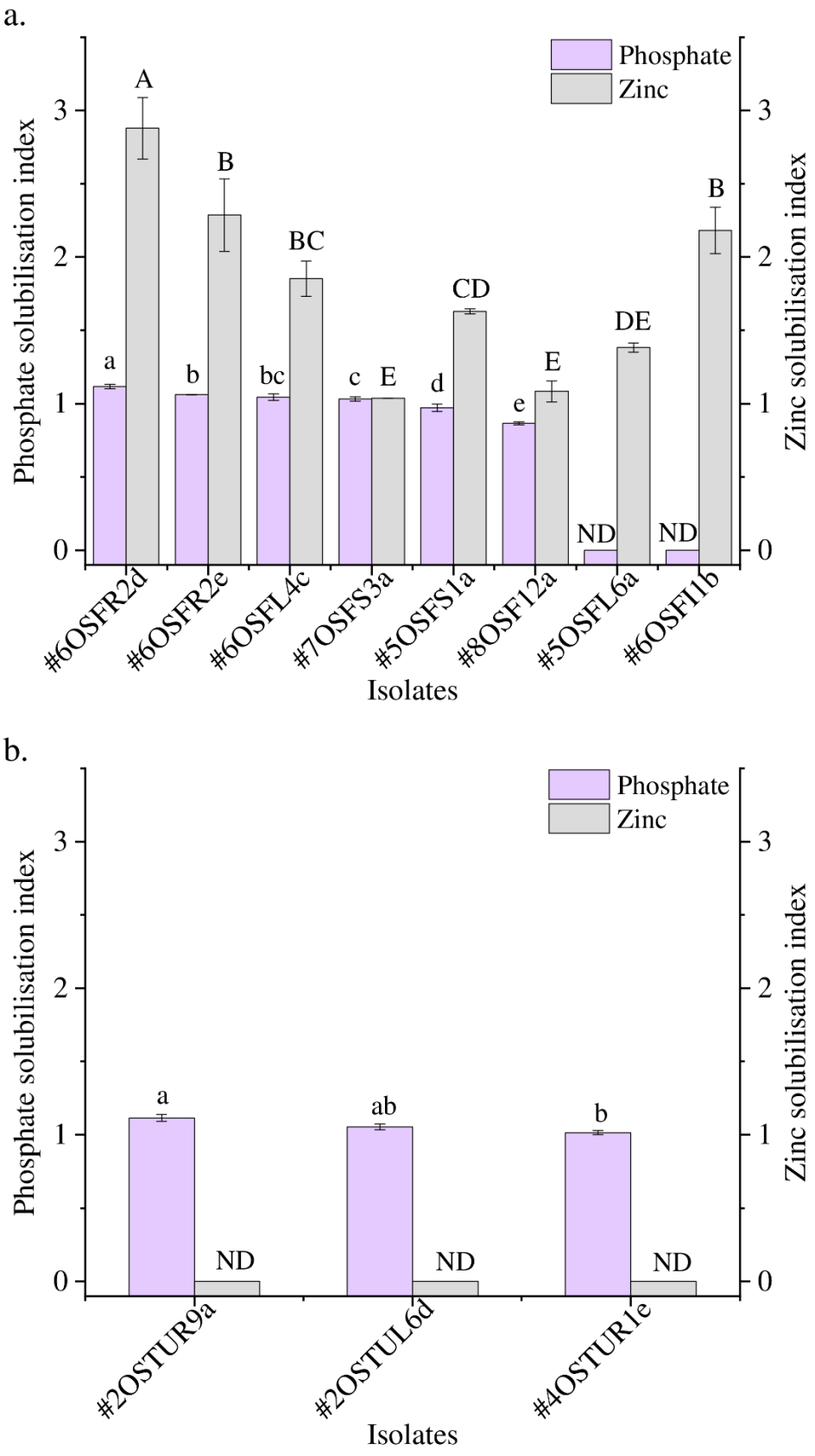

Fig. 5.40: Phosphate and zinc solubilisation potential of selected endophytic fungal isolates of a. rain-fed rice; b. drought resistant rice. The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$) and ND indicates not detected.

5.13.8 Production of extracellular lytic enzymes

The selected endophytic fungi of rain-fed and drought-resistant varieties exhibited varying extracellular lytic enzyme production. Fig. 5.41 shows the amylase, cellulase, laccase and pectinase activity of selected endophytic fungi. In the case of rain-fed rice isolates, #5OSFL6a exhibited the highest amylase activity with an EI of 1.0 ± 0.0, followed by isolate #6OSFR2e with an EI of 0.9 ± 0.0. The isolate #6OSFR2d exhibited the lowest amylase activity with an EI of 0.6 ± 0.0. Similarly, isolates #6OSFR2e, 5OSFL6a and #7OSFS3a exhibited the highest cellulase activity with an EI of 1.2 ± 0.0, 1.2 ± 0.0 and 1.1 ± 0.0. Here, with an EI of 1.0 ± 0.0, isolate #6OSFI1b exhibited the lowest cellulase activity (Fig. 5.42a). Furthermore, isolates #6OSFL4c, #6OSFR2d and #7OSFS3a exhibited the highest laccase activity with an EI of 1.2 ± 0.0, 1.1 ± 0.0 and 1.1 ± 0.0, respectively. The isolate #8OSFI2a had the lowest laccase activity with an EI of 0.7 ± 0.0. Whereas, in the case of pectinase activity, isolate #8OSFI2a exhibited exceptional results with an EI of 2.9 ± 0.2, followed by #6OSFSR2e with an EI of 1.8 ± 0.0. Here isolate #6OSFR2d exhibited the lowest pectinase activity with an EI of 1.0 ± 0.0 (Fig. 5.42b).

In the case of drought-resistant rice isolates, #2OSTUR9a exhibited the highest amylase activity with an EI of 1.1 ± 0.0, followed by #2OSTUL6d and #4OSTUR1e with an EI of 1.0 ± 0.0 by both isolates (Fig. 5.42c). Furthermore, isolate #2OSTUL6d exhibited the highest cellulase and laccase activity with an EI of 1.4 ± 0.0 and 2.4 ± 0.1. This was closely followed by isolate #2OSTUR9a with an EI of 1.3 ± 0.0 and 2.2 ± 0.1, respectively. The isolate #2OSTUR9a also exhibited the highest pectinase activity with an EI of 1.5 ± 0.1, followed by isolates #2OSTUL6d and #4OSTUR1e with an EI of 1.3 ± 0.1 and 1.1 ± 0.0 (Fig. 5.42d) (Table A5-Appendix).

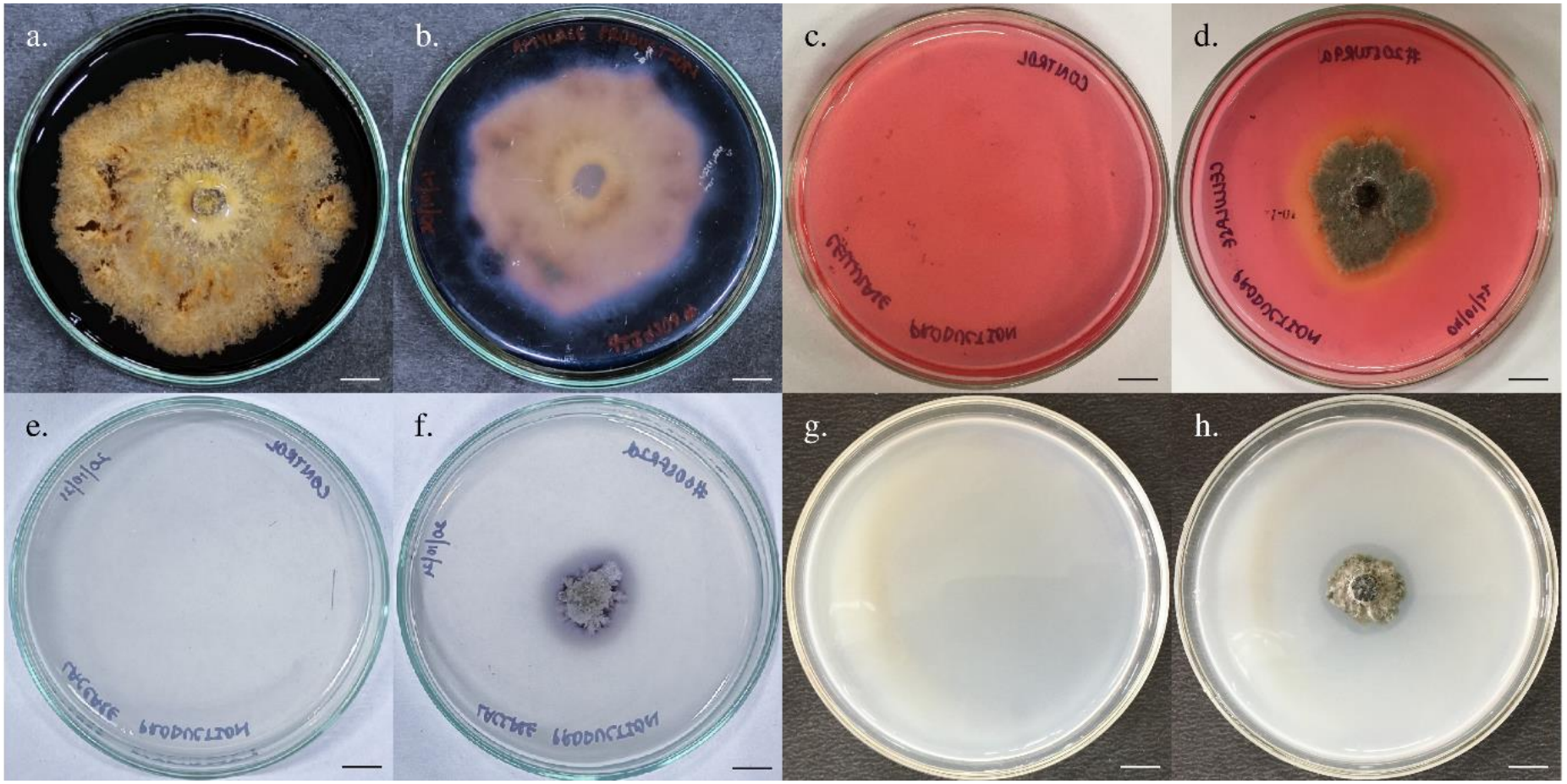

Fig. 5.41: The extracellular lytic enzyme production potential of selected endophytic fungal isolates denoted by the zone formation around the growing colony. Amylase production a. front and b. back view of the petri dish; Cellulase production c. control and d. test; Laccase production e. control and f. test; Pectinase production g. control and h. test (Bar: 10 mm).

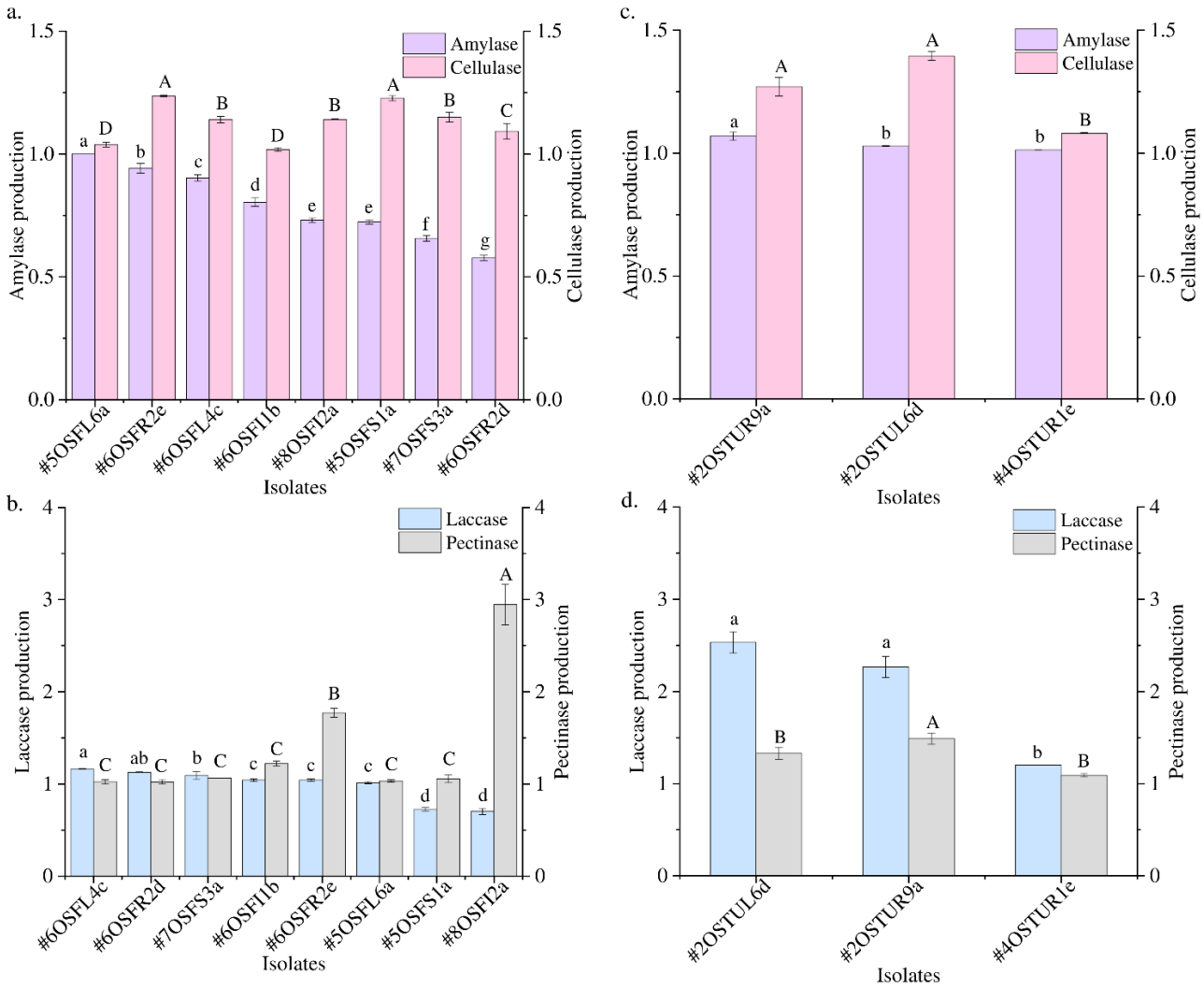

Fig. 5.42: Production of extracellular lytic enzymes by selected endophytic fungal isolates of rain-fed rice a. Amylase and Cellulase; b. Laccase and Pectinase; and drought resistant rice c. Amylase and Cellulase; d. Laccase and Pectinase. The values represent mean ± SD, n=3. Mean with different superscript letters are different by Tukey's post-hoc test ($p<0.05$).

5.13.9 Ammonia production

The selected isolates from rain-fed and drought-resistant rice varieties exhibited varying degrees of ammonia production qualitatively. Fig. 5.43 demonstrates the different intensities of ammonia production by selected endophytic fungal isolates (Table A6-Appendix). Among the rain-fed rice isolates, #6OSFL4c had the highest ammonia production. Followed by isolates #6OSFR2d, #6OSFR2e and #8OSFI2a. Whereas isolates #5OSFL6a and #6OSFI1b had the lowest ammonia production. Similarly, among the drought-resistant rice isolates, #2OSTUR9a and #4OSTUR1e exhibited the highest ammonia production, followed by #2OSTUL6d (Table 5.7).

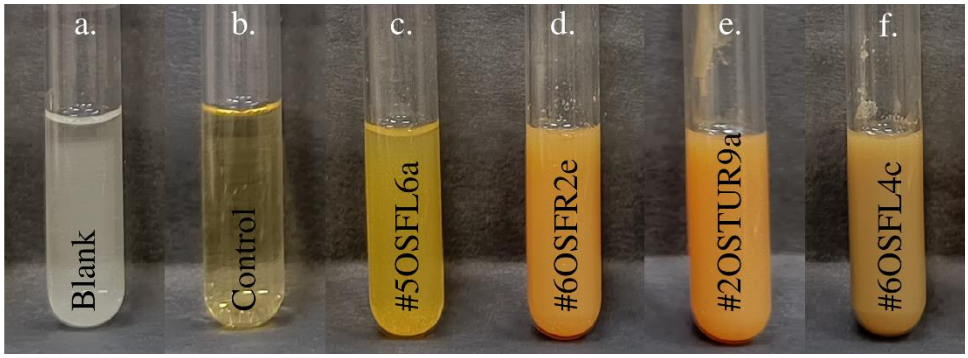

Fig. 5.43: Ammonia production using Nessler's reagent a. Blank; b. Control; c-f. different test sample.

Table 5.7: Ammonia production by selected endophytic fungal isolates of rain-fed and drought resistant rice varieties.

Variety/Isolate	Ammonia production
Rain-fed	
#5OSFS1a	++
#5OSFL6a	+
#6OSFL4c	++++
#6OSFI1b	+
#6OSFR2d	+++
#6OSFR2e	+++
#7OSFS3a	++
#8OSFI2a	+++
Drought-resistant	
#2OSTUL6d	++
#2OSTUR9a	+++
#4OSTUR1e	+++

−, indicates no ammonia production; +, indicates low ammonia production; ++, indicates moderate ammonia production; +++, indicates good ammonia production; ++++, indicates very high ammonia production; ND, indicates not detected.

5.13.10 Hydrogen cyanide production

The tested isolates of rain-fed and drought-resistant rice varieties did not exhibit HCN production. The picric acid dipped Whatman paper did not turn brown, as shown in Fig. 5.44. The test and control filter paper remains pale yellow even after ten days of endophyte growth, indicating no liberation of HCN by the selected isolates.

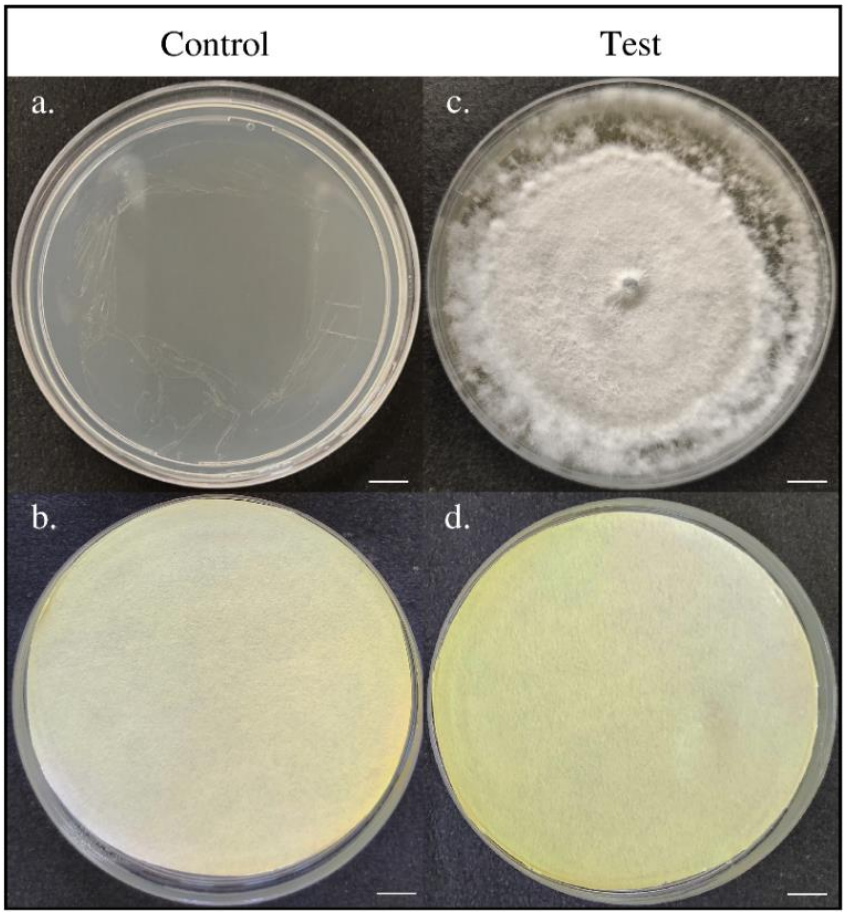

Fig. 5.44: Whatman paper indicating no HCN production in a-b. control; c-d. test (Bar: 10 mm).

5.14 Morphological and molecular identification of selected endophytic fungi

Based on the superior plant growth-promoting characteristics, endophytes #6OSFR2e of rain-fed and #2OSTUR9a of drought-resistant rice variety were selected. The isolates were identified using morphological and molecular identification tools.

The rain-fed isolate #6OSFR2e had fast-growing, raised, white colonies on PDA. Likewise, white slow-growing colonies were observed on MHA, CZD and SDA. On RBA, the isolate exhibited white slow-growing colonies with hues of pink at the centre, which increased on maturation. In addition, the colony was woolly with crateriform-like growth. Over WA, the isolate exhibited a sparse, flat colony with a brown filamentous form (Fig. 5.45). While examining #6OSFR2e under a microscope, septate hyphae were detected in the branches. Furthermore, grouped conidiophores were noted to exhibit subcylindrical shapes with terminal

conidiogenous cells. These cells appeared large, ampulliform, determinate, and smooth. Solitary conidia with ellipsoid shapes, smooth textures, and a dark brown colour were also observed (Fig. 5.45). Thus, the isolate #6OSFR2e was identified as *Nigrospora* sp.

The drought-resistant isolate #2OSTUR9a exhibited fast-growing white colony, which turned from grey to black as they matured. The colony was raised, round and had regular margins. The colonies were also fast-growing on MHA and RBA. In addition, hues of grey were observed on the white colonies on MHA. On SDA and CZD, white medium growing colonies were observed with irregular margins (Fig. 5.46). The microscopic characteristics of #2OSTUR9a revealed septate hyphae. The smooth and branched hyphae were brown. The hyaline conidiophore exhibited branching, aggregation, and bore a black conidium measuring less than 10 µm in diameter, featuring an ampulliform shape. The conidiogenous cells were subspherical in shape, whereas globose to subglobose conidia were observed (Fig. 5.46). Based on observations, isolate #2OSTUR9a was also identified as *Nigrospora* sp.

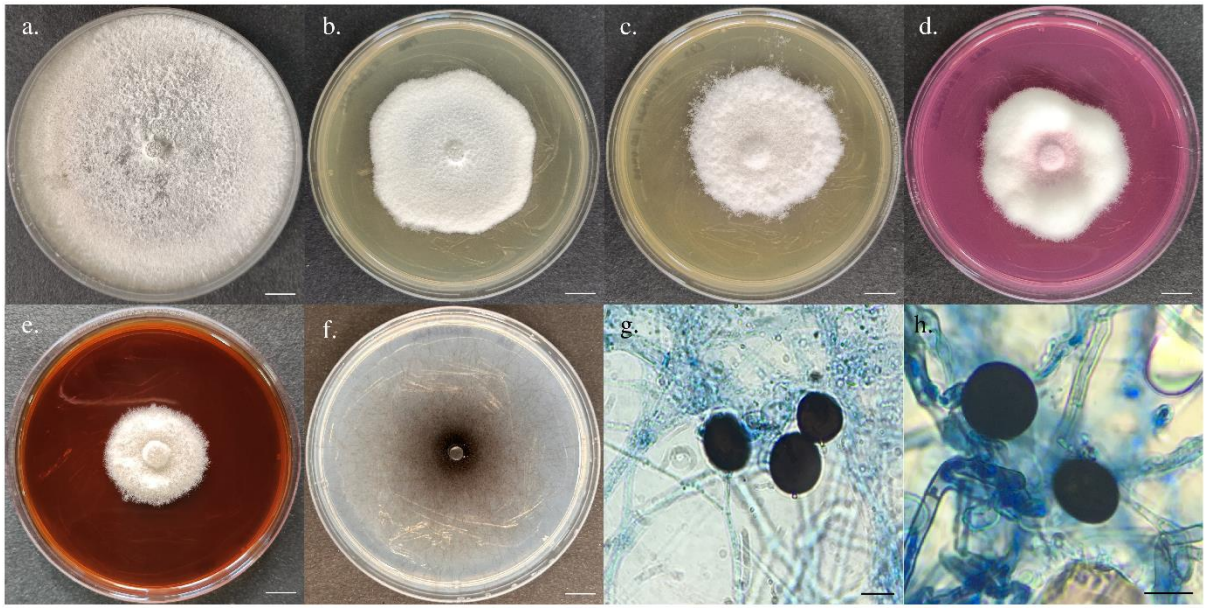

Fig. 5.45: Colonies of #6OSFR2e on a. PDA; b. MHA; c. CZD; d. RBA; e. SDA; f. WA; g-h. conidiogenous cells of #6OSFR2e.

On BLAST analysis of amplified ITS region, isolates #6OSFR2e and #2OSTUR9a revealed close homology with *Nigrospora zimmermanii* and *Nigrospora oryzae*, respectively (Fig. 5.47). Both the isolate belonged to the same phylum level clade Ascomycota. Similar results were observed on BLAST analysis of amplified TUB2 region where isolates #6OSFR2e and #2OSTUR9a displayed close homology with *Nigrospora zimmermanii* and *Nigrospora oryzae* (Fig. 5.48). A maximum-likelihood tree using the Tamura–Nei model was constructed with 1000 bootstraps for confirmation. The heuristic search's initial tree(s) were obtained using the Maximum Parsimony method. A discrete Gamma distribution was used to model

evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site.

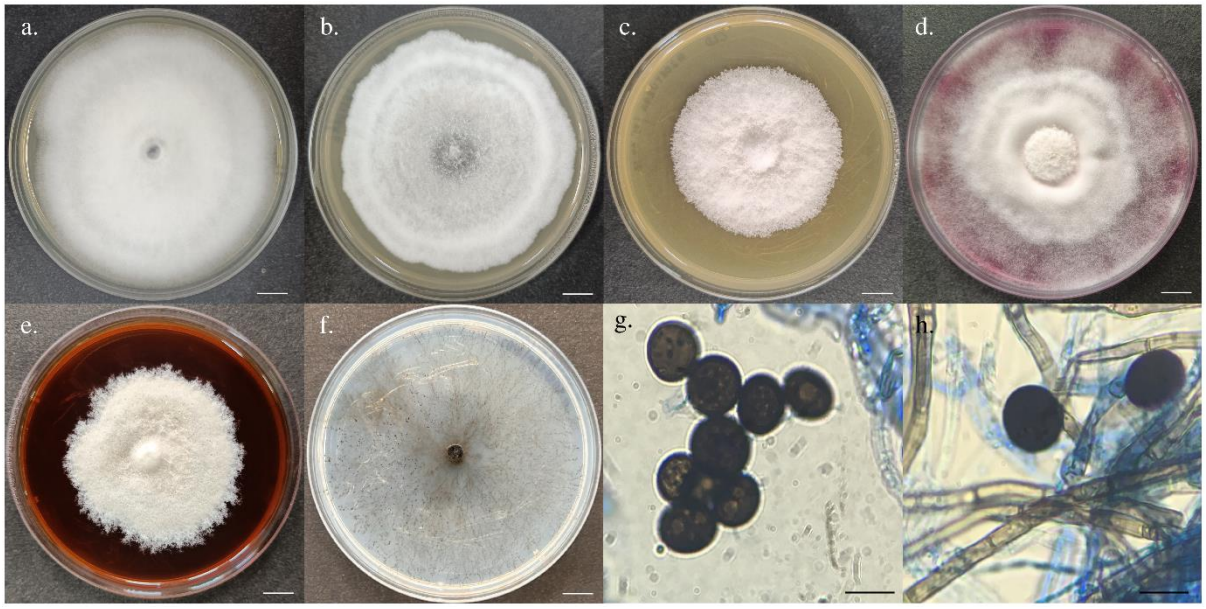

Fig. 5.46: Colonies of #2OSTUR9a on a. PDA; b. MHA; c. CZD; d. RBA; e. SDA; f. WA; g-h. conidiogenous cells of #2OSTUR9a.

In the case of the ITS region, the rain-fed isolate #6OSFR2e clustered with *Nigrospora zimmermanii* with 97.4% sequence identity. In contrast, the drought-resistant isolate #2OSTUR9a clustered with *Nigrospora oryzae* with 97.3% sequence identity (Fig. 5.47). The ITS sequences of #6OSFR2e and #2OSTUR9a have been submitted to GenBank with accession numbers ON392014 and ON392017, respectively.

In the TUB2 region, the rain-fed isolate #6OSFR2e clustered with *Nigrospora zimmermanii* with 100% sequence identity. In contrast, the drought-resistant isolate #2OSTUR9a clustered with *Nigrospora oryzae* with 99.1% sequence identity (Fig. 5.48). The TUB2 sequences of #6OSFR2e and #2OSTUR9a have been submitted in GenBank with accession numbers OR552398 and OR555818, respectively.

In the TEF-1α region, the rain-fed isolate #6OSFR2e clustered with *Nigrospora zimmermanii* with 100% sequence identity. In contrast, the drought-resistant isolate #2OSTUR9a clustered with *Nigrospora oryzae* with 100% sequence identity (Fig. 5.49). The TEF-1α sequences of #6OSFR2e and #2OSTUR9a have been submitted in GenBank with accession numbers PP183294 and PP183293, respectively.

The isolates #6OSFR2e and #2OSTUR9a have also been deposited in the National Fungal Culture Collection of India (NFCCI), Pune, under the accession numbers #5448 and #5447, respectively.

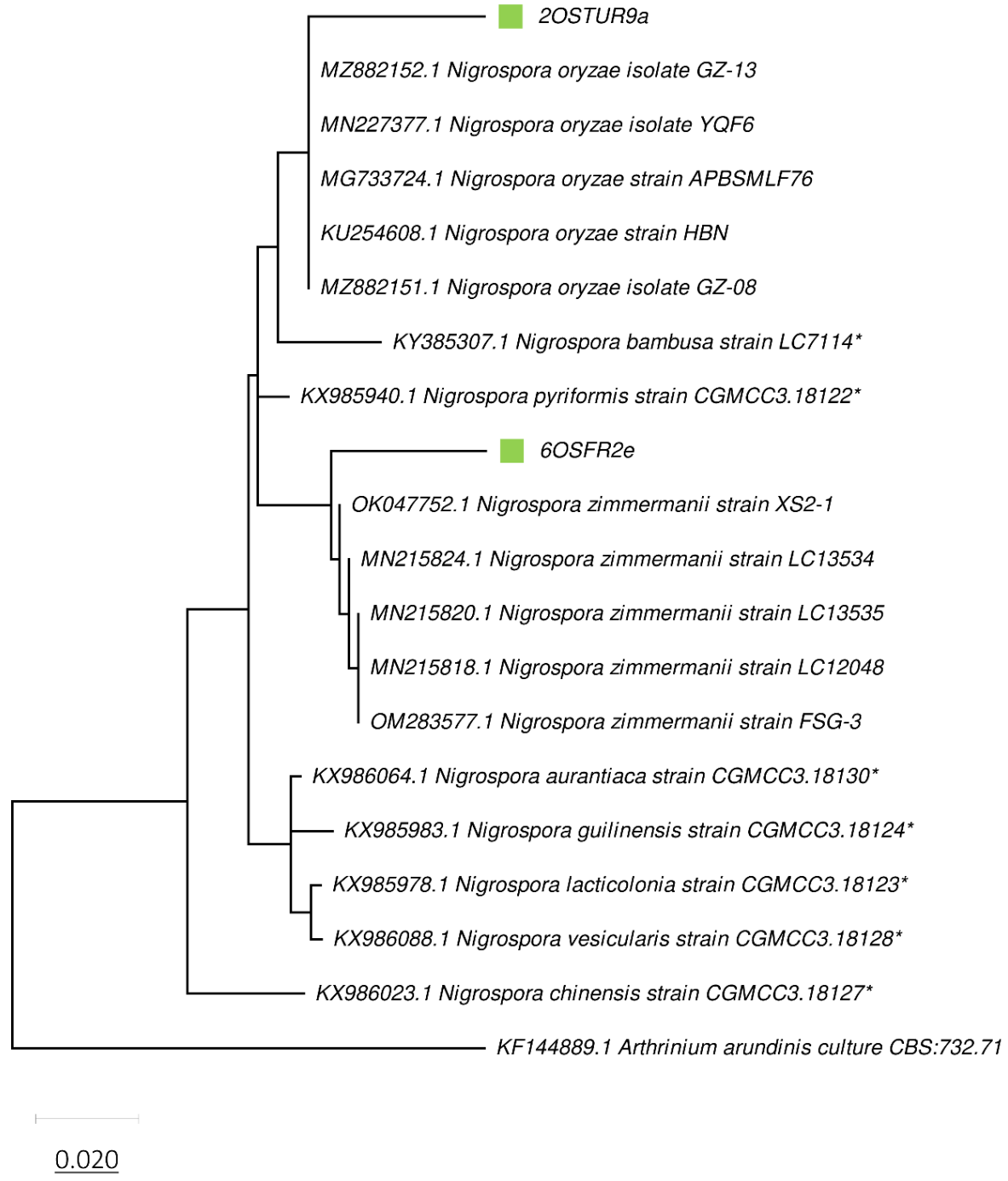

Fig. 5.47: Maximum-likelihood tree showing #6OSFR2e and #2OSTUR9a, based on the ITS1-5.8S-ITS2 region using Tamura and Nei model, indicating the associated taxa clustered together in the bootstrap test (1000 replicates). * Indicates Ex-type species.

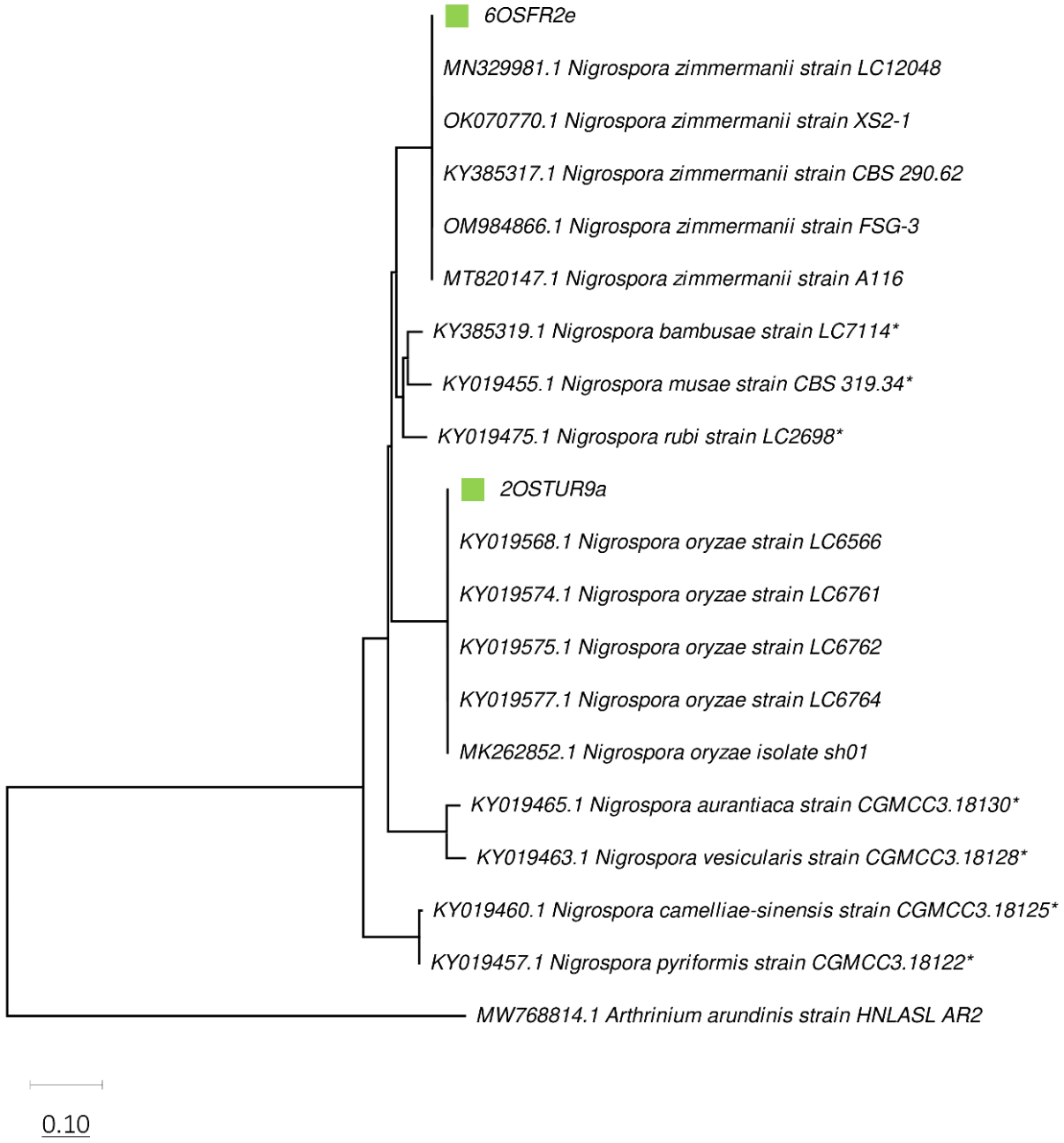

Fig. 5.48: Maximum-likelihood tree showing #6OSFR2e and #2OSTUR9a, based on the TUB2 region using Tamura and Nei model, indicating the associated taxa clustered together in the bootstrap test (1000 replicates). * Indicates Ex-type species.

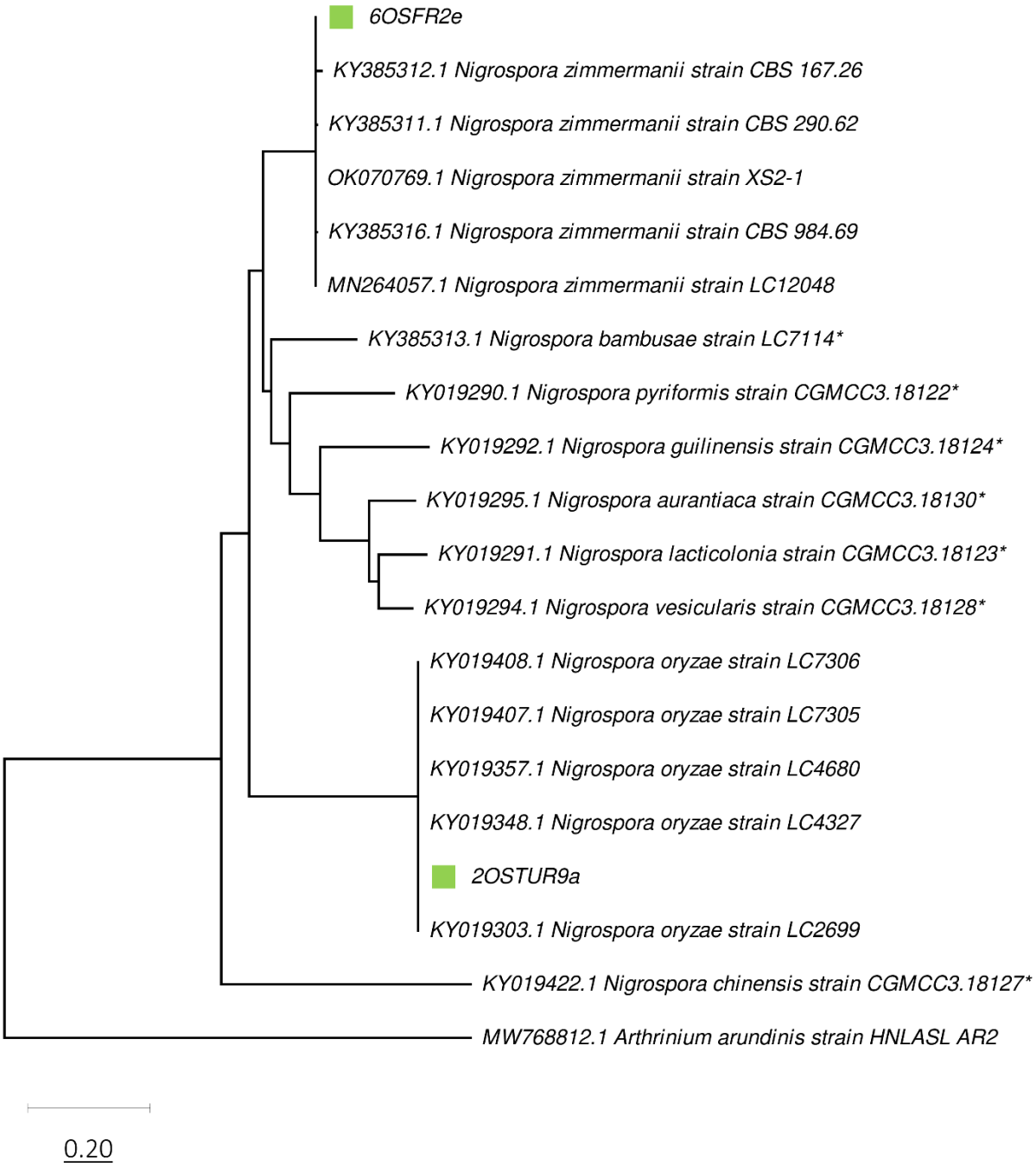

Fig. 5.49: Maximum-likelihood tree showing #6OSFR2e and #2OSTUR9a, based on the TEF-1α region using Tamura and Nei model, indicating the associated taxa clustered together in the bootstrap test (1000 replicates). * Indicates Ex-type species.

Chapter 6

Results (In-vivo)

6.1 Effect of endophyte inoculation on seed germination

The germination potential of seeds of *Oryza sativa* var. PUSA-44 under salinity and drought stress was tested. In comparison to the control (uninoculated) seeds, the inoculated seeds enhanced the probability of seed germination under the abiotic stress. Fig. 6.1a shows germinated seedlings of *Oryza sativa* var. PUSA-44. When *Nigrospora zimmermanii* #6OSFR2e (E1) was used as a bioinoculant, it enhanced the seed germination by 8.4 and 24.7% under salinity and drought stress, respectively, as compared to the control. Similarly, a 14.9% increase in seed germination was observed in inoculated plants under combined salinity and drought stress (Fig. 6.1b). Similarly, *Nigrospora oryzae* #2OSTUR9a (E2) inoculated plants exhibited a 19.1 and 27.2% enhanced seed germination under salinity and drought stress, respectively. Under combined salinity and drought stress, only 19.4% increase in seed germination was observed in E2-inoculated plants (Fig. 6.1b). Two-way ANOVA analysis revealed a significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A7-Appendix).

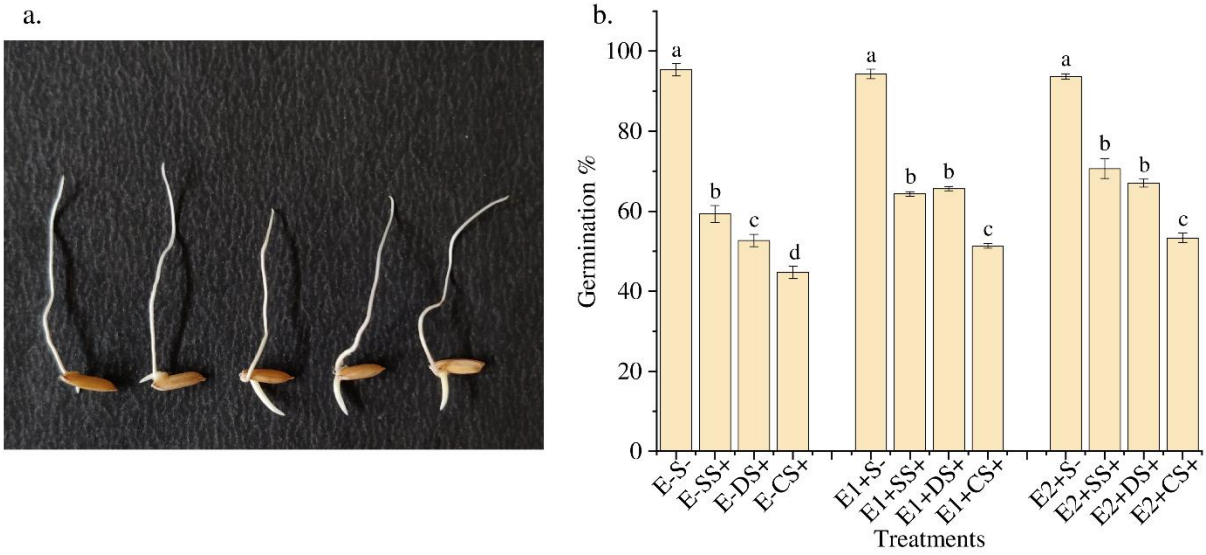

Fig. 6.1: a. Germinated seedlings of *Oryza sativa* var. PUSA-44; b. Germination (%) under different stress regimen (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.2 Pot trials under controlled environmental conditions

6.2.1 Effect of endophyte inoculation on physiological attributes

The salinity and drought stress conditions caused a drastic decrease in the physiological parameters of the rice plants. Fig. 6.2 shows the pots containing inoculated and uninoculated seedlings under trial in a controlled environment. The uninoculated plants displayed a shoot length of 13.6 ± 0.8, 7.3 ± 0.7, 7.0 ± 0.4 and 5.7 ± 0.5 cm under no stress, salinity, drought

and combined salinity and drought stress, respectively (Fig. 6.2). However, compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 39.1 and 35.3% increase in the shoot length under salinity and drought stress, respectively. Also, under combined salinity and drought stress, a 49.0% increase in the shoot length was observed. In the case of *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 43.5 and 55.5% increase in the shoot length was observed under salinity and drought stress, respectively. Whereas under combined salinity and drought stress, a 67.0% increase was seen in the shoot length (Fig. 6.3a).

Fig. 6.2: Pot experiment showing 14-day old rice seedlings under controlled environmental conditions.

A similar trend was seen in the case of fresh and dry weight of shoot. The uninoculated plants exhibited a shoot fresh weight of 243.2 ± 7.0, 131.8 ± 4.0, 126.2 ± 4.1 and 111.8 ± 4.8 mg and dry weight of 29.8 ± 1.9, 15.8 ± 1.7, 16.5 ± 2.6 and 14.2 ± 2.0 mg under no stress, salinity, drought and combined salinity and drought stress, respectively. Compared to the uninoculated plants, in *N. zimmermanii* #6OSFR2e (E1) inoculated plants, the fresh weight of shoot increased by 34.8, 47.2 and 48.3% under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.3b). Whereas the dry weight of the shoot increased by 50.5, 29.3 and 28.2% under salinity, drought and combined salinity and drought stress. In the case of *N. oryzae* #2OSTUR9a (E2) inoculated plants, the increase in fresh weight of shoot was 49.3, 45.7 and 54.8% under salinity, drought and combined salinity and drought stress, respectively. However, under salinity, drought and combined salinity and drought stress, the shoot dry weight increased by 42.1, 40.4 and 41.1%, respectively (Fig. 6.3b-c).

Furthermore, the uninoculated plants displayed a root length of 4.0 ± 0.2, 2.2 ± 0.2, 2.2 ± 0.3 and 1.9 ± 0.2 cm under no stress, salinity, drought and combined salinity and drought stress, respectively. However, compared to uninoculated plants, the root length of *N. zimmermanii* #6OSFR2e (E1) inoculated plants increased by 29.1 and 45.0% under salinity and drought stress. Moreover, a 30.1% increase was observed in the root length under combined salinity and drought stress. In the case of *N. oryzae* #2OSTUR9a (E2) inoculated plants, the root length was enhanced by 42.5 and 41.9% under salinity and drought stress. Whereas, under combined salinity and drought stress, a 47.8% increase in the root length was observed (Fig. 6.3d).

As observed in the case of fresh and dry weight of shoot, a similar trend was observed in the fresh and dry weight of roots. The uninoculated plants exhibited a root fresh weight of 61.5 ± 2.1, 34.3 ± 4.0, 35.0 ± 3.9 and 32.0 ± 4.6 mg and dry weight of 6.7 ± 0.3, 3.3 ± 0.4, 3.5 ± 0.3 and 3.2 ± 0.2 mg under no stress, salinity, drought and combined salinity and drought stress, respectively. The *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited an increase of 43.2 and 33.8% in fresh weight of roots under salinity and drought stress. Whereas an increase of 41.0 and 41.1% in root dry weight under salinity and drought stress compared to the respective controls was observed. Under combined salinity and drought stress, an increase of 30% in the root fresh weight and 36.6% in root dry weight was observed in E1 inoculated plants. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the root fresh weight increased by 45.7 and 51% under salinity and drought stress, whereas the root dry weight enhanced by 46.5 and 48.8%. Under combined salinity and drought stress, the root's fresh and dry weight were enhanced by 43.2 and 36.6%, respectively (Fig. 6.3e-f).

In the uninoculated plants, relative water content (RWC) of 93.3 ± 4.1, 56.4 ± 5.0, 58.8 ± 5.6 and 47.6 ± 3.7% was observed under no stress, salinity, drought and combined salinity and drought stress, respectively (Fig. 6.4a). On the contrary, the inoculated plants exhibited a tremendous increase in the RWC. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, the RWC increased by 29.5, 33.4 and 38.5% under salinity, drought and combined salinity and drought stress, respectively. Whereas in *N. oryzae* #2OSTUR9a (E2) inoculated plants, the same increased by 52.0, 40.6 and 56.9%.

Under no stress, salinity, drought and combined salinity and drought stress a chlorophyll content of 1022.5 ± 7.8, 715.7 ± 10.2, 689.0 ± 11.3 and 618.8 ± 6.6 µg/g FW was seen in the uninoculated plants (Fig. 6.4b). However, the inoculated plants exhibited enhanced chlorophyll production. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, the total chlorophyll content enhanced by 25.6 and 24.7% under salinity and drought stress. In

contrast, a 29.6% increase was observed under combined salinity and drought stress. Likewise, an increase in total chlorophyll content in *N. oryzae* #2OSTUR9a (E2) inoculated plants by 29.6, 38.6 and 21.8% was seen under salinity, drought and combined salinity and drought stress.

Furthermore, the uninoculated plants had a carotenoid content of 117.4 ± 5.5, 84.6 ± 2.7, 88.5 ± 4.3 and 73.3 ± 3.3 µg/g FW under no stress, salinity, drought and combined salinity and drought stress, respectively. However, both E1 and E2 inoculated plants exhibited enhanced carotenoid content. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, the carotenoid content increased by 20.7, 13.9 and 26.7% under salinity, drought and combined salinity and drought stress, respectively. This increase was 21.9, 17.6 and 9.1% under salinity, drought, combined salinity, and drought stress in *N. oryzae* #2OSTUR9a (E2) inoculated plants (Fig. 6.4c). Furthermore, two-way ANOVA analysis revealed significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A8-Appendix).

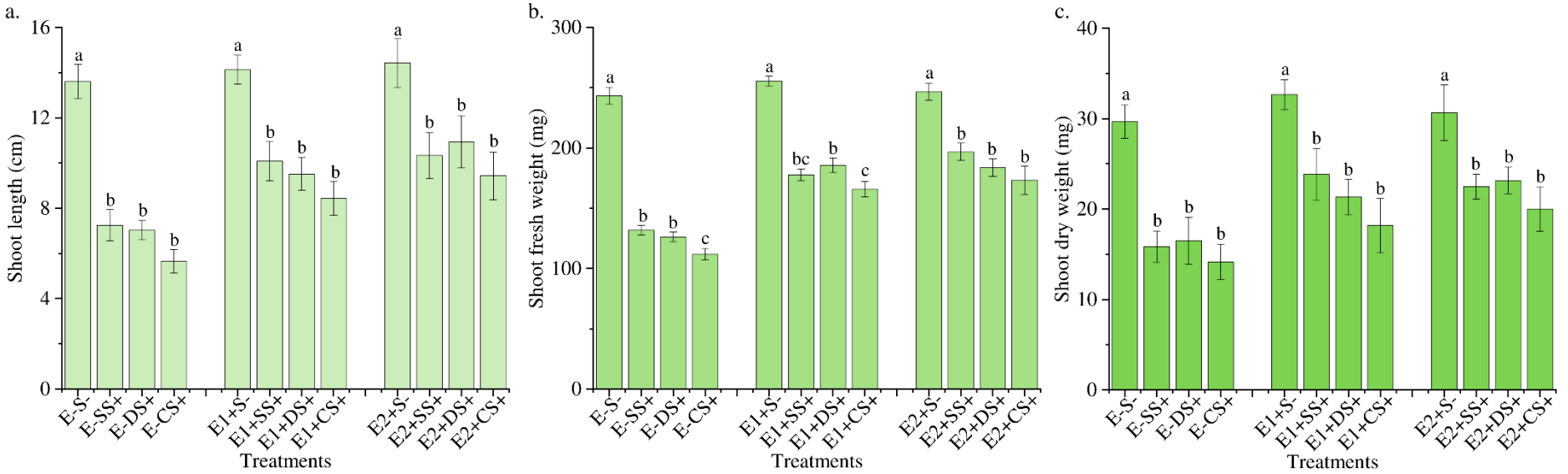

Fig. 6.3: Effect of endophyte inoculation on a. Shoot length; b. Shoot fresh weight; c. Shoot dry weight of rice plant under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

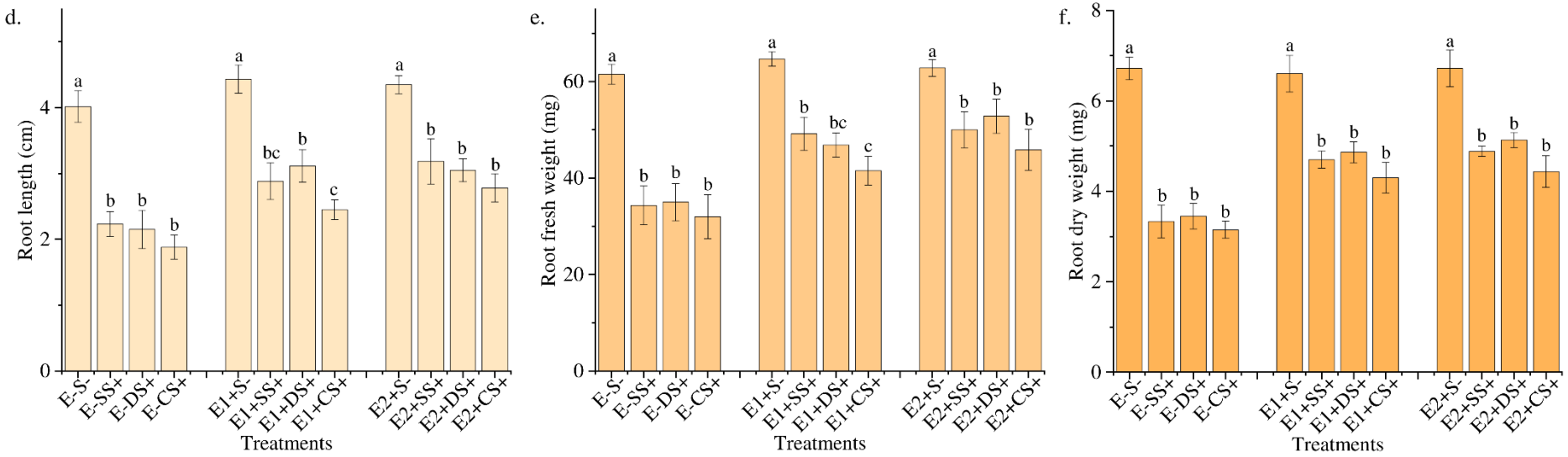

Fig. 6.3: Effect of endophyte inoculation on d. Root length; e. Root fresh weight; f. Root dry weight of rice plant under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

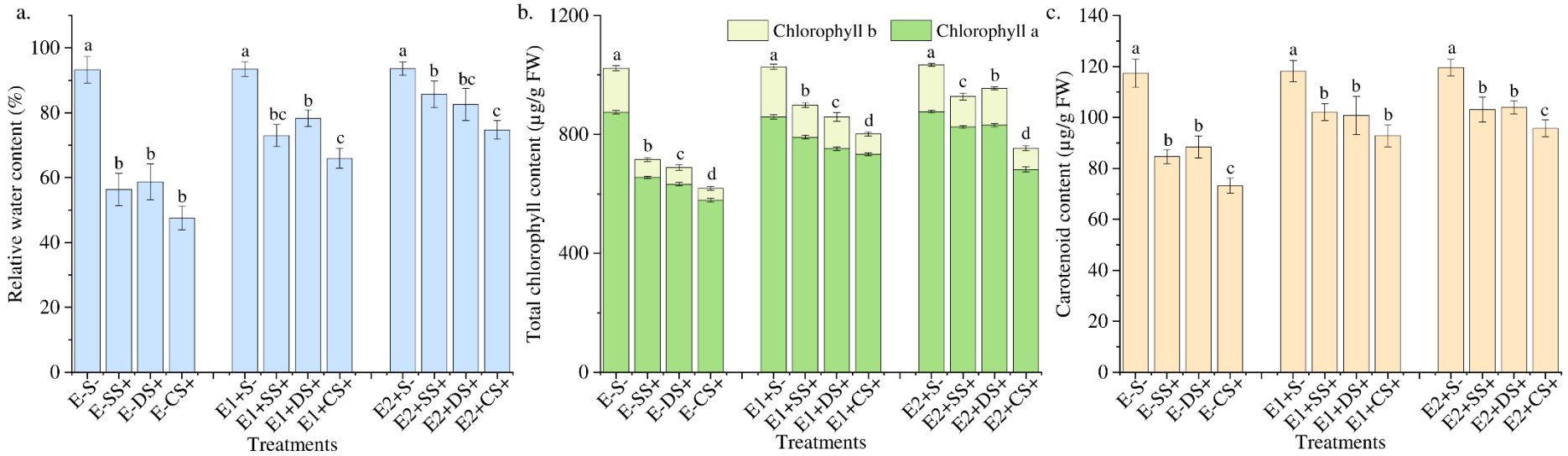

Fig. 6.4: Effect of endophyte inoculation on a. Relative water content; b. Total Chlorophyll content; c. Carotenoid content under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.2.2 Effect of endophyte inoculation on biochemical attributes

The different stress regimens also affected the plants' total phenolic and flavonoid content (Fig. 6.5). The uninoculated plants exhibited a total phenolic content of 251.5 ± 6.2, 334.4 ± 7.3, 355.2 ± 7.0 and 379.6 ± 8.4 µg/g FW under no stress, salinity, drought and combined salinity and drought stress, respectively. However, in *N. zimmermanii* #6OSFR2e (E1) inoculated plants, an increase in phenolic content by 91.6, 74.5 and 61.6% was seen under salinity, drought and combined salinity and drought stress, respectively. Whereas, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, the phenolic content increased by 109.9, 102.7 and 77.6% under salinity, drought, combined salinity, and drought stress (Fig. 6.5a).

Similarly, the uninoculated plants demonstrated a total flavonoid content of 364.7 ± 6.4, 485.3 ± 6.5, 522.8 ± 7.9 and 458.9 ± 5.7 µg/g FW under no stress, salinity, drought and combined salinity and drought stress, respectively. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, 60.7, 68.1 and 48.1% increase was observed under salinity, drought, combined salinity, and drought stress. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the flavonoid content increased by 74.4 and 90.9% under salinity and drought stress. An increase of 68.5% under combined salinity and drought stress was seen (Fig. 6.5b).

The different stress regimens also trigged the accumulation of osmolytes, such as sugars and proline (Fig. 6.6). The uninoculated plants had a total sugar content of 39.9 ± 1.9, 61.4 ± 2.0, 57.7 ± 1.7 and 65.5 ± 3.5 mg/g DW under no stress, salinity, drought and combined salinity and drought stress, respectively. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 105.2, 136.0 and 124.9% increase in the total sugar content under salinity, drought and combined salinity and drought stress. In contrast, the increase in *N. oryzae* #2OSTUR9a (E2) inoculated plants was 129.3, 143.7 and 160.8%, respectively (Fig. 6.6a).

The trend was also evident in the content of reducing sugar. The uninoculated plants exhibited a reducing sugar content of 25.3 ± 1.9, 39.6 ± 2.8, 36.6 ± 2 and 41.9 ± 2.3 mg/g DW under no stress, salinity, drought and combined salinity and drought stress, respectively. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited a 119.3, 136.8 and 128.5% increase in reducing sugar content under salinity, drought and combined salinity and drought stress. Whereas, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, this increase was 122.5, 143.5 and 174.9%, respectively (Fig. 6.6b).

Similarly, under no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited a proline content of 3.7 ± 0.1, 6.0 ± 0.3, 6.8 ± 0.2 and 8.6 ± 0.2 µM/g of sample. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, compared to the

uninoculated plants, this increase in proline accumulation was 51.4, 28.6 and 30.4% under salinity, drought and combined salinity and drought stress. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 58.0, 56.6 and 53.8% was observed (Fig. 6.6c).

The induction of different stress regimens caused an increase in malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) content in the plants (Fig. 6.7). The uninoculated plants exhibited MDA content of 60.2 ± 5.8, 129.0 ± 6.5, 135.5 ± 10.2 and 165.6 ± 5.4 nM/g FW under no stress, salinity, drought and combined salinity and drought stress, respectively. However, in *N. zimmermanii* #6OSFR2e (E1) inoculated plants, 36.7, 32.5 and 30.5% reduction in MDA content was seen compared to the uninoculated plants under salinity, drought and combined salinity and drought stress. Likewise, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, the MDA content was reduced by 33.3, 42.1 and 42.2%, respectively (Fig. 6.7a).

Likewise, under no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited a H_2O_2 content of 0.5 ± 0.0, 1.4 ± 0.0, 1.6 ± 0.0 and 1.8 ± 0.0 µM/g FW, respectively. In contrast, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited a reduction in H_2O_2 content by 37.3 and 32.5% under salinity and drought stress. Whereas under combined salinity and drought stress, a 28.6% reduction was observed. Furthermore, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, the H_2O_2 content decreased by 33.7, 46.5 and 31.4% under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.7b). Two-way ANOVA analysis revealed a significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A8-Appendix).

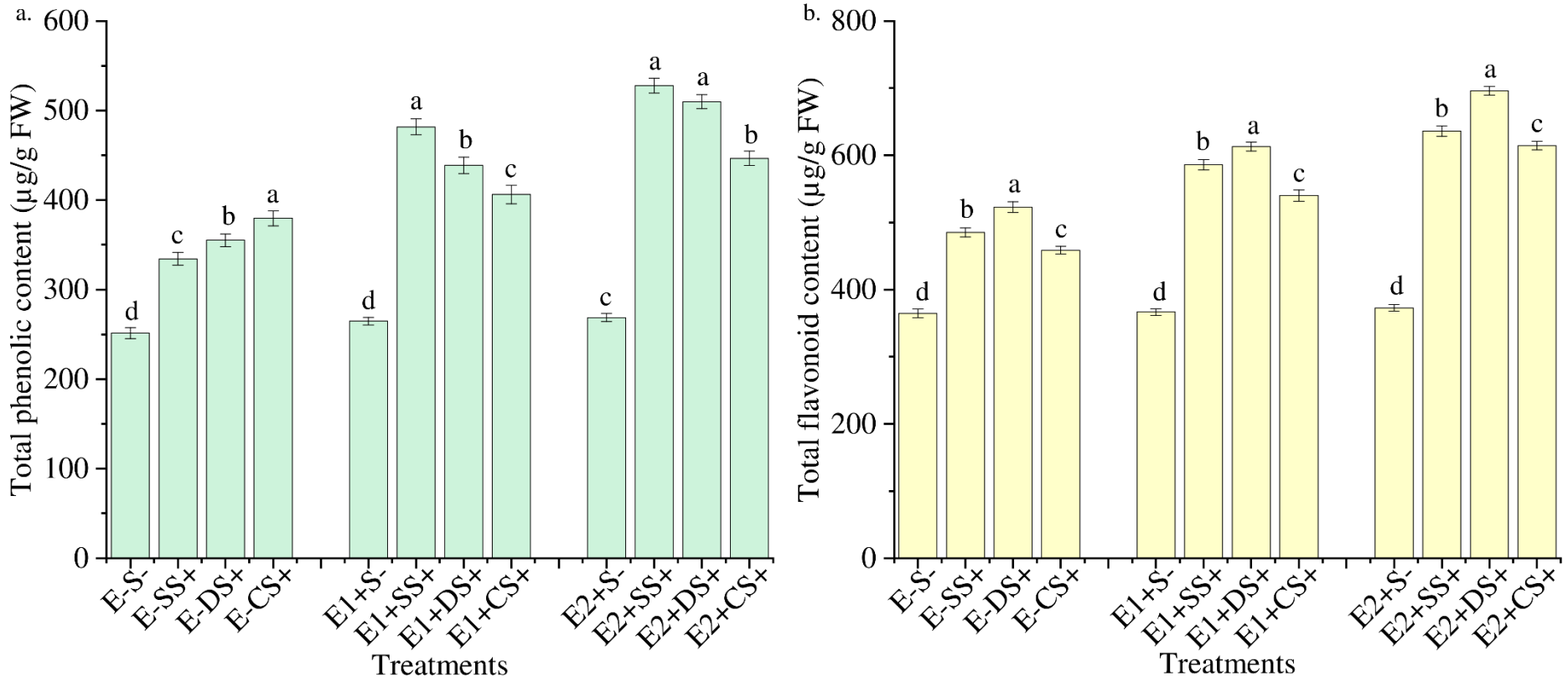

Fig. 6.5: Effect of endophyte inoculation on a. Total phenolic content; b. Total flavonoid content under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

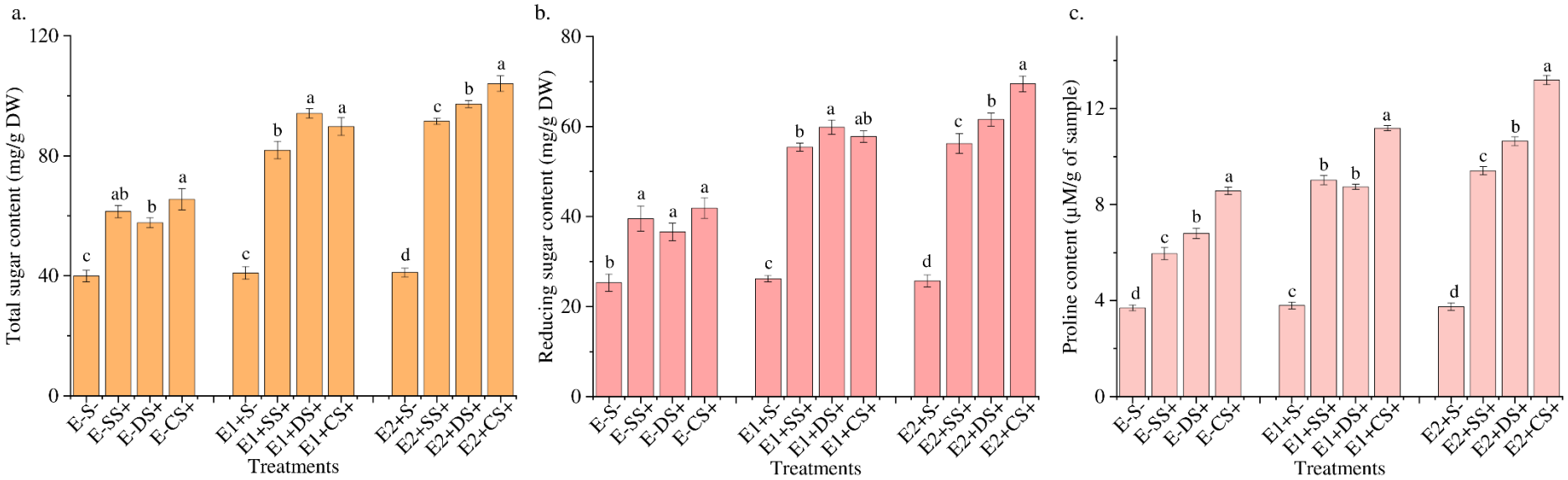

Fig. 6.6: Effect of endophyte inoculation on a. Total sugar; b. Reducing sugar; c. Proline content under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

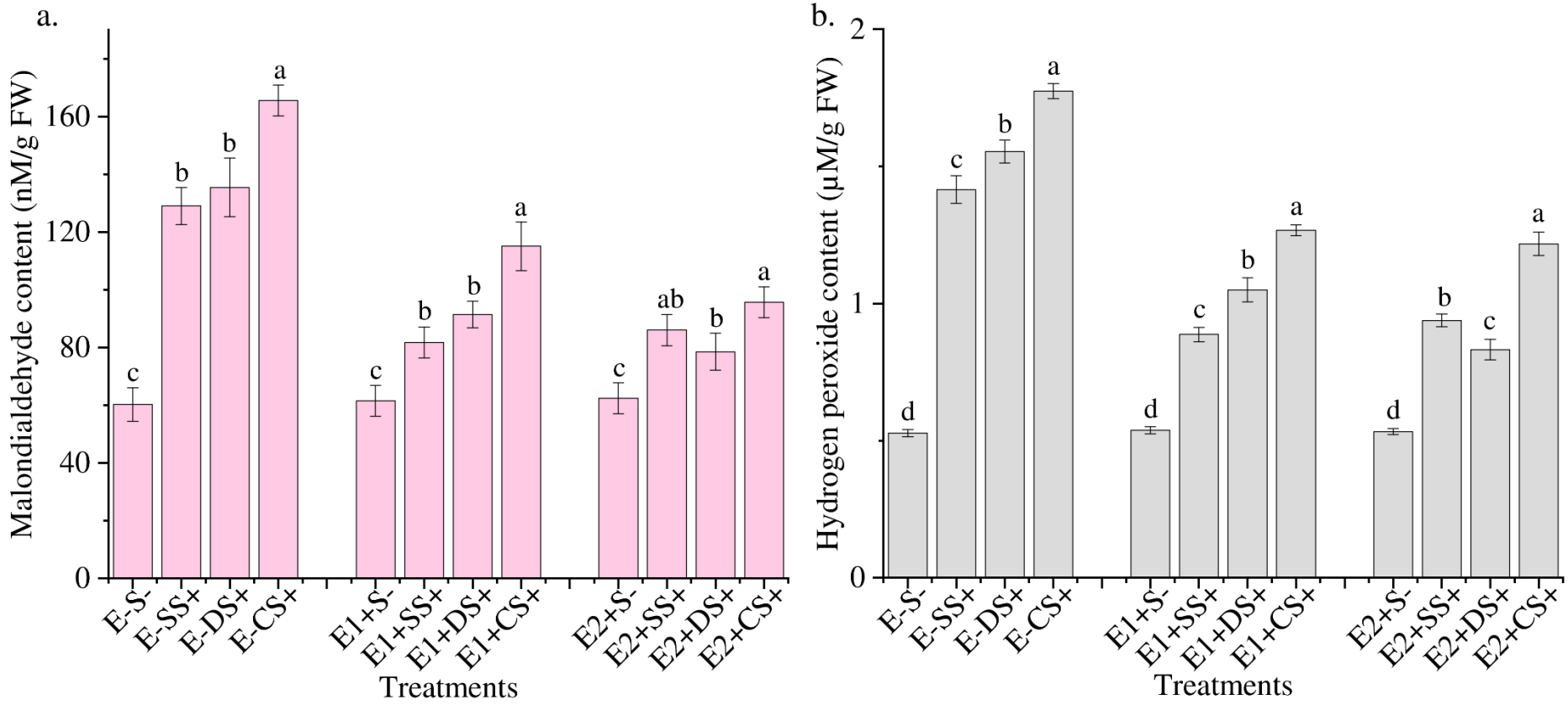

Fig. 6.7: Effect of endophyte inoculation on Malondialdehyde content under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.2.3 Effect of endophyte inoculation on enzymatic attributes

The induction of different stress regimens led to an increase in the antioxidant enzyme activity in both inoculated and uninoculated plants. The uninoculated plants exhibited ascorbate peroxidase (APX) activity of 0.7 ± 0.0, 1.2 ± 0.0, 1.3 ± 0.0 and 1.1 ± 0.0 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively (Fig. 6.8). However, in *N. zimmermanii* #6OSFR2e (E1) inoculated plants, the APX activity enhanced by 59.7, 21.1 and 32.5% under salinity, drought, combined salinity, and drought stress. Whereas in *N. oryzae* #2OSTUR9a (E2) plants, there was an increase of 48.6, 57.7 and 43.7%, respectively (Fig. 6.8a).

In the case of catalase (CAT), the uninoculated plants exhibited an activity of 0.05 ± 0.0, 0.1 ± 0.0, 0.1 ± 0.0 and 0.09 ± 0.0 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively. However, an increase of 31.3, 30.2 and 25.5% in *N. zimmermanii* #6OSFR2e (E1) inoculated plants and 28.3, 43.9 and 35.8% in *N. oryzae* #2OSTUR9a (E2) inoculated plants was observed under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.8b).

Similar results were obtained in peroxidase (POX) and superoxide dismutase (SOD) activity. The uninoculated plants exhibited POX activity of 0.1 ± 0.0, 0.3 ± 0.0, 0.3 ± 0.0 and 0.3 ± 0.0 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively. The POX activity increased by 36.2, 35.4 and 19.1% in *N. zimmermanii* #6OSFR2e (E1) inoculated plants and by 55.5, 39.3 and 29.4% in *N. oryzae* #2OSTUR9a (E2) inoculated plants under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.8c).

In the case of SOD, the uninoculated plants exhibited an activity of 4.1 ± 0.1, 5.3 ± 0.1, 5.8 ± 0.1 and 6.0 ± 0.1 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively. Whereas, *N. zimmermanii* #6OSFR2e (E1) plants exhibited an increase of 33.3, 18.8 and 8.6% under salinity, drought and combined salinity and drought stress. In contrast, the *N. oryzae* #2OSTUR9a (E2) inoculated plants exhibited an increase of 38.0, 35.1 and 13.7%, respectively (Fig. 6.8d). Two-way ANOVA analysis revealed a significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A8-Appendix).

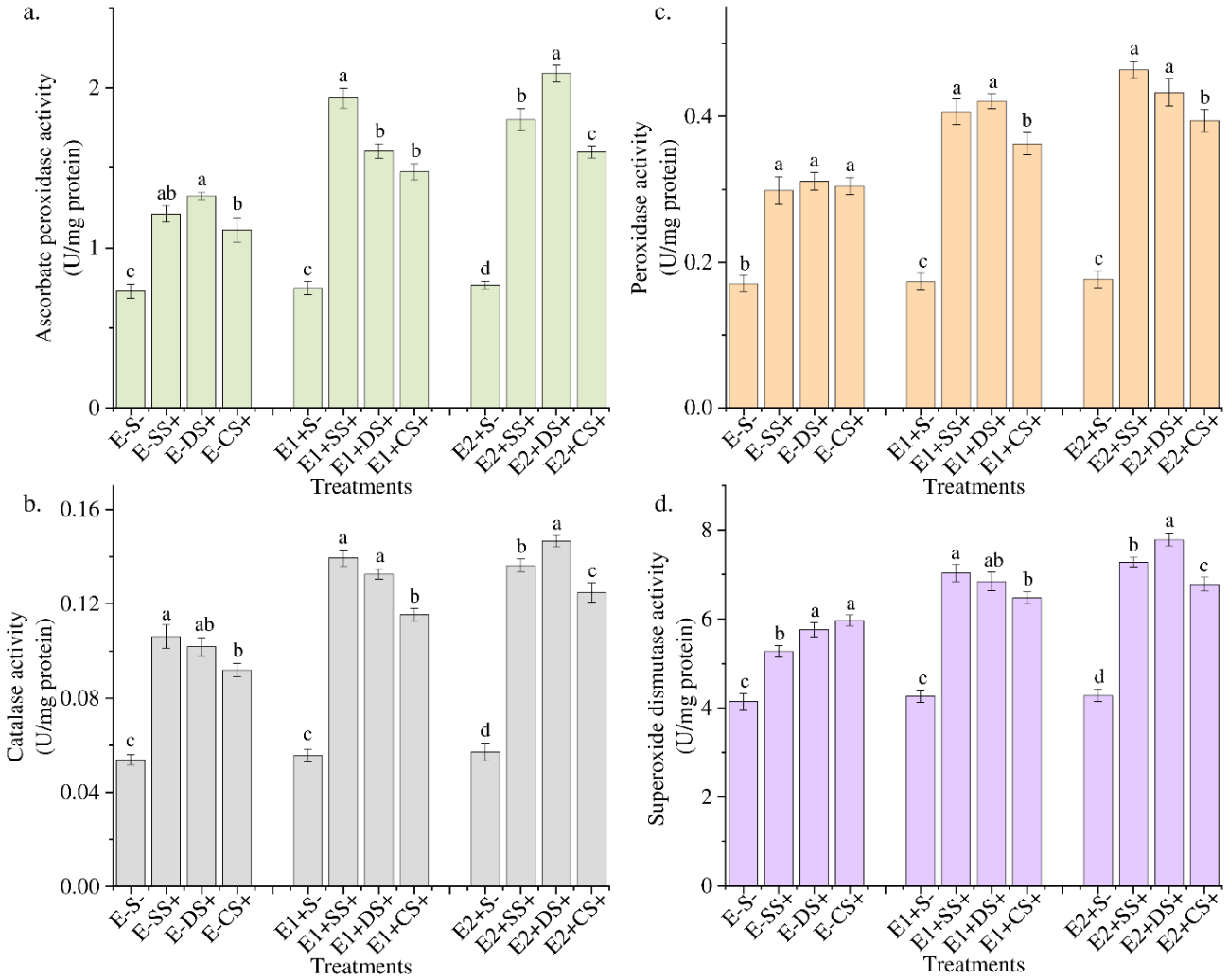

Fig. 6.8: Effect of endophyte inoculation on a. Ascorbate peroxidase; b. Catalase; c. Peroxidase; d. Superoxide dismutase activity under controlled conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.3 Pot trials under ambient environmental conditions

6.3.1 Effect of endophyte inoculation on physiological attributes

Under the ambient environment, the stress conditions caused a significant reduction in the physiological parameters of the rice plant. Fig. 6.9 shows the uninoculated and inoculated plants under different stresses. Under different regimens, such as no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited a shoot length of 24.5 ± 2.4, 10.9 ± 1.6, 10.8 ± 1.3 and 9.0 ± 1.0 cm, respectively (Fig. 6.10). However, the inoculated plants exhibited increased physiological parameters under tested stress conditions. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 32.0 and 28.8% increase in shoot length under salinity and drought stress, respectively. Whereas under combined salinity and drought stress, an increase of 38.9% was observed. Similarly, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 34.1 and 39.9% was seen under salinity and drought stress. In addition, an increase of 54.4% under combined salinity and drought stress was observed in *N. oryzae* #2OSTUR9a (E2) inoculated plants (Fig. 6.10a).

In the case of shoot fresh weight, the uninoculated plants had 351.1 ± 8.0, 189.7 ± 8.1, 180.3 ± 8.3 and 156.8 ± 10.1 mg fresh weight under as no stress, salinity, drought and combined salinity and drought stress, respectively. However, compared to the uninoculated plants, the shoot fresh weight of *N. zimmermanii* #6OSFR2e (E1) inoculated plants increased by 33.7, 29.2 and 40.2% under salinity, drought and combined salinity and drought stress, respectively. Likewise, this increase in *N. oryzae* #2OSTUR9a (E2) inoculated plants was 33.0, 43.8 and 51.7%, respectively (Fig. 6.10b).

Similarly, the uninoculated plants exhibited 39.8 ± 2.4, 20.0 ± 2.3, 21.5 ± 2.7 and 18.1 ± 3.2 mg dry weight under as no stress, salinity, drought and combined salinity and drought stress, respectively. On the contrary, compared to the uninoculated plants, the shoot dry weight of *N. zimmermanii* #6OSFR2e (E1) inoculated plants enhanced by 52.5 and 24.8% under salinity and drought stress. Under combined salinity and drought stress, this increase was 33.9%. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 36.7 and 35.7% increase was observed under salinity and drought stress. And 44.0% under combined salinity and drought stress (Fig. 6.10c).

Under different regimens, such as no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited a root length of 5.6 ± 0.4, 2.8 ± 0.3, 2.9 ± 0.3 and 2.5 ± 0.4 cm, respectively. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 35.5, 21.0 and 22.5% increase in

the root length under salinity, drought and combined salinity and drought stress. In the case of *N. oryzae* #2OSTUR9a (E2) inoculated plants, this increase was 43.4, 43.2 and 46.3%, respectively (Fig. 6.10d).

Furthermore, a decline in the root fresh and dry weight was also recorded in the uninoculated plants. Under no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited root fresh weight of 74.0 ± 3.7, 39.8 ± 2.0, 40.7 ± 2.0 and 35.8 ± 5.0 mg, respectively. However, the inoculated plants exhibited an increase in the fresh weight. Compared to the uninoculated plants, *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 30.5, 21.3 and 23.4% increase in the root fresh weight under salinity, drought and combined salinity and drought stress. Whereas in *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 26.4 and 35.3% increase was seen under salinity and drought stress. Under combined salinity and drought stress, an increase of 34.4% was observed (Fig. 6.10e).

Similarly, the uninoculated plants exhibited a root dry weight of 7.6 ± 0.6, 3.9 ± 0.1, 4.1 ± 0.1 and 3.4 ± 0.4 mg under no stress, salinity, drought and combined salinity and drought stress, respectively. Whereas, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 36.3 and 21.5% increase in the root dry weight under salinity and drought stress compared to the uninoculated plants. Under combined salinity and drought stress, an increase of 26.7% was observed. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the root dry weight enhanced by 38.0 and 34.4% under salinity and drought stress. This increase was 43.1% under combined salinity and drought stress (Fig. 6.10f). Furthermore, two-way ANOVA analysis revealed significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A9-Appendix).

Fig. 6.9: Pot experiment showing 21-day old rice seedlings under ambient environmental conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress).

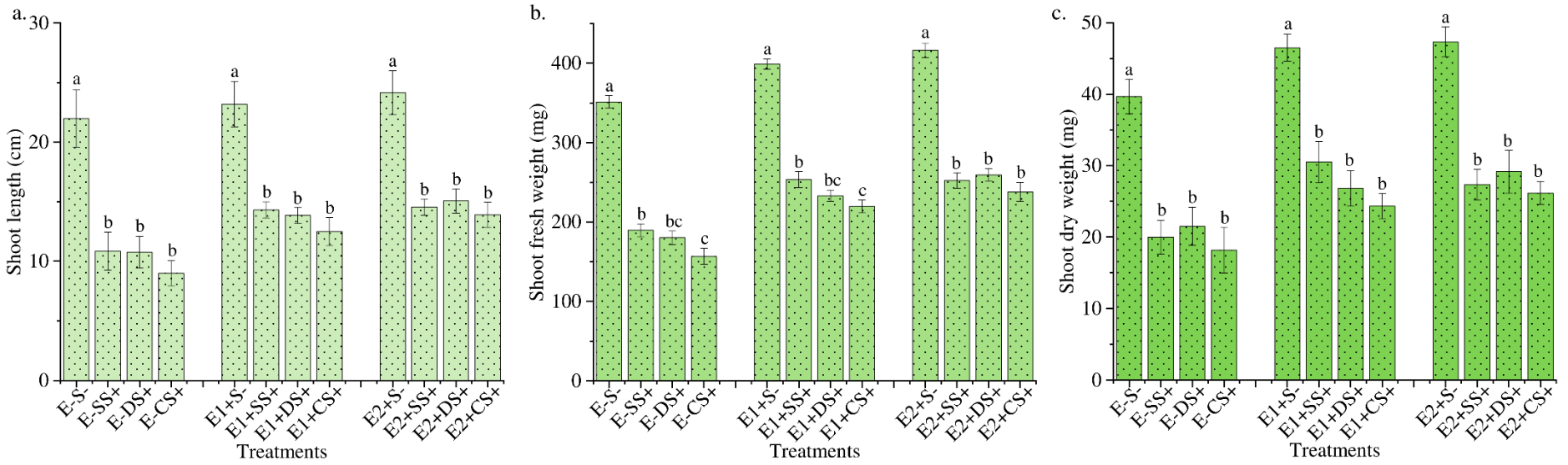

Fig. 6.10: Effect of endophyte inoculation on a. Shoot length; b. Shoot fresh weight; c. Shoot dry weight of rice plant under ambient conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

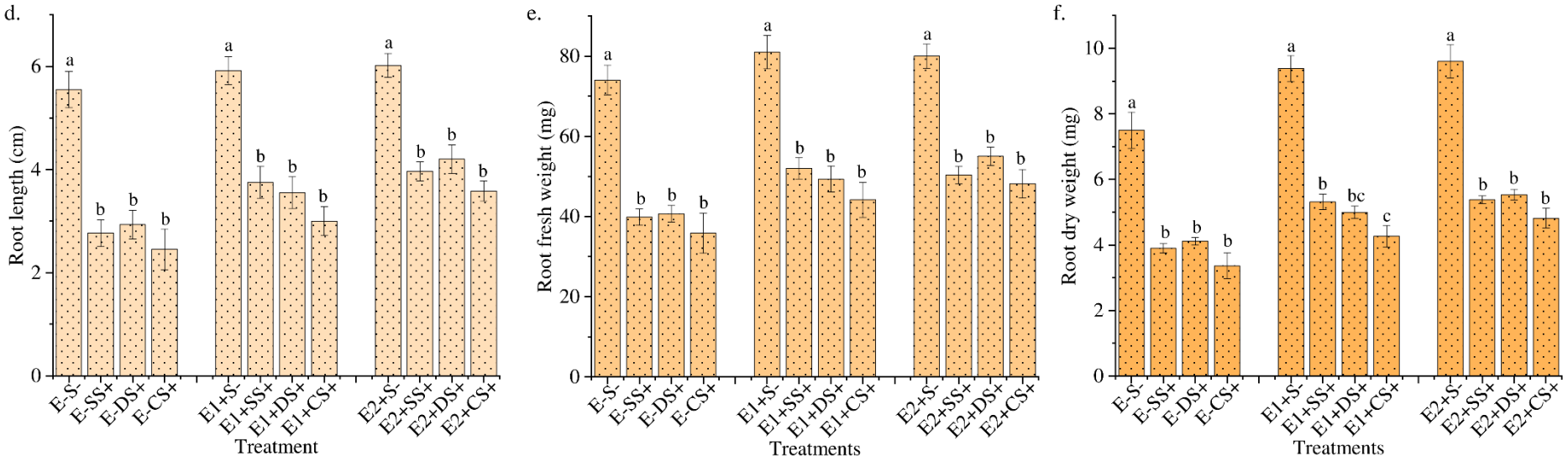

Fig. 6.10: Effect of endophyte inoculation on a. Shoot length; b. Shoot fresh weight; c. Shoot dry weight; d. Root length; e. Root fresh weight; f. Root dry weight of rice plant under ambient conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

An overall reduction in the RWC was seen under different stress protocols (Fig. 6.11a). The uninoculated plants exhibited a RWC of 92.1 ± 1.3, 55.4 ± 2.9, 54.5 ± 1.3 and 47.6 ± 3.7% under no stress, slainity, drought and combined salinity and drought stress, respectively. However, in the inoculated plants a significant increase in the RWC was seen. Compared to the uninoculated plants, *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 30.3 and 40.6% increase in the RWC under salinity and drought stress. Under combined salinity and drought, this increase was 33%. Similarly, the *N. oryzae* #2OSTUR9a (E2) inoculated plants exhibited a 48.4% increase in the RWC under salinity stress, a 50.3% increase under drought stress and a 50.4% increase under combined salinity and drought stress (Fig. 6.11a).

A similar trend was evident in the case of total chlorophyll content. The uninoculated plants exhibited a total chlorophyll content of 1392.3 ± 10.4, 950.5 ± 1.7, 891.8 ± 6.0 and 788.8 ± 8.1 µg/g FW under no stress, salinity, drought and combined slainity and drought stress, respectively. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 23.1 and 27.4% increase in the total chlorophyll content under salinity and drought stress. Likewise, an increase of 25.2% was observed under the combined salinity and drought stress. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 30.9 and 39.5% in the total chlorophyll content was observed under salinity and drought stress. Under combined salinity and drought stress, this increase was 35.2% (Fig. 6.11b).

In the case of carotenoid content, the uninoculated plants exhibited a content of 162.0 ±6.4, 112.9 ± 1.6, 120.7 ± 2.0 and 96.5 ± 5.8 µg/g FW under no stress, salinity, drought and combined slainity and drought stress, respectively. In comparison, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 20.5 and 9.3% increase in the carotenoid content under salinity and drought stress. Whereas under combined salinity and drought stress, a 26.8% increase was seen. Furthermore, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 24.0 and 15.7% increase in carotenoid content were observed under salinity and drought stress. Additionally, a 32.8% increase under combined salinity and drought stress was seen (Fig. 6.11c).

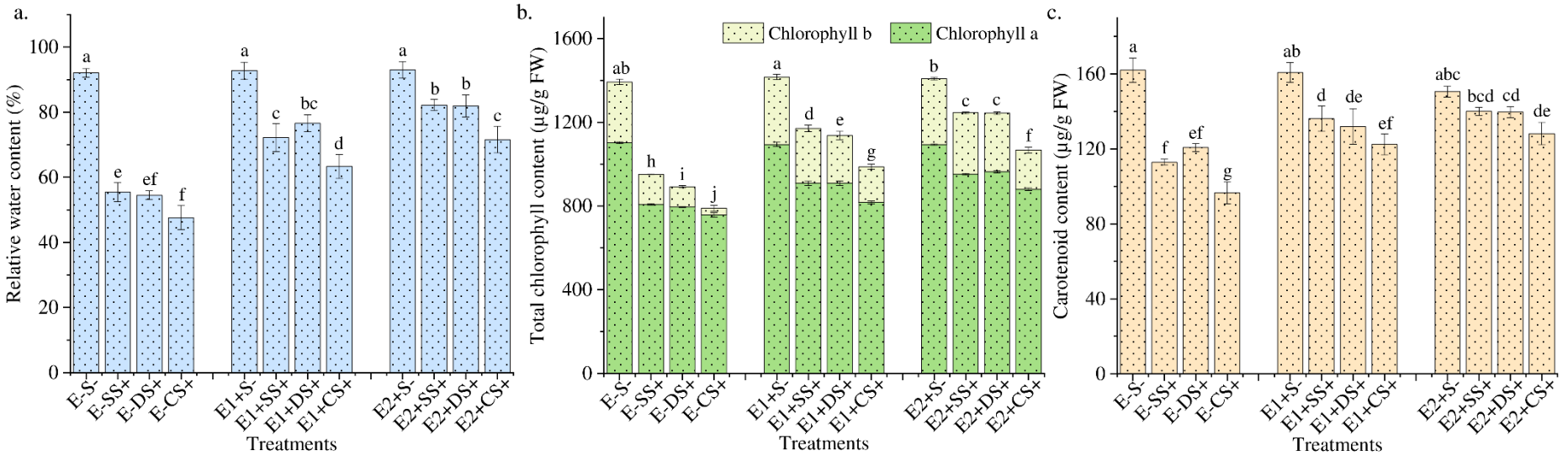

Fig. 6.11: Effect of endophyte inoculation on a. Relative water content; b. Total Chlorophyll content; c. Carotenoid content under ambient conditions (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.3.2 Effect of endophyte inoculation on biochemical attributes

An overall increase in the total phenolic and the total flavonoid content was observed under different stress protocols (Fig. 6.12). The uninoculated plants exhibited a total phenolic content of 366.6 ± 5.8, 476.0 ± 4.4, 501.9 ± 4.1 and 516.2 ± 10.7 µg/g FW under no stress, salinity, drought and combined salinity and drought stress, respectively. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, compared to the uninoculated plants, the total phenolic content enhanced by 27.0 and 15.3% under salinity and drought stress, respectively. Under combined salinity and drought stress, an increase of 8.1% was seen. Similarly, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 25.3 and 33.0% was seen under salinity and drought stress. Whereas under combined salinity and drought stress, an increase of 21.6% was observed (Fig. 6.12a).

In the case of total flavonoid content, the uninoculated plants exhibited 614.7 ± 8.6, 790.3 ± 8.5, 851.9 ± 6.5, 717.8 ± 13.7 µg/g FW flavonoid content under no stress, salinity, drought and combined salinity and drought stress, respectively. Whereas, in comparison, *N. zimmermanii* #6OSFR2e (E1) plants exhibited an increase of 19.4 and 7.6% under salinity and drought stress. And under combined salinity and drought stress, an increase of 13.7% was seen compared to the uninoculated plants. Similarly, under salinity and drought stress, the *N. oryzae* #2OSTUR9a (E2) inoculated plants exhibited 22.7 and 20.9% increase, respectively. While under combined salinity and drought stress, an increase of 28.8% was seen (Fig. 6.12b).

Furthermore, the stress conditions caused an increase in the accumulation of osmolytes, such as sugars and proline. Under different stress regimens, the uninoculated plants exhibited an overall increase in the total sugar, reducing sugar and proline content (Fig. 6.13). Here, the uninoculated plants exhibited a total sugar content of 56.6 ± 1.6, 83.3 ± 2.0, 94.2 ± 1.7 and 107.0 ± 2.5 mg/g DW under no stress, salinity, drought and combined salinity and drought stress, respectively. Compared to the uninoculated plants, *N. zimmermanii* #6OSFR2e (E1) inoculated plants enhanced the total sugar content by 33.3 and 23.2% under salinity and drought stress. Under combined salinity and drought stress, this increase was 15.9%. Similarly, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 50.7, 42.0 and 20.7% was seen under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.13a).

In the case of reducing sugar content, the uninoculated plants exhibited 39.6 ± 1.1, 53.8 ± 2.4, 57.7 ± 2.0 and 64.1 ± 2.0 mg/g DW reducing sugar content under no stress, salinity, drought and combined salinity and drought stress, respectively. Whereas, *N.*

zimmermanii #6OSFR2e (E1) inoculated plants exhibited a 35.7 and 35.0% increase compared to the uninoculated plants under salinity and drought stress. Whereas under combined salinity and drought stress, this increase was 29.5%. Likewise, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, an increase of 54.9 and 56.1% was seen under salinity and drought stress. And an increase of 32.6% was seen under combined salinity and drought stress (Fig. 6.13b).

Furthermore, under no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited 5.1 ± 0.1, 8.6 ± 0.1, 9.0 ± 0.1 and 12.3 ± 0.2 µM/g FW proline content, respectively. However, in comparison the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 39.0, 22.4 and 12.9% increase in the proline content under salinity, drought and combined salinity and drought stress. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 43.7 and 50.4% increase was seen under salinity and drought stress. Under combined salinity and drought stress, this increase was 28.7% (Fig. 6.13c).

The stress conditions also caused an increase in the MDA and H_2O_2 content. The uninoculated plants exhibited 74.2 ± 3.5, 174.2 ± 5.4, 182.8 ± 7.4 and 221.5 ± 14.7 nM/g FW MDA content under no stress, salinity, drought and combined salinity and drought stress, respectively (Fig. 6.14). Compared to the uninoculated plants, *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 35.8 and 25.3% reductions in MDA content under salinity and drought stress. Under combined salinity and drought stress, this decrease was 24.8%. Likewise, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, a 29.6, 37.1 and 40.3% reduction in MDA content was observed under salinity, drought and combined salinity and drought stress (Fig. 6.14a).

Likewise, the uninoculated plants exhibited 0.6 ± 0.0, 1.8 ± 0.0, 1.9 ± 0.0 and 2.2 ± 0.1 µM/g FW H_2O_2 content under no stress, salinity, drought and combined salinity and drought stress, respectively. Compared to the uninoculated plants, the *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited 32.7 and 31.1% reduction in the H_2O_2 content under salinity and drought stress. Whereas under combined salinity and drought stress, a reduction of 26.3% was seen. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the H_2O_2 content decreased by 27.9, 39.7 and 30.2% under salinity, drought, combined salinity, and drought stress (Fig. 6.14b). Furthermore, two-way ANOVA analysis revealed significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A9-Appendix).

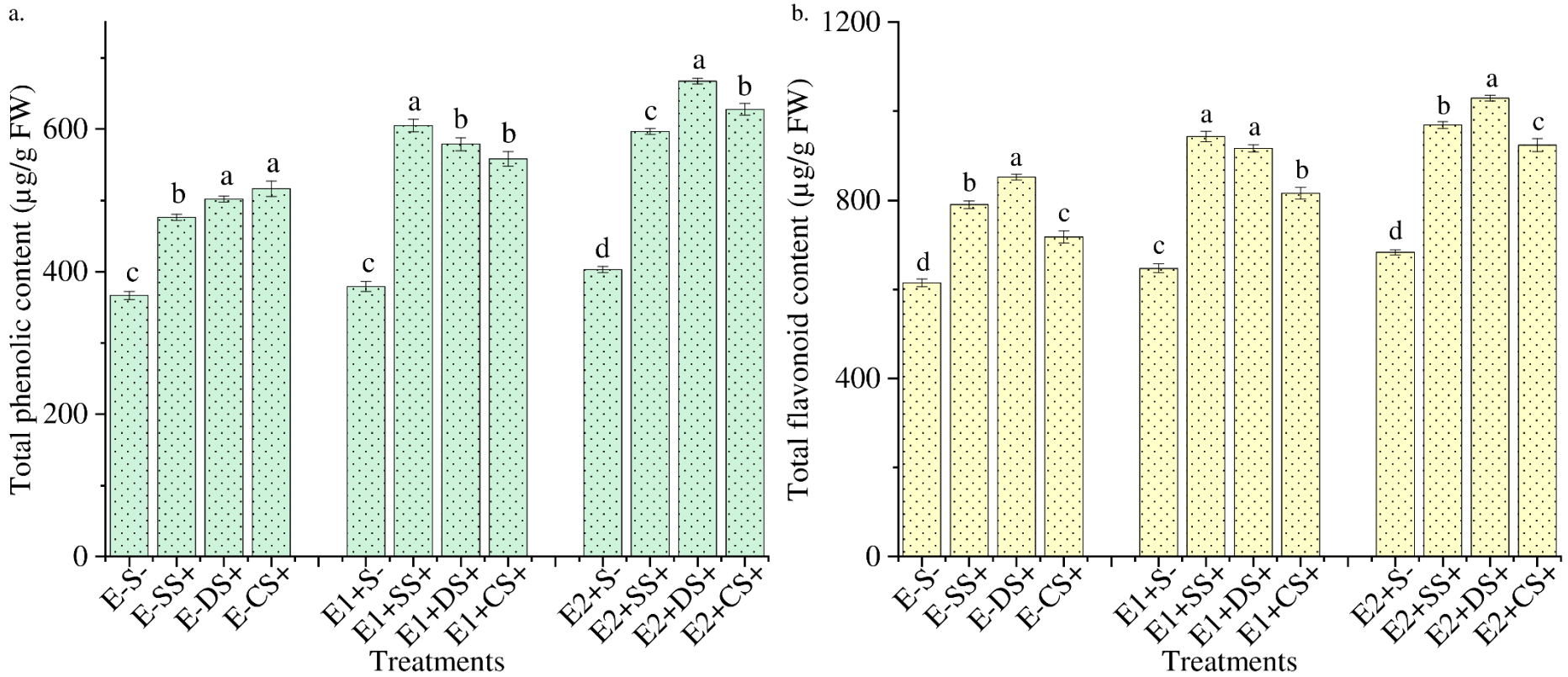

Fig. 6.12: Effect of endophyte inoculation on a. Total phenolic; b. Total flavonoid content under ambient environment (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

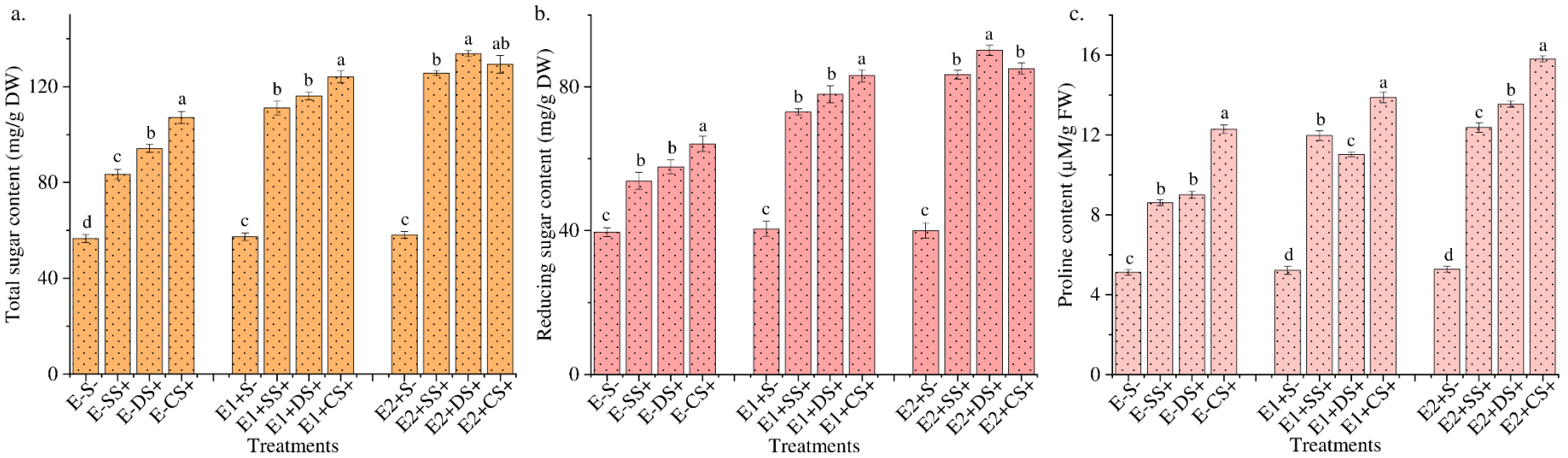

Fig. 6.13: Effect of endophyte inoculation on a. Total sugar content; b. Total reducing content; c. Proline content under ambient environment (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined salinity and drought stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

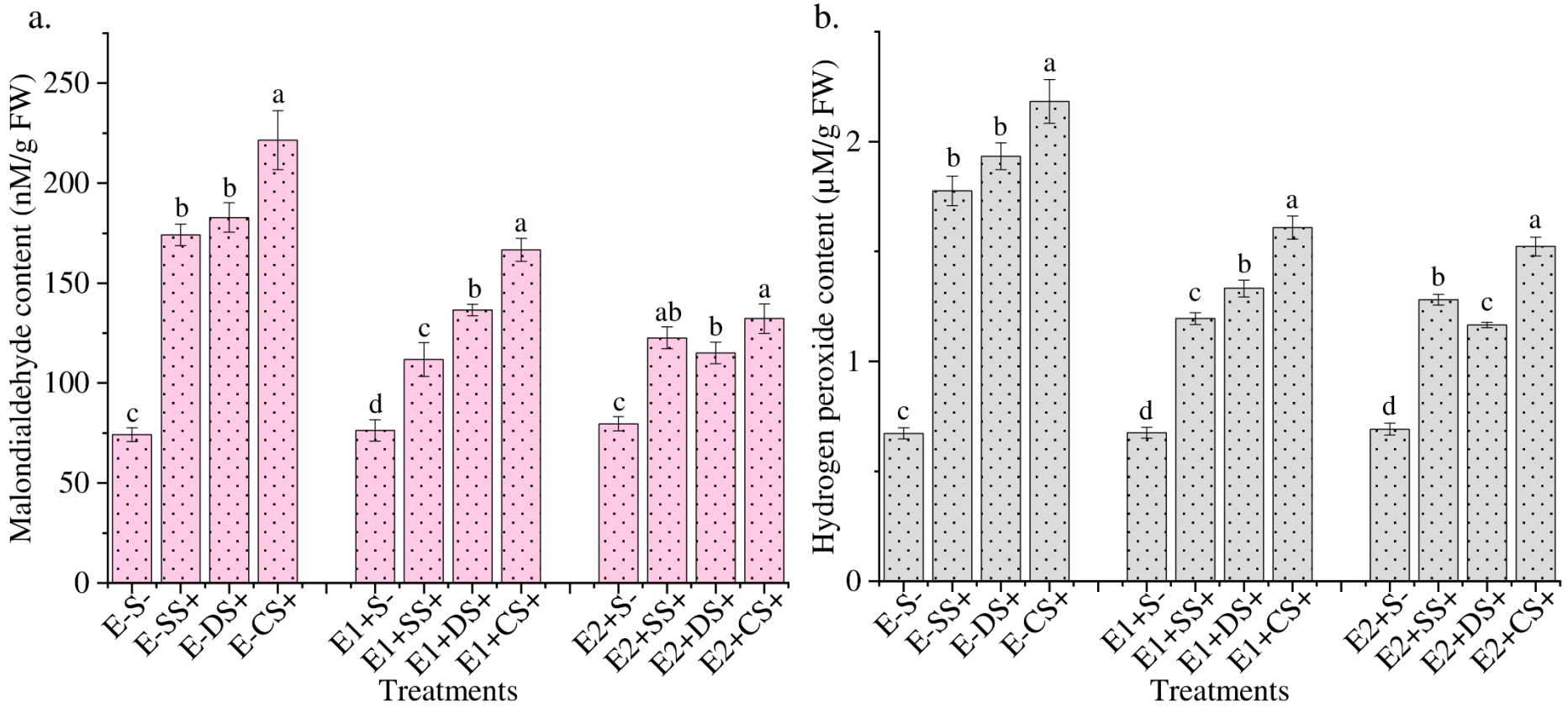

Fig. 6.14: Effect of endophyte inoculation on a. Malondialdehyde; b. Hydrogen peroxide content under ambient environment (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.6.3 Effect of endophyte inoculation on enzymatic attributes

The stress conditions caused an increase in the activity of antioxidant enzymes (Fig 6.15). Under different regimens like no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited APX activity of 1.0 ± 0.0, 1.7 ± 0.0, 1.9 ± 0.0 and 1.6 ± 0.0 U/mg protein, respectively. Compared to the uninoculated plants, the APX activity of *N. zimmermanii* #6OSFR2e (E1) inoculated plants enhanced by 56.7 and 19.8% under salinity and drought stress. Whereas under combined salinity and drought stress, this increase was 32.7%. Likewise, in *N. oryzae* #2OSTUR9a (E2) inoculated plants, the APX activity enhanced by 46.9, 57.1 and 40.3% under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.15a).

Similarly, the uninoculated plants exhibited CAT activity of 0.07 ± 0.0, 0.1 ± 0.0, 0.1 ± 0.0 and 0.1 ± 0.0 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively. In *N. zimmermanii* #6OSFR2e (E1) inoculated plants, a 30.6 and 33.0% increase in the CAT activity was seen under salinity and drought stress. In comparison, an increase of 28.5% was observed under combined salinity and drought stress. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the CAT activity enhanced by 25.5, 41.0 and 36.1% under salinity, drought and combined salinity and drought stress (Fig. 6.15b).

In the case of POX, the uninoculated plants exhibited activity of 0.3 ± 0.0, 0.5 ± 0.0, 0.5 ± 0.0 and 0.5 ± 0.0 U/mg protein under no stress, salinity, drought and combined salinity and drought stress, respectively *N. zimmermanii* #6OSFR2e (E1) inoculated plants exhibited a 34.8 and 32.9% increase under salinity and drought stress, compared to the uninoculated plants. Under combined salinity and drought stress, the activity enhanced by 16.1%. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the POX activity increased by 57.5 and 36.8% under salinity and drought stress. Moreover, an increase of 27.8% was observed under the combined salinity and drought stress (Fig. 6.15c).

Likewise, under no stress, salinity, drought and combined salinity and drought stress, the uninoculated plants exhibited SOD activity of 6.7 ± 0.1, 8.2 ± 0.1, 9.0 ± 0.1 and 9.4 ± 0.2 U/mg protein. The SOD activity of *N. zimmermanii* #6OSFR2e (E1) inoculated plants enhanced by 36.3 and 18.8% compared to the uninoculated plants under salinity and drought stress. Whereas under combined salinity and drought stress, this increase was 10.1%. In *N. oryzae* #2OSTUR9a (E2) inoculated plants, the SOD activity enhanced by 37.8, 24.3 and 14.6% under salinity, drought and combined salinity and drought stress, respectively (Fig. 6.15d). Furthermore, two-way ANOVA analysis revealed significant ($p < 0.001$) interaction between endophyte inoculation and different abiotic stresses (Table A9-Appendix).

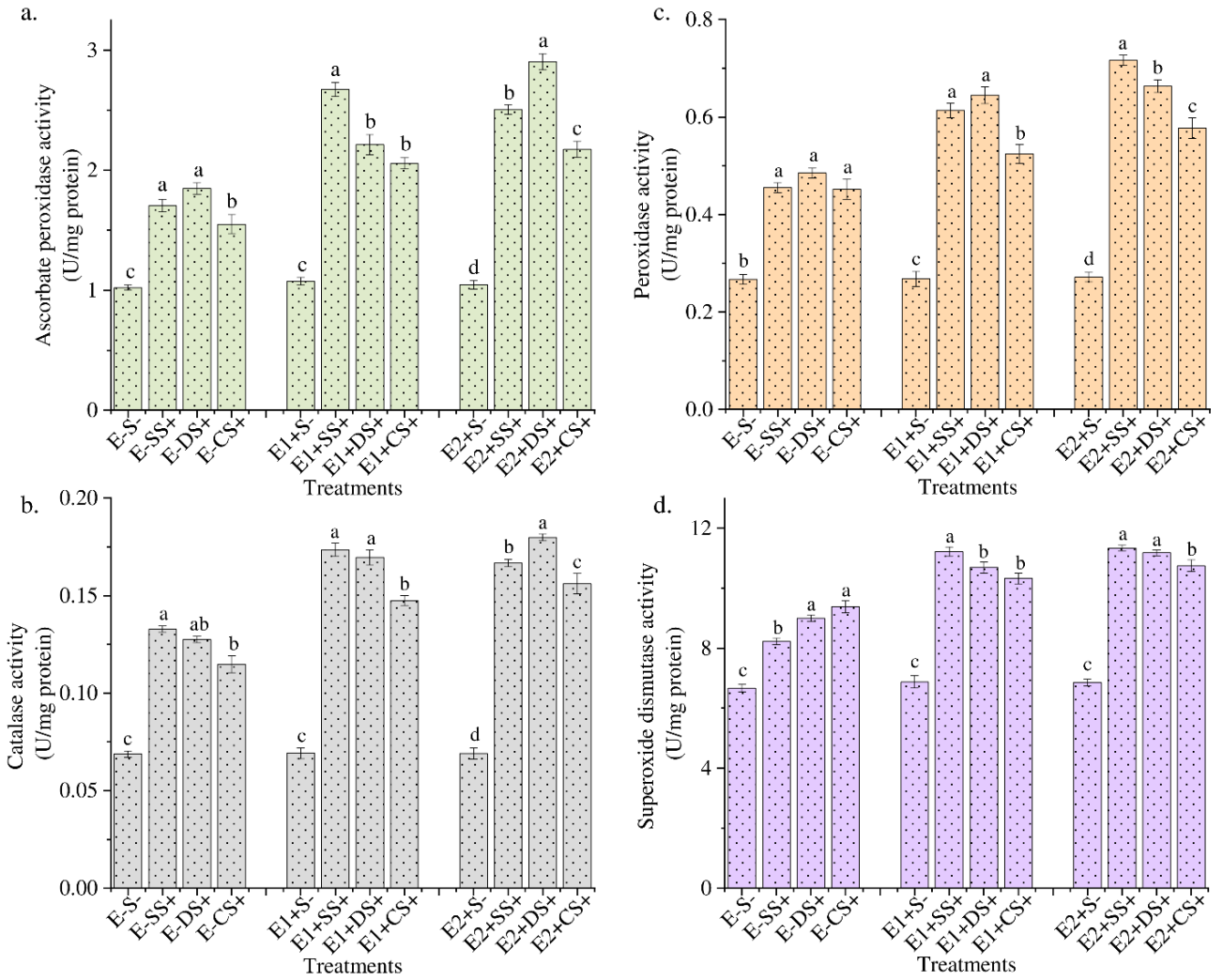

Fig. 6.15: Effect of endophyte inoculation on a. Ascorbate peroxidase; b. Catalase; c. Peroxidase; d. Superoxide dismutase activity under ambient environment (where, E- = Uninoculated, S- = No stress, E1 = *N. zimmermanii* #6OSFR2e, E2 = *N. oryzae* #2OSTUR9a, SS = salinity stress, DS = drought stress, CS = combined stress). The values represent mean ± SD, n=3. Significant difference within subsets is estimated using one-way ANOVA where superscript letters are different by Tukey's post-hoc test ($p<0.05$).

6.4 Confirmation of colonisation by root staining

The microscopic analysis revealed successful *Oryza sativa* var. PUSA-44 root colonisation by the selected endophytic fungi (Fig. 6.16). Under controlled conditions, 86.1 ± 5.5 and 83.3 ± 6.4% colonisation frequency was observed in plants inoculated with *N. zimmermanii* #6OSFR2e (E1) and *N. oryzae* #2OSTUR9a (E2), respectively. Whereas under ambient conditions, 80.6 ± 5.6 and 80.6 ± 10.6% colonisation frequency was observed in E1 and E2 inoculated plants, respectively.

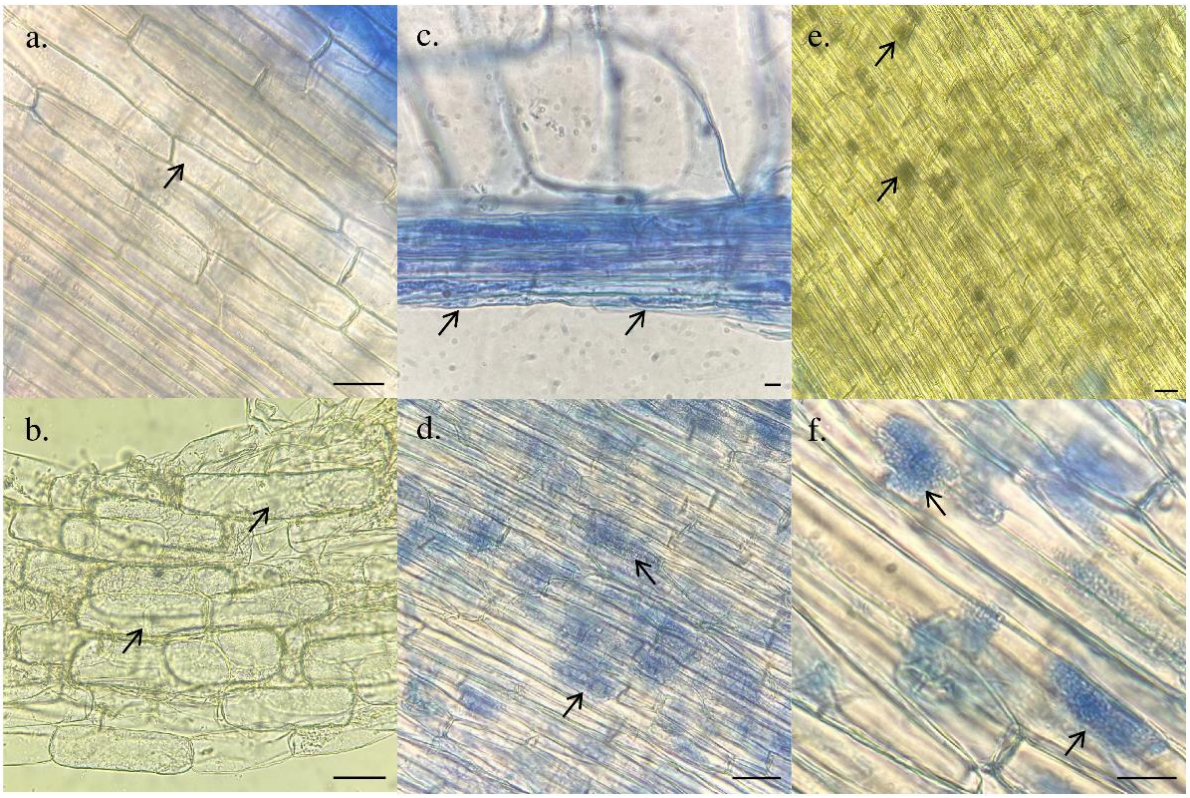

Fig. 6.16: a-b. Uncolonized roots of control rice plant; Detection of intracellular colonization by endophytic c-d. *Nigrospora zimmermanii* (#6OSFR2e); e-f. *Nigrospora oryzae* (#2OSTUR9a), indicated by arrowheads (Bar: 30 µm).

Chapter 7

Discussion

Since the dawn of time, scientists have recorded significant changes in the Earth's climate. These changes were majorly attributed to celestial and environmental catastrophes. However, the ongoing climate change is predominantly marked by anthropogenic activities (Ortiz-Bobea et al., 2021; Scotese et al., 2021; Bhardwaj et al., 2022). With the expansion of cities, the arable land is decreasing, whereas industrialisation has led to unfavourable agricultural conditions. The rising temperatures, poor irrigation practices, deforestation, and excessive use of agrochemicals have caused abiotic stresses at across the earth (Hossain et al., 2020; Duchenne-Moutien et al., 2021; Majedul, 2022; Hu et al., 2023). Salinity and drought have become common abiotic stresses which are potential threats to food security (Eswar et al., 2021; Orimoloye et al., 2022). Staple crops such as rice are a source of nutrition for much of the global population. However, the sensitivity of such crops to the unprecedented climate conditions hampers their growth and productivity (Razzaq et al., 2020; Panda et al., 2021).

Given the tremendous health benefits of rice consumption, it is imperative to sustainably enhance its production to keep up with the demand of the growing population (Chen et al., 2022; SubbuThavamurugan et al., 2023). Previous studies have reported adverse effects of salinity and drought on rice cultivation (Thu et al., 2017; Gao et al., 2019; Ishimaru et al., 2022). The stresses cause a reduction in the osmotic potential of the plants, thereby affecting the photosynthetic rates. These changes affect rice's overall nutrition and integrity, hence depreciating its sensory quality (Panda et al., 2021; Ishimaru et al., 2022). Traditional interventions for the intensification of agriculture, such as agrochemicals, saw a boom during the Green Revolution. Advanced techniques such as cross-hybridisation and genetically modified crops also presented a ray of hope. However, prolonged exposure to agrochemicals causes wide array of health problems (Elahi et al., 2019; Lee and Choi, 2020; Rani et al., 2021; Devi et al., 2022; Nath et al., 2023). Similarly, the hybrid varieties require extra inputs, such as fertilisers and herbicides, which is an added cost and has environmental implications (Meena et al., 2020; Pedroso et al., 2022; Nath et al., 2023). And lastly, the ethical concern related to GM and negative implications associated with these techniques discourage their extensive application (Adlak et al., 2019; Mueller and Flachs, 2022; Rezende et al., 2022).

Thus, employing sustainable techniques to protect food security is the need of the hour. One such method is using fungal endophytes, also known as endosymbionts, these microbes are ubiquitous (Verma et al., 2017; Fontana et al., 2021). The fossilized remains date back the association of plants and endophytes to 400 million years ago (Rodriguez and Redman, 2008). Moreover, the symbiosis is believed to be the cause for plants transitioning to the land (Remy et al., 1994; Lipnicki, 2015). Throughout the literature, studies have

extensively explored the symbiotic relationship of fungal endophytes and plants (Pang et al., 2020; Baron and Rigobelo 2022; Ganie et al., 2022). These endophytes have exhibited plant growth-promoting (PGP) attributes and mitigated the negative effect of stress conditions (Tsai et al., 2020; Hu et al., 2021; Rezaei et al., 2022; Gateta et al., 2023).

In this investigation, fungal endophytes were isolated from two rice varieties, namely, PUSA-44 (rain-fed) and Sahbhagi Dhan (drought-resistant). PUSA-44 is a traditional rice variety extensively cultivated in Punjab. In contrast, Sahbhagi Dhan is explicitly designed by the Indian Council of Agricultural Research-National Rice Research Institute (ICAR-NRRI) to withstand drought stress in the lowlands of Orrisa and Jharkhand (Dar et al., 2020). A total of 182 fungal endophytes were isolated from leaves, roots, internode and spikes of the two rice varieties. Of the total isolates, 120 belonged to the rain-fed and 62 belonged to the drought-resistant rice variety. Past studies have isolated endophytic fungi from rice. For instance, Tian et al. (2004) isolated 72 endophytic fungi from different plant parts of four rice cultivars grown in South China. In another study, Naik et al. (2009) reported 570 endophytic fungi isolated from three different rice cultivars of Karnataka, India. Potshangbam et al. (2017) isolated endophytic fungi from the late reproductive to early ripening stage of an indigenous rice variety grown in eastern India. However, this is the first investigation where PUSA-44 and Sahbhagi Dhan associated endophytic fungi have been isolated from all plant parts at different stages of the crop cycle.

It is a well-known fact that, the presence and abundance of endophytes can vary across different plant species, as well as within the same plant species across different growth phases (Pang et al., 2020; Sampangi-Ramaiah et al., 2020). Isolation frequency as a measure of endophytes presence in different plant parts and different growth phases was investigated in this study. Interestingly, during the reproductive stage, roots of both rain-fed and drought-resistant rice offered the highest number of isolates with isolation frequency of 11.3 and 8.8%, respectively. Studies in the past have also stated similar observations where roots and leaves of rice plants have shown high colonisation of fungal endophytes (Tian et al., 2004; Naik et al., 2009; Zakaria et al., 2010). Furthermore, the identification of genera to which these isolates belong can provide an idea regarding their characteristic nature. The previous studies focused on endophytes of rice plants have reported *Aspergillus*, *Chaetomium*, *Curvularia*, *Fusarium*, *Nigrospora* and *Penicillium* as the dominant genera (Tian et al., 2004; Naik et al., 2009; Zakaria et al., 2010; Atugala et al., 2015; Sampangi-Ramaiah et al., 2019). Our investigation also yielded similar results as reported in the past (Potshangbam et al., 2017; Sampangi-Ramaiah et al., 2020; Fernández-Pastor et al., 2021). The 120 isolates of rain-fed

rice represented 11 genera whereas the 62 isolates of drought resistant variety represented 8 genera. The majority of rain-fed variety isolates, belonged to the genera *Nigrospora*, followed by *Fusarium* and *Alternaria*. Likewise, *Fusarium* and *Nigrospora* were also the dominant genera isolated from the drought-resistant rice variety. The presence of various genera in different plant parts imply that the isolated endophytic fungi are not tissue or host specific assuring application over wide array of host plants (Potshangbam et al., 2017).

Talking about the different plant parts, *Nigrospora* was also the dominant genera isolated from all plant parts of the rain-fed rice, closely followed by *Fusarium*. Whereas *Fusarium* was the dominant genera isolated from all plant parts of the drought-resistant rice variety closely followed by *Nigrospora*. Additionally, these genera were also isolated from all stages of the crop cycle in both varieties. Recently, Sampangi-Ramaiah et al. (2020) reported *Fusarium* sp. isolated from salt adapted rice variety. Moreover, Fernández-Pastor et al. (2021) reported *Nigrospora* sp. as the dominant genera seen in other plants of the Poaceae family. Overall, the rain-fed rice variety had higher diversity of endophytic fungi, although 8 common genera were seen in both the varieties. Among plant parts, the roots and internode exhibited highest Shannon-Weiner Diversity Index (H') of 2.13 and 1.79 in the rain-fed and drought resistant rice, respectively. The results are in accordance with previous reports. Potshangbam et al. (2017) reported diversity of endophytic fungi in rice plants with Shannon-Weiner Diversity Index (H') of 2.37. A high prevalence of endophytes was seen in roots of rice plant with a H' of 1.56.

Taking into consideration the *in-vitro* screening and exploration of plant growth promoting traits, isolate #6OSFR2e from the rain-fed rice variety and #2OSTUR9a from the drought resistant rice were selected as the best performing isolates. Further tentative analysis of these isolates using morphological and microscopic techniques revealed close resemblance to *Nigrospora* species (Pryce et al., 2003; Wang et al., 2017; Hao et al., 2020). Post-sequencing, the BLAST analysis of ITS, TUB2 and TEF-1α regions of #6OSFR2e and #2OSTUR9a displayed close homology with *Nigrospora zimmermanii* and *Nigrospora oryzae*, respectively. The maximum-likelihood tree using the Tamura–Nei model (1000 bootstraps) gave similar results where #6OSFR2e and #2OSTUR9a clustered with *Nigrospora zimmermanii* and *Nigrospora oryzae*, respectively. The ITS, TUB2 and TEF-1α sequences of the isolates are submitted in GenBank and the isolates have also been added to the repository of NFCCI, Pune. In a past study, a salt tolerant *Nigrospora chinensis* isolated from *Aeluropus littoralis* was reported by Tarroum et al. (2021). However, the present study is the first report

on salinity, drought-tolerant *Nigrospora* species from the PUSA-44 (rain-fed) and Sahbhagi Dhan (drought resistant) variety of rice, along with plant growth promoting attributes.

Stresses like salinity and drought frequently co-occur in nature thereby causing severe harm to rice plants. Soils having electrical conductivities of 2 dS/m or higher (~0.02 M NaCl) are termed as saline. This build-up of sodium and calcium ions because of these stresses hinder the rice plants water intake which hampers its growth (Ma et al., 2020; FAO, 2021). In the present study, a reduction in radial growth and biomass of fungal endophytes was seen under different salt concentrations. This is because of the poor nutrient transport brought on by the reduction in water activity in the fungal cells. However, selected fungal endophytes in the present study demonstrated high salt tolerance capabilities. Eight rain-fed rice isolates (#5OSFS1a, #5OSFL6a, #6OSFL4c, #6OSFI1b, #6OSFR2d, #6OSFR2e, #7OSFS3a and #8OSFI2a) could sustain 2 M NaCl stress. Whereas, three isolates (#2OSTUL6d, #2OSTUR9a and #4OSTUR1e) from drought-resistant rice exhibited growth at 1.5 M NaCl stress. The findings are similar to other studies, like Sampangi-Ramaiah et al. (2020) observed salt tolerance of *Fusarium* sp. The endophyte isolated from salt-tolerant Pokkali rice (*Oryza sativa*) exhibited 78.0 and 48.3% growth at 1.5 and 2M NaCl, respectively. Likewise, Ripa et al. (2019) reported salt tolerance of endophytic *Trichoderma* isolates from wheat plants. The isolates were able to grow at 10% NaCl concentration. Another study reported, endophytic *Fusarium clavum* exhibiting growth at 0.5 M NaCl stress (Meshram et al., 2023). Nevertheless, the isolates in the current study demonstrated higher growth at similar salinity concentration.

Furthermore, polyethylene glycol (PEG-6000) was used to influence the osmotic potential of growth medium. The high molecular weight of PEG inhibits its absorption by fungal cells. It creates a low potential in the medium which affects the cellular homeostasis (Saddique et al., 2018). In the present investigation, six rain-fed rice isolates (#5OSFS1a, #6OSFL4c, #6OSFR2d, #6OSFR2e, #7OSFS3a and #8OSFI2a) and three isolates (#2OSTUL6d, #2OSTUR9a and #4OSTUR1e) from drought-resistant rice demonstrated growth potential at 20% PEG (-1.25 ± 0.01 MPa) stress. Other studies have shown similar findings, for instance, a study reported species of *Trichoderma*, *Aspergillus*, *Fusarium* and *Alternaria* exhibiting growth under different concentrations of PEG stress (Ripa et al., 2019). In another study, *Oryza sativa* associated endophytic *Talaromyces purpureogenus* could sustain 10% PEG stress. On testing the endophyte as inoculum, increase in physiological characteristics like root and shoot length, fresh and dry weight were seen (Pang et al., 2020). The present study also observed a positive correlation between the plate and broth assay employed for testing salinity

and drought tolerance. As well as a positive correlation between salinity and drought tolerance capability of fungal endophytes.

Rice plant is adversely affected by drought stress. The dry spell further increases soil salinity, especially in low-lying coastal locations. Altogether, these stresses affect plants in two ways: the water deficient effect, which reduces the water absorption, and the ion excess effect, which reduces growth by damaging the cells in the transpiring leaves (dos Santos et al., 2022). In terms of physiological characteristics, both salinity and drought cause a reduction in root and shoot length of rice plants. As a consequence, the overall biomass of the plant is affected (Iqbal et al., 2020; Kim et al., 2020; Zheng et al., 2023). The inefficient water uptake because of smaller roots coupled with build-up of NaCl in soil gravely reduces the relative turgidity of the rice plants. As the water uptake declines, the plant in unable to acquire proper nutrition (Huang et al., 2020; Dietz et al., 2021). In adverse cases it may also lead to plant death. India reports annual losses amounting to US$ 800 million, majorly because of abiotic stresses (Sharma et al., 2019; Wassmann, 2019). Both salinity and drought also cause a reduction in photosynthesis. It damages the thylakoid membranes which affects the photosynthetic machinery of the plants (Iqbal et al., 2020; Dietz et al., 2021). The poor nutrition absorption leads to ineffective regulation of enzymes involved in biosynthesis of chlorophyll (Thu et al., 2017; Chaudhry and Sidhu 2022).

In this study, on testing endophytic *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a as inoculum in *Oryza sativa* var. PUSA-44, the shoot and root length, fresh and dry weights of the plant enhanced significantly in comparison to the uninoculated plants under salinity, drought and combined salinity and drought stress. Among the two, *N. zimmermanii* #6OSFR2e inoculated plants exhibited better growth. Previous studies have observed similar findings, for instance, Santos et al. (2017) documented enhanced biomass and height of Nipponbare and Piauí rice varieties on inoculating dark septate endophytes under drought stress. A similar study reported enhanced biomass and plant height of Piauí rice variety on inoculation of fungal endophytes (Vergara et al., 2018). In another investigation, a *Fusarium* sp. enhanced the biomass of IR-64 rice variety by ~34.4% when subjected to salt stress (Sampangi-Ramaiah et al., 2020). Studies have observed similar outcomes on utilisation of endophytes in other plants as well (Qiang et al., 2019, Bilal et al., 2020, Hosseyni et al., 2022; Moghaddam et al., 2022).

In addition to the enhanced physical traits, the *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a inoculated plants also had higher relative water content and pigment production (Chlorophyll a, b and carotenoid) in comparison to the uninoculated plants under

stress conditions. The accumulation of water-soluble salts reduces the water availability for rice plant (Yang et al., 2019; Ishimaru et al., 2022). However, the improved RWC indicate efficient translocation of water. Previous literature shows enhanced physiological traits of different rice cultivars on association with rice under abiotic stresses. A study reported, GA3 producing endophytic *Fusarium* and *Aspergillus* sp. which improved the shoot and root length of Waito-C rice (Bilal et al., 2018). Mukherjee et al. (2022) observed that the phytohormones produced by endophytes stimulate growth, plant vigor, nutrient assimilation which aid plant to withstand stress conditions. Other studies have reported increase in chlorophyll content in rice plants associated with endophytic *Fusarium*, *Phomopsis* and *Serendipita* species under salinity and drought stress (Khalid and Aftab, 2020; Sampangi-Ramaiah et al., 2020; Hu et al., 2021; Khalvandi et al., 2021).

Salinity and the mineral nutrition of crops have a complicated relationship. Because of the interaction between Na^+ and NH_4^+, increased salinity results in decreased nitrogen absorption (Huang et al., 2020; Paul et al., 2020; Zheng et al., 2023). Minerals such as zinc and phosphorous are imperative for growth of rice and make up ~0.2% of the plant's dry weight. However, ~95-99% of the soil mineral content exists in insoluble form (Prabhu et al., 2019; Hnamte et al., 2021). Our study reports mineral solubilising potential endophytic *Nigrospora* sp. Here, *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a exhibited 1.0 ± 00 and 1.1 ± 0.0 phosphate solubilisation index. In addition, *N. zimmermanii* #6OSFR2e also demonstrated zinc solubilisation potential with an index of 2.3 ± 0.2. Similarly, Pang et al. (2020) and Tandon et al. (2020) reported phosphate solubilising species of *Chaetomium*, *Penicillium*, *Talaromyces* and *Trichoderma* associated with roots of rice plants. In a recent study, Chaudhary et al. (2023) reported endophytic *Aspergillus* and *Lecanicillium* sp. exhibiting solubilisation of different zinc salts. The enhanced physiological traits of the rice plants could be attributed to the plant growth promoting effect of fungal endophytes. Hydrogen cyanide is another compound which when produced in minute quantities by the endophytic fungi can positively impact the host plant (Ripa et al., 2019; Chauhan et al., 2024). Besides supressing the harmful microbes and initiating induced systemic response in plants, the HCN can solubilise certain minerals thereby enhancing the nutrient availability to the plants, especially under stress (Meena et al., 2020; Akhtar et al., 2022). However, none of the selected isolates of rain-fed and drought resistant varieties exhibited HCN production.

In addition, the endophytes in the current investigation demonstrated siderophore production. Endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a exhibited 67.9 ± 0.6 and 72.6 ± 0.1% siderophore production. The findings are in line with previous studies

which have reported siderophore producing *Aspergillus*, *Alternaria*, *Fusarium* and *Trichoderma* species (Ripa et al., 2019; Turbat et al., 2020; Taheri et al., 2022). Card et al. (2016) also mentioned the potential role of endophytic siderophores in inducing systemic resistance. Since excess salt disrupts the ionic homeostasis of rice, the plant in unable to absorb water. Consequently, the physiological traits of the plants are affected (Safdar et al., 2019; dos Santos et al., 2022). However, the mineral solubilisation and siderophore production capability demonstrated by the tested *Nigrospora* sp. fulfils a significant task by improving the nutrition and iron absorption in adverse conditions. As a result, the physiological parameters of the tested rice plant were significantly improved.

Furthermore, in addition to tolerating salinity and drought stress, the *Nigrospora* sp. reported in this study also exhibited phytohormone production potential. An absorption-maxima similar to that of standard indole acetic acid (IAA) and gibberellic acid (GA3) was observed on analysing the culture filtrate of selected fungal endophytes. The endophytes demonstrated IAA production in presence and absence of tryptophan, indicating the potential to utilise tryptophan by plants in natural environment (Sharma et al., 2018; Turbat et al., 2020; Badawy et al., 2021). On quantification, *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a produced the highest IAA production of 320.3 ± 5.5 and 351.0 ± 7.1 µg/mL. And GA3 production of 19.2 ± 0.1 and 26.8 ± 0.9 µg/mL, respectively. Previous studies have observed IAA and GA3 production from endophytic *Alternaria alternata*, *Aspergillus awamori*, *Aspergillus fumigatus*, *Chaetomium globosum*, *Galactomyces geotrichum*, *Fusarium proliferatum*, *Fusarium oxysporum*, *Penicillium* sp., and *Phoma glomerata* and among many others (Waqas et al., 2012, Waqas et al., 2014; Al-Hosni et al., 2018; Bilal et al., 2018; Mehmood et al., 2019, Qiang et al., 2019; Bao et al., 2020; Ben Rhouma et al., 2020). The phytohormones play a crucial role in seed germination, cell elongation and development of the rice (Bhatt et al., 2020; Rhaman et al., 2020; Pal et al., 2023). The increase of physiological attributes observed in this study could be attributed to the synergistic effect of mineral solubilisation, siderophore production and phytohormone production by endophytes. The endophyte association improves nutrition accessibility which enhances the plant's root system. Since roots physically interact with soil, this would allow better uptake of water and protect the photosynthetic machinery. While the exact mechanism is still unknown, the literature suggests that endophytes regulate genes related to maintenance of cell wall and aerenchyma formation. This facilitates exchange of gases and provides an energy reservoir for the distressed plant (Sun et al., 2019; Hu et al., 2021).

Besides affecting the photosynthetic machinery, the low water and nutrition conditions caused salinity and drought also affect the ionic and osmotic balance of rice plants. Biochemically, the NaCl causes disbalance of the osmotic potential which dehydrates the plants. Consequently, the plants start producing stress mediated ethylene which triggers a cascade of effects. From inhibiting root growth to disruption of ionic homeostasis, ethylene exacerbates the effects of salinity and drought stresses. While testing the endophytic *Nigrospora* sp. as inoculum in the present study an increase in accumulation of osmolytes such as, total sugar, reducing sugar and proline was seen. The findings are in line with previous reports. Rawat et al. (2016) observed high proline accumulation in *Trichoderma* associated rice seedings under salt stress. Similarly, proline content enhanced in rice plants on inoculation of fungal endophytes as seen in a study by Pang et al. (2020). The enhanced osmolyte accumulation seen in inoculated plants could be attributed to different PGP traits of the endophytes. For instance, both *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a endophytes reported in this investigation also exhibited ammonia production potential. Past research has documented species of endophytic *Aspergillus* and *Agaricus* among others exhibiting ammonia production (Mehmood et al., 2019; Ripa et al., 2019; Chand et al., 2020).

The selected endophytes in the present study also displayed ACC deaminase biosynthesis potential. The enzyme converts ACC (1-aminocyclopropane-1-carboxylic acid) into α-ketobutyrate liberating ammonia. The endophyte *N. zimmermanii* #6OSFR2e exhibited activity of 317.4 ± 0.4 nmol α-ketobutyrate/mg/hour followed by endophyte *N. oryzae* #2OSTUR9a with 305.4 ± 0.8 nmol α-ketobutyrate/mg/hour. A previous study demonstrated *Penicillium purpurogenum* with ACC deaminase activity of 355 nmol α-ketobutyrate/mg/hour (Ali et al., 2021). Likewise in another study ACC deaminase activity of 330 nmol α-ketobutyrate/mg/hour was observed in *Trichoderma asperellum* (Rauf et al., 2021). The nitrogen acquired in the form of ammonia enables the plants to synthesise proteins, amino acids and osmolytes. Whereas the biosynthesis of ACC deaminase enumerates the ethylene mediated damage in nutrient devoid conditions (Wang et al., 2022; Ikram et al., 2023). The accumulation of osmolytes maintains an osmotic equilibrium and provide an energy source to keep up the metabolic processes. Thus, there is a lower influx of ions which counters the oxidative burst (Jogawat et al., 2016; Kord et al., 2019).

Another critical effect of salinity and drought stress in rice plants is the oxidative damage. It is primarily caused by the reactive oxygen species (ROS), such as free radical and non-radicals. Plants carefully regulate a stable equilibrium of ROS; however, this equilibrium is disturbed during stress conditions (Qin et al., 2019; Tsai et al., 2020). Oxidative stress,

resulting from an excess of ROS, is the initial indicator observed in plants when exposed to abiotic stress. Disruption of this balance leads to cellular harm and significantly decreases crop yield (Czarnocka and Karpiński 2018; Hasanuzzaman et al., 2020). While testing the endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a as inoculum, an increase in the total phenolic and flavonoid content of the rice plant was observed under different stress regimens. In past studies, Jan et al. (2022) observed increase in TPC and TFC of *Candida membranifaciens* inoculated maize plants in presence of salt stress. Likewise, a 66% increase in phenolic content of endophyte inoculated *Moringa oleifera* seedlings was reported by Javed et al. (2022). Furthermore, in this study the activity of antioxidant enzymes also increased in *Nigrospora* associated rice plants. Both, *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a enhanced the activity of APX, CAT, POX and SOD under stress environment. Such findings have been reported by other researchers (Javed et al., 2022; Li et al., 2023). For instance, Asaf et al. (2018) noted enhanced salt tolerance of *Aspergillus flavus* associated Glycine max plants because of the high antioxidant enzyme activity. In another report, *Aspergillus ochraceus* inoculated barley plants demonstrated an increase of 21.4 and 36.6% in POX and SOD activity under salinity stress (Badawy et al., 2021). Qin et al., 2019 reported (Z)-N-(4-hydroxyethyl) formamide (NFA), a coumarin analogue which reduced the adverse effects of drought stress in rice plants by upregulating the antioxidant enzymes. In another study, Tsai et al. (2020) saw elevated POX and SOD activity of *P. indica* inoculated rice seedlings under drought conditions. Similarly, Siddiqui et al. (2022) reported enhanced SOD, CAT and APX activity in rice on inoculation of endophytic fungi.

To corroborate these findings, indicators of oxidative damage like malondialdehyde and hydrogen peroxide content were analysed. The oxidative stress disrupts the polyunsaturated lipids which compromises the structural integrity of the plants (Xue et al., 2021; Reshna et al., 2022). The present study revealed, a reduction in MDA content in rice plants inoculated with *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a. Previous studies indicate a similar pattern, for instance a study reported reduction in MDA content of rice by using endophyte derived coumarin derivative (Qin et al., 2019). Likewise, Tsai et al. (2020) observed reduction in MDA content on inoculating *P. indica* in rice subjected to drought stress. Another study documented a 19% decrease in MDA content of *Aspergillus terreus* inoculated rice seedlings under salt stress. Similarly, H_2O_2 is primarily produced by plant cells during photosynthesis, photorespiration, and to a lesser extent, during respiration. However, an excessive amount of H_2O_2 is produced in stressful circumstances, resulting in programmed cell death (Hamayun et al., 2017; Rehman et al., 2022). In this study, under stress conditions, a decrease in H_2O_2 content of inoculated plants was observed. Similar results were observed by

Siddiqui et al. (2022); the H_2O_2 content was reduced by 14% in rice plants inoculated with *A. terreus*. 249 In another study, *A. ochraceus* reduced H_2O_2 content in barley plants under salinity stress (Badawy et al., 2021). Likewise, Ghabooli and Kaboosi (2022) reported a reduction in H_2O_2 content by 20% on inoculation of *Serendipity indica* under drought stress. The findings imply the upregulation of the antioxidant enzyme system in inoculated plants by virtue of endophytic fungi. It highlights the potential of the tested endophytes in ameliorating oxidative damage.

In such atypical circumstances, antioxidant compounds such as phenols and flavonoids aid the plant by scavenging the free radicals. In our investigation, the selected endophytes of both the varieties exhibited varying concentrations of total phenolic and flavonoid content. Endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a exhibited the highest total phenolic content of 241.5 ± 4.9 and 211.7 ± 0.3 GAE/mg of sample, respectively. Previously, Khalil et al. (2020) reported endophytic *Aspergillus terreus* exhibiting 204.5 mg/mL phenolic content. In another study endophytic *Nigrospora sphaerica* exhibiting 43.7 ± 0.2 mg GAE/g DW phenolic content was documented (Kumar et al., 2021). Various other studies have reported extracellular phenolic content production by endophytic fungi (Gautam et al., 2021; Husna et al., 2021). Likewise, in this investigation endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a also exhibited the highest flavonoid content of 464.4 ± 4.4 and 408.4 ± 21.7 GAE/mg of sample, respectively. The findings are in accordance with previous studies which have reported flavonoid production by endophytic fungi (Khalil et al., 2020; Gautam et al., 2021; Husna et al., 2021; Kumar et al., 2021).

Further, on testing the scavenging of free radicals, the endophyte *N. zimmermanii* #6OSFR2e exhibited the highest % FRS of 88.8 ± 3.0% against DPPH radical. Whereas endophyte *N. oryzae* #2OSTUR9a exhibited 82.4 ± 0.3% scavenging. The endophytes exhibited an IC_{50} value of of 391.6 ± 20.7 and 365.5 ± 10.0 µg/mL. Other studies have documented similar reports, for instance Caicedo et al. (2019) reported 51.5% scavenging of DPPH radical by endophytic *Fusarium oxysporum*. In another study, endophytic *Curvularia geniculata* exhibiting 81.3 ± 3.4% FRS activity against DPPH radical was reported (Kalimuthu et al., 2022). The endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a also demonstrated Trolox equivalent antioxidant capacity. An IC_{50} value of 366.0 ± 5.9 and 357.5 ± 7.0 µg/mL was seen by endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a, respectively. Previous studies have also reported $ABTS^+$ scavenging capabilities of fungal endophytes. Recently, Tan et al., 2022 reported $ABTS^+$ scavenging capability of endophytic *Diplodia fraxini* with 37.8 ± 6.1 TE/mg of extract. Kalimuthu et al. (2022) reported *Curvularia*

geniculata with 79.5 ± 3.1% FRS activity against $ABTS^+$ radical. Mishra et al. (2022) reported endophytic *Aspergillus* sp. exhibiting IC_{50} of 14.33 µg/mL against $ABTS^+$.

The abiotic stresses are known to cause lipid peroxidation which destabilises the cell membrane of the plants (Tsai et al., 2020; Chaudhry and Sidhu 2022). Thus, the isolates endophytes were also tested using Fenton's reagent. The endophytes *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a exhibited best IC_{50} value of 227.8 ± 3.8 and 231.5 ± 7.7 µg/mL. The findings are similar to those reported earlier. For instance, Khalil et al., 2020 reported hydrogen peroxide scavenging ranging from 93-99% exhibited by various species of *Aspergillus*, *Fusarium*, *Alternaria* and *Mucor*. In another study endophytic *Arcopilus aureus* was documented exhibiting H_2O_2 scavenging with an IC_{50} of 0.1 ± 0.1 mg/mL (Dwibedi and Saxena 2020). In order to scavenge the free radicals, the endophytic fungi must possess the ability to simply act as electron donors and terminate radical chain reactions (Rani et al., 2021). This makes the reducing power assessment a vital parameter to be assessed. In this investigation, the reducing power of endophytic fungi was assessed by analysing the reduction of ferric ion. The highest activity of 78.9 ± 0.5 and 75.0 ± 0.5 µg Fe (II) equivalent/mg of extract was seen in *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a, respectively. Previously, Khalil et al. (2020) reported FRAP of various endophytic fungi with highest activity of 12.1 ± 0.1 mg/mL exhibited by *Aspergillus terreus*. Another study reported 0.1 ± 0.0 µM Fe_2SO_4/mg FRAP by endophytic fungi isolated from *Brunfelsia uniflora*, a flowering plant (Marsola et al., 2022).

The PGP traits of fungal endophytes are vital for the sustenance of plants. However, for the fungal endophytes to enter and establish a functional relationship with the host plant, production of extracellular lytic enzymes is a must (Sornakili et al., 2020; Ratul et al., 2023). All the selected isolates of rain-fed and drought resistant rice exhibited lytic enzyme production of varying degrees. The findings are in accordance with previous reports. Sornakili et al. (2020) reported cellulase and laccase producing *Nigrospora* species isolated from leaves of rice plant. Production of lytic enzymes such as cellulase and laccase have been reported from various endophytic isolates of rice plants. The major species in such cases were found to be *Absidia*, *Acremonium*, *Aspergillus*, *Cladosporium*, *Penicillium* and *Rhizopus* to name a few (Atugala and Deshappriya 2015). These lytic enzymes catalyse the hydrolysis of polysaccharides components, which can be assimilated by fungal endophyte and its host (Sunitha et al., 2013). The mentioned enzymes also provide inhibits pathogenic activities, protect plant against biotic stress prevalent in nature (Khan et al., 2016).

In addition, the selected endophytes also demonstrated antimicrobial potential. The ability of endophytic fungi to inhibit certain disease-causing microbes aids the plants by suppression of these microorganisms (Rho et al., 2018). The maximum inhibition % against *Escherichia coli* (18.3 ± 0.6), *Enterobacter aerogenes* (15.3 ± 1.2), *Bacillus subtilis* (15.7 ± 0.6) and *Staphylococcus aureus* (13.7 ± 0.6) was observed by endophyte *N. zimmermanii* #6OSFR2e among rain-fed rice variety isolates. Whereas endophyte *N. oryzae* #2OSTUR9a from drought resistant rice exhibited maximum inhibition against *Escherichia coli* (17.3 ± 1.2), *Enterobacter aerogenes* (13.3 ± 0.6), *Bacillus subtilis* (16.3 ± 0.6) and *Staphylococcus aureus* (14.3 ± 0.6). Similarly, the endophyte *N. zimmermanii* #6OSFR2e also exhibited highest inhibition % against *Alternaria alternata* (42.5 ± 0.8), *Botrytis cinerea* (45.0 ± 1.9), *Colletotrichum gloeosoriodes* (49.6 ± 1.9) and *Fusarium moniliforme* (35.2 ± 2.3). Whereas endophyte *N. oryzae* #2OSTUR9a showed the highest inhibition % against *Alternaria alternata* (29.1 ± 1.6), *Botrytis cinerea* (61.1 ± 1.0), *Colletotrichum gloeosoriodes* (42.2 ± 2.7) and *Fusarium moniliforme* (30.0 ± 0.5).

Previous studies have reported similar findings where endophytic fungi from numerous plants have exhibited antimicrobial potential. de Carvalho et al. (2021) reported endophytic *Alternaria* and *Diaporthe* sp. exhibiting inhibition against *E. coli* and *Staphylococcus aureus*. In another study, Du et al. (2020) reported species of endophytic *Chaetomium*, *Fusarium*, *Cladosporium* and *Penicillium* exhibiting inhibition of *E. coli*, *Enterococcus faecalis* and *Staphylococcus aureus*. Additionally, the endophytes also exhibited antifungal potential against *Fusarium oxysporum* and *Colletotrichum siamense*. Here, the effective antagonism of harmful microbes indicates the possibility of successful colonisation of the host plant by the selected fungal endophyres. This also serves as one of the crucial aspects by which the selected endophytes would prove to be useful.

For confirmation of root colonisation by selected fungal endophytes in the present study, the roots were stained using trypan blue. Both *N. zimmermanii* #6OSFR2e and *N. oryzae* #2OSTUR9a successfully colonised the host plant with more than 80% colonisation frequency. Previously, various studies have observed colonisation of plant roots by endophytic fungi using this method (Yuan et al., 2010; Rani et al., 2016; Wijesooriya and Deshappriya 2016; Getachew et al., 2019). Overall, the enzymatic and non-enzymatic antioxidants are necessary for ionic balance and stress resistance. The results suggest a connection between increased photosynthesis and enhanced activity of antioxidant enzymes. It also supports the idea that fungal endophytes promote photosynthesis in the host plant because of the reduction in ROS build-up. Moreover, the mineral solubilisation potential of the endophyte could also be

interlinked with the enhanced physiological attributes of the plants. As a result, the inoculated plants were able to thrive in complex environmental settings. Thus, in this research work we prove that *Nigrospora zimmermanii* #6OSFR2e and *Nigrospora oryzae* #2OSTUR9a hold tremendous potential to be developed as bioinoculants to ameliorate salinity and drought stress in *Oryza sativa* var. PUSA-44, a rain-fed rice variety.

Chapter 8

Conclusion

The global decline in crop productivity has become evident over time. These losses, primarily caused by anthropogenic activities, have brought many high-yielding crop varieties, such as PUSA-44, to the verge of discontinuation. Endophytic fungi exhibit the intrinsic property of adapting to the host plant's environment and exhibiting similar characteristics.

- To the best of our knowledge, this is the first study to isolate endophytic fungi from different plant parts of PUSA-44 and Sahbhagi Dhan rice variety throughout the crop cycle in India.
- The isolation frequency of the endophytic fungi throughout the crop cycle followed an asymmetrical bell-shaped curve trend in both the rice varieties, indicating high colonisation during the reproductive phase.
- The study also observed the highest diversity of endophytic fungi in the roots and internodes of the rice plants. In addition, an increase in diversity of endophytic fungi was seen during the transition from late reproductive to maturation stage.
- Furthermore, the study found a positive correlation between salinity and drought tolerance of endophytic fungi.
- The selected isolates from both rice varieties exhibited phenomenal antioxidant, antimicrobial potential, phytohormone, siderophore, ACC deaminase production, mineral solubilisation and extracellular lytic enzyme production.
- The tested isolates from both rice varieties demonstrated successful colonisation of the rain-fed rice, indicating the non-host-specific nature of endophytic fungi.
- Moreover, the tested isolates effectively mitigated the impact of salinity, drought, combined salinity, and drought stress under controlled and ambient growth conditions.
- This is the first study to report plant growth promoting and abiotic stress mitigation potential of *Nigrospora zimmermanii* and *Nigrospora oryzae*.

The intent to sustainably intensify agricultural production is a strenuous task considering the vulnerability of agriculture to climate change. Pursuing beneficial microorganisms to boost plant growth and development, especially for staple crops, could guide towards more green solutions. Considering the current findings, further studies should explore omics tools to understand the cross-talk and mechanism between the symbionts and the eventual application of the technology under field conditions to elucidate its full potential.

Chapter 9

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Chapter 10

Appendix

Appendix-A

Details of instruments and chemicals used

Instruments

• Autoclave	Equitron, India.
• Centrifuge	Sigma 1-15PK, 2-16KL, USA.
• ELISA plate reader	BioTek Synergy, Agilent Technologies, USA.
• Fourier Transform Infrared Spectroscopy	IRTracer-100, Shimadzu Corp., Japan.
• High Performance Liquid Chromatography	UFLC HPLC System, Shimazdu Corp., Japan.
• Incubator	Metrex BOD incubator, Delhi, India.
• Laminar flow cabinet	Thermadyne Biosafety Cabinet-I, India.
• Oven	Metrex oven, Delhi, India.
• pH meter	Cole-Parmer P200, Antylia Scientific, USA.
• Rotary evaporator	DLAB RE100-Pro, China.
• Shaking incubator	Lab-Therm incubator shaker, Kuhner, Switzerland.
• Ultrasonic bath	Ultrasonic bath: PCI analytics-9L, India.
• UV-Viz spectrophotometer	UV-1900, Shimadzu Corp., Japan.
• Vortex	Vortex: Tarson Spinix 3002, India.
• Water bath	Stuart SBS40 Water Bath, Cole-Parmer, Antylia Scientific, USA
• Weighing balance	Analytical Balance-TW223L, Shimazdu Corp., Japan.

Chemicals

The chemicals used for experimentation were of the highest analytical grade and purchased from Loba-Chemie Pvt. Ltd, India, unless otherwise specified. The media components were sterilised in an autoclave at 121°C (15 psi) for 15 min unless otherwise specified.

Hi Media Laboratories Pvt. Ltd. (Mumbai, India): Agar-agar, Czapek dox agar, Potato dextrose agar, Potato dextrose broth, Rose Bengal agar, Sabouraud dextrose agar.

Sigam Aldrich (USA): 1-aminocyclopropane-1-carboxylic acid, α-ketobutyrate.

Appendix-B

List of isolates and ANOVA analysis

Table A1: List of endophytic fungi isolated from rain-fed rice variety-PUSA-44.

Isolation	Culture code	Plant part	Tentative identification
1st isolation (15th day)	1OSFI1a	Internode	*Nigrospora sp.*
	1OSFI2a	Internode	*Paecilomyces sp.*
	1OSFL1a	Leaf	*Colletotrichum sp.*
	1OSFL2a	Leaf	Non-sporulating
	1OSFR1a	Root	Unidentified
	1OSFR2a	Root	*Nigrospora sp.*
2nd isolation (30th day)	2OSFI1a	Internode	*Alternaria sp.*
	2OSFL1a	Leaf	*Nigrospora sp.*
	2OSFL1b	Leaf	*Alternaria sp.*
	2OSFL2a	Leaf	Unidentified
	2OSFR2a	Root	*Fusarium sp.*
	2OSFR2b	Root	*Fusarium sp.*
3rd isolation (45th day)	3OSFI1a	Internode	*Aspergillus sp.*
	3OSFI2b	Internode	*Alternaria sp.*
	3OSFL1a	Leaf	Unidentified
	3OSFL2a	Leaf	*Fusarium sp.*
	3OSFL4b	Leaf	*Fusarium sp.*
	3OSFR1a	Root	*Cladosporium sp.*
	3OSFR2a	Root	Non-sporulating
	3OSFR2b	Root	Non-sporulating
4th isolation (60th day)	4OSFI1a	Internode	*Penicillium sp.*
	4OSFI2a	Internode	*Penicillium sp.*
	4OSFI2b	Internode	*Nigrospora sp.*
	4OSFL1a	Leaf	*Nigrospora sp.*
	4OSFL2a	Leaf	*Nigrospora sp.*
	4OSFL3a	Leaf	*Nigrospora sp.*
	4OSFL3b	Leaf	*Rhizopus sp.*
	4OSFL4a	Leaf	*Aspergillus sp.*
	4OSFL4b	Leaf	Non-sporulating
	4OSFL5a	Leaf	*Nigrospora sp.*
	4OSFR1a	Root	*Curvularia sp.*
	4OSFR1b	Root	*Penicillium sp.*
	4OSFR2a	Root	*Nigrospora sp.*

	4OSFR2b	Root	*Fusarium sp.*
5th isolation (75th day)	5OSFL1a	Leaf	*Fusarium sp.*
	5OSFL2a	Leaf	*Aspergillus sp.*
	5OSFL3a	Leaf	*Nigrospora sp.*
	5OSFL3b	Leaf	*Penicillium sp.*
	5OSFL4a	Leaf	*Nigrospora sp.*
	5OSFL5a	Leaf	*Alternaria sp.*
	5OSFL6a	Leaf	*Nigrospora sp.*
	5OSFL6b	Leaf	*Aspergillus sp.*
	5OSFR1a	Root	*Pestalotiopsis sp.*
	5OSFR2a	Root	Non-sporulating
	5OSFR2b	Root	Non-sporulating
	5OSFR3a	Root	*Rhizopus sp.*
	5OSFR3b	Root	*Pestalotiopsis sp.*
	5OSFR4b	Root	Unidentified
	5OSFS1a	Spike	*Nigrospora sp.*
	5OSFS2a	Spike	Unidentified
	5OSFS2b	Spike	*Nigrospora sp.*
	5OSFS3a	Spike	Unidentified
6th isolation (90th day)	6OSFI1a	Internode	*Aspergillus sp.*
	6OSFI1b	Internode	*Nigrospora sp.*
	6OSFI1c	Internode	*Fusarium sp.*
	6OSFI2b	Internode	Unidentified
	6OSFI2c	Internode	*Alternaria sp.*
	6OSFI3a	Internode	Unidentified
	6OSFI3b	Internode	Unidentified
	6OSFL1a	Leaf	*Penicillium sp.*
	6OSFL1b	Leaf	*Fusarium sp.*
	6OSFL2a	Leaf	*Alternaria sp.*
	6OSFL2b	Leaf	Unidentified
	6OSFL3a	Leaf	*Nigrospora sp.*
	6OSFL4a	Leaf	*Aspergillus sp.*
	6OSFL4b	Leaf	*Fusarium sp.*
	6OSFL4c	Leaf	*Nigrospora sp.*
	6OSFR1a	Root	Unidentified
	6OSFR2a	Root	*Nigrospora sp.*
	6OSFR2b	Root	Unidentified
	6OSFR2c	Root	Unidentified

	6OSFR2d	Root	*Nigrospora sp.*
	6OSFR2e	Root	*Nigrospora sp.*
	6OSFR3a	Root	*Fusarium sp.*
	6OSFR3b	Root	*Curvularia sp.*
	6OSFR3c	Root	*Fusarium sp.*
	6OSFS1a	Spike	*Rhizopus sp.*
	6OSFS1b	Spike	*Pestalotiopsis sp.*
	6OSFS1c	Spike	*Fusarium sp.*
	6OSFS2a	Spike	*Nigrospora sp.*
	6OSFS3a	Spike	*Penicillium sp.*
	6OSFS4a	Spike	*Curvularia sp.*
7^{th} isolation (105^{th} day)	7OSFI1a	Internode	*Alternaria sp.*
	7OSFI2a	Internode	Unidentified
	7OSFI3a	Internode	*Penicillium sp.*
	7OSFL1a	Leaf	*Nigrospora sp.*
	7OSFL2a	Leaf	*Nigrospora sp.*
	7OSFL3a	Leaf	*Alternaria sp.*
	7OSFL4a	Leaf	*Fusarium sp.*
	7OSFR1a	Root	Unidentified
	7OSFR1b	Root	*Penicillium sp.*
	7OSFR2a	Root	*Alternaria sp.*
	7OSFR3a	Root	Unidentified
	7OSFR4a	Root	Penicillium sp.
	7OSFS1a	Spike	*Nigrospora sp.*
	7OSFS2a	Spike	Unidentified
	7OSFS3a	Spike	*Nigrospora sp.*
	7OSFS4a	Spike	Unidentified
	7OSFS5a	Spike	*Alternaria sp.*
	7OSFS5b	Spike	*Alternaria sp.*
8^{th} isolation (120^{th} day)	8OSFI1a	Internode	Unidentified
	8OSFI2a	Internode	*Nigrospora sp.*
	8OSFI2b	Internode	Non-sporulating
	8OSFI3a	Internode	*Fusarium sp.*
	8OSFI4a	Internode	*Nigrospora sp.*
	8OSFI5a	Internode	*Alternaria sp.*
	8OSFI6a	Internode	*Nigrospora sp.*
	8OSFL1a	Leaf	*Nigrospora sp.*
	8OSFL2a	Leaf	*Colletotrichum sp.*

8OSFL3a	Leaf	*Fusarium sp.*
8OSFL4a	Leaf	*Alternaria sp.*
8OSFL5a	Leaf	*Fusarium sp.*
8OSFR1a	Root	*Alternaria sp.*
8OSFS1a	Spike	*Alternaria sp.*
8OSFS2a	Spike	*Nigrospora sp.*
8OSFS3a	Spike	*Fusarium sp.*
8OSFS3b	Spike	*Fusarium sp.*
8OSFS4a	Spike	*Alternaria sp.*
8OSFS5a	Spike	*Fusarium sp.*
8OSFS6a	Spike	Unidentified

Table A2: List of endophytic fungi isolated from drought-resistant rice variety-Sahbhagi Dhan.

Isolation	Culture code	Plant part	Tentative identification
1st isolation (15th day)	#1OSTUL2a	Leaf	Unidentified
	#1OSTUR1c	Root	*Fusarium* sp.
	#1OSTUR2a	Root	*Fusarium* sp.
	#1OSTUI1d	Internode	*Cladosporium* sp.
	#1OSTUI10a	Internode	*Fusarium* sp.
2nd isolation (30th day)	#2OSTUL4a	Leaf	*Alternaria* sp.
	#2OSTUL4b	Leaf	*Nigrospora* sp.
	#2OSTUL5a	Leaf	*Nigrospora* sp.
	#2OSTUL6d	Leaf	*Nigrospora* sp.
	#2OSTUR9a	Root	*Nigrospora* sp.
	#2OSTUI2c	Root	Unidentified
	#2OSTUI2d	Internode	Unidentified
3rd isolation (45th day)	#3OSTUL2a	Leaf	Non-sporulating
	#3OSTUL2b	Leaf	*Nigrospora sp.*
	#3OSTUL2d	Leaf	Unidentified
	#3OSTUL2e	Leaf	*Nigrospora* sp.
	#3OSTUR2d	Root	*Paecilomyces* sp.
	#3OSTUR3b	Root	*Aspergillus* sp.
	#3OSTUR7a	Root	*Collectotrichum* sp.
	#3OSTUR7e	Root	*Nigrospora* sp.
	#3OSTUI4d	Internode	*Collectotrichum* sp.
	#3OSTUI9a	Internode	*Nigrospora* sp.
4th isolation (60th day)	#4OSTUL3b	Leaf	*Fusarium* sp.
	#4OSTUL5a	Leaf	Non-sporulating
	#4OSTUL8a	Leaf	Non-sporulating
	#4OSTUR1a	Root	*Cladosporium* sp.
	#4OSTUR1e	Root	*Nigrospora* sp.
	#4OSTUR2e	Root	*Fusarium* sp.
	#4OSTUR3a	Root	*Fusarium* sp.
	#4OSTUR8a	Root	*Fusarium* sp.
	#4OSTUR8b	Root	*Fusarium* sp.
	#4OSTUR8d	Root	*Fusarium* sp.
	#4OSTUI3a	Internode	*Cladosporium* sp.
	#4OSTUI4a	Internode	*Cladosporium* sp.

	#4OSTUI4b	Internode	Non-sporulating
	#4OSTUS1b	Spike	Unidentified
	#4OSTUS2a	Spike	*Fusarium* sp.
	#4OSTUS2c	Spike	Unidentified
	#4OSTUS9c	Spike	Unidentified
5th isolation (75th day)	#5OSTUL4a	Leaf	*Fusarium* sp.
	#5OSTUL4b	Leaf	*Nigrospora* sp.
	#5OSTUL4d	Leaf	Non-sporulating
	#5OSTUR1a	Root	*Fusarium* sp.
	#5OSTUR2b	Root	Rhizopus sp.
	#5OSTUR3d	Root	*Fusarium* sp.
	#5OSTUR4b	Root	*Fusarium* sp.
	#5OSTUR4c	Root	*Nigrospora* sp.
	#5OSTUS3a	Spike	*Cladosporium* sp.
	#5OSTUS3b	Spike	*Nigrospora* sp.
	#5OSTUS6c	Spike	Non-sporulating
6th isolation (90th day)	#6OSTUL1a	Leaf	*Nigrospora* sp.
	#6OSTUL7a	Leaf	Unidentified
	#6OSTUR10b	Root	Unidentified
	#6OSTUI2a	Internode	*Fusarium* sp.
	#6OSTUI4b	Internode	*Aspergillus* sp.
	#6OSTUI4d	Internode	*Cladosporium* sp.
	#6OSTUS3a	Spike	Non-sporulating
7th isolation (105th day)	#7OSTUL3b	Leaf	*Fusarium* sp.
	#7OSTUL8a	Leaf	*Fusarium sp.*
	#7OSTUR1e	Root	*Rhizopus* sp.
	#7OSTUR2a	Root	*Alternaria* sp.
	#7OSTUS7e	Spike	*Fusarium* sp.

Table A3: One-way ANOVA analysis of salinity and drought stress tolerance using plate assay.

	Rain-fed rice isolates				Drought-resistant rice isolates			
	Internode isolates at 0.5 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	44910	24	1871	839.5	29730	11	2702	843.4
Residual	111.5	50	2.229	***	76.90	24	3.204	***
Total	45020	74			29800	35		
	Internode isolates at 1 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	40110	24	1671	1076	34970	11	3179	832.1
Residual	77.67	50	1.553	***	91.68	24	3.820	***
Total	40190	74			35060	35		
	Internode isolates at 1.5 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	44880	24	1870	1383	22630	11	2058	2947
Residual	67.60	50	1.352	***	16.76	24	0.6982	***
Total	44950	74			22650	35		
	Internode isolates at 2 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	35960	24	1498	1176	10480	11	952.9	845.4
Residual	63.70	50	1.274	***	27.05	24	1.127	***
Total	36020	74			10510	35		
	Internode isolates at 5% PEG-6000 (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**

Treatments	35590	23	1547	610.8	38050	11	3459	1298
Residual	121.6	48	2.533	***	63.96	24	2.665	***
Total	35710	71			38110	35		
				Internode isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	32700	23	1422	435.7	27530	11	2502	1237
Residual	156.6	48	3.263	***	48.54	24	2.022	***
Total	32860	71			27580	35		
				Internode isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	42940	23	1867	1072	23690	11	2153	785.8
Residual	83.59	48	1.741	***	65.77	24	2.740	
Total	43030	71			23750	35		
				Internode isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	33220	23	1444	690.6	21820	11	1984	738.0
Residual	100.4	48	2.092	***	64.52	24	2.688	***
Total	33320	71			21890	35		
				Root isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	23660	30	788.6	289.7	60150	21	2864	936.1
Residual	168.7	62	2.722	***	134.6	44	3.060	***
Total	23830	92			60290	65		
				Root isolates at 1 M NaCl (w/v)				

	SS	df	MS	F value	SS	df	MS	F value
Treatments	33220	23	1444	471.7	56940	21	2712	403.7
Residual	100.4	48	2.092	***	295.6	44	6.717	***
Total	33320	71			57240	65		

Root isolates at 1.5 M NaCl (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	61680	30	2056	947.1	40440	21	1926	1503
Residual	134.6	62	2.171	***	56.38	44	1.281	***
Total	61820	92			40500	65		

Root isolates at 2 M NaCl (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	55100	30	1837	1353	25960	21	1236	1853
Residual	84.14	62	1.357	***	29.35	44	0.6671	***
Total	55180	92			25990	65		

Root isolates at 5% PEG-6000 (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	54710	30	1824	446.8	61360	21	2922	1167
Residual	253.1	62	4.081	***	110.2	44	2.504	***
Total	54960	92			61470	65		

Root isolates at 10% PEG-6000 (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	47730	30	1591	575.7	47880	21	2280	1192
Residual	171.4	62	2.764	***	84.18	44	1.913	***
Total	47900	92			47960	65		

				Root isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	61750	30	2058	942.9	42770	21	2037	891.9
Residual	135.3	62	2.183	***	100.5	44	2.284	***
Total	61880	92			42870	65		
				Root isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	60310	30	2010	980.5	36610	21	1743	1857
Residual	127.1	62	2.050	***	41.30	44	0.9385	***
Total	60430	92			36650	65		
				Leaf isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	45250	35	1293	407.6	15480	18	859.9	52.98
Residual	228.4	72	3.172	***	616.7	38	16.23	***
Total	45480	107			16090	56		
				Leaf isolates at 1 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	22510	35	643.2	330.3	14430	18	801.9	196.6
Residual	140.2	72	1.947	***	155.0	38	4.079	***
Total	22650	107			14590	56		
				Leaf isolates at 1.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	23760	35	678.8	377.8	9494	18	527.4	436.2
Residual	129.4	72	1.797	***	45.95	38	1.209	***

Total	23890	107			9540	56		
				Leaf isolates at 2 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	30210	35	863.2	637.1	8389	18	466.1	386.0
Residual	97.54	72	1.355	***	45.89	38	1.208	***
Total	30310	107			8435	56		
				Leaf isolates at 5% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	65170	35	1862	442.6	12210	18	678.1	253.1
Residual	302.9	72	4.207	***	101.8	38	2.679	***
Total	65470	107			12310	56		
				Leaf isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	49900	35	1426	522.8	10910	18	606.1	218.2
Residual	196.3	72	2.727	***	105.6	38	2.778	***
Total	50090	107			11020	56		
				Leaf isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	27680	35	791.0	396.9	9573	18	531.8	324.4
Residual	143.5	72	1.993	***	62.30	38	1.639	***
Total	27830	107			9635	56		
				Leaf isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	33450	35	955.8	386.8	8743	18	485.7	307.5

Residual	177.9	72	2.471	***	60.02	38	1.579	***
Total	33630	107			8803	56		
				Spike isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	19130	20	956.3	213.6	4787	8	598.4	291.2
Residual	188.0	42	4.476	***	36.99	18	2.055	***
Total	19310	62			4824	26		
				Spike isolates at 1 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	19830	20	991.7	309.2	2749	8	343.6	187.9
Residual	134.7	42	3.207	***	32.92	18	1.829	***
Total	19970	62			2782	26		
				Spike isolates at 1.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	31040	20	1552	629.1	2488	8	311.0	119.0
Residual	103.6	42	2.467	***	47.06	18	2.614	***
Total	31140	62			2535	26		
				Spike isolates at 2 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	30170	20	1508	694.5	555.9	8	69.49	35.91
Residual	91.22	42	2.172	***	34.83	18	1.935	***
Total	30260	62			590.8	26		
				Spike isolates at 5% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**

Treatments	58940	20	2947	960.3	9844	8	1230	633.6
Residual	128.9	42	3.069	***	34.96	18	1.942	***
Total	59070	62			9879	26		
				Spike isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	38030	20	1901	457.5	9948	8	1243	579.9
Residual	174.5	42	4.156	***	38.59	18	2.144	***
Total	38200	62			9986	26		
				Spike isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	30720	20	1536	568.7	4482	8	560.2	272.4
Residual	113.4	42	2.701	***	37.01	18	2.056	***
Total	30830	62			4519	26		
				Spike isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	37210	20	1860	710.6	3052	8	381.5	43.49
Residual	110.0	42	2.618	***	157.9	18	8.772	***
Total	37320	62			3210	26		

ND = Activity not detected; *** $p<0.0001$

Table A4: One-way ANOVA analysis of salinity and drought stress tolerance using broth assay.

	Rain-fed rice isolates				Drought-resistant rice isolates			
	Internode isolates at 0.5 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	8215	10	821.5	15180000	2391	4	597.8	9585000
Residual	0.001190	22	0.00005411	***	0.0006237	10	0.00006237	***
Total	8215	32			2391	14		
	Internode isolates at 1 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9136	10	913.6	13290000	720.6	4	180.2	2907000
Residual	0.001512	22	0.00006874	***	0.0006197	10	0.00006197	***
Total	9136	32			720.6	14		
	Internode isolates at 1.5 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	5353	10	535.3	11690000	48.01	4	12.00	294800
Residual	0.001007	22	0.00004578	***	0.0004072	10	0.00004072	***
Total	5353	32			48.01	14		
	Internode isolates at 2 M NaCl (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	2377	10	237.7	35090	308.9	4	77.22	2643000
Residual	0.1491	22	0.006775	***	0.0002922	10	0.00002922	***
Total	2378	32			308.9	14		
	Internode isolates at 5% PEG-6000 (w/v)							
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**

Treatments	2056	10	205.6	11680000	15.35	4	3.838	103400
Residual	0.0003873	22	0.00001760	***	0.0003713	10	0.00003713	***
Total	2056	32			15.35	14		
				Internode isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	2655	10	265.5	11100	124.9	4	31.23	677400
Residual	0.5264	22	0.02393	***	0.0004610	10	0.00004610	***
Total	2656	32			124.9	14		
				Internode isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9412	10	941.2	24460	302.0	4	75.50	3650000
Residual	0.8467	22	0.03849	***	0.0002069	10	0.00002069	***
Total	9413	32			302.0	14		
				Internode isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1637	10	163.7	508.1	395.9	4	98.98	1986000
Residual	7.087	22	0.3221	***	0.0004983	10	0.00004983	***
Total	1644	32			395.9	14		
				Root isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9829	11	893.6	1624000	7261	8	907.6	20760000
Residual	0.01320	24	0.0005502	***	0.0007871	18	0.00004373	***
Total	9829	35			7261	26		
				Root isolates at 1 M NaCl (w/v)				

	SS	df	MS	F value	SS	df	MS	F value
Treatments	15990	11	1453	22110	4017	8	502.1	11790000
Residual	1.577	24	0.06572	***	0.0007667	18	0.00004259	***
Total	15990	35			4017	26		

Root isolates at 1.5 M NaCl (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	16940	11	1540	29050000	1811	8	226.3	4887000
Residual	0.001272	24	0.00005301	***	0.0008336	18	0.00004631	***
Total	16940	35			1811	26		

Root isolates at 2 M NaCl (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	3667	11	333.4	6420	1244	8	155.4	3329000
Residual	1.246	24	0.05193	***	0.0008405	18	0.00004669	***
Total	3669	35			1244	26		

Root isolates at 5% PEG-6000 (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	14100	11	1282	24190000	1751	8	218.9	4018000
Residual	0.001272	24	0.00005299	***	0.0009806	18	0.00005448	***
Total	14100	35			1751	26		

Root isolates at 10% PEG-6000 (w/v)

	SS	df	MS	F value	SS	df	MS	F value
Treatments	12870	11	1170	18970000	3590	8	448.7	8669000
Residual	0.001480	24	0.00006165	***	0.0009317	18	0.00005176	***
Total	12870	35			3590	26		

				Root isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	5681	11	516.5	10460000	4688	8	586.1	9299000
Residual	0.001185	24	0.00004939	***	0.001134	18	0.00006302	***
Total	5681	35			4688	26		
				Root isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	108.5	11	9.861	245700	3412	8	426.5	10410000
Residual	0.0009634	24	0.00004014	***	0.0007374	18	0.00004097	***
Total	108.5	35			3412	26		
				Leaf isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	36050	19	1897	66320	6774	13	521.1	18510000
Residual	1.144	40	0.02861	***	0.0007881	28	0.00002815	***
Total	36050	59			6774	41		
				Leaf isolates at 1 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	11490	19	604.6	7319000	3984	13	306.5	5258000
Residual	0.003304	40	0.00008261	***	0.001632	28	0.00005828	***
Total	11490	59			3984	41		
				Leaf isolates at 1.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9131	19	480.6	11480000	1752	13	134.8	6036000
Residual	0.001675	40	0.00004188	***	0.0006254	28	0.00002233	***

Total	9131	59			1752	41		
				Leaf isolates at 2 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	3215	19	169.2	29050	2058	13	158.3	3989000
Residual	0.2330	40	0.005825	***	0.001111	28	0.00003968	***
Total	3215	59			2058	41		
				Leaf isolates at 5% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	31450	20	1572	28030000	5524	15	368.3	10770000
Residual	0.002356	42	0.00005609	***	0.001094	32	0.00003419	***
Total	31450	62			5524	47		
				Leaf isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	20190	20	1009	15440000	5188	15	345.9	7577000
Residual	0.002745	42	0.00006535	***	0.001461	32	0.00004565	***
Total	20190	62			5188	47		
				Leaf isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	10820	20	541.2	8470000	3522	15	234.8	6141000
Residual	0.002684	42	0.00006389	***	0.001224	32	0.00003824	***
Total	10820	62			3522	47		
				Leaf isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	621.2	20	31.06	417800	2541	15	169.4	3679000

Residual	0.003123	42	0.00007435	***	0.001473	32	0.00004604	***
Total	621.2	62			2541	47		
				Spike isolates at 0.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	7865	8	983.1	10310000	24.05	2	12.03	341600
Residual	0.001717	18	0.00009536	***	0.0002112	6	0.00003520	***
Total	7865	26			24.05	8		
				Spike isolates at 1 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	3738	8	467.3	7648000	34.25	2	17.12	141800
Residual	0.001126	18	0.00006257	***	0.0007245	6	0.0001207	***
Total	3738	26			34.25	8		
				Spike isolates at 1.5 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	3312	8	414.0	258200	313.4	2	156.7	2758000
Residual	0.02886	18	0.001603	***	0.0003410	6	0.00005683	***
Total	3312	26			313.4	8		
				Spike isolates at 2 M NaCl (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1840	8	230.0	177800	222.5	2	111.3	10780000
Residual	0.02328	18	0.001294	***	0.00006192	6	0.00001032	***
Total	1840	26			222.5	8		
				Spike isolates at 5% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**

Treatments	9558	8	1195	2986	235.6	3	78.52	3355000
Residual	7.202	18	0.4001	***	0.0001873	8	0.00002341	***
Total	9565	26			235.6	11		
				Spike isolates at 10% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	3586	8	448.2	1408	238.3	3	79.44	6429000
Residual	5.729	18	0.3183	***	0.00009885	8	0.00001236	***
Total	3591	26			238.3	11		
				Spike isolates at 15% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1962	8	245.2	614.9	157.0	3	52.35	1146000
Residual	7.179	18	0.3988	***	0.0003655	8	0.00004569	***
Total	1969	26			157.0	11		
				Spike isolates at 20% PEG-6000 (w/v)				
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9.952	8	1.244	4.893	136.1	3	45.38	440200
Residual	4.577	18	0.2543	***	0.0008246	8	0.0001031	
Total	14.53	26			136.1	11		

ND = Activity not detected; *** $p<0.0001$

Table A5: One-way ANOVA analysis of plant growth promoting attributes of fugal isolates.

	Rain-fed rice isolates				Drought-resistant rice isolates			
Total phenolic content								
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	49820	7	7117	572.4	7584	2	3792	10020
Residual	198.9	16	12.43	***	2.270	6	0.3783	***
Total	50020	23			7587	8		
Total flavonoid content								
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	374200	7	53460	354.8	81360	2	40680	124.6
Residual	2411	16	150.7	***	1959	6	326.5	***
Total	376600	23			83320	8		
DPPH scavenging assay								
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1200000	8	150100	311.0	34520	3	11510	95.62
Residual	8684	18	482.4	***	962.8	8	120.3	***
Total	1209000	26			35490	11		
Trolox equivalent antioxidant capacity								
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1225000	11	111300	635.4	22500	3	7500	233.6
Residual	4205	24	175.2	***	256.8	8	32.10	***
Total	1229000	35			22760	11		
H_2O_2 scavenging assay								
	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1683000	8	210400	1646	42670	3	14220	141.9

Residual	2301	18	127.8	***	802.0	8	100.2	***
Total	1686000	26			43470	11		

Ferric ion reducing antioxidant power

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	2661	7	380.1	2074	5205	2	2602	1917
Residual	2.932	16	0.1833	***	8.146	6	1.358	***
Total	2664	23			5213	8		

Antibacterial activity against *Escherichia coli*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	1006	8	125.7	212.2	371.6	3	123.9	135.1
Residual	10.67	18	0.5926	***	7.333	8	0.9167	***
Total	1017	26			378.9	11		

Antibacterial activity against *Enterobacter aerogenes*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	681.9	8	85.23	104.6	258.7	3	86.22	86.22
Residual	14.67	18	0.8148	***	8.000	8	1.000	***
Total	696.5	26			266.7	11		

Antibacterial activity against *Bacillus subtilis*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	568.7	8	71.08	106.6	420.3	3	140.1	420.3
Residual	12.00	18	0.6667	***	2.667	8	0.3333	***
Total	580.7	26			423.0	11		

Antibacterial activity against *Staphylococcus aureus*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	434.7	8	54.33	63.78	408.3	3	136.1	544.3

Residual	15.33	18	0.8519	***	2.000	8	0.2500	***
Total	450.0	26			410.3	11		

Antifungal activity against *Alternaria alternata*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	4370	7	624.2	589.8	841.2	2	420.6	196.0
Residual	16.93	16	1.058	***	12.88	6	2.146	***
Total	4387	23			854.1	8		

Antifungal activity against *Botrytis cinerea*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	2167	7	309.5	334.3	5880	2	2940	1905
Residual	14.81	16	0.9259	***	9.260	6	1.543	***
Total	2181	23			5889	8		

Antifungal activity against *Colletotrichum gloeosporioides*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	3099	7	442.7	80.33	829.4	2	414.7	66.42
Residual	88.18	16	5.511	***	37.46	6	6.244	***
Total	3187	23			866.9	8		

Antifungal activity against *Fusarium moniliforme*

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	2694	7	384.8	231.4	671.9	2	336.0	415.8
Residual	26.61	16	1.663	***	4.848	6	0.8081	***
Total	2720	23			676.8	8		

Production of indole acetic acid

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	29770	7	4253	**622.4**	4405	2	2203	**476.3**

Residual	109.3	16	6.834	***	27.75	6	4.625	***
Total	29880	23			4433	8		

Production of indole acetic acid (in presence of tryptophan)

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	96160	7	13740	638.6	38490	2	19250	648.5
Residual	344.2	16	21.51	***	178.1	6	29.68	***
Total	96510	23			38670	8		

Production of gibberellic acid

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	734.1	7	104.9	383.0	688.4	2	344.2	1079
Residual	4.381	16	0.2738	***	1.914	6	0.3190	***
Total	738.5	23			690.3	8		

Production of ACC deaminase at 1 mM substrate concentration

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.1008	7	0.01439	43180	0.02796	2	0.01398	25160
Residual	0.000005333	16	0.0000003333	***	0.000003333	6	0.0000005556	***
Total	0.1008	23			0.02796	8		

Production of ACC deaminase at 2 mM substrate concentration

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.2111	7	0.03016	60330	0.07591	2	0.03796	68320
Residual	0.000008000	16	0.0000005000	***	0.000003333	6	0.0000005556	***
Total	0.2112	23			0.07592	8		

Production of ACC deaminase at 3 mM substrate concentration

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.2954	7	0.04220	144700	0.1321	2	0.06603	118800

Residual	0.000004667	16	0.0000002917	***	0.000003333	6	0.0000005556	***
Total	0.2954	23			0.1321	8		

Production of ACC deaminase at 4 mM substrate concentration

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.3078	7	0.04397	75380	0.1388	2	0.06938	208100
Residual	0.000009333	16	0.0000005833	***	0.000002000	6	0.0000003333	***
Total	0.3078	23			0.1388	8		

Production of ACC deaminase at 5 mM substrate concentration

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.3180	7	0.04543	181700	0.1450	2	0.07249	217500
Residual	0.000004000	16	0.0000002500	***	0.000002000	6	0.0000003333	***
Total	0.3180	23			0.1450	8		

Production of siderophores

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	25180	7	3597	24890	9930	2	4965	77060
Residual	5.781	40	0.1445	***	0.9664	15	0.06443	***
Total	25180	47			9930	17		

Phosphate solubilisation

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	4.752	7	0.6789	3149	0.01540	2	0.007700	19.30
Residual	0.003449	16	0.0002156	***	0.002393	6	0.0003989	***
Total	4.756	23			0.01779	8		

Zinc solubilisation

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	8.952	7	1.279	68.12			ND	

Residual	0.3004	16	0.01877	***				
Total	9.252	23						

Amylase production assay

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.4535	7	0.06478	423.4	0.01669	2	0.008346	81.28
Residual	0.002448	16	0.0001530	***	0.0006161	6	0.0001027	***
Total	0.4559	23			0.01731	8		

Cellulase production assay

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.1304	7	0.01863	80.01	0.1597	2	0.07985	119.2
Residual	0.003725	16	0.0002328	***	0.004021	6	0.0006701	***
Total	0.1341	23			0.1637	8		

Laccase production assay

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	0.6578	7	0.09397	198.4	2.987	2	1.493	168.0
Residual	0.007579	16	0.0004737	***	0.05333	6	0.008889	***
Total	0.6653	23			3.040	8		

Pectinase production assay

	SS	**df**	**MS**	**F value**	**SS**	**df**	**MS**	**F value**
Treatments	9.632	7	1.376	201.6	0.2385	2	0.1193	72.96
Residual	0.1092	16	0.006825	***	0.009808	6	0.001635	***
Total	9.741	23			0.2483	8		

ND = Activity not detected; *** $p<0.0001$

Table A6: Reference table for ammonia production using Nessler's reagent.

Absorbance range at 450 nm	Activity	Indication
0-0.25	Low	+
0.25-0.5	Moderate	++
0.5-0.75	High	+++
More than 0.75	Intense	++++

Table A7: Two-way ANOVA analysis on effect of endophytic fungi on seed germination under different abiotic stresses.

Seed germination				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	270.5	45.08	23.87***
Abiotic stress	3	9721	3240	1715***
Prescence of endophytic fungi	2	427.1	213.5	113.0***
Error	24	45.33	1.889	

ns: not significant; * $p<0.05$; ** $p<0.01$; *** $p<0.001$

Table A8: Two-way ANOVA analysis on effect of endophytic fungi on physiological, biochemical and enzymatic attributes of rice plant under different abiotic stresses in controlled environmental conditions.

Shoot length				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	21.55	3.591	5.163***
Abiotic stress	3	403.7	134.6	193.5***
Prescence of endophytic fungi	2	108.7	54.35	78.15***
Error	60	41.73	0.6955	
Shoot fresh weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	8634	1439	33.54***
Abiotic stress	3	105700	35230	821.2***
Prescence of endophytic fungi	2	32440	16220	378.0***
Error	60	2574	42.90	
Shoot dry weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	97.47	16.25	3.244**
Abiotic stress	3	1901	633.6	126.5***
Prescence of endophytic fungi	2	400.1	200.0	39.94***
Error	60	300.5	5.008	
Root length				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	1.145	0.1909	3.628**
Abiotic stress	3	37.75	12.58	239.1***
Prescence of endophytic fungi	2	8.250	4.125	78.41***
Error	60	3.157	0.05261	
Root fresh weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	550.2	91.70	8.339***
Abiotic stress	3	5661	1887	171.6***
Prescence of endophytic fungi	2	2001	1001	90.99***
Error	60	659.8	11.00	
Root dry weight				

Source of variation	df	SS	MS	F value
Interaction	6	6.731	1.122	13.49***
Abiotic stress	3	82.11	27.37	329.2***
Prescence of endophytic fungi	2	17.73	8.864	106.6***
Error	60	4.988	0.08314	

Relative water content

Source of variation	df	SS	MS	F value
Interaction	6	1761	293.4	20.34***
Abiotic stress	3	9085	3028	209.9***
Prescence of endophytic fungi	2	5083	2542	176.1***
Error	60	865.8	14.43	

Total Chlorophyll content

Source of variation	df	SS	MS	F value
Interaction	6	139600	23260	272.9***
Abiotic stress	3	852600	284200	3334***
Prescence of endophytic fungi	2	344900	172500	2023***
Error	60	5115	85.25	

Carotenoid content

Source of variation	df	SS	MS	F value
Interaction	6	1099	183.2	10.17***
Abiotic stress	3	12080	4026	223.4***
Prescence of endophytic fungi	2	2209	1104	61.28***
Error	60	1081	18.02	

Total phenolic content

Source of variation	df	SS	MS	F value
Interaction	6	65810	10970	184.0***
Abiotic stress	3	400400	133500	2239***
Prescence of endophytic fungi	2	143200	71590	1201***
Error	60	3577	59.62	

Total flavonoid content

Source of variation	df	SS	MS	F value
Interaction	6	54520	9086	199.6***
Abiotic stress	3	612400	204100	4484***

Prescence of endophytic fungi	2	179400	89680	1970***
Error	60	2731	45.52	

Total sugar content

Source of variation	df	SS	MS	F value
Interaction	6	3477	579.5	119.4***
Abiotic stress	3	24350	8116	1672***
Prescence of endophytic fungi	2	9734	4867	1002***
Error	60	291.3	4.855	

Reducing sugar content

Source of variation	df	SS	MS	F value
Interaction	6	1619	269.8	86.75***
Abiotic stress	3	10490	3496	1124***
Prescence of endophytic fungi	2	4091	2045	657.8***
Error	60	186.6	3.110	

Proline content

Source of variation	df	SS	MS	F value
Interaction	6	41.15	6.858	238.7***
Abiotic stress	3	494.7	164.9	5739***
Prescence of endophytic fungi	2	110.5	55.23	1922***
Error	60	1.724	0.02873	

Malondialdehyde content

Source of variation	df	SS	MS	F value
Interaction	6	11990	1998	56.10***
Abiotic stress	3	33160	11050	310.4***
Prescence of endophytic fungi	2	23140	11570	324.9***
Error	60	2137	35.61	

Hydrogen peroxide content

Source of variation	df	SS	MS	F value
Interaction	6	1.080	0.1800	175.5***
Abiotic stress	3	7.455	2.485	2423***
Prescence of endophytic fungi	2	2.732	1.366	1332***
Error	60	0.06153	0.001026	

Ascorbate peroxidase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	1.494	0.2490	97.30***
Abiotic stress	3	10.01	3.336	1304***
Prescence of endophytic fungi	2	2.846	1.423	556.1***
Error	60	0.1535	0.002559	
Catalase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	0.003443	0.0005739	53.44***
Abiotic stress	3	0.06226	0.02075	1933***
Prescence of endophytic fungi	2	0.01036	0.005181	482.4***
Error	60	0.0006444	0.00001074	
Peroxidase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	0.04628	0.007713	39.96***
Abiotic stress	3	0.5762	0.1921	995.1***
Prescence of endophytic fungi	2	0.1177	0.05884	304.8***
Error	60	0.01158	0.0001930	
Superoxide dismutase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	9.130	1.522	63.62***
Abiotic stress	3	75.89	25.30	1058***
Prescence of endophytic fungi	2	19.59	9.796	409.6***
Error	60	1.435	0.02392	

ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Table A9: Two-way ANOVA analysis on effect of endophytic fungi on physiological, biochemical and enzymatic attributes of rice plant under different abiotic stresses in ambient environmental conditions.

Shoot length				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	16.99	2.832	1.460^{ns}
Abiotic stress	3	1470	490.1	252.7***
Prescence of endophytic fungi	2	184.2	92.10	47.48***
Error	60	116.4	1.940	
Shoot fresh weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	1745	290.9	3.695**
Abiotic stress	3	389500	129800	1649***
Prescence of endophytic fungi	2	68860	34430	437.4***
Error	60	4723	78.72	
Shoot dry weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	68.97	11.50	1.964^{ns}
Abiotic stress	3	5300	1767	301.9***
Prescence of endophytic fungi	2	887.6	443.8	75.83***
Error	60	351.2	5.853	
Root length				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	1.664	0.2774	3.435**
Abiotic stress	3	85.75	28.58	354.0***
Prescence of endophytic fungi	2	12.64	6.318	78.25***
Error	60	4.845	0.08075	
Root fresh weight				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	184.4	30.73	2.763*
Abiotic stress	3	14310	4770	428.9***
Prescence of endophytic fungi	2	1610	805.1	72.39***
Error	60	667.3	11.12	
Root dry weight				

Source of variation	df	SS	MS	F value
Interaction	6	2.349	0.3915	3.800**
Abiotic stress	3	243.8	81.27	788.9***
Prescence of endophytic fungi	2	34.66	17.33	168.2***
Error	60	6.182	0.1030	

Relative water content

Source of variation	df	SS	MS	F value
Interaction	6	1582	263.7	29.14***
Abiotic stress	3	9798	3266	360.9***
Prescence of endophytic fungi	2	4950	2475	273.5***
Error	60	543.0	9.049	

Total Chlorophyll content

Source of variation	df	SS	MS	F value
Interaction	6	209300	34890	363.1***
Abiotic stress	3	1984000	661400	6884***
Prescence of endophytic fungi	2	709200	354600	3691***
Error	60	5765	96.09	

Carotenoid content

Source of variation	df	SS	MS	F value
Interaction	6	3573	595.6	21.49***
Abiotic stress	3	16700	5567	200.9***
Prescence of endophytic fungi	2	3966	1983	71.54***
Error	60	1663	27.72	

Total phenolic content

Source of variation	df	SS	MS	F value
Interaction	6	43760	7294	139.6***
Abiotic stress	3	477000	159000	3042***
Prescence of endophytic fungi	2	143000	71510	1368***
Error	60	3136	52.27	

Total flavonoid content

Source of variation	df	SS	MS	F value
Interaction	6	51030	8505	85.18***
Abiotic stress	3	874600	291500	2920***

Prescence of endophytic fungi	2	300300	150200	1504***
Error	60	5990	99.84	

Total sugar content

Source of variation	df	SS	MS	F value
Interaction	6	3296	549.3	120.7***
Abiotic stress	3	44780	14930	3279***
Prescence of endophytic fungi	2	8581	4291	942.5***
Error	60	273.1	4.552	

Reducing sugar content

Source of variation	df	SS	MS	F value
Interaction	6	1999	333.2	102.3***
Abiotic stress	3	16360	5452	1675***
Prescence of endophytic fungi	2	5513	2756	846.6***
Error	60	195.4	3.256	

Proline content

Source of variation	df	SS	MS	F value
Interaction	6	42.01	7.001	199.5***
Abiotic stress	3	736.7	245.6	6997***
Prescence of endophytic fungi	2	108.8	54.39	1550***
Error	60	2.106	0.03509	

Malondialdehyde content

Source of variation	df	SS	MS	F value
Interaction	6	17580	2930	60.63***
Abiotic stress	3	89230	29740	615.4***
Prescence of endophytic fungi	2	34540	17270	357.3***
Error	60	2900	48.33	

Hydrogen peroxide content

Source of variation	df	SS	MS	F value
Interaction	6	1.326	0.2210	95.85***
Abiotic stress	3	11.63	3.877	1681***
Prescence of endophytic fungi	2	3.361	1.681	728.8***
Error	60	0.1384	0.002306	

Ascorbate peroxidase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	2.869	0.4782	155.3***
Abiotic stress	3	19.11	6.371	2068***
Prescence of endophytic fungi	2	5.111	2.556	829.7***
Error	60	0.1848	0.00308	
Catalase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	0.005661	0.0009436	104.5***
Abiotic stress	3	0.09747	0.03249	3600***
Prescence of endophytic fungi	2	0.01496	0.007482	828.9***
Error	60	0.0005416	0.000009026	
Peroxidase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	0.1162	0.01937	85.74***
Abiotic stress	3	1.301	0.4335	1919***
Prescence of endophytic fungi	2	0.2552	0.1276	564.8***
Error	60	0.01356	0.0002259	
Superoxide dismutase activity				
Source of variation	**df**	**SS**	**MS**	**F value**
Interaction	6	18.00	3.000	125.2***
Abiotic stress	3	159.3	53.08	2216***
Prescence of endophytic fungi	2	41.06	20.53	857.1***
Error	60	1.437	0.02395	

ns: not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$

Appendix-C

Details of reagents prepared

- **Cetyltrimethylammonium bromide (CTAB):** For 100 mL, 10 mL of 1 M Tris-HCl, 28 mL of 5 M NaCl, 4 mL of 0.5 M EDTA and 2 g CTAB were added. The pH was set to 8.0 and the final volume was made 100 mL using distilled water.
- **Dinitrosalicyclic acid (DNSA) reagent:** To 50 mL distilled water 2.5 g DNS and 4 g NaOH were added at 70 °C. Then, 75 g disodium tartarate was added and stirred until complete dissolution. Final volume was made up to 250 mL using distilled water.
- **Dworkin and Foster (DF media):** For 1000 mL;

Macronutrients	**Quantity (g/L)**
Ammonium sulfate	2
Magnesium sulfate heptahydrate	2
Potassium phosphate dibasic	6
Potassium phosphate monobasic	4
Trace elements	**Quantity (µg/L)**
Ferrous sulfate	1000
Boric acid	10
Manganese sulphate	10
Zinc sulfate	70
Copper sulfate	50
Manganese oxide	10

- **Hoagland solution:** For 1000 mL of half-strength solution;

Macronutrients	**Quantity (g/L)**
Calcium nitrate	236
Magnesium sulphate	246.5
Potassium monophosphate	68
Potassium nitrate	106
Micronutrients	
Boric acid	1.43
Copper sulphate	0.04
Ferric tartrate	2.5
Manganese chloride	0.905
Molybdic acid	0.045
Zinc sulphate	0.11

- **Phosphate buffer:** The following components were added to 80 mL distilled water. Upon dissolution, the volume was made to 100 mL using distilled water.

Molarity (M)	Potassium phosphate dibasic (mg)	Potassium phosphate monobasic (mg)	pH
0.05	467.1	315.4	7
0.1	1211.9	414	7.4
1	12119	4140	7.4

- **Tris- Ethylenediaminetetraacetic acid (TE buffer):** For 100 mL buffer, 1 mL 1 M Tris-HCl and 2 mL of 0.5 M EDTA were added. The pH was set to 8.0 and the final volume was made 100 mL using distilled water.

Appendix-D

Standard curves

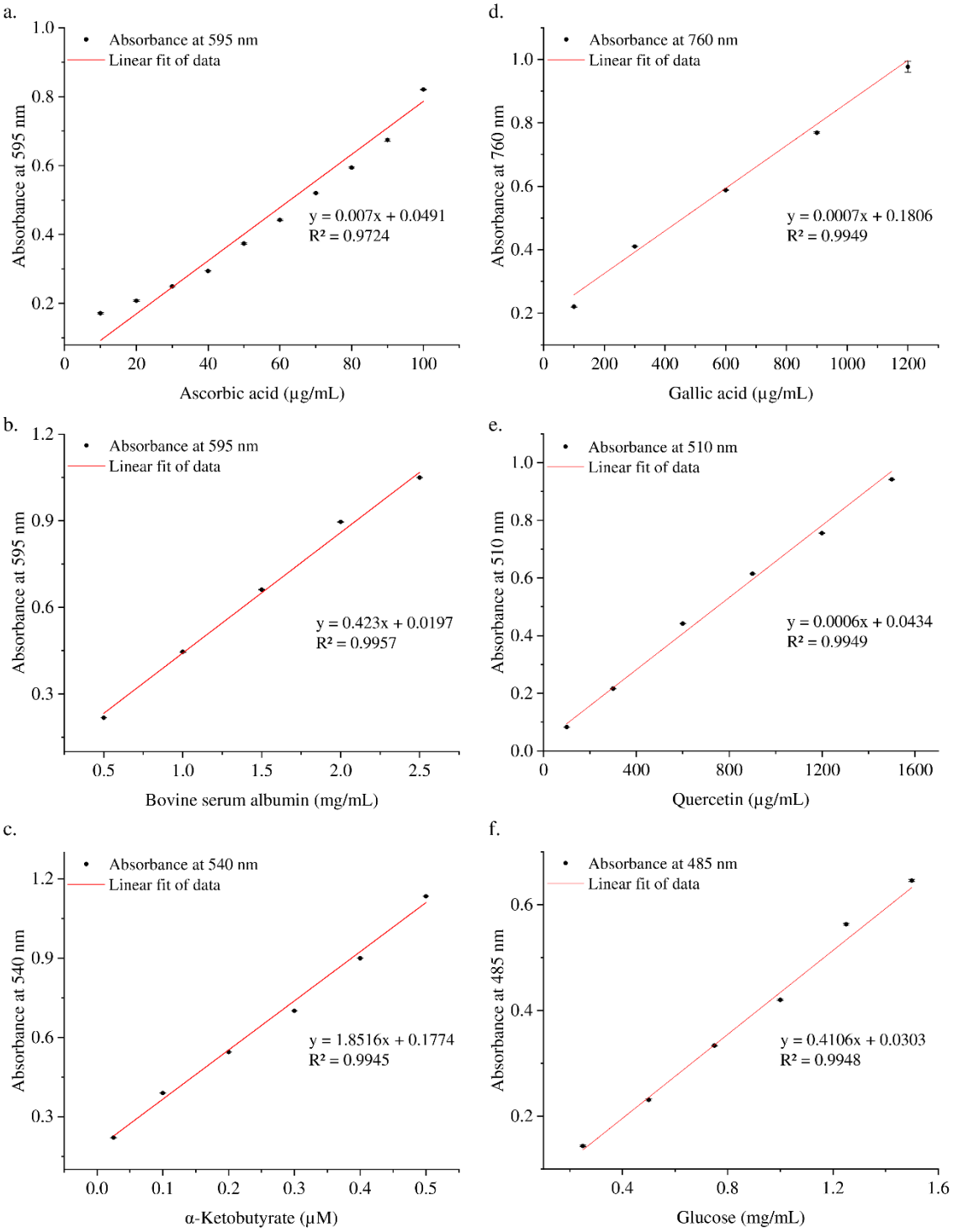

Fig. A1: Standard curve of a. Ascorbic acid-FRAP assay; b. Bovine serum albumin-Bradford assay; c. α-Ketobutyrate-ACC deaminase production; d. Gallic Acid-Total phenolic content; e. Quercetin-Total flavonoid content; f. Glucose-Total sugar content. The data represents mean ± SD, n=3.

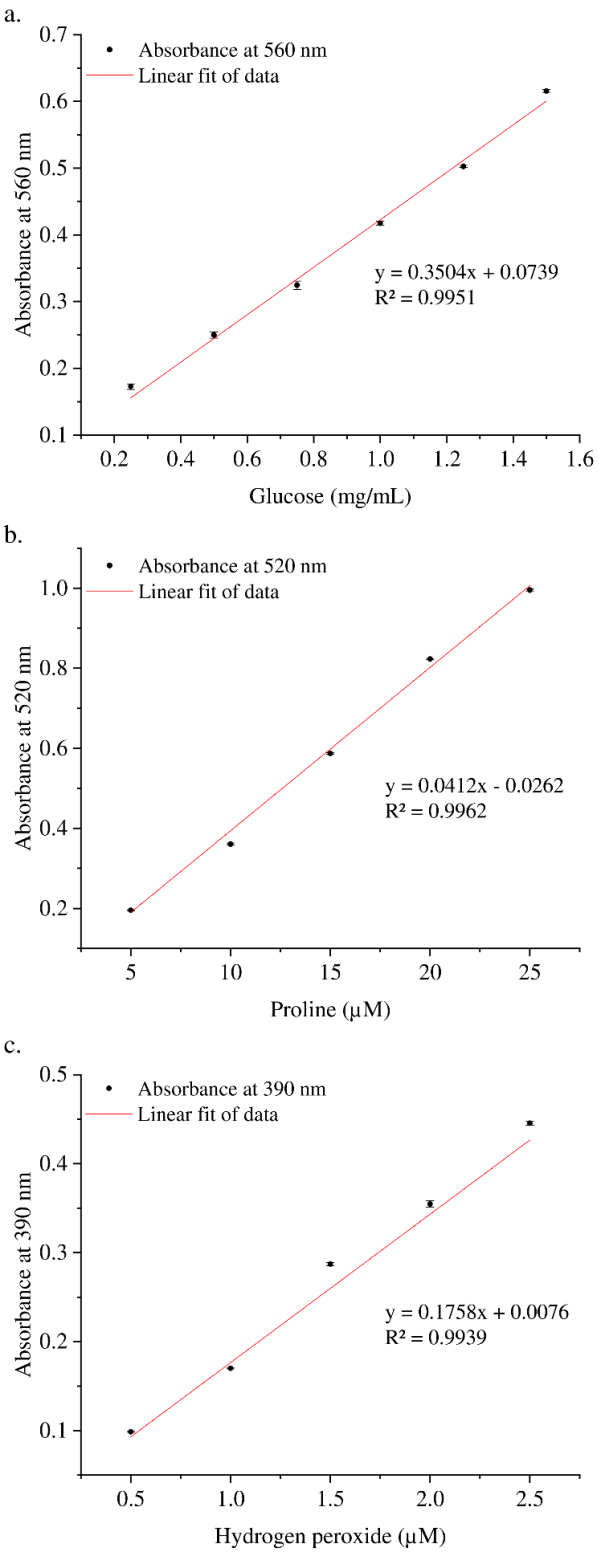

Fig. A2: Standard curve a. Glucose-Reducing sugar content; b. Proline-Acid Ninhydrin assay; c. Hydrogen peroxide. The data represents mean ± SD, n=3.

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ORIGINAL ARTICLE

Promising drought and salinity tolerance features of *Nigrospora* species existing as endophytes in *Oryza sativa*

Gurleen Kaur Sodhi[1] · **Sanjai Saxena**[1]

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Abstract

In this study, we report the discovery of novel *Nigrospora* species isolated from the extensively cultivated PUSA 44 rice variety in Punjab, India. Out of the 120 isolates examined, 6.6% and 5% isolates exhibited tolerance to high salinity and drought stress. Isolates 6OSFR2e and 7OSFS3a exhibited the highest indole acetic acid and gibberellic acid production, with 268.32 ± 08.10 and 25.72 ± 0.04 µg/mL. Additionally, isolates 7OSFS3a, 6OSFR2e and 6OSFL4c had highest antioxidant potential with IC_{50} 345.45 ± 11.66, 391.58 ± 10.66, and 474.529 ± 11.08 µg/mL. The isolates 6OSFR2e and 6OSFL4c also exhibited phosphate solubilisation with a PI of 1.06 ± 0.00 and 1.04 ± 0.02. The highest cellulase and laccase production with EI 1.24 ± 0.00 and 1.16 ± 0.00 was observed by isolates 6OSFR2e and 6OSFL4c. Promising results were observed in the case of ammonia production. The isolates belonged to the same phylum, Ascomycota and were identified as *Nigrospora zimmermanii* (6OSFR2e) and *Nigrospora oryzae* (7OSFS3a), and *Nigrospora sphaerica* (6OSFL4c) using morpho-taxonomic and molecular identification. The present study provides a critical insight into the characteristics of these *Nigrospora* species, which could be used to develop a bio-consortium for the rejuvenation of PUSA-44 cultivation.

Keywords Climate change · Abiotic stress · Plant growth promotion · Phytohormone · Phosphate solubilisation

Introduction

Since beginning of the last century, the earth has witnessed fundamental climate changes predominantly marked by the rise of global temperature, scarcity of water, salinisation of fresh water and soil, and reduction of arable land (Del Buono 2021). These changes pose a serious threat to sustainable agriculture and impact global food availability. According to Food and Agriculture Organisation of United Nations (FAO), feeding the ever-growing population which is likely to reach 2.3 billion by the year 2050 would require an estimated enhancement by 70% of the current food production (Van Dijk et al. 2021). Therefore, humanity's greatest challenge is to achieve higher food production while mitigating the negative environmental impacts on crops due to anthropogenic activities.

Abiotic stresses, such as salinity and drought, lead to significant reduction in crop yield by over 50%. These stresses affect primarily growth and productivity by bringing physiological, biochemical, and molecular changes in the plants (Chaudhry and Sidhu 2021). As a staple food, rice is a widely consumed cereal grain, particularly in Asia. In terms of production, it is the third most highly produced agricultural commodity, with ~ 496.1 million metric tonnes produced in 2019 (Parvin et al. 2021). Rice reportedly contributes one-fifth of the total calories consumed by humans, contributing to around 20% of the world's dietary energy supply. Hence, it forms an integral part of culinary traditions in several countries (Sangeetha et al. 2020). As per IRRI (International Rice Research Institute), India's prominent rice-producing states, viz., Punjab, Uttar Pradesh, and West Bengal, position India as a leading rice producer globally (Mishra et al. 2021).

Rice, as a kharif crop, requires ample water for growth and reproduction (Wang et al. 2022). In Punjab, India, highly intensive rice and wheat cropping system, increased fertiliser usage, and heavy reliance on irrigation have resulted

Accession no: GenBank—ON392014, ON392015, and ON392016.

✉ Sanjai Saxena
sanjaibiotech@yahoo.com

[1] Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala, Punjab 147004, India

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in overall instability of the ecosystem (Jalota et al. 2018; Nazir et al. 2020). PUSA-44, developed by Indian Council for Agriculture Research (ICAR), is a long-duration rice variety, extensively cultivated in Punjab despite its extensive water consumption due to its high yield as compared to its counterpart varieties (Dwivedi et al. 2021). Intensive cropping PUSA-44 has impacted the water table in Punjab drastically leading to an increase in soil salinity (Kumar and Kaur 2019a, b).

In India, ~ 6.72-million-hectare arable land is salt affected, of which the Indo-Gangetic alluvial tract of Punjab, Haryana, and Uttar Pradesh account for 2.34-million-hectare. Studies have also reported that parts of Punjab are under high-to-severe drought hazard conditions (Sahana et al. 2021). The rising temperatures bring on these drought conditions. For every 1 °C rise in temperature, a 10% increase in the crop's water requirement is estimated. Punjab has witnessed a 0.9–3.7% rise in maximum temperature during the kharif season. In addition, a 6.92% reduction in rainfall has been observed in the state. Altogether these factors could cause a significant loss in rice yield by as much as 8.10% (Kumar and Kaur 2019a, b; Kumar and Sharma 2020). This affects the gross crop productivity of not only PUSA-44 but also other rice varieties (Krishan et al. 2021; Singh et al. 2022). Therefore, developing sustainable strategies to enhance tolerability/adaptability of PUSA-44 and other rice varieties to salinity and drought-like conditions has become imminent to maintain their productivity. Globally, drought and salinity stress have been recognised as abiotic stressors that grossly affect crop plants' growth and productivity (Verma et al. 2021a).

Plants harbour microbial communities as epiphytes or endophytes and generally comprise fungi, eubacteria, and archaebacteria. When the microbes reside within the plant tissues without apparent signatures, they are known as endophytes (Verma et al. 2021b). Today, it is well documented that fungi ubiquitously exist as endophytes in all plants, providing habitat-adapted symbiotic fitness benefits to plants and critical plant growth processes (Poveda et al. 2021). Endophytes modulate the plant responses to stressors using different mechanisms, from signal molecules to modifying host gene regulation (Jia et al. 2016). Endophytic isolates from the roots of plants growing in the extreme environment have been tested for their presumptive role in combating abiotic stresses, such as drought and salinity. Studies have evaluated the salinity and drought tolerance of endophytic fungi from various plants using different levels of sodium chloride (0.05–3 M w/v) and polyethylene glycol (1–40% w/v) supplemented growth media (Ripa et al. 2019; Sampangi-Ramaiah et al. 2020; Tarroum et al. 2021; Javed et al. 2022; Khan et al. 2022). Species of fungi, such as *Talaromyces*, *Penicillium*, *Aspergillus,* and *Chaetomium* isolated from rice plant roots, have exhibited tolerance to abiotic stresses (Bilal et al. 2018; Pang et al. 2020; Sampangi-Ramaiah et al. 2020; Ganie et al. 2021). Studies have also reported the successful colonisation of salinity-enduring endophytic fungi in rice, improving its growth under abiotic stress (Mohd et al. 2017; Lata et al. 2018; Qin et al. 2019).

Although our understanding of the intricate interactions/cross-talk between endophytic fungi and plants is limited, research shows that the relationship is particularly close-knit (Mattoo and Nonzom 2021). The co-evolution leading to horizontal gene transfer between the two symbionts has enabled the endophytic fungi to mimic plant-like characteristics. For instance, endophytic fungi can produce all classes of phytohormones, otherwise produced only by plants. These phytohormones play diverse roles, from seed development to root formation. Several studies have shown that the exogenous application of phytohormones can help the plant adapt to extreme stress conditions (Baron and Rigobelo 2022; Sabagh et al. 2022). Thus, the utilisation of phytohormone-producing endophytic fungi could prove beneficial. Other plant growth-promoting (PGP) attributes, such as antioxidant potential, mineral solubilisation, and production of extracellular lytic enzymes, are also exhibited by endophytic fungi (Sodhi and Saxena 2023). They help the plants by reducing oxidative damage, enhancing nutrient uptake, and providing adequate energy for growth under stressful environments. Various genera of fungal endophytes, such as *Aspergillus*, *Penicillium*, and *Trichoderma*, have been reported to exhibit these PGP traits (Bilal et al. 2018; Chand et al. 2020; Turbat et al. 2020; Poveda et al. 2021).

Based on the previous research, this work is a novel attempt to explore the culturable endophytic fungi of PUSA-44, a high-irrigation variety of rice, from sowing to crop harvest. All the isolates obtained at different stages of the crop cycle were tested for their endurance to salinity and drought under in vitro conditions. The isolates that exhibited tolerance to these stressors were further evaluated for their plant growth-promoting properties and identified using morpho-taxonomic and molecular identification techniques.

Materials and methods

Sample collection

Healthy plant parts (leaf, spike, internode, and roots) of *Oryza sativa*, PUSA 44 variety, were collected from a farm in Fatehgarh Sahib, Punjab, India (30.6174° N, 94 76.3888° E). The samples were collected at intervals of 15 days from the sowing of the rice variety till maturity. These were stored in zip-lock bags and transported to the laboratory for processing.

Isolation of endophytic fungi

Endophytic fungi were isolated using the method of Schulz et al. (1993) with minor modifications to the sterilisation time of each component used. Samples were cleaned under the running tap water and cut into small pieces measuring 4–5 cm. These were surface sterilised using 0.1% sodium hypochlorite (Hi-media, AS102) for 1–2 min, followed by 70% ethanol for 1 min and 30% ethanol for 45 s. After surface sterilisation, the samples were aseptically cut into small 2–3 mm fragments using a sterile scalpel. The fragments were then plated on ¼ strength potato dextrose agar (PDA) and water agar plates with the ventral side of the sample facing the medium. The petri plates were incubated in a BOD incubator at 26 ± 2 °C for 10–15 days with 12-h light/dark cycles to enable the emergence of fungal endophytes. Subsequently, individual colonies were picked from the colony's edge and transferred to PDA. Pure cultures were stored in PDA slants and vials containing 10% glycerol (Hi-media, GRM1027).

Isolation frequency

The isolation frequency (IF) of endophytic fungi was calculated for every isolation and different plant parts using the formula (Ikram et al. 2022)

$$\mathrm{IF}\% = \frac{\text{No. of individual fungi recorded}}{\text{Total no. of segments}} \times 100.$$

Screening of endophytic fungi for salinity and drought tolerance

The isolates were screened using plate and broth assay to assess the effect of stress conditions on the radial growth rate and biomass of endophytic fungi.

Plate assay

A broad range of salinity and drought stress were employed to screen the potent isolates and check the tolerance limit. For in vitro plate assay, PDA plates supplemented with different concentrations of NaCl ranging from 0.5–2 M (w/v) were prepared to induce salinity stress (Sampangi-Ramaiah et al. 2020). Likewise, 5–20% (w/v) of PEG-6000 amended PDA plates were prepared to induce drought stress (Ripa et al. 2019). A mycelial disc of 5–7 day old actively growing culture was inoculated facing the media surface, and the plates were kept at 26 ± 2 °C for 10 days. The growth rate of fungi was measured by noting the mean diameter every day till 10 days. It was compared against a control having no NaCl in salinity stress and no PEG in drought stress.

Broth assay

Based on the above screening protocol, the isolates exhibiting more than 50% growth compared to the control were then subjected to salinity and drought tolerance screening under broth conditions at the same concentrations described above. The growth rate of fungi was measured by noting the wet and dry biomass after 10 days. It was compared against the control with no NaCl in salinity stress and no PEG in drought stress. The isolates exhibiting the least reduction in biomass weight were selected to evaluate the plant growth-promoting attributes.

A correlation analysis was carried out to explore the quantitative relationship between plate and broth assay for salinity and drought tolerance. The relationship was statistically evaluated using Statistical Package for Social Sciences (IBM SPSS Statistics, ver. 28.0.1.1 (15)).

Evaluation of plant growth-promoting attributes of selected fungal endophytes

In vitro antioxidant potential

The free radical scavenging activity of the cell-free culture filtrates of selected endophytic isolates was performed using DPPH assay as per the method of Dhayanithy et al. (2019). DPPH in methanol produces a violet/purple colour which becomes yellow in the presence of antioxidants. This colour change is recorded spectrophotometrically at 517 nm. Briefly, this test comprises 20 µL of the 1 mg/mL test sample (concentration range 200–1000 µg/mL) to which 230 µL of DPPH solution (prepared in methanol) was added. The mixture was incubated for 30 min at room temperature in the dark, and then, absorbance was measured at 517 nm using a microplate reader (Biotek, USA).

Quercetin (concentration range 200–1000 µg/mL) was used as standard, and working DPPH as the control. The DPPH radical scavenging capacity was expressed as micrograms of quercetin equivalents per milligram of extract. The percentage of free radical scavenging activity was calculated as follows:

$$\%\mathrm{FRS} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100.$$

Production of phytohormones

The production of two phytohormones, i.e., Indole Acetic acid and Gibberellic acid, by selected endophytes were assessed.

Indole acetic acid (IAA) production

Salkowski's reagent was used to assess the in vitro production of IAA by the selected isolates (Wary et al. 2022). Briefly, the selected endophytic fungi were grown in Czapek Dox Broth with and without L-tryptophan. The culture was incubated at 120 rpm at 26 ± 2 °C under dark conditions for 10 days. Subsequently, cell-free supernatant was obtained by harvesting fungal biomass. This cell-free supernatant was subsequently mixed with Salkowski's reagent in a ratio of 1:2 and incubated for 30 min in the dark at room temperature. After the incubation, the absorbance of the reaction mixture was taken at 530 nm using a microplate reader (Biotek, USA). A standard curve of IAA was prepared to quantify IAA production.

Gibberellic acid (GA) production

The ability of the endophytic isolates to produce Gibberellic acid (GA3) extracellularly was done by the method of Holbrook et al. (1961) with minor modifications in the volume of reagents used. Briefly, 7-day-old mycelial plugs of selected endophytic fungi were aseptically inoculated in pre-sterilised Czapek Dox broth in Erlenmeyer flasks and then incubated at 26 ± 2 °C, 120 rpm for 10 days. To estimate GA3 production, 10 mL of culture filtrate and 0.5 mL of 1 M zinc acetate were mixed thoroughly for 3 min. Subsequently, 0.5 mL of 1 M potassium ferrocyanide solution was added, and the mixture was centrifuged at 10,000 rpm for 15 min. After centrifugation, 2.5 mL of the supernatant was withdrawn, and 8 mL of absolute alcohol and 90 mL of 30% HCl were added. The control comprised 35 mL of 5% HCl solution made up to 100 mL using 65 mL of distilled water in a 250 mL Erlenmeyer flask. The reaction mixtures were incubated at 26 ± 2 °C for 75 min, and then, absorbance was recorded at 254 nm. The standard curve of GA3 was prepared using defined concentrations to establish linearity between the concentration of GA and absorbance at 254 nm.

Phosphate solubilisation

The selected endophytic isolates of fungi were tested for their potential to convert insoluble phosphate compounds into a soluble form and make it available for the plants, which helps in plant nutrition, growth, and reproduction. Briefly, Pikovskaya's Agar medium [composition (g/L): 0.5 g $(NH_4)_2SO_4$, 0.1 g $MgSO_4 \cdot 7H_2O$, 0.02 g NaCl, 0.02 g KCl, 0.003 g $FeSO_4 \cdot 7H_2O$, 0.003 g $MnSO_4 \cdot H_2O$, 5 g $Ca_3(PO_4)_2$, 10 g glucose, 0.5 g yeast extract, 15 g agar, and 1000 mL distilled water] was supplemented with bromophenol blue. Subsequently, 5 mm mycelial plugs of 7-day-old culture of selected isolates were inoculated on the agar media and incubated at 26 ± 2 °C for 07 days. The control set comprised sterile agar plugs with no culture. Three replicates were tested for each fungal isolate. A yellow-coloured halo around the colony indicated the presence of phosphate-solubilising activity on 7th day (Jasim et al. 2014). The phosphate-solubilisation index (PSI) was calculated by the formula (Edi-Premono et al. 1996)

$$\mathrm{PSI} = \frac{\text{Colony diameter (mm)} + \text{Halo diameter (mm)}}{\text{Colony diameter (mm)}}.$$

Ammonia production

The capacity for ammonia production by the selected endophytic isolates was assessed by inoculating mycelial plugs of 5 mm in 10 mL of sterile peptone water in test tubes and incubated at 26 ± 2 °C, 120 rpm for 7 days. To 5 mL of culture filtrate, 1 mL of Nessler's reagent was added. A change in colour to deep yellow/brown indicated ammonia production (Chand et al. 2020).

Extracellular enzyme production

The ability to produce extracellular enzymes of the selected isolates was defined by the Enzymatic Index (EI) calculated by the formula (Florencio et al. 2012)

$$\mathrm{EI} = \frac{\text{Colony diameter (mm)} + \text{Halo diameter (mm)}}{\text{Colony diameter (mm)}}.$$

Cellulase activity

Glucose yeast peptone (GYP) medium [composition: 1 g/L glucose, 0.1 g/L yeast extract and 0.5 g/L peptone, 15 g Agar, and 1000 mL distilled water] was supplemented with 1% Carboxymethyl Cellulose as a substrate for cellulase production. Briefly, 5 mm mycelial plugs were prepared from 7-day-old cultures of selected endophytic fungi on a PDA medium. These were aseptically placed on the sterile GYP plates supplemented with CMC, and incubated at 26 ± 2 °C for 7 days—the control comprised of an uninoculated 5 mm PDA plug. After 7 days of incubation, the plates were flooded with 0.5% Congo red solution for 20 min. These were de-stained with 1 M NaCl for 15 min. Subsequently, the de-staining solution was decanted. The production of cellulase was indicated by a yellow halo (Lee et al. 2014). The enzyme activity was ascertained by calculating the EI, indicating the potential of endophytic fungi to produce the enzyme.

Laccase activity

For assessing the laccase production by the selected fungal endophytes, 0.005% 1-naphthol was mixed with the GYP medium and autoclaved. Subsequently, the medium was used to prepare culture plates using pre-sterilised Petri dishes. Briefly, 5 mm mycelial plugs prepared from 7-day-old cultures of selected endophytic fungi were seeded in the centre of the GYP + 1-naphthol plates and incubated at 26 ± 2 °C for 7 days. The control comprised of uninoculated PDA plug only. The change of colour from colourless to purple/violet coloured zone/halo was formed due to laccase activity (Lee et al. 2014). The EI for laccase activity was estimated using the formula mentioned in the previous section.

Morphological and phylogenetic identification of selected fungal endophytes

The fungal endophytic isolates, which expressed abiotic stress tolerance potential and plant growth-promoting bioactivities, were identified using morphological as well as molecular phylogenetic methods. These isolates were cultured on different media, such as potato dextrose agar (PDA), water agar (WA), and corn meal agar (CMA) at 26 ± 2 °C for 10 days, with a photoperiod of 12 h. Distinctive culture features on the petri dish, such as the colony texture, colour, colony growth rate, and pigment production, were recorded. The microscopic features of hyphal characteristics, conidia, conidiophores, phialides, and other cellular bodies were minutely observed, recorded, and photographed using a Nikon microscope (Nikon E200, Tokyo, Japan). The micrometric observations were performed using the Image J software (National Institutes of Health, Bethesda, MD, USA) with at least 30 observations per structure (Wang et al. 2017).

Molecular identification involved the isolation of genomic DNA of the selected endophytes by the CTAB (Cetyl trimethylammonium bromide) as per the method of Van Burik et al. (1998) with minor modifications in the composition of extraction buffer. Briefly, 0.5 g of fungal mycelia was scrapped off from the 4- to 5-day-old culture of the selected endophytes and crushed using liquid nitrogen. Further cell lysis was performed by the addition of 1 mL extraction buffer [composition: 1% CTAB, 1 M Tris HCl, 0.5 M EDTA, 5 M NaCl, and 2% cetrimide] and incubated at 60 °C for 30 min. After that, 1.5 µL of RNase solution was added and the reaction mixture was incubated at 37 °C for 15 min. The lysate extraction was done using phenol:chloroform:isoamyl alcohol (25:24:1) and centrifuged at 12,000 rpm for 10 min. The precipitation of genomic DNA from the aqueous layer was done using chilled isopropanol. The resulting pellet was washed using 80% ethanol, air dried, dissolved in TE buffer, and stored at -20 °C till further use.

The amplification of the ITS (Internal Transcriber Spacer) region 1, 5.8S, ITS 4 of the genomic DNA was done using ITS 1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS 4 (5′TCCTCCGCTTA TTGATATGC-3′) primers. Briefly, 25 µL of reaction mixture containing 25 ng of extracted fungal DNA, 0.8 µM of each primer, 2.5 mM of dNTP (Bangalore GeNei), 1.5 mM $MgCl_2$ (Bangalore, GeNei), and 1.5 U of Taq DNA Polymerase (Bangalore GeNei) in 10 X Taq buffer (Bangalore, GeNei) was prepared. The conditions for the thermal cycler consisted of initial denaturation at 96 °C for 5 min followed by 39 cycles of 95 °C for 1 min, 58 °C for 1.30 min, 72 °C for 1 min, and final extension at 72 °C for 5 min (White et al. 1990). The PCR amplicons were examined using gel electrophoresis in a 1.5% agarose gel at 40 V. Gel imaging was performed under UV light in the Bio-Rad Gel documentation system.

The purified amplicons were sent for sequencing to Biokart, India. The sequences were analysed using Sequencher ver. 5.4.6 (www.genecodes.com) for their purity (> 90%), aligned and then submitted to GenBank. nBLAST algorithm software was used to search for similarity for the final ITS sequences of 6OSFR2e, 7OSFS3a, and 6OSFL4c. Furthermore, a sequence similarity matrix was generated using the new multiple sequence alignment program, Clustal Omega (Sievers and Higgins 2014). MEGA 11 was used to align the reference sequences obtained from the nBLAST analysis, and the alignment file comprised 20 sequences, 14 type species, and the sequences under study. *Arthrinium arundinis* KF114889 served as the outgroup in the tree formation. The maximum-likelihood method based on the Tamura–Nei model with 1000 bootstraps was used for the phylogenetic tree construction.

Statistical analysis

All the tests were performed in triplicate, representing the data as mean ± SD. One-way ANOVA analysis was done, followed by Tuckey's post hoc test (considering $p < 0.05$ as significant) using Graph Pad Prism software. Linear regression was used to calculate the IC_{50} value (concentration at which 50% scavenging occurs) of the antioxidant activities.

Results and discussion

Isolation of endophytic fungi and isolation frequency

The evolution of plants onto land led to the omnipresent symbiosis with microorganisms. This co-evolution shaped the new niche of microorganisms in host plants in exchange for the positive impact on the overall health and fitness of the plants (Baron and Rigobelo 2022). Considering the

nutritional importance and the amount of global consumption over the years, many studies have explored the symbiotic relationship between endophytes and rice plants. This study isolated endophytic fungi from PUSA-44, a traditional rice variety grown extensively in Punjab. A total of eight plant samples were collected over 17 weeks. The physicochemical properties of soil from the sampling site had the following characteristics: pH 7.34 ± 0.076, organic carbon 0.51 ± 0.017%, total nitrogen 0.12 ± 0.006%, total phosphorous 987.00 ± 4.583 mg/kg, and available phosphorous 94.67 ± 2.887 mg/kg. Of the total samples collected, 120 endophytic fungal isolates were obtained from healthy plant parts (leaf, internode, spike, and roots) during the different growth stages of the plant cycle. Some of the endophytic fungi isolated during the study are shown in Fig. 1.

Of the total isolates, 40 were isolated from leaves, 32 from roots, 25 from internode, and 23 from spikes. The day-wise and plant-part-wise isolation frequency is shown in Fig. 2. The isolation frequency from leaves and roots remained the same during the vegetative stage of the plant (from first to the third isolation), whereas comparatively lesser isolates were recovered from the internode. However, during the initiation of the reproductive stage (fifth isolation) in the plant, no endophytic fungi were obtained from the internode. In contrast, four isolates with an isolation frequency of 5% were isolated from the spike. The highest isolation frequency of 11.25% was seen in roots during the reproductive stage of the plant, followed by 10% in the leaves. The results are in accordance with the previous reports, which have reported a high colonisation rate of endophytic fungi in the roots and leaves of the rice plant (Tian et al. 2004; Naik et al. 2009; Zakaria et al. 2010). With further progression in the growth cycle, the isolation frequency of endophytic fungi from roots declined from 6.25% around the 105th day to only 1.25% near the end of the growth cycle. At the same time, the isolation frequency of spikes increased from 7.5% around the 105th day to 8.75% near the end of the growth cycle. Considering these findings, it can be postulated that endophytic fungi colonised the newer plant parts with advancement

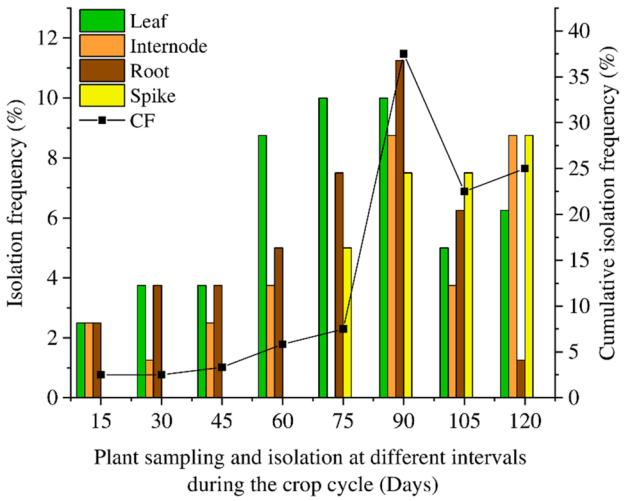


Fig. 2 Isolation frequency and cumulative isolation frequency (CF) of endophytic fungi isolated from different plant parts of PUSA-44 over a period of 120 days

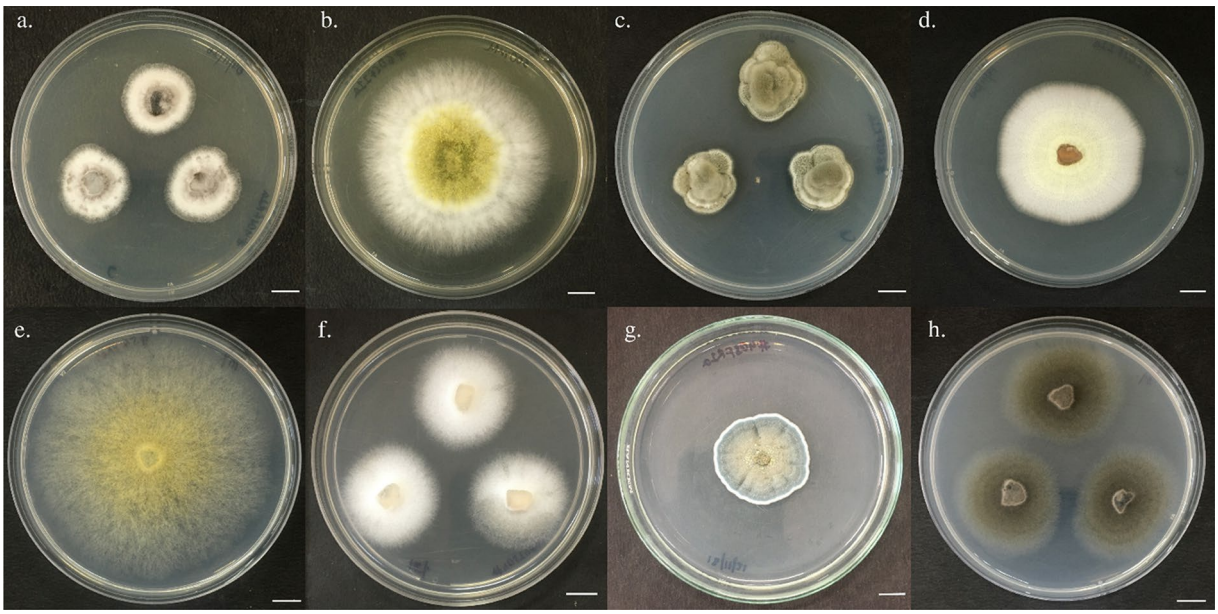


Fig. 1 Some of the endophytic fungi isolated during the study: **a** *Nigrospora* sp., **b** *Aspergillus* sp., **c** non-sporulating, **d** *Paecilomyces* sp., **e** *Rhizopus* sp., **f** *Fusarium* sp., **g** *Penicillium* sp., **h** unidentified (bar: 10 mm)

in the growth cycle, eventually colonising the spikes. The findings indicate the possibility of a vertical transmission pattern adopted by the endophytic fungi, although further studies need to be undertaken for an irrefutable conclusion. This method of transmission is especially intriguing as the beneficial endophytic population is passed on from the plant to its progeny.

Furthermore, the isolates were tentatively identified based on their morphological and microscopic characteristics. Of the identified isolates, 45% belonged to Sordariomycetes class, whereas 16.66% and 13.33% belonged to Dothideomycetes and Eurotiomycetes. Likewise, 2.5% of isolates belonged to Zygomycetes. The 120 endophytic isolates belonged to 11 genera: *Nigrospora*, *Fusarium*, *Alternaria*, *Penicillium*, *Aspergillus*, *Curvularia*, *Rhizopus*, *Colletotrichum*, *Paecilomyces*, *Cladosporium*, and *Pestalotiopsis*. In contrast, some isolates were unidentified because of their non-sporulating nature. Previous studies have reported *Fusarium*, *Aspergillus*, *Penicillium*, *Chaetomium,* and *Curvularia* among the dominant genera isolated from different rice varieties (Tian et al. 2004; Naik et al. 2009; Zakaria et al. 2010; Atugala and Deshappriya 2015). *Nigrospora* sp. has also been reported from other plants of the Poaceae family (Fernández-Pastor et al. 2021). In the current study, the majority of isolates, i.e., 25%, belonged to *Nigrospora*, 15.8% to *Fusarium,* and 13.3% to *Alternaria*. Similarly, 7.5 and 5% of isolates belonged to *Penicillium* and *Aspergillus*, while less than 3% belonged to *Curvularia*, *Rhizopus*, *Colletotrichum*, *Paecilomyces*, *Cladosporium,* and *Pestalotiopsis*.

Screening of endophytic fungi for salinity and drought tolerance

In nature, stresses, such as salinity and drought, often occur together and can plants suffer severe physiological and biochemical harm from them. Soils with electrical conductivities of 2 dS/m or higher (~ 0.02 M NaCl) are termed saline. Salinity stress causes a build-up of sodium and calcium ions which leads to reduction in the water accumulation by plants, as well as their water potential (Ma et al. 2020; FAO 2021). During the salinity screening assay of endophytic fungi, a reduction in fungal growth and biomass was observed with an increase in the salinity concentration. Out of 120 isolates, 84 exhibited over 70% growth at 0.5 M NaCl concentration. This number decreased to 53 and 24 isolates at 1 and 1.5 M NaCl concentration, respectively. Only eight isolates, namely 5OSFS1a, 5OSFL6a, 6OSFR2e, 6OSFR2d, 6OSFL4c, 6OSFI1b, 7OSFS3a, and 8OSFI2a exhibited more than 70% growth at 2 M NaCl concentration (Fig. 3a–d). The high NaCl concentrations reduce the water activity in fungal cells, thereby reducing the transportation rate of nutrients in and out of the cells. This increase in NaCl concentrations gravely hampers their growth. However, the current findings indicate that a select few isolates possess the ability to exhibit tolerance to high salt stress. In a recent study, *Fusarium* sp. (V-4J isolated from salt-tolerant Pokkali rice) exhibited 78.01% and 48.34% growth at 1.5 and 2 M NaCl concentrations, respectively (Sampangi-Ramaiah et al. 2020). Nevertheless, the isolates screened in this investigation exhibited higher growth at the same salinity concentration.

Drought stress was induced using PEG-6000, which has an inert nature and high molecular weight that helps influence the medium's osmotic potential without getting absorbed by fungal cells. In this study, out of 120 isolates tested, 91 exhibited over 70% growth at 5% PEG concentration (-0.45 ± 0.01 MPa), followed by 62 and 29 isolates at 10% and 15% PEG concentration (-0.65 ± 0.01 and -0.89 ± 0.01 MPa, respectively). Only six isolates, namely 5OSFS1a, 6OSFR2e, 6OSFR2d, 6OSFL4c, 7OSFS3a, and 8OSFI2a, exhibited more than 70% growth at 20% PEG concentration (-1.25 ± 0.01 MPa) (Fig. 3e–h). As low water potential disrupts cellular homeostasis, it leads to the inhibition of the growth of endophytic fungi. Our findings are in accordance with the other studies. For instance, Pang et al. (2020) reported an endophytic *Talaromyces purpureogenus* isolated from the roots of *Oryza sativa* that exhibited tolerance to drought stress induced by 10% PEG. The isolate also enhanced the root length, root, and shoot fresh weight of the host plant under drought stress. However, in this investigation, the isolates exhibiting salinity and drought stress tolerance were recovered from different plant parts at various stages of the crop cycle. For instance, isolates 5OSFS1a and 5OSFL6a were isolated during the early reproductive stage and isolates 6OSFR2e, 6OSFR2d, 6OSFL4c, 6OSFI1b were isolated from the late reproductive stage of the plant. Likewise, isolates 7OSFS3a and 8OSFI2a were isolated during the plant's ripening (grain-filling) stage.

Furthermore, the correlation analysis revealed a positive correlation between plate and broth assay at different concentrations of NaCl and PEG-6000. A Pearson's correlation coefficient (PCC) of 0.989, 0.939, 0.988, and 0.998 (p value < 0.001) at 0.5, 1, 1.5, and 2 M NaCl concentration, respectively, was observed (Fig. 4a–d). The linear correlation between two variables ranges from −1 to 1, where a value close to 1 denotes a positive correlation implying that the data points of the two variables lie on the same line. In the case of plate and broth assay using different PEG concentrations, a positive correlation with PCC of 0.884, 0.861, 0.796, and 0.795 (p value < 0.001) at 5, 10, 15, and 20% PEG concentration was observed (Fig. 4e–h). Overall, the positive correlation depicts the efficiency of both plate and broth methods in predicting the tolerance of isolates for different stressors. Considering the growth of selected isolates under both salinity and drought stress, they were further evaluated for their PGP attributes.

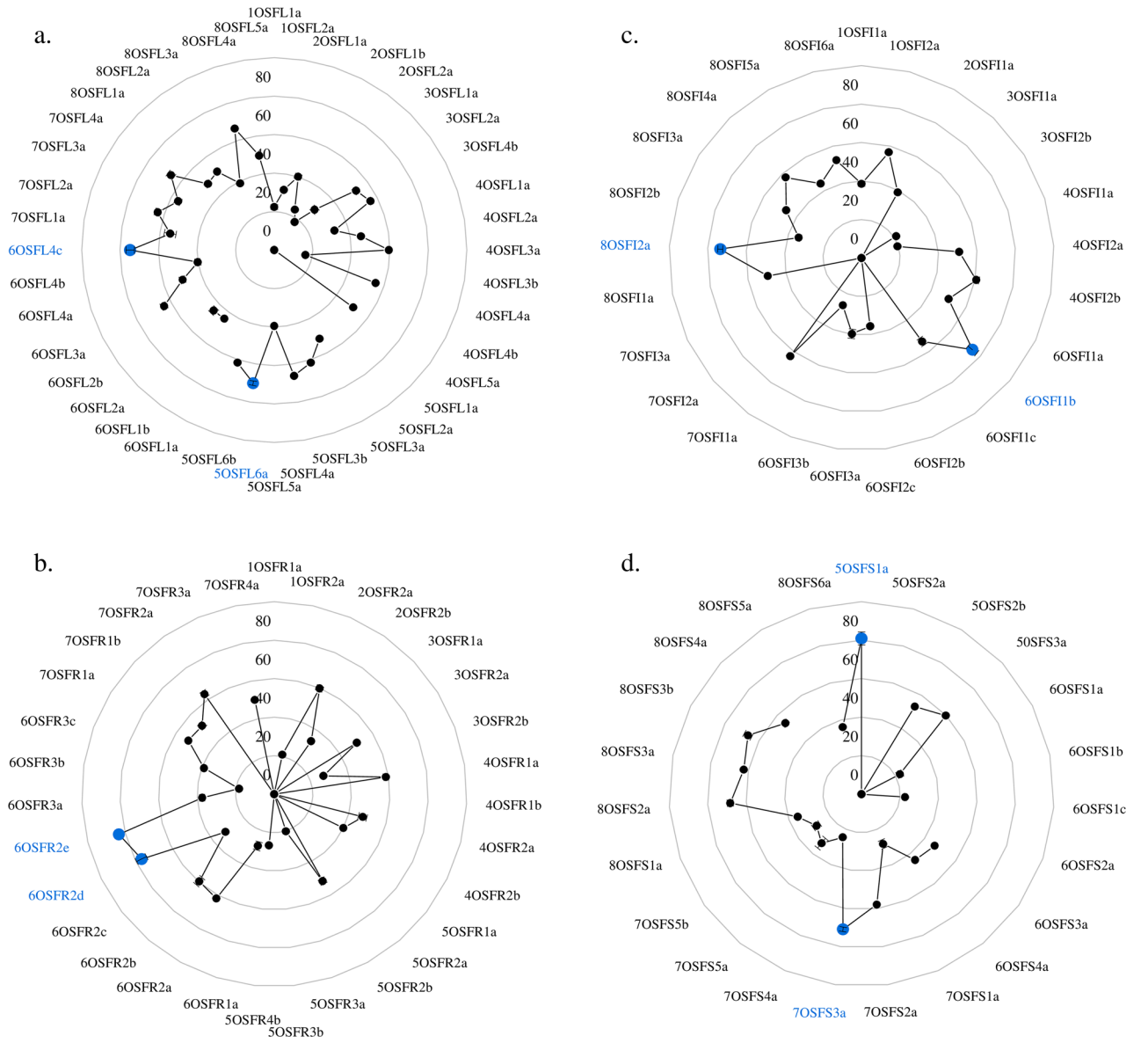


Fig. 3 Isolates from different plant parts exhibiting growth under salinity and drought stress. **a** Leaf isolates at 2 M NaCl concentration. **b** Root isolates at 2 M NaCl concentration. **c** Internode isolates at 2 M NaCl concentration. **d** Spike isolates at 2 M NaCl concentration. **e** Leaf isolates at 20% PEG-6000 concentration. **f** Root isolates at 20% PEG-6000 concentration. **g** Internode isolates at 20% PEG-6000 concentration. **h** Spike isolates at 20% PEG-6000 concentration. The values represent mean ± SD, $n = 3$; mean with different superscript letters are different by Tukey's post hoc test ($p < 0.05$)

Plant growth-promoting attributes of selected fungal endophytes

Reactive oxygen species (ROS), including free radicals and non-radical molecules, are primarily formed in cellular metabolism at various plant sites such as mitochondria, chloroplast, and apoplast (Czarnocka and Karpiński 2018). Plants intricately maintain a steady-state balance and optimum ROS levels through a diverse endogenous defence mechanism involving antioxidant enzymes. However, this fine-tuned balance between the production and processing of ROS is disrupted during stress conditions. Overaccumulation of ROS is known as oxidative stress, and it is the first sign seen in plants under abiotic stress. The imbalance of this equilibrium causes cellular damage and gravely reduces crop productivity (Hasanuzzaman et al. 2020). This investigation evaluated the free radical scavenging (%FRS) capacity of selected isolates using DPPH radical. The methanolic extract of isolate 6OSFR2e exhibited the highest %FRS capacity of 88.78% followed by isolate 7OSFS3a at 79.67%, 6OSFL4c

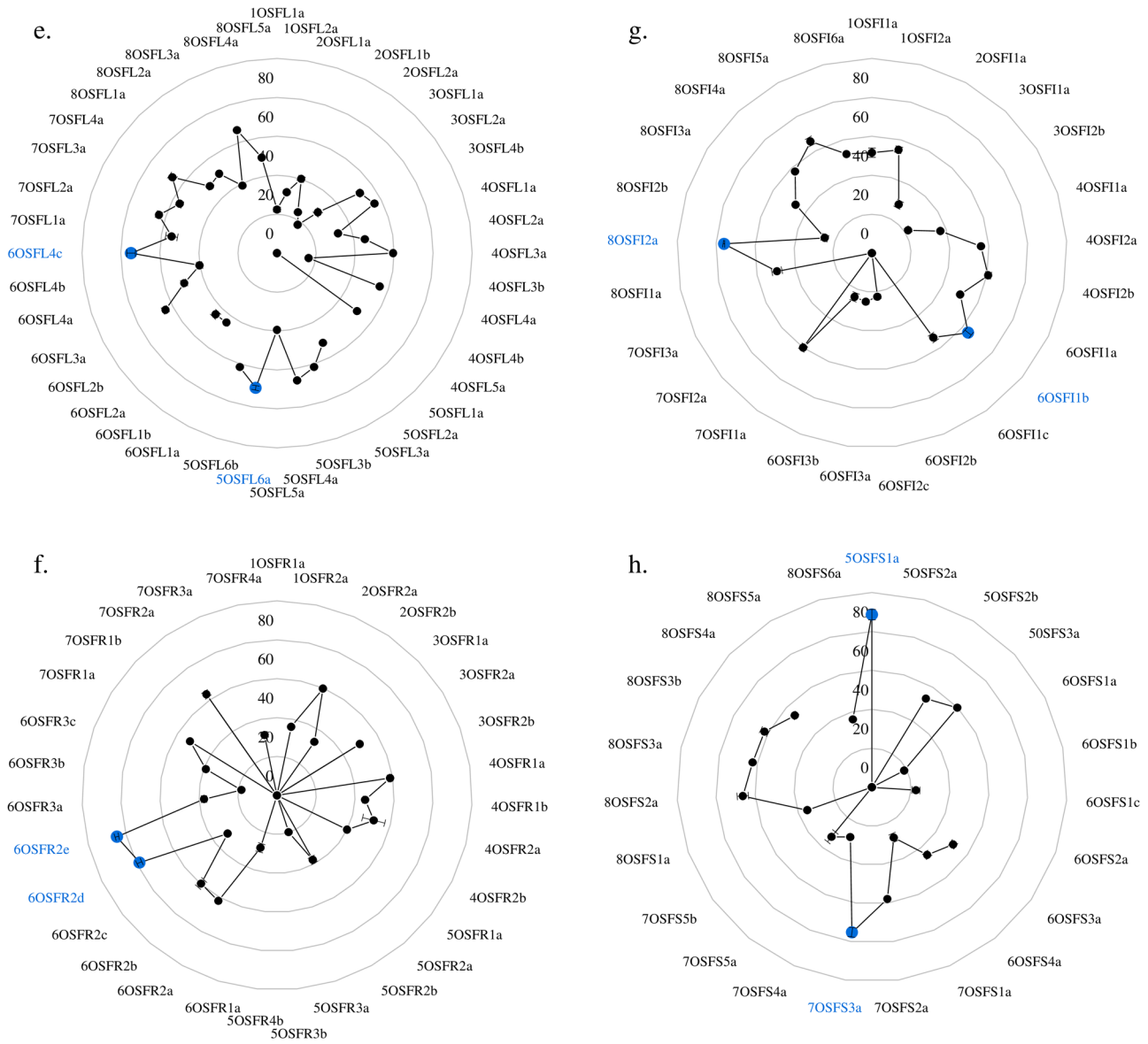
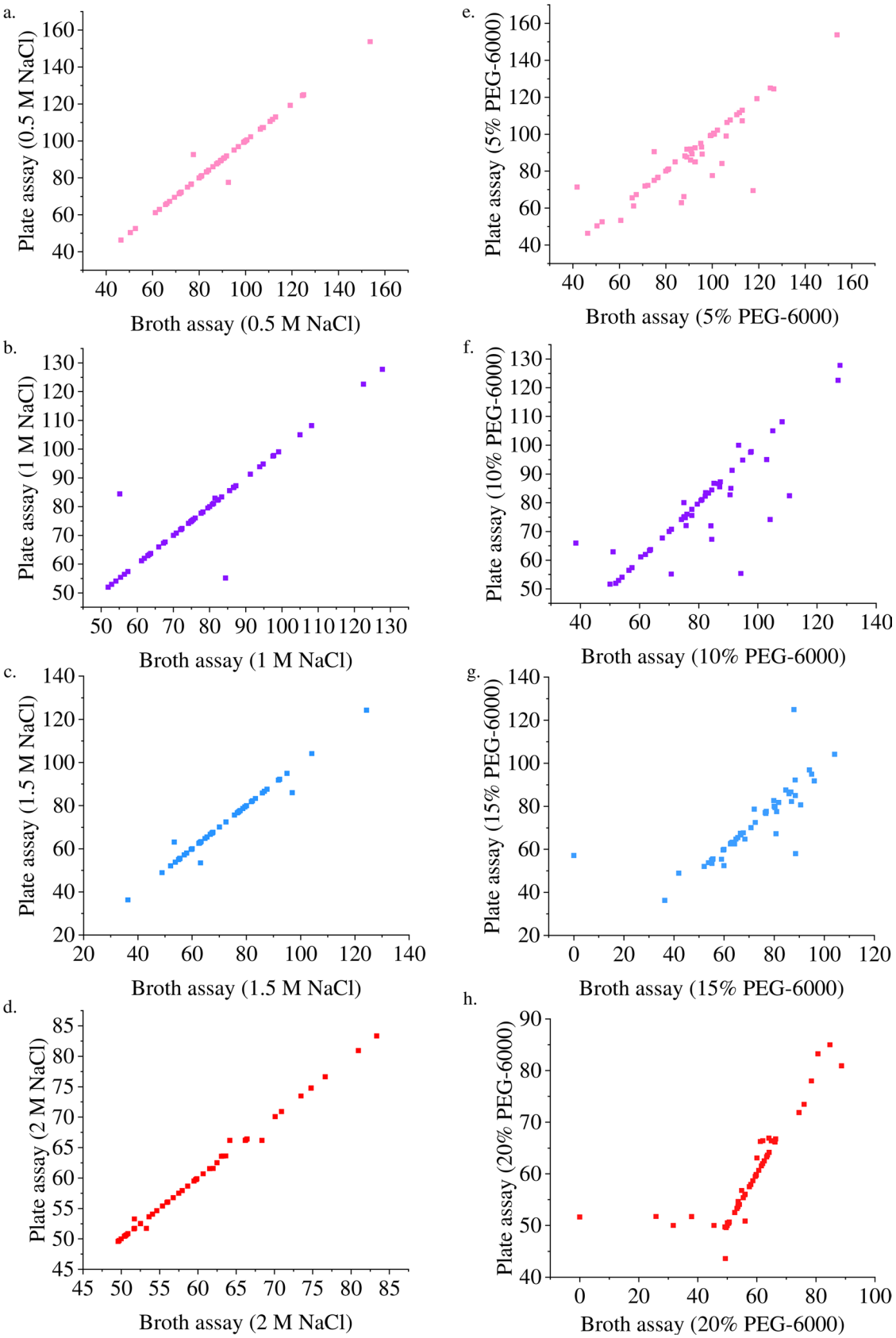


Fig. 3 (continued)

at 72.52% and 6OSFR2d at 72.90%. In a recent study, *Penicillium citrinum* (isolated from *Digitaria bicornis*, a plant of the Poaceae family) exhibited 52.3% FRS. Likewise, *Nigrospora sphaerica* EHL2 and *Nigrospora oryzae* exhibiting 43.54 and 59.6% FRS, respectively, have been reported (Gautam et al. 2022; Nischitha and Shivanna 2022; Vig et al. 2022). However, the %FRS of the isolates investigated in this study is higher than the latest reports. The least %FRS of 50% was observed in isolate 5OSFS1a (Fig. 5a).

DPPH is a robust method based on electron transfer and is extensively used to analyse the free radical scavenging potential of microorganisms and plant extracts. The electron is donated by the antioxidant molecule, in this case, the endophytic fungi, which reduces this free radical to give a colourless solution (Dwibedi and Saxena 2020). Further, linear regression was used to calculate the IC_{50} value for each fungal extract. IC_{50} value denotes the half-maximal inhibitory concentration, where low values indicate the extract's effectiveness at an even lower concentration. The lowest IC_{50} value of 345.45 ± 11.66 µg/mL was observed in isolate 7OSFS3a, followed by 391.58 ± 10.66 µg/mL by isolate 6OSFR2e. The highest IC_{50} value of 1024.02 ± 46.24 µg/mL was observed in isolate 5OSFS1a (Fig. 5b). The results indicate the efficiency of endophytic fungi in the possible detoxification of free radicals generated during stress conditions. Although plants have an elaborate defence system of enzymatic and non-enzymatic antioxidants, it is generally overburdened under stress conditions. Studies have


a.
Plate assay (0.5 M NaCl)
Broth assay (0.5 M NaCl)
b.
Plate assay (1 M NaCl)
Broth assay (1 M NaCl)
c.
Plate assay (1.5 M NaCl)
Broth assay (1.5 M NaCl)
d.
Plate assay (2 M NaCl)
Broth assay (2 M NaCl)
e.
Plate assay (5% PEG-6000)
Broth assay (5% PEG-6000)
f.
Plate assay (10% PEG-6000)
Broth assay (10% PEG-6000)
g.
Plate assay (15% PEG-6000)
Broth assay (15% PEG-6000)
h.
Plate assay (20% PEG-6000)
Broth assay (20% PEG-6000)

◂**Fig. 4** Correlation analysis between plate and broth screening assay at **a** 0.5 M NaCl, **b** 1 M NaCl, **c** 1.5 M NaCl, **d** 2 M NaCl, **e** 5% PEG-6000, **f** 10% PEG-6000, **g** 15% PEG-6000, and **h** 20% PEG-6000. The values represent mean ± SD, $n = 3$; mean with different superscript letters are different by Tukey's post hoc test ($p < 0.05$)

also reported the enhanced antioxidant potential of plants inoculated with endophytic fungi (Javed et al. 2022; Li et al. 2023). Thus, it presents the endophytic fungi as a promising candidate for mitigating oxidative damage in host plants under abiotic stresses.

As discussed earlier, the evolutionary phenomenon of horizontal gene transfer enables the endophytic fungi to produce various phytochemicals. Production of phytohormones is another crucial characteristic exhibited by plant growth-promoting endophytic fungi. The selected endophytic fungi's culture filtrate was analysed for IAA production using Salkowski's reagent. It is a widely used method comprising $FeCl_3$, which on reduction, forms a complex with IAA and yields pink colour (Suebrasri et al. 2020). IAA is the chief auxin responsible for cell elongation, apical dominance, and tissue differentiation. Though phytohormone plays no prominent role in endophytic fungi, they have been known to facilitate the interaction between the endophytes and host plants. In this investigation, all the isolates produced IAA ranging from 133.56 ± 05.85 to 268.32 ± 08.01 µg/mL. Isolate 6OSFR2e exhibited the highest IAA production of 268.32 ± 08.01 µg/mL, followed by 210.30 ± 08.46 µg/mL by isolate 7OSFS3a and 172.68 ± 05.86 µg/mL by isolate 6OSFL4c, which is comparatively higher than many previously reported studies (*Galactomyces geotrichum* WLL1 76.89 ± 2.35 µg/mL, *Alternaria alternata* LQ1230 40.12 ± 8.59 µg/mL, *Penicillium* sp. LWL3 29.8 µg/mL, *Aspergillus awamori* wl1 24.2 µg/mL, and *Phoma glomerata* LWL2 3.89 µg/mL) (Waqas et al. 2012, 2014; Mehmood et al. 2019; Qiang et al. 2019).

All the tested isolates exhibited IAA production in the absence of tryptophan. Moreover, all the isolates exhibited increased IAA production on evaluating the culture filtrate supplemented with tryptophan. Isolates 6OSFR2e and 7OSFS3a exhibited the highest IAA production of 359.27 ± 07.35 and 346.65 ± 13.98 µg/mL, followed by isolate 6OSFL4c with 276.65 ± 7.47 µg/mL (Fig. 5c). In the natural environment, the endophytic fungi can utilise tryptophan secreted by the plant roots to produce IAA and promote plant health. Studies have reported a reduction in IAA production in plants under stress conditions. However, the exogenous application of IAA can mitigate the adverse effects of abiotic stress in rice resulting in enhanced plant biomass, spike viability, yield, and reduction in ROS accumulation (Sharma et al. 2018). In addition, as indicated by the results, the production of IAA by both tryptophan-dependent and independent pathways presents the isolates as promising candidates. The findings are in accordance with the previous studies that reported endophytic fungi producing IAA by both pathways (Turbat et al. 2020; Badawy et al. 2021). Thus, using such endophytic fungi as exogenous IAA can help the plant by strengthening its immune response, especially under stressed conditions (Khalid and Aftab 2020).

Similarly, gibberellic acid is another significant phytohormone crucial for the growth and development of plants. GAs, as diterpene phytohormones, play a vital role in the germination of seeds and the plant's transition from the vegetative to the reproductive stage. Moreover, various plant growth processes are regulated by the distribution pattern of GA. The detection of GA in culture filtrate of selected endophytic fungi was done using the traditional spectrophotometric method based on the conversion of gibberellic acid to gibberellenic acid in the presence of strong acid. Isolate 7OSFS3a exhibited the highest GA production of 25.72 ± 0.04 µg/mL followed by 12.67 ± 0.01 and 12.66 ± 0.02 µg/mL by isolates 6OSFL4c and 6OSFR2e, respectively (Fig. 5d). Previous studies have reported GA-producing endophytic fungi for rice cultivation. For instance, Bilal et al. (2018) reported GA-producing *Fusarium proliferatum* BRL1 and *Aspergillus fumigatus* TS1. On inoculation, the isolates enhanced physiological attributes of the Waito-C rice plant, viz., a GA mutant rice variety. Similarly, Al-Hosni et al. (2018) reported GA-producing *Preussia* sp. BSL significantly enhanced the physiological attributes of Waito-C rice and the 'Jin so mi cultivar' of rice. Like IAA, exogenous GA application helps the plant attain height and biomass, which helps the plant to combat hostile environmental conditions. Due to endophytic fungi's capacity to produce phytohormones and provide supplements to regulate various physiological processes in plants, they present a sustainable substitute for biofertilisers to intensify crop production.

Plants require various minerals, such as phosphorous, vital for growth. It is one of the most crucial micronutrients, making up 0.2% of a plant's dry weight. However, more than 95–99% of phosphorous is present in insoluble form in the soil, which makes it difficult for the plant to utilise it. Thus, the association of plants with such microbes that can solubilise these nutrients is an added advantage. Many phosphate-solubilising endophytic fungi have been reported, which can hydrolyse the organic and inorganic phosphate into free form by producing phosphatase and phytase enzymes (Prabhu et al. 2019). The detection method uses Pikovskaya's agar media which comprises yeast extract and dextrose as energy sources for the endophytic fungi. The phosphate-solubilising microorganisms grow to form a clear zone around the periphery because of phosphate-solubilisation activity (Hnamte et al. 2021). In this study, isolate 6OSFR2d exhibited the highest phosphate-solubilisation activity of 1.11 ± 0.01, followed by isolates 6OSFR2e and

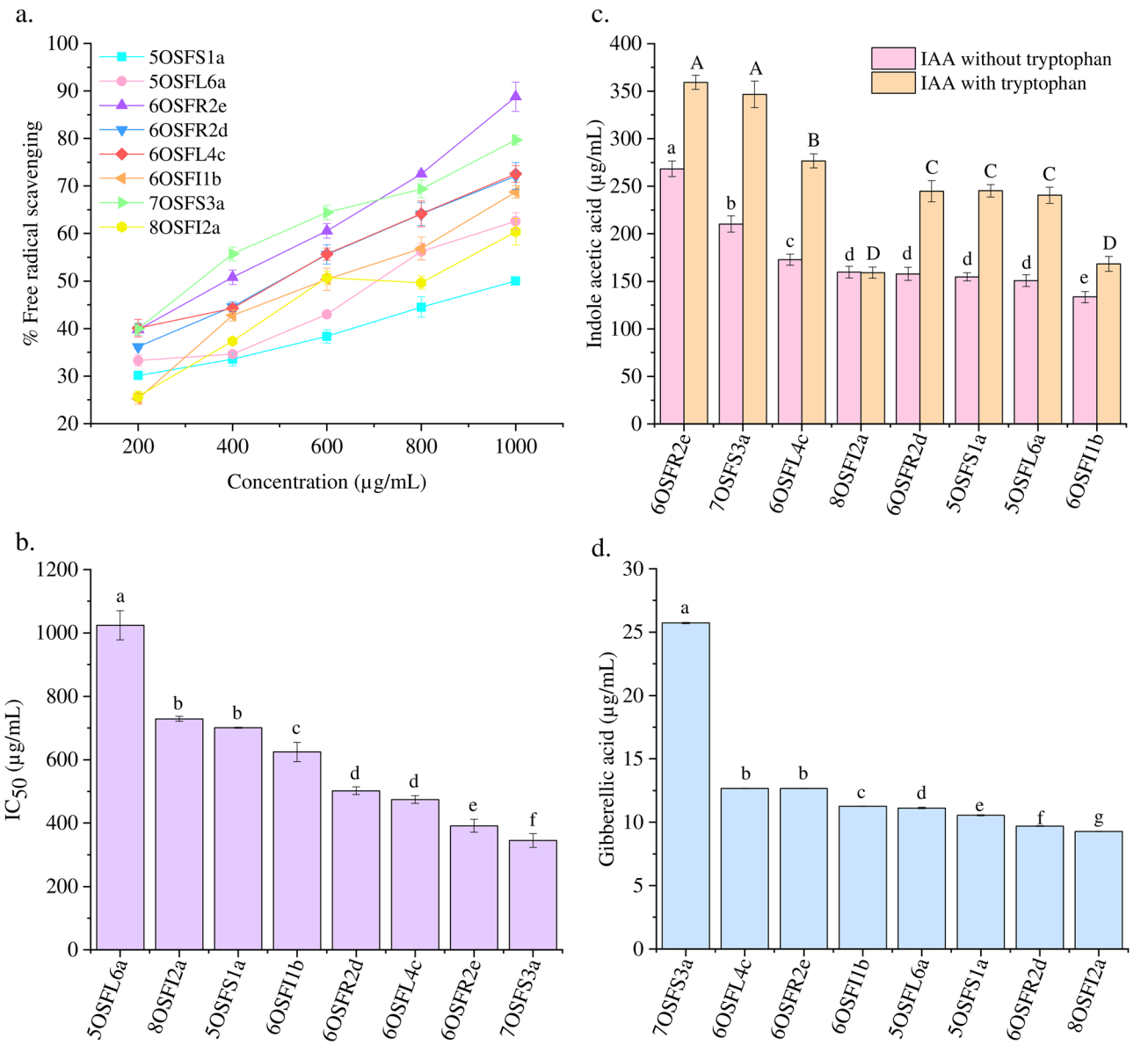


Fig. 5 Plant growth-promoting attributes of selected endophytic fungi. **a**, **b** Free radical scavenging % and IC50 values evaluated using DPPH assay. **c** Indole acetic acid production. **d** Gibberellic acid production. The values represent mean of value ± SD, $n = 3$; mean with different superscript letters are different by Tukey's post hoc test ($p < 0.05$)

6OSFL4c with a PI of 1.06 ± 0.00 and 1.04 ± 0.02, respectively (Table 1). Previously, many species of *Talaromyces*, *Chaetomium* and *Penicillium* isolated from roots of *Oryza sativa* have been reported for their phosphate-solubilisation potential. The isolates could solubilise organic and inorganic phosphorous (Pang et al. 2020). In addition, Tandon et al. 2020 reported a *Trichoderma* strain NBRI-PR5, isolated from rice plants for its phosphate-solubilisation abilities. Similar findings have been reported from other plants of the Poaceae family, such as *Triticum aestivum* (Ripa et al. 2019). These traits exhibited by the endophytic fungi are often termed indirect growth-promoting properties. The association of plants with such microorganisms strengthens soil fertility, nutrient absorption, and plant growth. Thus, their employment is imperative under stressful environments.

Another PGP trait exhibited by endophytic fungi is the production of ammonia. The ammonia-producing endophytic fungi bind the air-borne nitrogen making it readily available for the plant. The culture filtrate of the majority of the tested isolates exhibited ammonia production. The intensity of the yellow colour on adding Nessler's reagent to the culture filtrate indicates the amount of ammonia production. In this study, the highest intensity of ammonia production

Table 1 Ammonia production, phosphate-solubilisation index, and extracellular enzyme production of selected endophytic fungi

Isolate	Ammonia production	Phosphate solubilisation index[a]	Enzyme index[a]	
			Cellulase[a]	Laccase[a]
5OSFS1a	+ +	0.97 ± 0.02^{d}	1.23 ± 0.01^{a}	0.73 ± 0.02^{d}
5OSFL6a	+	ND	1.04 ± 0.01^{d}	1.01 ± 0.01^{c}
6OSFR2e	+ + +	1.06 ± 0.00^{b}	1.24 ± 0.00^{a}	1.04 ± 0.01^{c}
6OSFR2d	+ + +	1.11 ± 0.01^{a}	1.09 ± 0.03^{c}	1.13 ± 0.00^{ab}
6OSFL4c	+ + + +	1.04 ± 0.02^{bc}	1.14 ± 0.01^{b}	1.16 ± 0.00^{a}
6OSFI1b	+	ND	1.02 ± 0.01^{d}	1.04 ± 0.01^{c}
7OSFS3a	+ +	1.03 ± 0.01^{c}	1.15 ± 0.02^{b}	1.09 ± 0.04^{b}
8OSF12a	+ + +	0.86 ± 0.00^{e}	1.14 ± 0.00^{b}	0.70 ± 0.03^{d}

−, indicates no ammonia production; + indicates low ammonia production + +, indicates moderate ammonia production; + + +, indicates good ammonia production; + + + +, indicates very high ammonia production; ND, indicates not detected

[a]The values represent mean ± SD, $n = 3$; mean with different superscript letters are different by Tukey's post hoc test ($p < 0.05$)

was seen in 6OSFL4c, followed by 6OSFR2e and 6OSFR2d (Table 1). Previous studies have reported ammonia-producing endophytic fungi *Aspergillus flavus* 582PDA5, *Agaricus bisporus* (PVS2), and *Aspergillus awamori* wl1 from different plants (Mehmood et al. 2019; Ripa et al. 2019; Chand et al. 2020). Plants must acquire nitrogen in the form of ammonia from the organic matter in the soil. Nitrogen, a vital component of the chlorophyll molecule, helps plants by providing adequate energy. The capacity of endophytic fungi to liberate ammonia would increase the nitrogen content in plant tissues and, subsequently, the biosynthesis of chlorophyll. A direct impact of the same is evident in the inoculated plants' enhanced root and shoot biomass (Sun et al. 2019; Paul et al. 2020). Thus, an association of plants with such microorganisms, especially under stress conditions, would help the plants thrive and enhance crop production.

The endophytic fungi must produce extracellular lytic enzymes to enter and establish a functional relationship with the host plant. The detection of two lytic enzymes, namely, cellulase and laccase, was evaluated in the current investigation. The detection method involves halo formation around the growing colony. In this study, highest cellulase production of 1.24 ± 0.00 and 1.23 ± 0.01 was seen by isolates 6OSFR2e and 5OSFS1a, respectively. Similarly, the highest laccase production with EI of 1.16 ± 0.00, 1.13 ± 0.00, and 1.09 ± 0.04 was exhibited by isolates 6OSFL4c, 6OSFR2d, and 7OSFS3a, respectively (Table 1). In a recent study, *Nigrospora sphaerica* and *Nigrospora oryzae*, isolated from rice plant leaves, were reported for cellulase and laccase production (Sornakili et al. 2020). Likewise, species of *Cylindrocladium*, *Absidia*, *Acremonium*, *Penicillium*, *Cladosporium*, *Phoma*, *Gliocladium*, *Arthroderma*, *Paecilomyces*, *Rhizophus*, *Rhizoctonia,* and *Aspergillus* isolated from different varieties of rice plant have been reported for the production of cellulase and laccase (Atugala and Deshappriya 2015). Extracellular enzymes, such as cellulase and laccase, are responsible for the lysis of starch, cellulose, and lignocellulosic materials, which can be assimilated by both endophytic fungi and the host (Sunitha et al. 2013). These enzymes also protect plants against biotic stress by suppressing pathogenic activities (Khan et al. 2016). Overall, the findings indicate the great potential of isolated endophytic fungi in plant growth promotion and their potential use as an environment-friendly bioinoculant to enhance crop yield leading to sustainable agriculture.

Morphological and phylogenetic identification of selected fungal endophytes

Based on in vitro analysis of PGP traits, isolates 6OSFR2e, 7OSFS3a, and 6OSFL4c were predominantly the top performers and were hence identified using morphological and molecular identification techniques. The culture characteristics of isolates 6OSFR2e, 7OSFS3a, and 6OSFL4c had woolly colonies on PDA with round and regular margins. The floccose-like colonies of 6OSFL4c and 7OSFS3a turned grey/black on maturation. During microscopic observations of 6OSFR2e, septate hyphae were observed in branches. In addition, aggregated conidiophores were observed to have subcylindrical shapes and terminal conidiogenous cells. The conidiogenous cells were large, ampulliform, determinate, and smooth, whereas solitary conidia having ellipsoid, smooth, and dark brown colour were seen (Fig. 6a–c). Further, the microscopic observations of 7OSFS3a revealed smooth, branched, septate, and brown hyphae. The conidiophores were aggregated, extensively branched, and smooth. Aggregated conidiogenous cells were observed having ampulliform-to-subspherical shapes. In contrast, the conidia were globose-to-subglobose, smooth, shiny, and black (Fig. 6d–f). In the case of 6OSFL4c, branched and

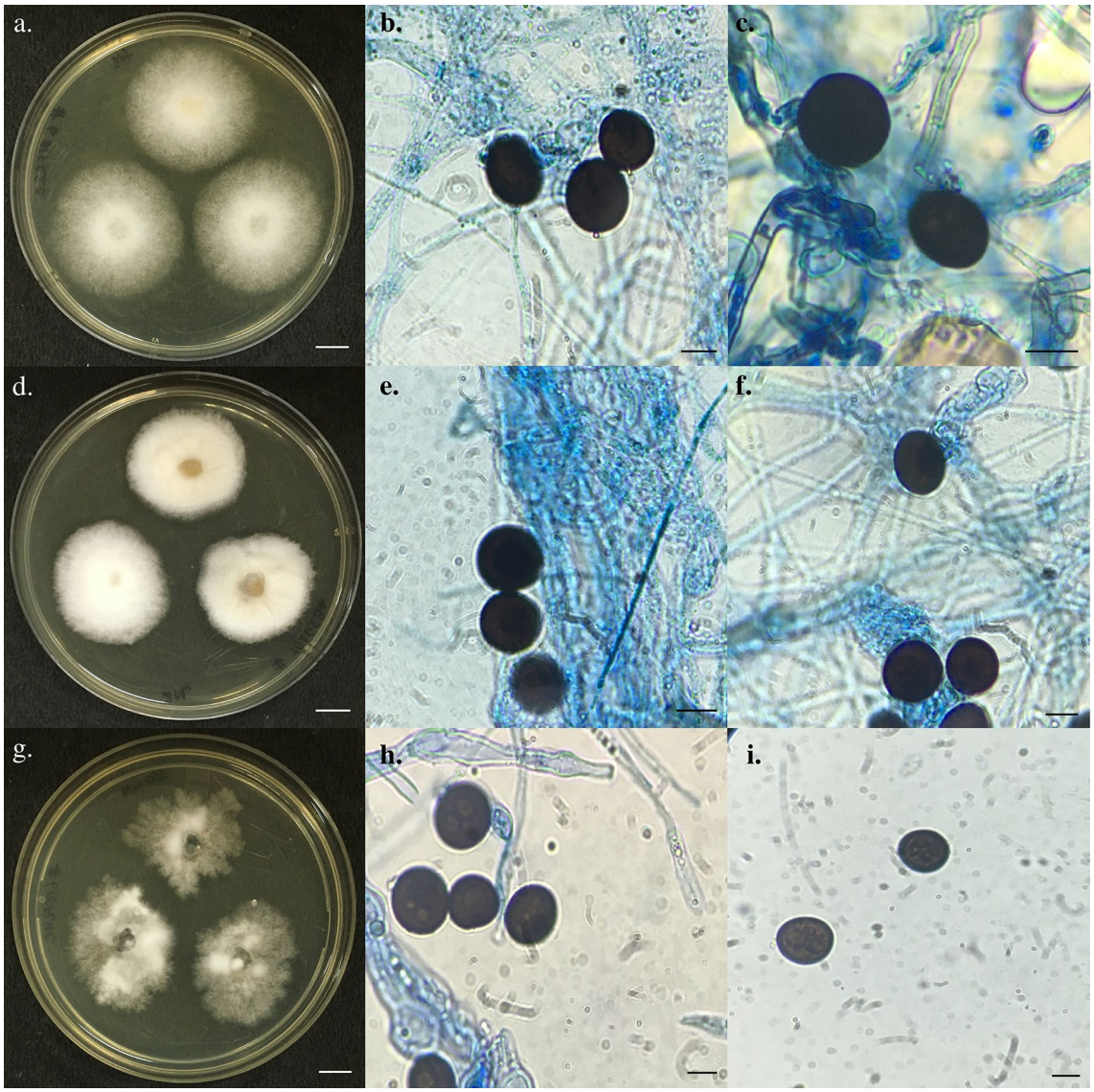


Fig. 6 **a** Colony morphology of 6OSFR2e on PDA. **b**, **c** Conidiogenous cells of 6OSFR2e. **d** Colony morphology of 7OSFS3a on PDA. **e**, **f** Conidiogenous cells of 7OSFS3a. **g** Colony morphology of 6OSFLS4c on PDA. **h**, **i** Conidiogenous cells of 6OSFL4c (**a**, **d**, **g** Bar: 10 mm; **b**, **c**, **e**, **f**, **h**, **i** Bar: 10 µm)

septate hyphae were seen. The conidiophores were smooth, semi-macronematous, highly branched, and surrounded with conidiogenous cells. The conidiogenous cells were determinate, subspherical and pale brown. Abundant globose-to-subglobose, solitary conidia were observed (Fig. 6 g–i). Based on these features, the isolates were tentatively identified as *Nigrospora* species (Wang et al. 2017; Hao et al. 2020).

The amount and pace at which sequence variation occurs in the ITS region make it one of the most extensively sequenced DNA regions for molecular systematics, even at the species level (Pryce et al. 2003). On amplification, PCR amplicons of ~ 500 bp were obtained. Post-sequencing, the BLAST analysis of 6OSFR2e, 7OSFS3a and 6OSFL4c exhibited close homology with *Nigrospora zimmermanii*, *Nigrospora oryzae*, and *Nigrospora sphaerica*. An identity matrix was generated using guide trees and a hidden Markov model. Here, the three isolates 6OSFR2e, 6OSFL4c, and 7OSFS3a exhibited > 95% identity. Isolate 6OSFR2e exhibited 97.37% similarity with *Nigrospora zimmermanii* strain LC13534 (MN215824.1), closely followed by 97.25% with *Nigrospora zimmermanii* strain XS2-1 (OK047752.1) (Table 2). Similarly, isolate 6OSFL4c exhibited 98.92% similarity with *Nigrospora sphaerica* isolate BRN-02 (OQ377130.1) (Table 2). Whereas isolate 7OSFS3a exhibited 97.80% similarity with *Nigrospora oryzae*, isolate

Table 2 Sequence identity matrix showing sequence identity for isolate 6OSFR2e (*N. zimmermanii*), 6OSFL4c (*N. sphaerica*), and 7OSFS3a (*N. oryzae*)

Isolate/accession number								
6OSFR2e	100							
OK047752.1	97.25	100						
MN215818.1	97.2	99.63	100					
OM283577.1	97.18	99.62	100	100				
MN215820.1	97	98.99	99.25	99.62	100			
MN215824.1	97.37	99.94	99.44	99.62	99.62	100		
MN215822.1	96.62	98.69	99.06	99.05	99.87	99.06	100	
MN215819.1	96.44	98.5	98.87	99.05	99.06	99.06	100	100
6OSFL4c	100							
KM999230.1	98.72	100						
MN795540.1	98.74	99.82	100					
MN215799.1	98.9	99.82	100	100				
MN215808.1	98.9	100	100	100	100			
MN215807.1	98.88	99.81	100	100	100	100		
OQ377130.1	98.92	99.82	99.82	100	100	100	100	
KT192261.1	98.9	99.82	100	100	100	100	100	100
7OSFS3a	100							
MT732051.1	97.79	100						
MT732026.1	97.61	100	100					
MT732017.1	97.65	100	100	100				
MZ882152.1	97.64	99.82	100	100	100			
MT732015.1	97.8	100	100	100	100	100		
MT732028.1	97.79	100	100	100	100	100	100	
KX985944.1	97.79	100	100	100	100	100	100	100

PGM1-3 (MT732015.1) (Table 2). Here, in the progressive multiple sequence alignment heuristic, the seeded guide trees decide the order of sequence alignment. On the other hand, the probabilistic HMM technique uses position-specific information to capture evolutionary changes in a set of related sequences.

The three isolates belonged to the same phylum level clade Ascomycota. A maximum-likelihood tree using the Tamura–Nei model was constructed with 1000 bootstraps for confirmation. The heuristic search's initial tree(s) were obtained by applying the Maximum Parsimony method. A discrete Gamma distribution was used to model evolutionary rate differences among sites. The rate variation model allowed for some sites to be evolutionarily invariable. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Here, isolate 6OSFR2e clustered with *Nigrospora zimmermanii* with 97.38% sequence identity, 6OSFL4c clustered with *Nigrospora sphaerica* with 98.92% sequence identity and isolate 7OSFS3a clustered with *Nigrospora oryzae* with 97.80% sequence identity (Fig. 7). The ITS sequences of 6OSFR2e, 7OSFS3a, and 6OSFL4c have been submitted in GenBank with accession numbers ON392014, ON392015, and ON392016, respectively. Previously, Tarroum et al. (2021) reported *Nigrospora chinensis* (KX985947) from *Aeluropus littoralis*, a perineal herb of the Poaceae family. The isolate exhibited tolerance to 1 M NaCl stress. However, this investigation is the first report on salinity, drought-tolerant *Nigrospora* species from the PUSA-44 variety of rice, along with PGP attributes.

Conclusion

The global decline in crop productivity has become evident over time. These losses, primarily caused by anthropogenic activities, have brought many high-yielding crop varieties, such as PUSA-44, to the verge of discontinuation. This study divulged that rice provides an ecological niche for diverse endophytic fungi. Further, the quest of screening salinity and drought-tolerant endophytic fungi from this variety resulted in the isolation of *Nigrospora* species. To our knowledge, this is the first report on abiotic stress tolerance (salinity and drought) and PGP traits of *N. zimmermanii*, *N oryzae*, and *N. sphaerica*. The selected isolates surpassed the others in the in vitro screening tests with promising results at high salinity and drought concentrations. Moreover, PGP features, such as antioxidant activity, phytohormone production, such as IAA, GA, phosphate solubilisation, and extracellular enzyme production, including cellulase and laccase, further validate their promising nature. Using endophytic fungi is a nascent

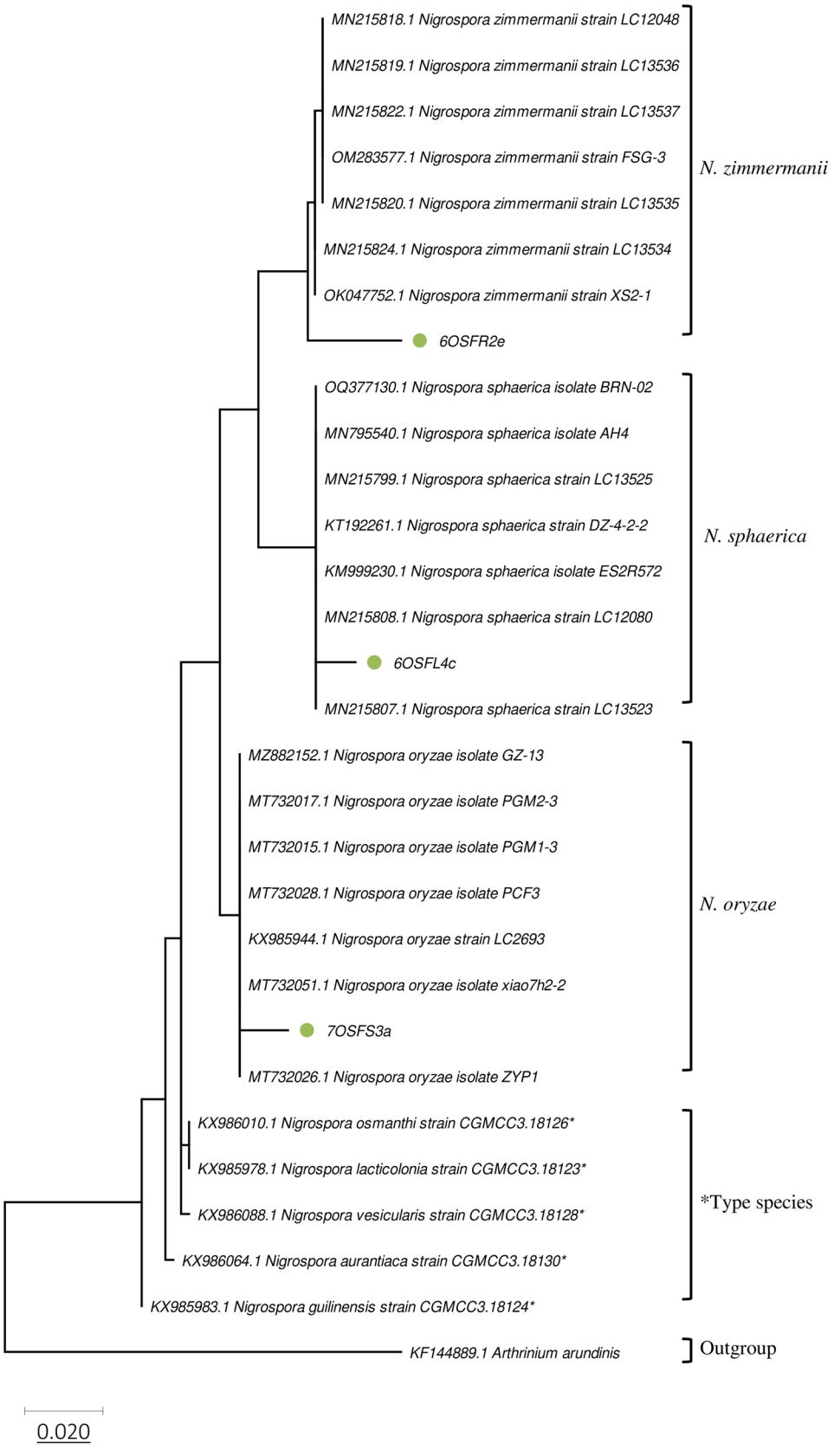


Fig. 7 Maximum-likelihood tree showing 6OSFR2e, 6OSFL4c, and 7OSFS3a based on the ITS1-5.8S-ITS2 region using Tamura and Nei model, indicating the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates)

technology that aligns well with the current global need for green solutions for sustainable agriculture. Considering the current findings, further studies involving the implementation of salient properties of these endophytic fungi for real-scale applications to elucidate their full potential in stress environments are under investigation.

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Author contributions Conceptualisation: SS and GKS; methodology, formal analysis and investigation, writing—original draft preparation: GKS; writing—review and editing, and supervision: SS. All authors read and approved the final manuscript.

Data availability All data supporting the findings of this study are available within the published article and its Supplementary Information.

Declarations

Conflict of interest The authors declare no conflict of interest in the publication.

Ethical approval This study does not involve experiments on animal or human subjects.

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Research Article

Plant growth-promoting endophyte *Nigrospora oryzae* mitigates abiotic stress in rice (*Oryza sativa* L.)

Gurleen Kaur Sodhi and Sanjai Saxena*

Department of Biotechnology, Thapar Institute of Engineering and Technology, Patiala-147004, Punjab, India
*Corresponding author. Department of Biotechnology, Thapar Institute of Engineering & Technology, Bhadson Road, Patiala 147004 Punjab, India.
Email: ssaxena@thapar.edu; sanjaibiotech@gmail.com
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Abstract

Climate change has severely impacted crop productivity. Nascent technologies, such as employing endophytic fungi to induce crop adaptogenic changes, are being explored. In this study, 62 isolates of fungi existing as endophytes were recovered from different parts of a drought-resistant rice variety and screened for salinity and drought tolerance. *Nigrospora oryzae* #2OSTUR9a exhibited *in vitro* antioxidant potential, indole acetic acid (351.01 ± 7.11 μg/mL), phosphate solubilisation (PI 1.115 ± 0.02), siderophore (72.57% ± 0.19%) and 1-aminocyclopropane-1-carboxylate deaminase production (305.36 ± 0.80 nmol α-ketobutyrate/mg/h). To the best of our knowledge, this is the first report on salinity and drought stress mitigation in rice plants by endophytic *N. oryzae*. In treated plants under salinity stress, the relative water, chlorophyll, phenolic and osmolyte content increased by 48.39%, 30.94%, 25.32% and 43.67%, respectively, compared with their respective controls. A similar trend was observed under drought stress, where the above parameters increased by 50.31%, 39.47%, 32.95% and 50.42%, respectively. Additionally, the antioxidant status of the treated plants was much higher because of the enhanced antioxidant enzymes and reduced lipid peroxidation. Our findings indicate the ability of *N. oryzae* to effectively mitigate the impact of stress, thereby enabling the rice plant to sustain stress conditions.

Keywords: climate change, drought, plant growth promotion, pot trials, salinity

Introduction

Staple crops, such as rice, are the third highest produced agricultural commodity. According to the Agriculture Department of the USA, ∼509.9 million tons of rice were produced in 2021/2022 (Rathna et al. 2019, Rice outlook 2022). Depending upon the variety, rice harbours different nutritional content; altogether, it constitutes 20% of the calories of the human diet (Custodio et al. 2019, Sangeetha et al. 2020). As a kharif crop, rice requires ample water for production, and the ongoing catastrophe, i.e. climate change, has made its cultivation difficult. There are many ways to view how climate change is affecting agriculture in particular. The increasing temperatures speed up crop development and hamper their ability to attain moisture, thereby decreasing grain production (Arora 2019, Guntukula 2020). There is an increase in soil salinisation accompanied by depleting water table levels across the globe. Because plants are static, they are prone to these diverse climate conditions. These adverse conditions cause physiological, biochemical and molecular damage in the plants, which has resulted in a decline in the global crop yield by nearly 50%. They also negatively affect the attributes of the soil, thereby decreasing the amount of arable land worldwide (Pulido et al. 2018, Corwin 2021, Imran et al. 2021, Malhi et al. 2021). The intention to keep food production on a par with the Fast paced human growth population is one of the most significant societal challenges.

Although plants have diverse coping mechanisms for stress, studies have reported promising results by exploring the symbiotic relationship with fungal endophytes (Yan et al. 2019, Lu et al. 2021). Endophytes are ubiquitous, and all crops harbour a unique diversity of endophytic fungi. First described by Johann Heinrich Friedrich Link, the endophytic fungi obtain nutrition from their host plants and provide support to their defence systems. Some of the stress-tolerant endophytic fungi have also been used as an inoculum to resist abiotic stress, according to a number of studies (Rodriguez et al. 2009, Saddique et al. 2018, Baron and Rigobelo 2022). Endophytic fungi have evolved alongside plants, enabling them to imitate a variety of phytohormones, phenols and flavonoids that benefit the plant under stress. Species of *Aspergillus* and *Fusarium* that produce phytohormones have been found to colonise rice plants and successfully reduce the effect of salinity and drought conditions (Bilal et al. 2018, Qin et al. 2019, Poveda et al. 2021, Sodhi and Saxena 2023a).

The endophytic diversity is related to the edaphic factors where a particular species of plant is grown. This allows the symbiotic microorganisms to exhibit characteristics native to the plants. This habitat-adapted symbiosis predicts that the endophytes will show encouraging outcomes in terms of stress tolerance (Rodriguez et al. 2008). For instance, it has been noted that a *Fusarium* isolate from a salt-resistant rice variety can impart tolerance or induce adaptation to a salt-sensitive variety. According to reports (Pang et al. 2020, Sampangi et al. 2020), species of *Penicillium* and *Chaetomium* isolated from upland rice varieties can transfer salinity and drought stress resistance to salt-sensitive rice cultivars. Considering the findings, we selected a drought-tolerant rice variety Sabhagi-Dhan, designed explicitly by the Indian Council of Agricultural Research-National Rice Research Institute (ICAR-NRRI) to withstand drought stress in the lowlands of Orrisa and Jharkhand (Dar et al. 2020). After procurement and plantation of the seeds, isolation of fungal endophytes associated with distinct

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parts of rice and further evaluation of those isolates for salinity and drought tolerance were carried out. This investigation is the first report on exploration of endophytic fungi associated with *Oryza sativa* var Sabhagi Dhan. The isolates exhibiting salinity and drought stress tolerance were evaluated for their plant growth-promoting properties. A statistically selected isolate was tested as an inoculum for its potential to induce salinity and drought stress tolerance to PUSA-44, an irrigation-fed variety of rice prominently grown in northern India.

Materials and Methods

Seed procurement, sampling and isolation of endophytic fungi

The seeds of *Oryza sativa* var Sahbhagi-Dhan were procured from ICAR-NRRI, Cuttack, India. For plantation, the seeds were covered with a wet muslin cloth for 24 h before sowing in a selected area in Patiala, Punjab (30.3574°N, 76.3666°E). Samples of leaves, roots, internodes and spikes were collected every 15 days from germination until maturity. The isolation was carried out using the previously described method by Schulz et al. (1993), with slight modifications. After initial washing and air drying of plant samples, surface sterilisation was performed using 0.1% sodium hypochlorite for 1–2 min. Subsequently, 1-min treatments using 70% and 30% ethanol were performed. The surface-sterilised samples were plated onto one-quarter strength potato dextrose agar (PDA) and water agar plates. A 12-h light/dark cycle at 26 ± 2°C was maintained for incubation of the inoculated Petri plates. For the purification of isolates, growing colonies were picked from the edge and inoculated on full-strength PDA. Furthermore, PDA slants and vials containing 10% glycerol were prepared to preserve pure cultures. The isolation frequency (IF) for every isolation and different plant part was calculated as follows (Ikram et al. 2022):

$$\text{IF\%} = \frac{\text{No. of individual fungi recorded}}{\text{Total no. of segments}} \times 100$$

Evaluation of salinity and drought tolerance of endophytic fungi

The isolates were initially screened for salinity and drought tolerance using a plate assay. For salinity stress, NaCl concentrations ranging from 0.5 to 2 M (w/v) were utilised, whereas for drought stress, PDA plates with 5%–20% PEG-6000 amendments were prepared. The plates were subsequently inoculated with a 5 mm plug of a 5–7-day-old active culture. The plates were kept for 10 days at 28 ± 2°C, and the mean diameter was measured every day. This value was compared with a control having no NaCl or PEG-6000, respectively (Ripa et al. 2019, Sampangi et al. 2020).

In vitro antioxidant potential

The ability of the culture filtrate/solvent fractions to scavenge free radicals (%FRS) was assessed using 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2, 2-azino-bis-3-ethylbenzothiazoline 6-sulphonic acid (ABTS+) according to Dhayanithy et al. (2019) and Re et al. (1999), respectively. Briefly, to 80 µl of the 1 mg/mL test sample (concentration range 200–1000 µg/mL dilution), 920 µl of DPPH was added. The reaction conditions involved incubation for 30 min in the dark, following which the result was measured at 517 nm on a titer plate reader (Biotek, USA). Likewise, to 1 ml of this working solution of ABTS+, a 1 mg/mL test sample was added and kept undisturbed for 6 min and was accessed at 734 nm. Different concentrations of Quercetin and Trolox were used as standard, respectively. The %FRS was calculated as follows:

$$\%\text{FRS} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

Production and quantification of phytohormones using high-performance liquid chromatography

Czapek dox broth was used to grow the selected endophytes at 28 ± 2°C for 10 days for quantification of two major phytohormones, namely, indole acetic acid and gibberellic acid (GA3). A C18 column (250 × 4.6 mm) was used for the quantification with a diode-array detector absorbing at 280 nm (Shimazdu Corp., Japan).

Indole acetic acid

In this assay, constant composition of mobile phase comprising acetonitrile, deionised water and acetic acid (60 : 40 : 1) was run at 40°C column oven temperature and flow of 1 ml/min (Wary et al. 2022). An injection volume of 10 µL was inserted. Different concentrations (50–350 µg/mL) of standard indole acetic acid (IAA) (Hi-media PCT0803) were prepared in high-performance liquid chromatography (HPLC)-grade methanol to determine the concentration of IAA. The analytes and standard retention times were compared and quantified using the peak area.

Gibberellic acid

Similarly, a mobile phase comprising methanol and deionised water (4 : 1) was run at 35°C column oven temperature and flow of 1 ml/min (Ben Rhouma et al. 2020). An injection volume of 10 µL was inserted. To determine the concentration of GA3, different concentrations (5–30 µg/mL) of standard GA3 (Hi-media, PCT0830) were prepared in HPLC-grade methanol. The analytes and standard retention times were compared and quantified using the peak area.

Phosphate solubilisation, siderophore and 1-aminocyclopropane-1-carboxylate deaminase production

The selected isolates were grown on Pikovskaya's agar medium enriched with bromophenol blue. The detection method involves the clear zone formation on the medium, indicating the endophytic fungi's solubilisation potential. The phosphate solubilisation index (PI) was calculated as follows (Premono et al. 1996, Jasim et al. 2013):

$$\text{PI} = \frac{\text{Total diameter (Colony diameter + Halo zone)}}{\text{Colony diameter}}$$

However, an iron-deficient succinate medium (composition [g/L]: 0.2 g magnesium sulphate heptahydrate, 1 g ammonium sulphate, 3 g monopotassium phosphate, 4 g succinic acid and 6 g dipotassium phosphate) was used for accessing siderophore production. A chrome azurol reagent (containing CAS dye, $FeCl_3$ and cetrimonium bromide) was added to the culture filtrate (Schwyn and Neilands 1987). The reaction conditions involved incubation at an ambient temperature (25°C) for 20 min with the result being recorded at 630 nm. The sterile iron-deficient medium functioned as control. The percentage siderophore unit (psu) was calculated as follows (Arora and Verma 2017);

$$\text{Siderophore unit (psu)} = \frac{\text{Absorbance (Control)} - \text{Absorbance (Sample)}}{\text{Absorbance (Control)}} \times 100$$

The selected fungal isolates were cultivated in DF media as described by Dworkin and Foster (1958), then enriched using different concentrations of 1-aminocyclopropane-1-carboxylate (ACC) (concentration range 1–5 mM) for 10 days at 28 ± 2°C and 120 r/m to evaluate ACC deaminase production. The growing mycelial mass was procured by centrifugation and reconstituted in 0.1 M Tris hydrochloride buffers of pH 7.6 and 8.5. Further, 0.56 M HCl and 2,4-dinitrophenylhydrazine (DNPH) was introduced into toluene-containing cells. After 30 min at 30°C, addition of 2 M NaOH was carried out. For quantification, different concentrations of α-ketobutyrate (concentration range 0.1–0.5 μM) were prepared, with results being recorded at 540 nm (Zhang et al. 2019).

Morphological and molecular identification of the selected endophytic isolate

The isolate #2OSTUR9a was initially assessed by morphological followed by molecular tools. The isolate was grown on media such as PDA, corn meal agar and water agar for 10–12 days at 28 ± 2°C. Physical attributes, such as appearance, colour and diameter, were recorded to observe microscopic features such as hyphae, conidia and cellular bodies. A Nikon microscope (E200, Tokyo, Japan) was used. For micrometric observations, at least 50 observations per structure were registered using Image J software (Wang et al. 2017).

The procedure of Van Burik et al. (1998) was followed with a few minor adjustments in order to isolate the genomic DNA using cetyl trimethylammonium bromide. Briefly, 1 gram of fungal mycelia from 4–5-day culture was crushed in a mortar and pestle using liquid nitrogen. After crushing of the sample, 1 ml of extraction buffer (0.5 M EDTA, 1 M Tris-hydrochloride, 5 M NaCl and 2% cetrimide) and 1.5 μL RNase were used for the extraction process, at 37°C for 30 min. For lysate extraction, a combination of 50% phenol, 48% chloroform and 2% isoamyl was used. Then genomic DNA precipitation from the aqueous layer using chilled isopropanol was performed. Post centrifugation, the resulting pellet was ethanol-washed before resuspending in Tris-EDTA buffer.

Furthermore, a primer set of ITS 1 and ITS 4 was employed to amplify the internal transcribed spacer (ITS) region. A 25 μL mixture was prepared containing the following components: extracted DNA (25 ng), ITS1 and ITS4 primer (0.8 μM), magnesium chloride (1.5 mM), deoxynucleoside triphosphate (2.5 mM), Taq DNA Polymerase (1.5 U prepared in 10 X Taq buffer) (Bangalore, GeNei). Reaction involved 5-min denaturation at 96°C. Then 39 rounds of annealing at 95, 58 and 72°C for 1, 1.5 and 1 min, respectively, were repeated. The last 5 min at 72°C were utilised for the final extension. The amplicons were checked using 1.5% agarose at 40 V and analysed using Bio-Rad system.

The amplicons were sequenced by Biokart, India. For analysis of the final sequence, Sequencher version 5.4.6 was used. For sequence similarity, the search was performed using BLAST algorithm software, and alignment was constructed using MEGA 11. The alignment file consisted of the under-study sequence, 20 sequences from BLAST analysis and 14 ex-type sequences; *Arthrinium arundinis* KF114889 was used as the outgroup. A Tamura-Nei model-based maximum likelihood tree with 1000 bootstraps was constructed. The sequence was submitted to GenBank. The fungal isolate was also registered in National Fungal Culture Collection of India (NFCCI), Pune.

Inoculum preparation of #2OSTUR9a

Oryza sativa var. PUSA 44 was used as a model for evaluating the selected endophytic fungi. For inoculum preparation, spore suspension of endophytic fungi #2OSTUR9a was prepared. The number of spores was determined under a Nikon microscope (Nikon E200, Tokyo, Japan) using a haemocytometer (1×10^6 no. of spores). The seeds were surface sterilised using 1% sodium hypochloride and treated with the inoculum, whereas the control set was treated with deionised water.

Pot experiments under salinity and drought conditions

The plant growth promotion features of #2OSTUR9a under salinity and drought stress were assessed via pot trials under an ambient environment. The endophyte-treated and untreated seedlings were planted in pots (14 × 11 × 11.5 cm; top × base × height) containing sterile and moistened soil in a randomised block design (n = 3) for each of the following sets: E-S-, E+S-, E-SS+, E+SS+, E-DS+ and E+DS+ (where E = endophyte, SS = salinity stress, DS = drought stress). Fourteen days after planting, the uninoculated seedlings were watered using half-strength Hoagland solution, whereas half-strength Hoagland solution supplemented with 150 mM NaCl and 10% PEG-6000 was used for inducing salinity and drought stress, respectively (Hoagland and Arnon 1950). This regimen of stress induction was followed for the next 7 days. The pots were grown under a photoperiod of 12 h with an average temperature of 30/25°C. Finally, the plants' physical, biochemical and enzymatic attributes were assessed. For confirmation of colonisation by endophytic fungi, the root-staining method of Phillips and Hayman (1970) was employed. Freshly harvested roots were treated with 10% KOH and acidified using 1% HCl. The roots were stained using 0.5% trypan blue (w/v) and observed under a Nikon microscope (E200, Tokyo, Japan). The colonisation frequency was calculated as follows:

$$\text{Root colonisation (\%)} \frac{\textit{Number of roots colonised}}{\textit{Total number of roots examined}} \times 100 \quad (1)$$

Effect of endophytic fungi on rice seedlings

Relative water content

The harvested plants were cleaned using distilled water to clean off the dust particles. After recording fresh weight (FW), plants were introduced into distilled water until they attained constant turgid weight (TW), following which they were oven-dried dry weight (DW). The calculation of relative water content (RWC) was performed as follows (Smart and Bingham 1974):

$$\text{RWC} = \frac{\text{FW} - \text{DW}}{\text{FW} - \text{TW}} \times 100$$

Estimation of plant pigments

The harvested plants were washed using distilled water to estimate the chlorophyll and carotenoid content. The samples were then crushed in 80% chilled acetone for a homogenous mixture. Post centrifugation, the result was recorded at the following wavelengths: 480, 650 and 663 nm. For estimation of chlorophyll and carotenoid content, the equations by Arnon (1949) were used.

Total phenolic and flavonoid content

Here, Folin-Ciocalteau assay by González-Teuber et al. (2022) was adopted with slight modifications. Briefly, 1 N FC-reagent was added to the test sample, followed by 6% (w/v) sodium carbonate. The reaction was allowed to proceed for 1 h at an ambient condition with the result being recorded at 760 nm. The aluminium

chloride colorimetric method of Ali et al. (2021) was adopted with slight medications. Briefly, 5% sodium nitrite, 10% aluminium chloride and 1 N sodium hydroxide were mixed with the test sample. The reaction was maintained for 10 min under ambient conditions with the result being recorded at 510 nm. Gallic acid and quercetin were used as standard, respectively.

Total sugar and proline content

The protocol of Dubois et al. (1956) was used to measure the total sugar concentration. A reaction combination made up of a test sample, concentrated sulphuric acid and phenol reagent was maintained for 30 min under ambient conditions with the result being assessed at 485 nm. For analysing the proline content, the harvested leaf sample was crushed with liquid nitrogen and suspended in 3% (w/v) sulphosalicylic acid. Further estimation was performed using acid-ninhydrin assay with the result being observed at 520 nm (Bates et al. 1973). Glucose and L-proline were used as standard, respectively.

Malondialdehyde content

The protocol of Heath and Packer (1968) was adopted using minor modifications to assess the formation of malondialdehyde (MDA) content. The harvested plants were crushed to a fine powder and suspended in trichloroacetic acid followed by thiobarbituric acid. After removing the non-specific absorbance at 600 nm, the result was observed at 532 nm and quantified taking 155 1/mM.cm as the molar extinction coefficient.

Total protein content and activity of antioxidant enzymes

The harvested plants were ground in 100 mM phosphate buffer at pH 7 to estimate the enzyme assays. After centrifugation, the supernatant was utilised to assess the total protein using Bradford assay. A standard curve comprising various concentrations of bovine serum albumin was prepared, with the result being measured at 595 nm (Bradford 1976). Furthermore, the enzyme activity was assessed by using Nitroblue tetrazolium for superoxide dismutase (SOD), hydrogen peroxide for catalase (CAT) and o-dianisidine for peroxidase (POX) as substrates (Beauchamp and Fridovich 1971, McEwen Jr 1971, Aebi 1984). The result was recorded at 560, 240 and 470 nm, respectively. The result was denoted as U/mg protein, where one unit equals the amount of enzyme required to breakdown 1 μM substrate into product in 1 minute.

Statistical analysis

The results are represented as mean ± SD (n = 3). For the one-way ANOVA and Tukey's post hoc analysis (regarding $P < 0.05$ as significant), the software used was Graph Pad Prism. The IC_{50} value (i.e. the concentration at which 50% of the free radicals are scavenged) was determined using regression based on best fit. The extinction coefficients of 39.4 and 11.3 1/mM.cm were used for calculation of CAT and POX activity, respectively.

Results

Isolation of endophytic fungi

The fungal endophytes associated with four plant parts—leaves, roots, internodes and spikes—were isolated at intervals of 15 days for 105 days. As a result, 62 endophytic fungal strains were subsequently discovered. Out of the total number of isolates, 22 were found in roots, 19 in leaves, 12 in internodes and nine in spikes. Around the 60th day after germination, the maximum isolation frequency of 8.75% was recorded in the roots (Fig. S1a). Tentative identification of the isolates using morpho taxonomic tools revealed that 53.23% belonged to the Sordariomycetes class, whereas 12.90% belonged to the Dothideomycetes class, and less than 5% of isolates belonged to each of Eurotiomycetes and Zygomycetes. The 62 endophytic isolates belonged to eight genera: *Fusarium*, *Nigrospora*, *Cladosporium*, *Alternaria*, *Aspergillus*, *Colletotrichum*, *Rhizopus* and *Paecilomyces*. In the present investigation, *Fusarium* and *Nigrospora* were the dominant genera, with 29.03% and 20.97% of isolates, respectively. Furthermore, 9.68% of isolates were identified as *Cladosporium* species, whereas less than 5% were identified as each of *Alternaria*, *Aspergillus*, *Colletotrichum*, *Rhizopus* and *Paecilomyces* (Fig. S1b).

Screening of endophytic fungi for salinity and drought tolerance

On testing the endophytes for their ability to withstand salt, 24.1% of them showed greater growth rates than the control when exposed to 1.5 M NaCl. Under 1.5 M NaCl stress, only three of these isolates showed growth rates of more than 70% (Fig. 1a). Similar results were seen for isolates under 10% PEG-6000 (-0.650.01 MPa) drought stress, where 46.7% showed greater than 60% growth compared with the control. On further subjection to 15% PEG-6000 (-0.890.01 MPa) stress, only three isolates exhibited more than 80% growth in comparison with the control (Fig. 1b). Based on the screening assay, the isolates exhibiting promising growth under salinity and drought stress were statistically chosen for further research.

Plant growth-promoting attributes of fungal isolates

The *in vitro* analysis revealed the highest DPPH scavenging activity in isolate #2OSTUR9a with %FRS of 82.39% ± 0.334% and an IC_{50} value of 365.54 ± 9.96 μg/mL. The highest TEAC was also observed in isolate #2OSTUR9a with %FRS of 85.61% ± 0.33% and an IC_{50} value of 357.51 ± 7.06 μg/mL (Fig. 2a). The HPLC analysis revealed the retention time of the standard IAA and the crude extract from the selected endophytic fungi to be 9.93 min (Fig. S2a). On quantification, the highest auxin production of 109.78 ± 2.55 μg/mL was observed in isolate #2OSTUR9a. IAA production further increased to 351.01 ± 7.11 μg/mL on supplementation of tryptophan in the media (Fig. 2b). In this study, the retention time of standard GA3 and the crude extract from the selected endophytic fungi was 2.2 min (Fig. S2b). The highest GA3 production of 26.80 ± 0.87 μg/mL was observed in isolate #2OSTUR9a. Similarly, isolate #2OSTUR9a exhibited the highest phosphate solubilisation potential, with a PI of 1.115 ± 0.02 and siderophore production with a psu of 72.57% ± 0.19% (Fig. 2c). Furthermore, ACC deaminase production at different concentrations of ACC ranging from 1 to 5 mM was assessed. The highest ACC deaminase activity of 305.36 ± 0.80 nmol of α-ketobutyrate/mg/h was observed in isolate #2OSTUR9a at 5 mM substrate concentration (Fig. 2d). However, no significant increase in activity was observed beyond 3 mM substrate concentration.

Morphological and molecular identification of isolate #2OSTUR9a

Using morpho-taxonomic methods, isolate #2OSTUR9a was provisionally identified. The isolate #2OSTUR9a had fuzzy, white to grey colonies that, as they matured, became grey to black. The floccose colonies had regular and round margins. The microscopic

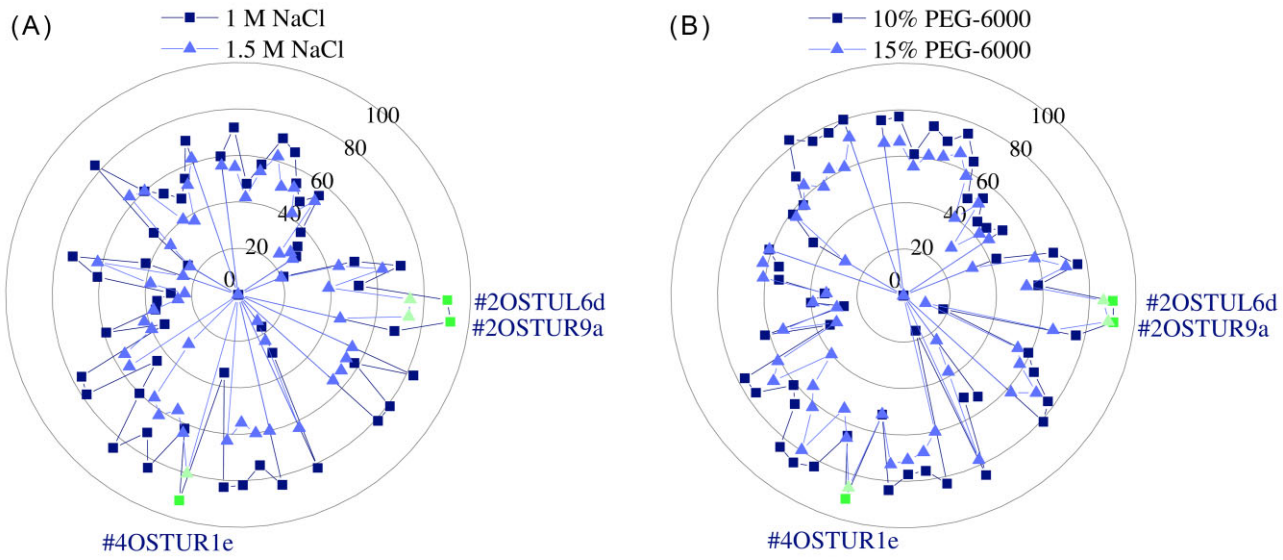

Figure 1. Growth percentage of isolates under (**A**) salinity stress conditions (1–1.5 M NaCl) and (**B**) drought stress conditions (10%–15% PEG-6000). The values represent mean of value ± SD, n = 3; mean values with different superscript letters are different by Tukey's post-hoc test ($P < 0.05$).

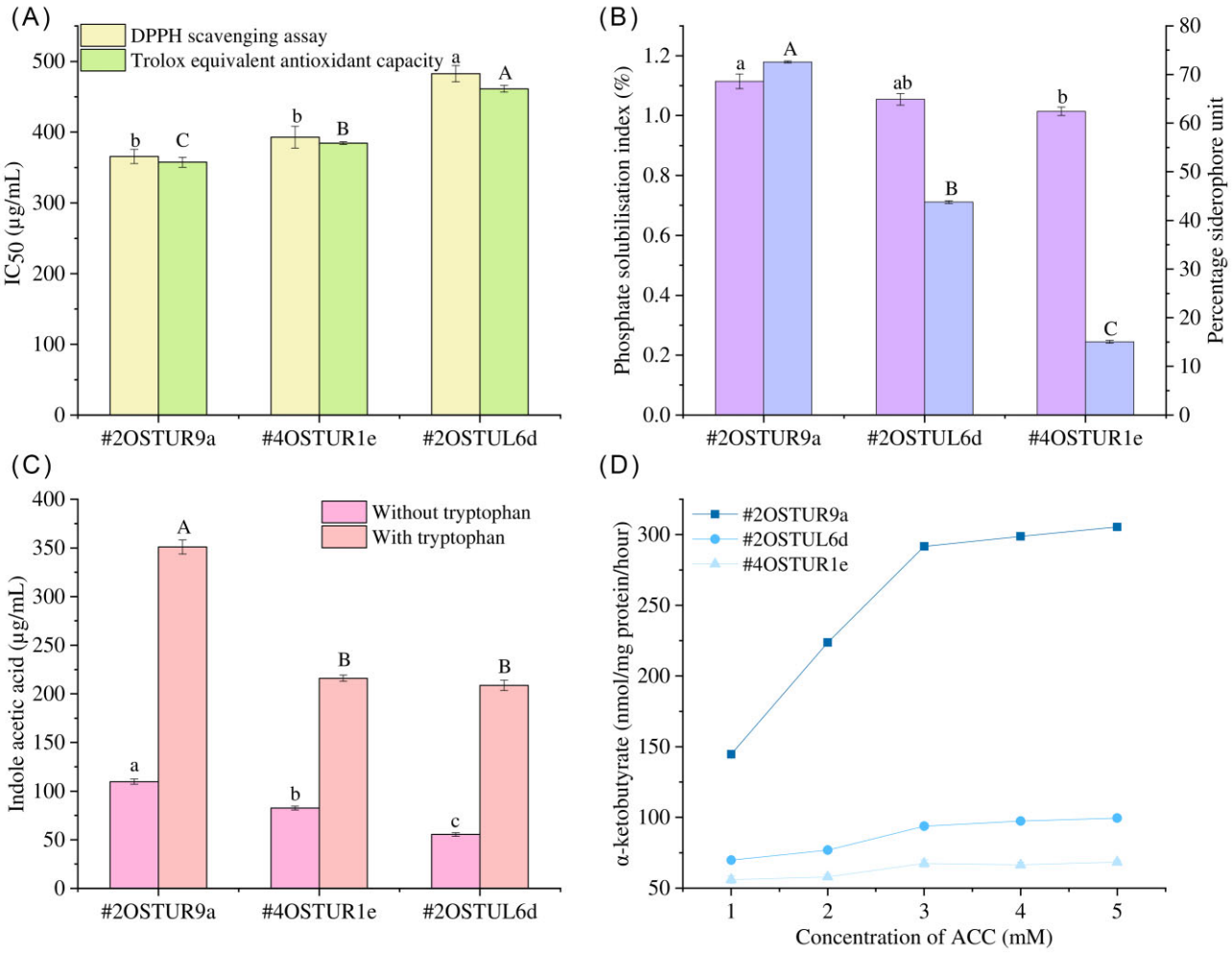

Figure 2. Plant growth-promoting attributes of selected endophytic fungi. (**A**) *In vitro* antioxidant potential using DPPH and ABTS radical; (**B**) indole acetic acid production (with and without supplementation of tryptophan); (**C**) phosphate solubilisation index and siderophore production potential; (**D**) ACC deaminase activity at different concentrations of ACC. The values represent mean of value ± SD, n = 3; mean values with different superscript letters are different by Tukey's post-hoc test ($P < 0.05$).

characteristics revealed septate hyphae brown in colour. The hyaline conidiophore was branched aggregated and had a black conidium with a diameter of less than 10 μm, and an ampulliform shape. Endophyte #2OSTUR9a was identified as *Nigrospora* sp., established using these characteristics. On amplification of the ITS region, PCR amplicons of ∼500 bp were obtained. After sequencing, the BLAST analysis of isolate #2OSTUR9a exhibited close similarity to *Nigrospora oryzae*. For verification, the Tamura-Nei model was used to build a maximum likelihood tree. The maximum parsimony method was applied for obtaining the heuristic search, whereas a discrete gamma distribution was used for evolutionary rates. The tree is drawn to scale with 1000 bootstraps where isolate #2OSTUR9a clustered with *Nigrospora oryzae* (Fig. 3a-c). The isolate is submitted under the GenBank accession number ON392017 and has also been added to the repository of NFCCI, Pune, under the accession number 5447.

Effect of #2OSTUR9a on physiological attributes of rice seedlings

Oryza sativa var. PUSA-44 seedlings were treated with an inoculum of isolate #2OSTUR9a and grown in pots to evaluate the response of chosen endophytic fungi on the agronomical characteristics of rice. The inoculum was put to the test under 150 mM NaCl and 10% PEG-6000 stress. The endophytic fungi successfully colonised the roots of the host plant with a frequency of 81.48% ± 6.41%, as confirmed through microscopic analysis (Fig. S3). Overall, the inoculated plants appeared to be flourishing under both conditions (Fig. 4a-f). Under salinity stress, the shoot length of uninoculated plants decreased, whereas the shoot length of treated plants increased tremendously by 34.10% (Fig. 5a). Additionally, the shoot FW and DW also increased by 32.95% and 36.66%, respectively, in comparison with the respective controls (Fig. 5b and c). Similar patterns were observed in root length, fresh and dry weight. The inoculated plants exhibited an increase of 43.37% in root length, 26.35% in root fresh weight and 38.03% in root dry weight under salinity stress (Fig. 5d-f). Under drought stress (10% PEG-6000), a decrease in the plant's growth parameters of the untreated plants was documented. However, the inoculated plants had an increase in shoot length of 39.93%. The weights of the shoots when they were fresh and dry also increased by 43.80% and 35.65%, respectively (Fig. 5a–c). Inoculated plants' root system, including length, FW and DW, also increased by 43.18%, 35.24% and 34.41% under drought stress (Fig. 5d-f). Furthermore, the inoculated plants showed an increase in RWC of 48.39% under salt and 50.31% under drought stress (Fig. 6a).

Effect of #2OSTUR9a inoculation on biochemical and enzymatic attributes of rice seedlings

The production of chlorophyll and carotenoid increased by 30.94% and 23.97%, respectively, compared with their respective controls under salt stress (Fig. 6b). The total phenolic, flavonoid, sugar and proline content of inoculated plants increased by 25.32%, 22.65%, 50.70% and 43.67%, respectively (Fig. 6c–e), whereas the inoculated plants exhibited a reduction in MDA content of 29.62% (Fig. 6f). In the case of SOD, CAT and POX activity, an increase of 37.78%, 25.46% and 57.46% in inoculated plants was documented, respectively (Fig. 7a–c).

Likewise, under drought stress, the total chlorophyll and carotenoid content of inoculated plants increased by 39.47% and 15.69%, respectively (Fig. 6b). Similarly, the inoculated plants' total phenolic, flavonoid, sugar and proline content increased by 32.95%, 20.85%, 42.00% and 50.42%, respectively (Fig. 6c–e). The inoculated plants exhibited a decrease of 37.05% in MDA content (Fig. 6f). Under drought stress conditions, the SOD, CAT and POX activity increased by 24.27%, 41.13% and 36.72% in inoculated compared with uninoculated plants (Fig. 7a–c), respectively.

Discussion

The investigation of alternative methods to boost rice production has increased significantly in response to climate change. To add to this, the continuously growing population is seriously hampering food security. Previous research on endophytic fungi from different rice varieties has demonstrated their usefulness as plant growth boosters under various abiotic stresses (Reshna et al. 2022, Santra and Banerjee 2023, Sodhi and Saxena 2023b). To understand the diversity of endophytic fungi, seeds of Sabhagi Dhan, a drought-resistant rice variety, were procured. Studies have reported *Fusarium*, *Nigrospora* and *Aspergillus* among the dominant genera of endophytes symbiotically associated with rice and other plants from the same family (Naik et al. 2009, Zakaria et al. 2010, Atugala and Deshappriya 2015, Fernández-Pastor 2021 et al. 2021). Both salinity and drought stress are known to cause significant physiological and biochemical damage in plants. These changes start at the seedling stage and gravely affect growth; they can cause plant death in adverse conditions. Salinity and drought stress disrupt the osmotic balance of the cells, thereby inhibiting growth. Thus, it is imperative to assess the efficiency of isolates under salinity and drought stress. Sampangi et al. (2020) reported isolate V-4 J identified as *Fusarium* sp. isolated from *Oryza sativa* L. var VTL-4, exhibited 90% growth compared with control under 1 M NaCl stress, which decreased to 78.01% under 1.5 M NaCl stress conditions. In another study by Pang et al. (2020), endophytic fungi *Talaromyces purpureogenus* exhibited growth under 10% PEG-induced drought stress. Upon inoculation in the host plant, the isolate also improved physiological indicators such as root length and fresh weight.

The endophytic fungi obtain nutrition from their respective host plant and, in return, confer benefits. During periods of stress, plants often exhibit early signs of free radical damage. It becomes crucial to scavenge these free radicals to minimise the detrimental effects caused by reactive oxygen species (ROS) (Qin et al. 2019, Baron and Rigobelo 2022). Both DPPH and TEAC are robust antioxidant detection methods. The polyphenolic compounds produced by the endophytic fungi act as electron donors, reducing free radicals like DPPH and $ABTS^+$. Similar to our findings, many studies have reported endophytic fungi's antioxidant potential. For instance, in a recent study, Kalimuthu et al. (2022) reported *Curvularia geniculata* exhibiting 81.34% ± 3.35% FRS activity against DPPH radical and 79.45% ± 3.13% FRS activity against $ABTS^+$ radical. Likewise, Dwibedi and Saxena (2020) reported *Arcopilus aureus* exhibiting IC_{50} of 0.11 ± 0.01 mg/mL against DPPH and 0.28 ± 0.0 mg/mL against $ABTS^+$. By mitigating the oxidative stress, endophytic fungi enhance the plant's resilience to the environmental stressors, thereby improving the overall health of the plant. Besides antioxidant potential, phytohormone production is a significant attribute of endophytic fungi, which promotes the growth of plants.

Auxin and other phytohormones are vital for plants to grow and yield produce. They are also known to facilitate the interaction between plants and the associated endophytes. The addition of tryptophan stimulates the formation of IAA, which was supported by the analysis, as tryptophan serves as a precursor for auxin synthesis. In comparison with earlier discoveries, isolate #2OSTUR9a exhibited higher IAA production. Recently, Tian

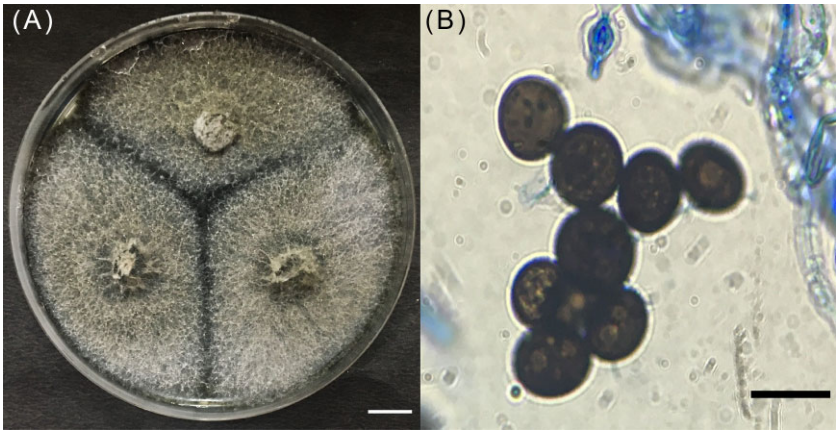

Figure 3. (**A**) Colony morphology of #2OSTUR9a on PDA (bar: 10 mm); (**B**) conidiogenous cells of #2OSTUR9a (bar: 10 μm). (**C**) Maximum likelihood tree of #2OSTUR9a, based on the ITS1-5.8S-ITS2 region indicating the percentage of replicate trees, in which the associated taxa clustered together in the bootstrap test with 1000 replicates (∗ indicate ex-type species).

et al. (2022) observed 13.70 mg/L IAA production by endophytic *Chaetomium globosum*. Likewise, *Alternaria*, *Aspergillus* and *Fusarium* species have also been described to exhibit IAA production. One of the direct benefits of utilising endophytes is the exogenous administration of phytohormones, which helps the plant under stress (Yan et al. 2019). Previously, researchers have also described synthesis of GA3 from symbiotic fungal endophytes. The culture filtrate of endophytic fungus contains GAs (different form of gibberellins) that are physiologically active, according to numerous recent investigations (Javed et al. 2022, Siddiqui et al. 2022). For instance, *Chaetomium globosum* exhibited 0.78 mg/L GA, whereas Ben Rhouma et al. (2020) reported GA-producing *Fusarium oxysporum*. GA-biosynthesis gene clusters from various species of *Fusarium* have also been reported (Bao et al. 2020). Phytohormone production is a critical attribute of endophytes. Various aspects of plant growth such as growth stimulation, enhanced plant vigour, nutrient uptake and assimilation are influenced by the phytohormones. By modulating the signalling pathways associated with hormone levels, endophytic fungi enables growth promotion of the host plants (Mukherjee et al. 2022). The results of this study are in line with plethora of previous investigations; however, the exact mechanism of action of the plant hormones that are excreted by endophytic fungi needs to be investigated further.

Improper nutrition or unavailability of major plant nutrients such as phosphorous in the soil can significantly affect crop production. Even when present in adequate amounts, these nutrients often exist in forms that are not readily available for the utilisation of plants. The unique characteristic of endophytes to solubilise minerals can improve the plant's nutrition uptake from the soil, especially under stress conditions. The mitigation of nutrient deficiencies allows healthier plant growth. Previously, the phosphate solubilisation ability of endophytic species of *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Talaromyces* and *Trichoderma* was documented (Ripa et al. 2019, Turbat et al. 2020). Besides mineral solubilisation, these microorganisms can also produce siderophores. These siderophores exhibit iron-chelation properties by binding to rhizospheric ferric ions. Because iron is essential for the survival of living forms, these small siderophore molecules produced by the endophytic fungi function as regulators of plant growth by inhibiting the accessibility of iron to various phytopathogens. Moreover, endophytic siderophores are also speculated to be involved in induced systemic resistance (Card et al. 2016). Recently, Taheri et al. (2022) reported endophytic *Aspergillus flavus* exhibiting 74.7% ± 0.16% siderophore production. Siderophore production was also observed in *Aspergillus*, *Trichoderma*, *Fusarium* and *Alternaria* species (Ripa et al. 2019, Turbat et al. 2020).

ACC deaminase biosynthesis is another crucial characteristic seen in endophytic fungi that support plant growth. A precursor to the stress hormone ethylene, ACC, is broken down by the enzyme generated by endophytic fungus into α-ketobutyrate and ammonia. The endophytic fungi's synthesis of this enzyme causes a drop in ethylene levels in the plants, which reduces the effects of ethylene-mediated growth inhibition. Ali et al. (2021) reported *Penicillium purpurogenum* exhibiting 355 nmol α-ketobutyrate/mg/h at 5 mM substrate concentration. Likewise, Rauf et al. (2021) reported *Trichoderma asperellum* exhibiting 330 nmol α-ketobutyrate/mg/h at 2 mM substrate concentration. The endophytic fungi's abilities to produce ACC deaminase improves root development and nutrient uptake, thereby promoting plant growth. This collectively shows promising possibilities for controlling the impacts of abiotic stress on the host plant.

Based on the morpho-taxonomic and ITS region, isolate #2OSTUR9a was identified as *Nigrospora oryzae*. The ITS region is a prominently sequenced genetic marker and barcode for fungal identification. Previous studies have reported *Nigrospora* sp. as endophytes associated with various plants (Gautam et al. 2022, Sodhi and Saxena 2023c). Recently, Tarroum et al. (2021) reported *Nigrospora chinensis* as a salinity-tolerant endophyte that also exhibited the ability to promote the growth of plants. The ongoing climate change has exposed plants to diverse hostile environments. The changes have gravely affected agricultural practices across the globe. Significant abiotic stress factors like salinity and drought interfere with the proper operation of rice plants and significantly reduce production. The high salt concentrations reduce the photosynthetic activity and other plant metabolic processes. In addition, the accumulated ions reduce nutrition uptake in the plants. Similarly, the onset of drought stress at an early vegetative state severely affects the rice plant because of its small root system. The change in turgor pressure and water potential affects the plant's normal functioning, causing leaf rolling, premature yellowing and restricted transport of nutrients (Kim et al. 2020, Razzaq et al. 2020, Chaudhry and Sidhu 2022). Consequently, the rice plant's physiological and morphological traits are affected and grain production is reduced.

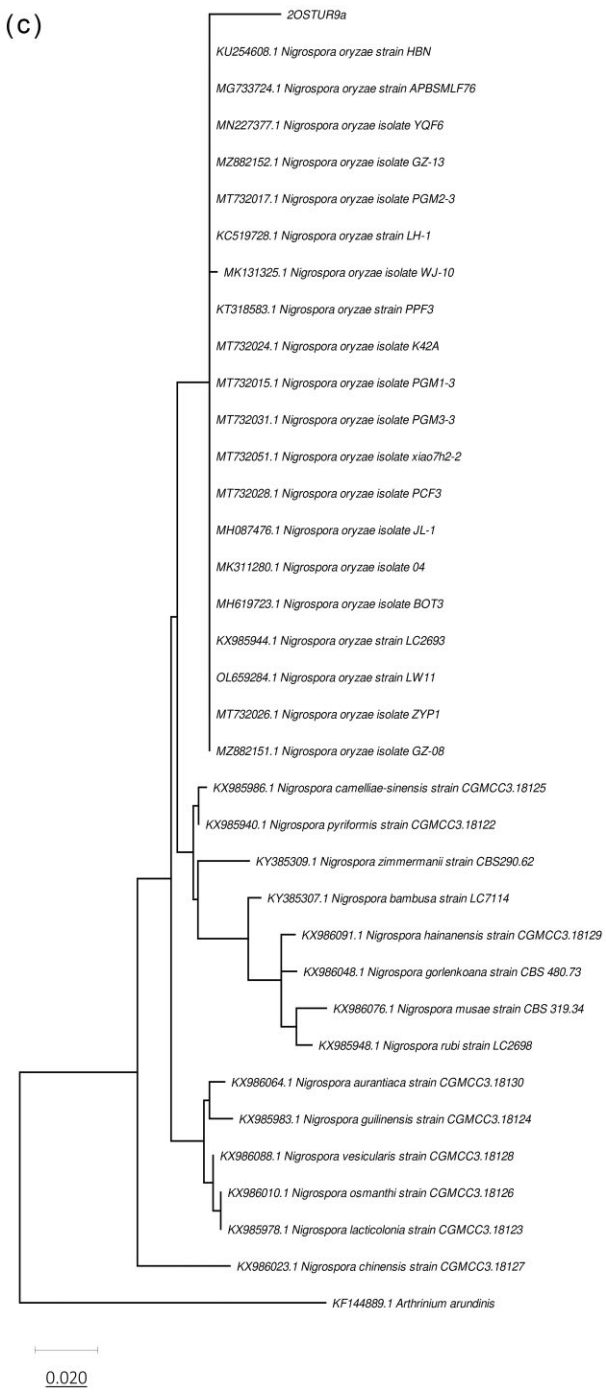

Figure 3. Continued.

The inoculated plants appeared to be flourishing under both conditions. The trend was evident regardless of the condition, envisaging the ability of endophyte to promote the productivity of plants and alleviate the negative effects of stress. Vergara et al. (2018) reported similar findings, where inoculation of fungal endophytes in the Piauí variety of rice enhanced the height and biomass of the host plant. It is well known that salt stress prevents plants from absorbing water and messes with their ionic homeostasis. The physical characteristics of the plants are impacted as a result. Numerous studies have also documented how endophytic fungi benefit plants under salt stress. For instance, Sampangi et al. (2020) reported a *Fusarium* sp. that conferred the ability to tolerate salt to IR-64, a variety highly sensitive to salt. Compared with the uninoculated plants, the inoculated plant's total biomass increased under stress by ∼34.4%. The endophytic fungi's exogenous phytohormone synthesis may be responsible for the improved shoot and root systems seen in treated plants. The ability of the fungal endophytes to solubilise nutrients fulfils a significant task in this situation, enhancing the plant's nutrition absorption ability. The inoculated plants' larger roots would enable higher water absorption from the soil. Because roots physically interact with the soil to absorb nutrients and water, plants must develop an efficient root system to withstand stress. Previously, Santos et al. (2017) reported dark septate endophytes, which improved the height, root and shoot DW of Nipponbare and Piauí varieties of rice under PEG-6000-induced drought conditions. Studies involving other plants under stress have shown similar findings of increased plant metrics using endophytic fungi (Qiang et al. 2019, Bilal et al. 2020, Moghaddam et al. 2022).

Abiotic stress causes the soil to accumulate water-soluble salts, reducing available water for plants. For plants to flourish, there must be a sufficient relative water content, also known as relative turgidity. Therefore, to study the equilibrium between the water supply to the plant and its transpiration rate, an assessment of the RWC of the plants is vital. The increased RWC observed in our investigation indicates efficient translocation of water from the soil and reduced cell damage in the inoculated plants. Although the exact mechanism of action has yet to be unveiled, there are speculations involving upregulation of the genes associated with the maintenance of cell wall structure and aerenchyma formation, leading to improved water conservation under stress conditions. The aerenchyma also relieves the respiration cost by ensuringadequate supply of oxygen and a pathway for efficient gas exchange, especially when surrounding soil or water has low oxygen. In addition, the aerenchyma also regulates carbohydrate distribution within the developing roots by providing an interconnected passage and reservoirs for energy needs (Hu et al. 2022).

Apart from the physiological attributes, abiotic stresses also affect the photosynthetic system of plants, as a result of which a reduction in chlorophyll content is observed. The stress conditions cause a decrease in nutrition uptake, disruption in the gaseous exchange and downregulation of the enzymes involved in chlorophyll biosynthesis (Chaudhry and Sidhu 2022). Thus, assessing the chlorophyll content of endophyte-inoculated plants under stress conditions could demonstrate their potential as plant growth promoters. The analysis revealed that the chlorophyll and carotenoid content increased in plants treated with fungal endophytes under stress and in a normal environment. The endophytic fungi inoculation improves nutrition availability, modulates phytohormone signalling and protects the photosynthetic machinery from oxidative damage. This improves the photosynthetic ability of plants under stress conditions. Our findings are similar to previous reports, where inoculation of endophytic *Fusarium* sp. enhanced the chlorophyll concentration in treated plants compared with their respective controls under salinity stress (Sampangi et al. 2020). Recent studies have also observed enhanced chlorophyll content in rice plants on inoculating endophytic *Phomopsis liquidambaris* and *Serendipita indica* (Khalvandi et al. 2021, Hu et al. 2022). The inoculated plants' enhanced photosynthetic potential was reflected in the improved biomass and root system of the plants, corroborating our findings.

Phytochemicals such as phenols and flavonoids are crucial during abiotic stress. They serve as non-enzymatic antioxidants and reduce irreversible oxidative damage by scavenging the radical

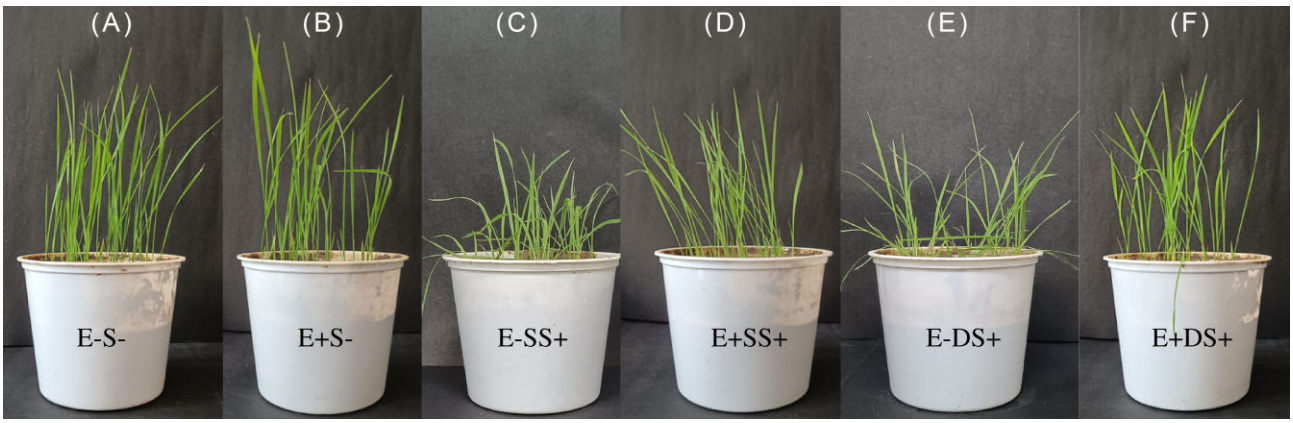

Figure 4. (**A**–**F**) Effect of endophytic *Nigrospora oryzae* #2OSTUR9a on 21-day-old rice seedlings under stress and non-stress conditions (where E = endophyte, SS = salinity stress and DS = drought stress).

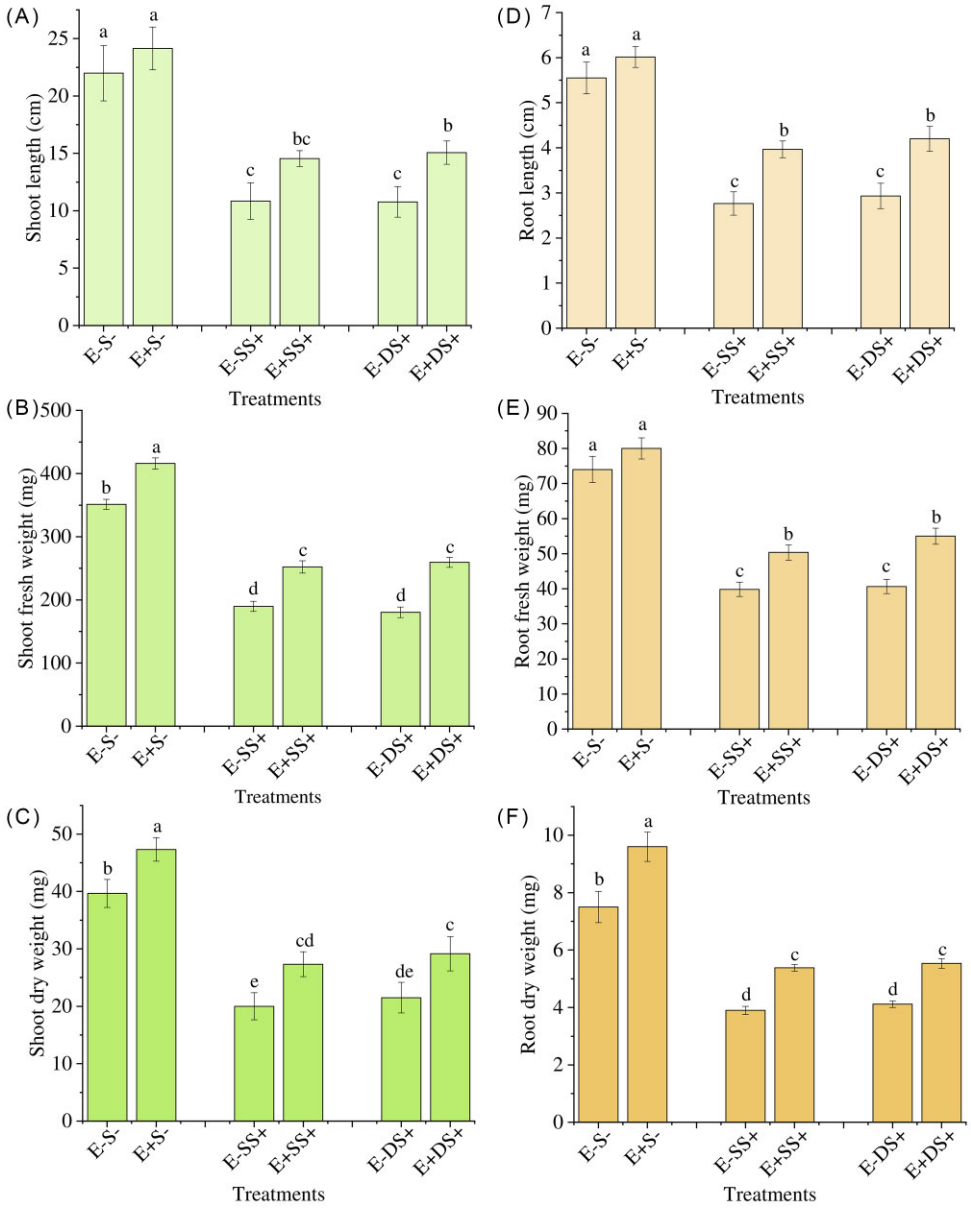

Figure 5. Effect of endophytic *Nigrospora oryzae* #2OSTUR9a on the physical attributes of rice plant including (**A**) shoot length, (**B**) shoot fresh weight, (**C**) shoot dry weight, (**D**) root length, (**E**) root fresh weight and (**F**) root dry weight. The values represent mean of value ± SD, n = 6; mean values with different superscript letters are different by Tukey's post-hoc test ($P < 0.05$).

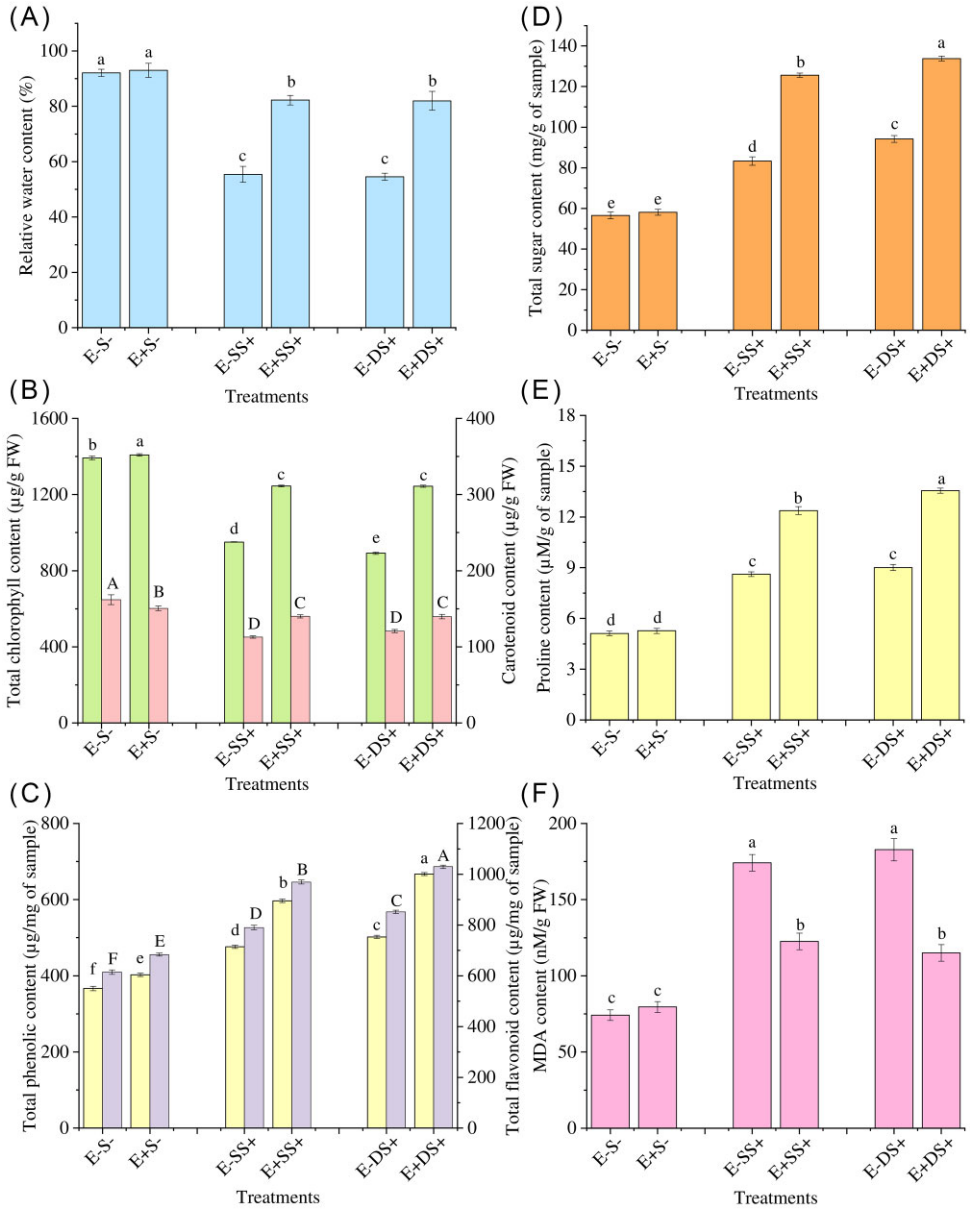

Figure 6. Effect of endophytic *Nigrospora oryzae* #2OSTUR9a on (**A**) relative water content, (**B**) total chlorophyll and carotenoid content, (**C**) total phenolic and flavonoid content, (**D**) total sugar content, (**E**) proline content and (**F**) malonaldehyde (MDA) content. The values represent mean of value ± SD, n = 6; mean values with different superscript letters are different by Tukey's post-hoc test ($P < 0.05$).

ions generated during a challenging environment. Compared with the uninoculated plants, the phenolic and flavonoid content of the endophyte-treated plants showed an increase under stress. Earlier, Ali et al. (2021) and Jan et al. (2022) observed analogous outcomes. Jan et al. (2022) found that endophytic *Candida membranifaciens* FH15 inoculation increased the phenolic and flavonoid content in maize under salinity stress, whereas Javed et al. (2022) found that inoculation of endophytic fungus into *Moringa oleifera* seedlings under drought stress resulted in a 0.66-fold increase in phenolic content.

Abiotic stress damages the thylakoid membranes of the plant, which reduces photosynthetic efficiency. It also causes ionic and osmotic imbalance, which, to combat, the plants accumulate low molecular weight osmolytes such as sugars and proline. In addition, the plants require ample energy to sustain the ongoing metabolic processes. Similar results with increased osmolyte build-up under stressful situations were observed in the current investigation. Prior to this, *Trichoderma* sp. inoculation under salinity stress was seen to upregulate the proline accumulation in rice seedlings by Rawat et al. (2016). Similarly, Pang et al. (2020) found that when rice plants were inoculated with endophytic fungus isolated from an upland rice variety, proline concentration increased under drought stress. The accumulating osmolytes maintain osmotic equilibrium, giving the plants stress tolerance, in addition to supplying them with energy. The findings suggest that enhanced osmolyte content in inoculated plants could have reduced the influx of ions and thus reduced the oxidative burst.

Furthermore, in this study, oxidative damage indicators such as lipid peroxidation were assessed by analysing the MDA content to corroborate the efficacy of endophytic fungi. The ROS

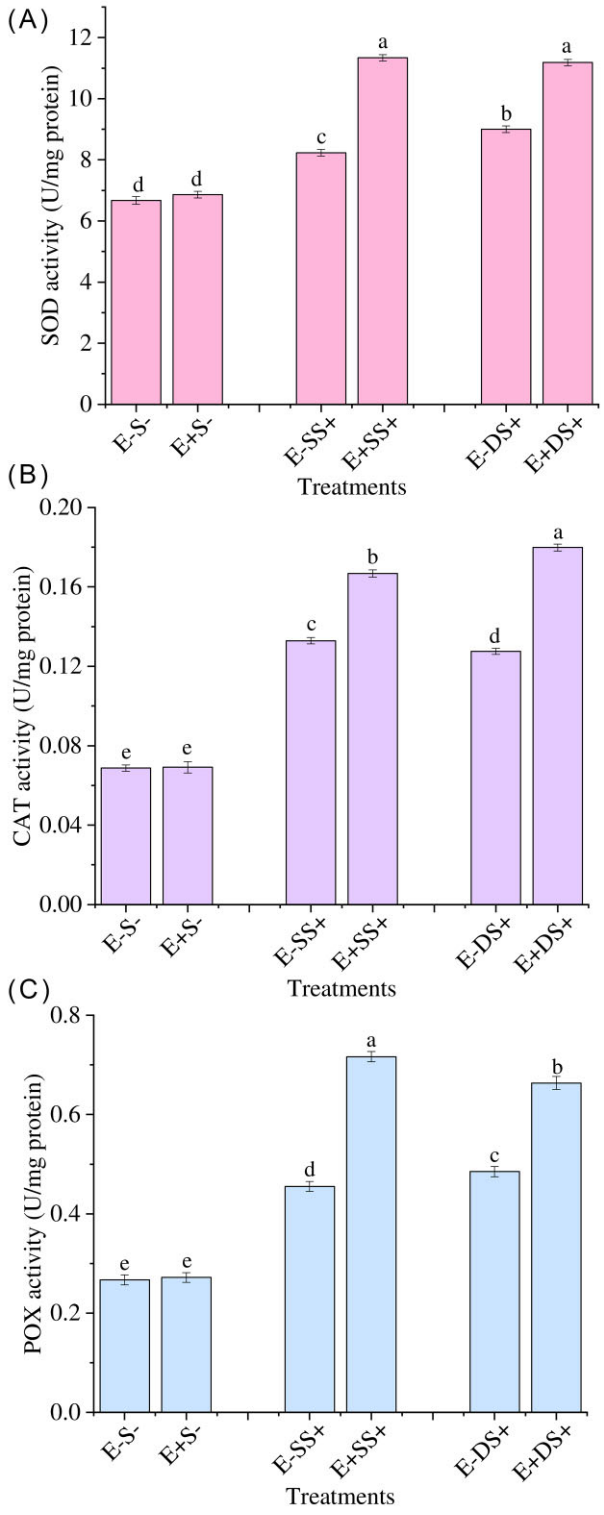

Figure 7. Effect of endophyte *Nigrospora oryzae* #2OSTUR9a on antioxidant enzymes of rice plant. (**A**) Superoxide dismutase (SOD); (**B**) catalase (CAT); (**C**) peroxidase (POX). The values represent mean of value ± SD, n = 6; mean values with different superscript letters are different by Tukey's post-hoc test ($P < 0.05$).

generated due to stress conditions degrade the polyunsaturated lipids, disrupting the structural components of the plant and increasing the MDA content. Under stress, it was observed that inoculated plants had lower MDA contents than uninoculated plants. Siddiqui et al. (2022) observed that under salinity stress, rice seedlings inoculated with *Aspergillus terreus* exhibited a 19% drop in MDA concentration. Reduced MDA content was found in rice seedlings inoculated with *Piriformospora indica* under drought (Tsai et al. 2020), while Qin et al. (2019) showed that using a coumarin derivative derived from endophytic fungi resulted in a reduction in the rice's MDA content. Similar results in other plants under salinity and drought have been observed by various scientists (Pang et al. 2020, Badawy et al. 2021). The decreased MDA concentration of the inoculated plants supports the performance of plants' antioxidant systems under abiotic stress. Because of these findings, it can be presumed that inoculation of endophytic fungi adequately reduces the breakdown of lipids in stressed plants.

Plants use the synthesis of antioxidant enzymes as a method to combat the ROS produced under stressful conditions. The available literature suggests that the plants inoculated with endophytic fungi have increased activity of antioxidant enzyme (Hosseyni Moghaddam et al. 2022, Javed et al. 2022). Similar results of high antioxidant enzyme activity were obtained in our study. Badawy et al. (2021) observed that *Aspergillus ochraceus*-inoculated barley had increased activity of SOD and POX of 36.61% and 21.35%, respectively, under salt stress. According to Asaf et al. (2018), increased antioxidant enzyme activity in inoculated plants led to greater salt tolerance in *Glycine max* plants after *Aspergillus flavus* inoculation. Elevated SOD and POX activity of rice seedlings inoculated with *Piriformospora indica* under PEG-induced drought was observed by Tsai et al. (2020) and is consistent with our findings. While CAT and POX cleave the peroxides, antioxidant enzymes like SOD catalyse the dismutation of superoxide ions into hydrogen peroxide. The joint action of the enzymatic and non-enzymatic antioxidants upholds the ionic homeostasis to combat stress. Studies have shown a correlation between the overexpression of antioxidant enzymes and enhanced photosynthesis (Ali et al. 2022, Meshram et al. 2023). Considering the reduction of ROS accumulation in inoculated plants, it was speculated that the endophytic fungi promote photosynthesis in the host plant, which was also corroborated by the findings of this study. As a result, the inoculated plants could thrive under stress conditions.

Conclusion

Anthropogenic activities have caused a severe drop in worldwide crop yields. Because these stress factors occur simultaneously, developing sustainable strategies for plants to combat various stress conditions is imperative. Endophytic fungi exhibit the intrinsic property of adapting to the host plant's environment and exhibiting similar characteristics. Hence, this study divulged in isolation and screening the culturable endophytic fungi associated with Sabhagi Dhan, a drought-resistant rice variety. Based on the superior plant growth promotion attributes, isolate #2OSTUR9a, identified as *Nigrospora oryzae*, was selected for further analysis. The isolate exhibited promising results in combating salinity and drought stress under pot trials. To the best of our knowledge, this is the first report on abiotic stress mitigation in rice plants by endophytic *N. oryzae*. It is conspicuous that salinity and drought stress cause sig-

nificant damage in the rice plant; however, the inoculated plants exhibited enhanced physiological attributes, osmolyte accumulation, antioxidant enzyme activity and reduced MDA levels. These effects enabled the rice plant to withstand abiotic stress. Considering the current findings, further studies involve exploring omics tools to understand the cross-talk and mechanism between the symbionts and the eventual application of the technology under field conditions to elucidate its full potential.

Author contributions

Gurleen Kaur Sodhi (Data curation, Formal analysis, Investigation, Methodology, Writing – original draft) and Sanjai Saxena (Conceptualisation, Project administration, Supervision, Validation, Writing – review & editing)

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Supplementary data

Supplementary data is available at *FEMSEC Journal* online.

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Availability of data and materials

Data shall be made available on viable and justifiable request.

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