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ORIGINAL ARTICLE

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

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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

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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

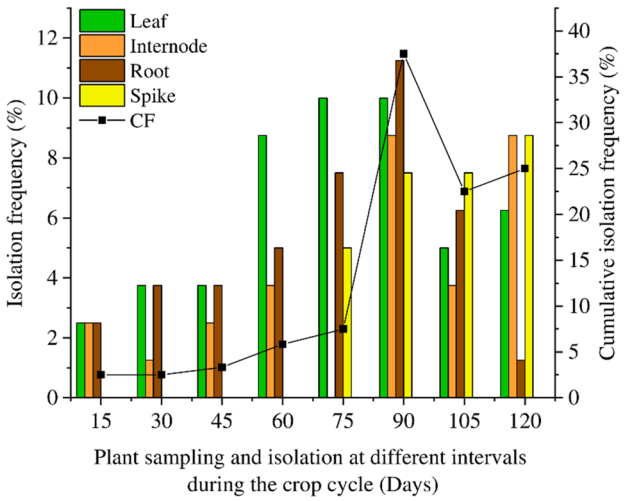


Fig. 2 Isolation frequency and cumulative isolation frequency (CF) of endophytic fungi isolated from different plant parts of PUSA-44 over a period of 120 days

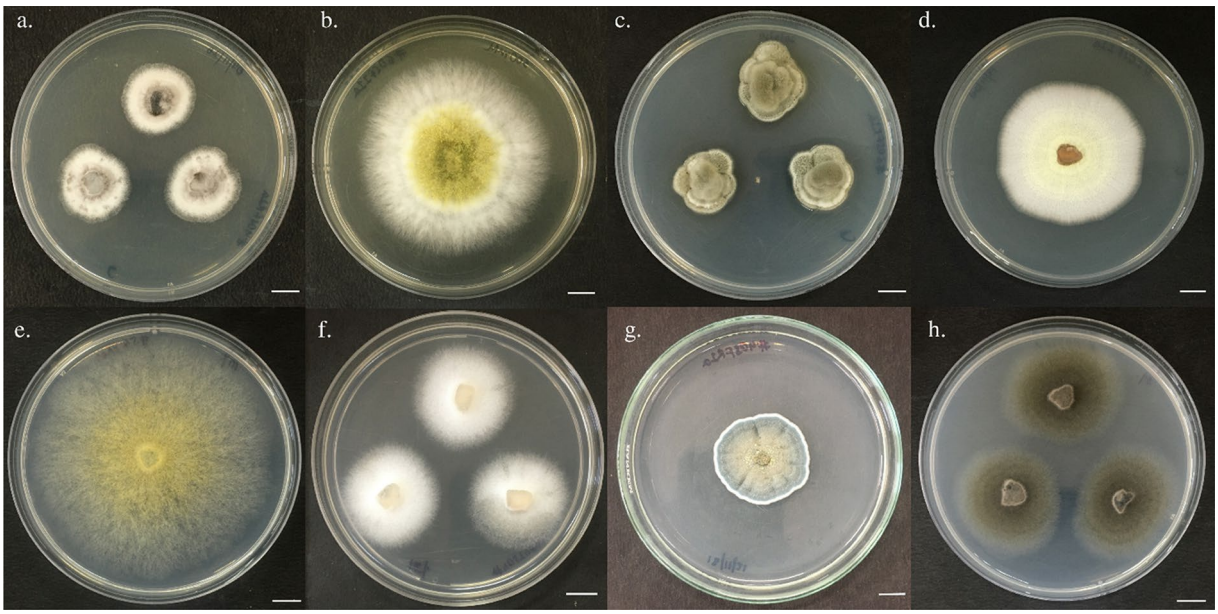


Fig. 1 Some of the endophytic fungi isolated during the study: **a** *Nigrospora* sp., **b** *Aspergillus* sp., **c** non-sporulating, **d** *Paecilomyces* sp., **e** *Rhizopus* sp., **f** *Fusarium* sp., **g** *Penicillium* sp., **h** unidentified (bar: 10 mm)

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.

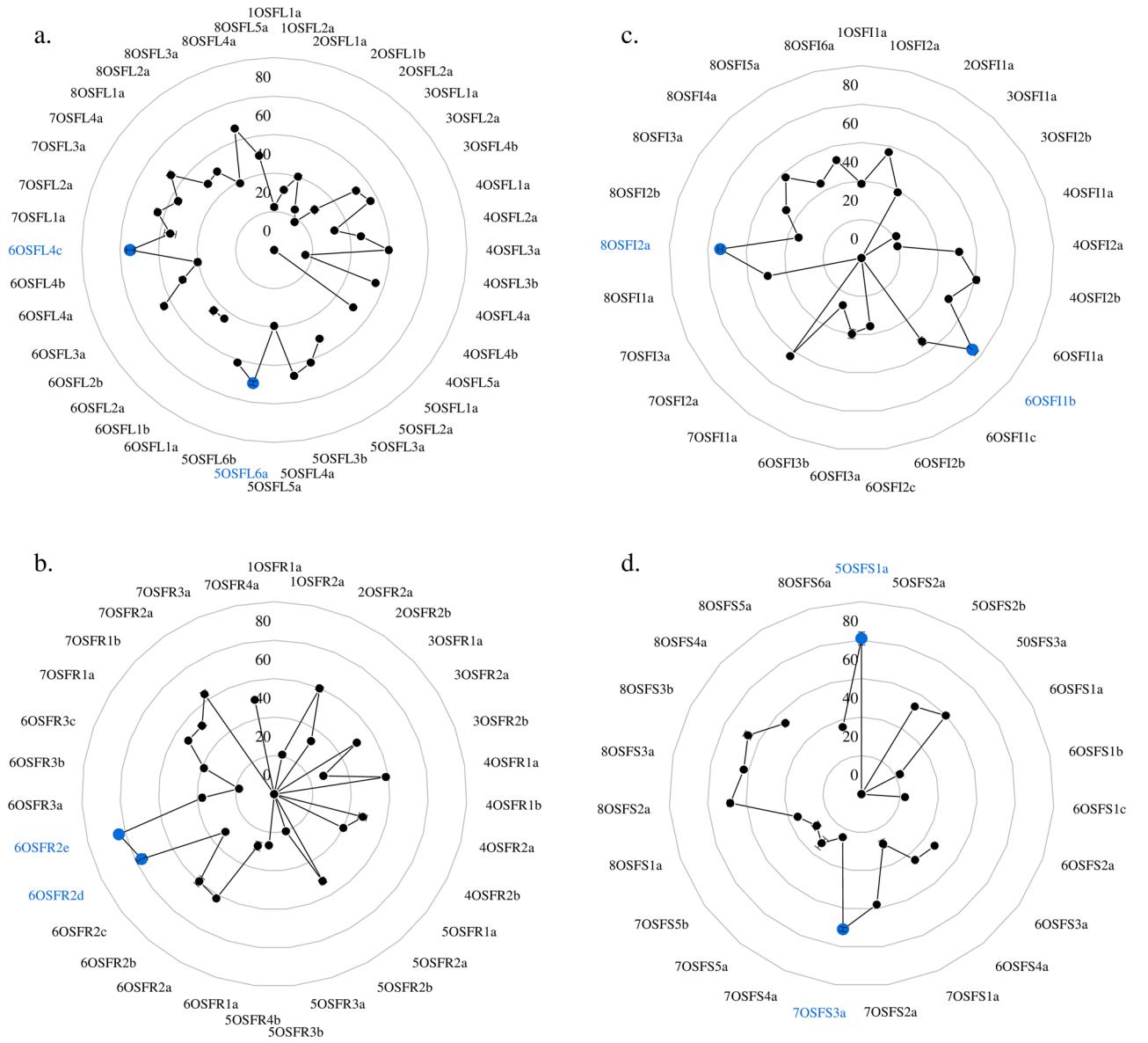


Fig. 3 Isolates from different plant parts exhibiting growth under salinity and drought stress. **a** Leaf isolates at 2 M NaCl concentration. **b** Root isolates at 2 M NaCl concentration. **c** Internode isolates at 2 M NaCl concentration. **d** Spike isolates at 2 M NaCl concentration. **e** Leaf 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

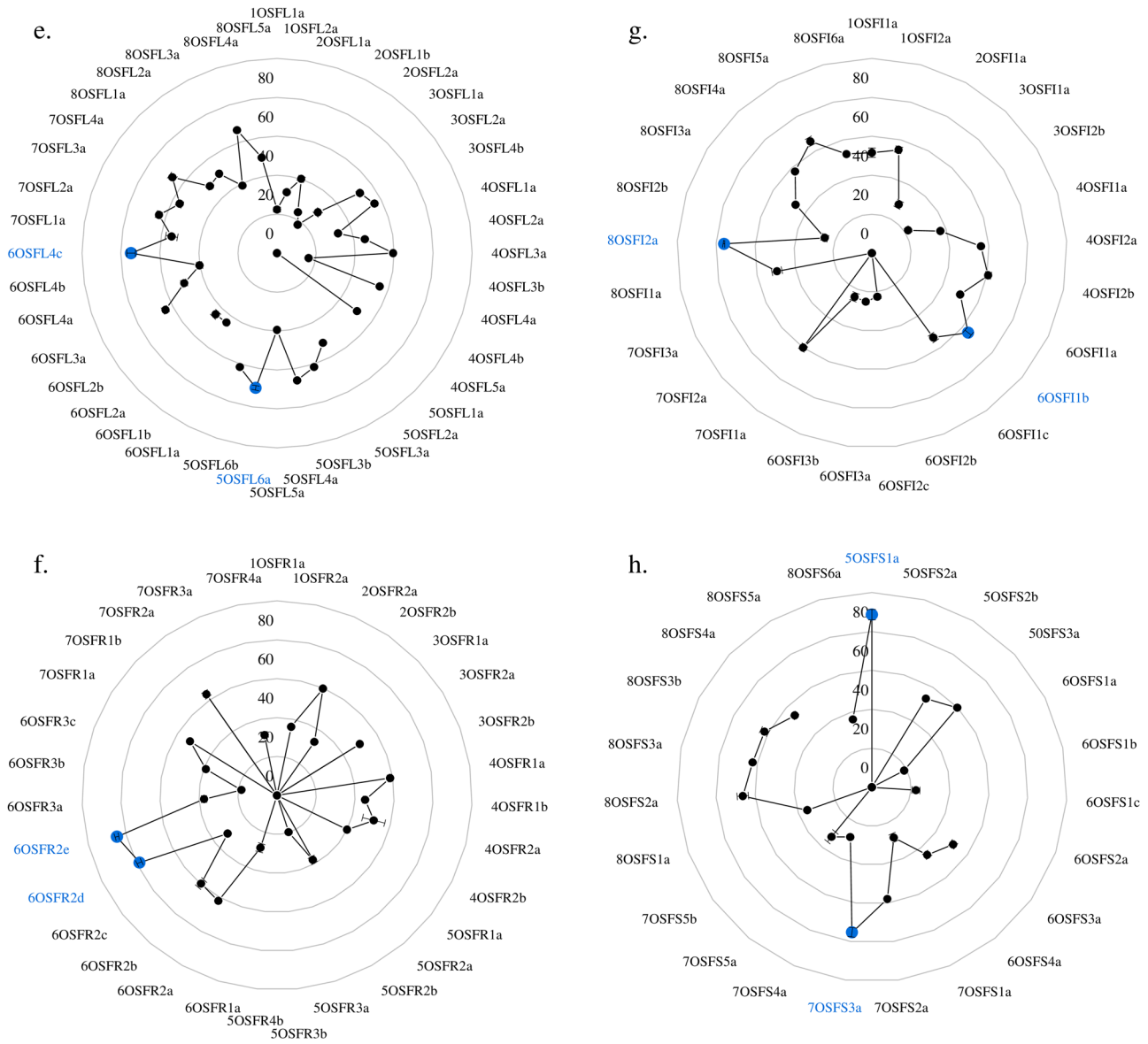
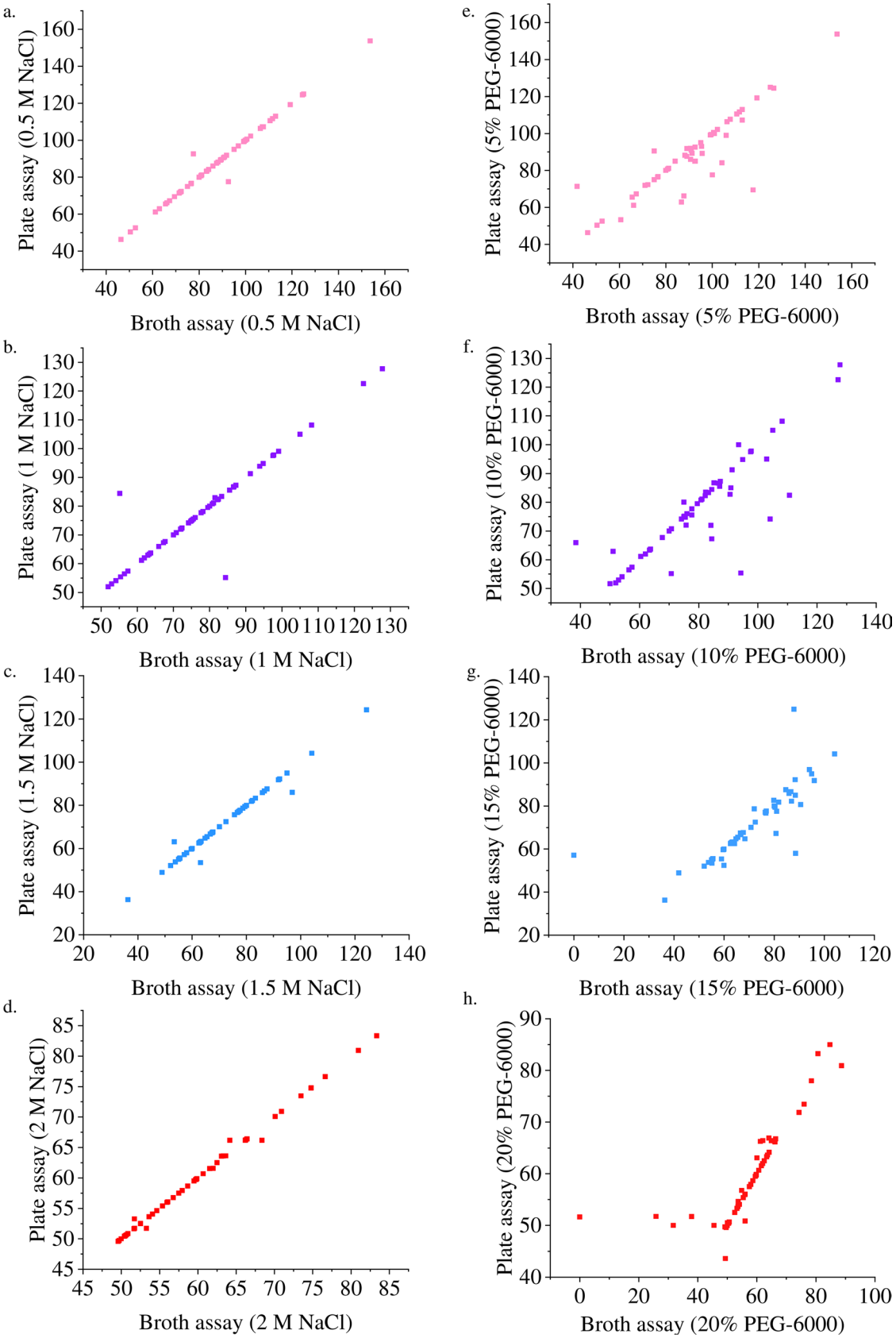


Fig. 3 (continued)

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

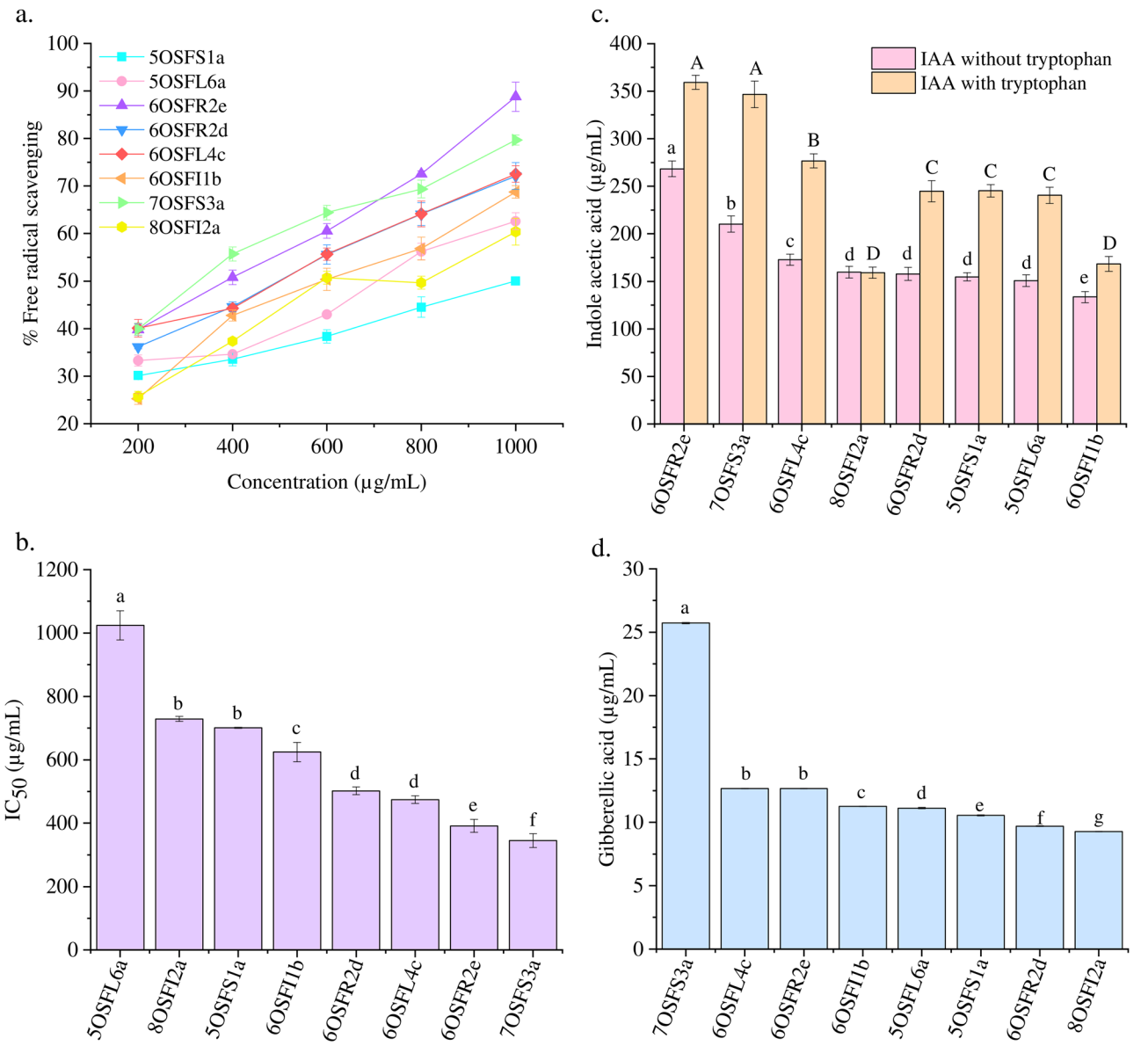


Fig. 5 Plant growth-promoting attributes of selected endophytic fungi. **a**, **b** Free radical scavenging % and IC50 values evaluated using DPPH assay. **c** Indole acetic acid production. **d** Gibberellic acid production. The values represent mean of value ± SD, $n = 3$; mean with different superscript 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

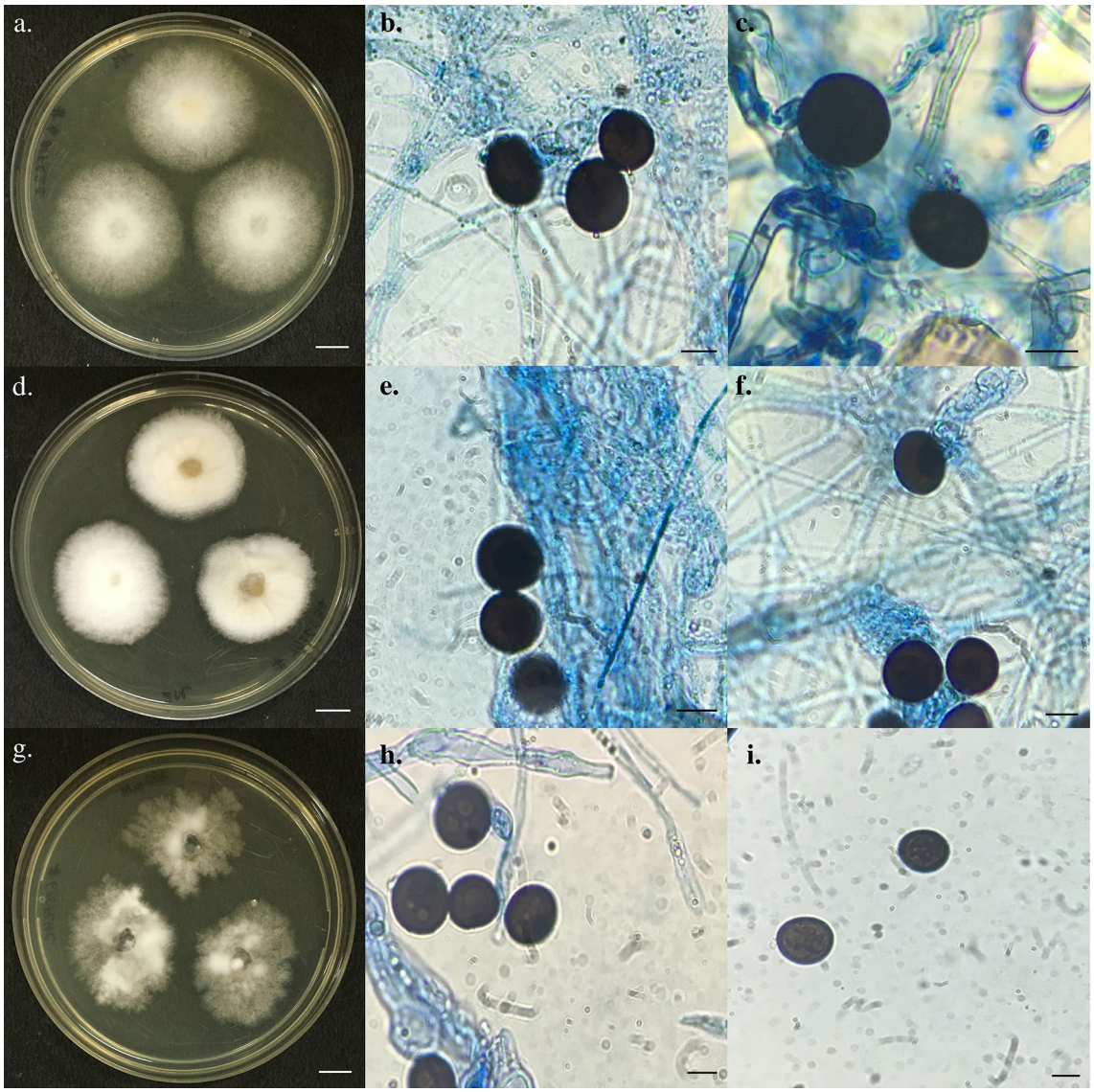


Fig. 6 **a** Colony morphology of 6OSFR2e on PDA. **b**, **c** Conidiogenous cells of 6OSFR2e. **d** Colony morphology of 7OSFS3a on PDA. **e**, **f** Conidiogenous cells of 7OSFS3a. **g** Colony morphology of 6OSFLS4c on PDA. **h**, **i** Conidiogenous cells of 6OSFL4c (**a**, **d**, **g** Bar: 10 mm;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

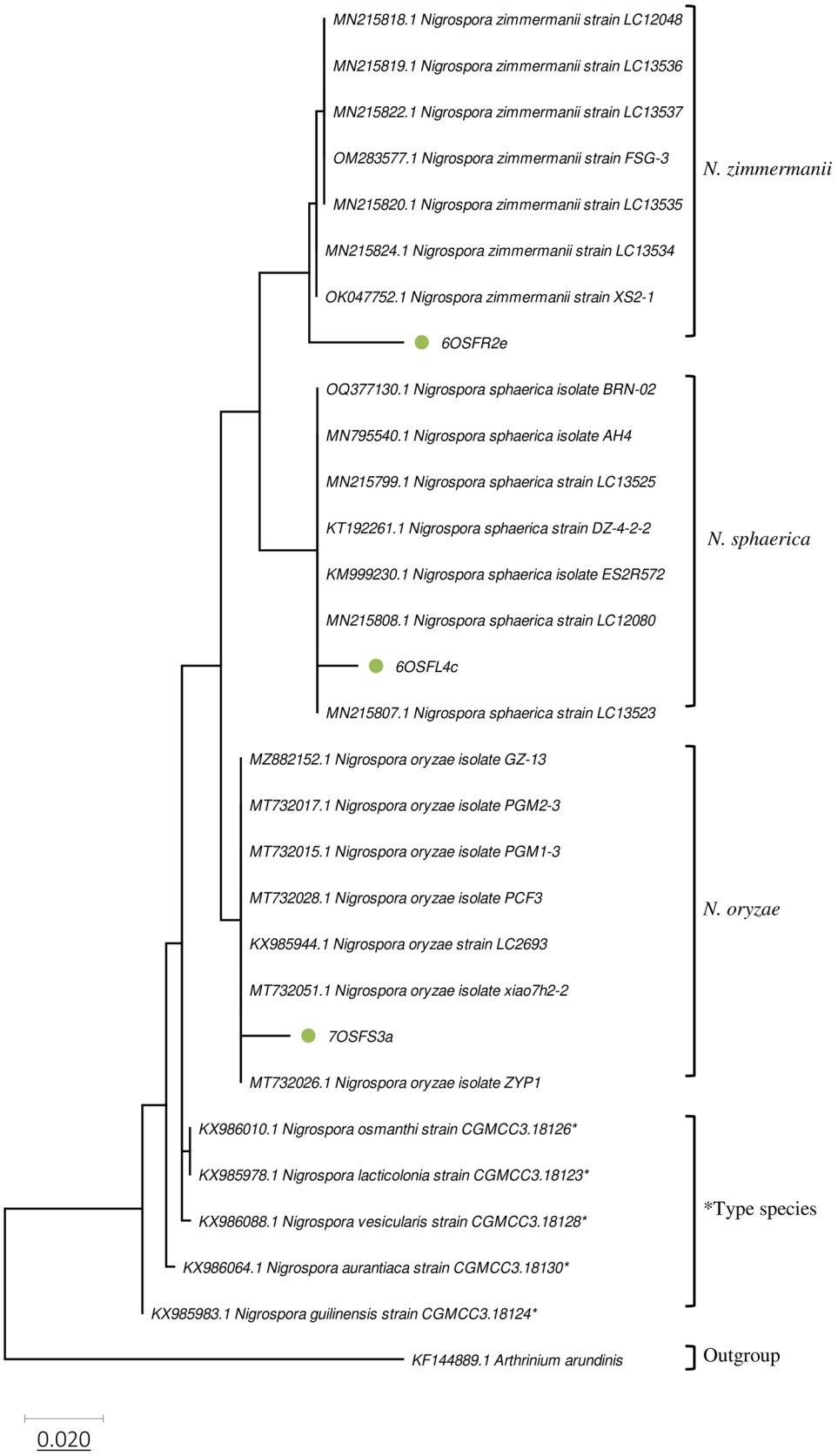


Fig. 7 Maximum-likelihood tree showing 6OSFR2e, 6OSFL4c, and 7OSFS3a based on the ITS1-5.8S-ITS2 region using Tamura and Nei model, indicating the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates)

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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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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