



ORIGINAL ARTICLE

Open Access



# Plant growth-promoting endophytic fungi of the wild halophytic rice *Oryza coarctata*

Arifa Akhter Airin<sup>1</sup>, Md Iyasir Arafat<sup>1</sup>, Rifat Ara Begum<sup>1</sup>, Md Rakibul Islam<sup>1</sup> and Zeba Islam Seraj<sup>1\*</sup>

## Abstract

**Background** Plant growth-promoting endophytic fungi (PGPEF) that are associated with halophytes have the potential to boost crop salinity tolerance and productivity. This in turn has the potential of enabling and improving cultivation practices in coastal lands affected by salt stress.

**Methods** Endophytic fungi from the wild halophytic rice *Oryza coarctata* were isolated, characterized, identified, and studied for their effects on all developing stages of rice plant growth and their yields both with and without salt stress.

**Key results** In this study, three different fungal endophytes were isolated from the halophytic wild rice *Oryza coarctata*. Two isolates were identified as *Talaromyces adpressus* (OPCRE2) and *Talaromyces argentinensis* (OPCRh1) by ITS region sequencing. The remaining isolate NPCRE2 was confirmed as a novel strain named *Aspergillus welwitschiae* Ocstreb1 (*AwOcstreb1*) by whole genome sequencing. These endophytes showed various plant growth-promoting (PGP) abilities in vitro (e.g., IAA, ACC-deaminase and siderophore production, phosphate, and zinc solubilization as well as nitrogen fixation), where *AwOcstreb1* was significantly more efficient compared to the other two isolates at high salinity (900 mM). Independent application of these fungi in commercial rice (*Oryza sativa*) showed significant elevation in plant growth, especially in the case of the *AwOcstreb1* inoculants, which had enhanced metabolite and chlorophyll content at the seedling stage in both no-salt control and 100-mM salt-stressed plants. At the same time, *AwOcstreb1*-treated plants had a significantly lower level of H<sub>2</sub>O<sub>2</sub>, electrolyte leakage, and Na<sup>+</sup>/K<sup>+</sup> ratio under saline conditions. Higher expression (1.6 folds) of the SOS1 (salt overly sensitive 1) gene was also observed in these plants under salinity stress. This strain also improved percent fertility, tillering, panicle number, and filled grain number in both no-salt control and 45-mM salt-stressed inoculated plants at the reproductive stage. Consequently, the differences in their yield was 125.16% and 203.96% ( $p < 0.05$ ) in colonized plants in normal and saline conditions, respectively, compared to uninoculated controls.

**Conclusions** We propose that *AwOcstreb1* is a potential candidate for an eco-friendly biofertilizer formula to improve the cultivation and yield of rice or any other crop in the highly saline coastal regions of Bangladesh.

**Keywords** Endophytic fungus, *Oryza coarctata*, Rice, *AwOcstreb1*, Salinity tolerance, *OsSOS1*, Yield, *Aspergillus welwitschiae*

## Introduction

Rice (*Oryza sativa*) is the staple food of approximately 135 million people in Bangladesh (Mia et al. 2022). Among the most vital cereals produced globally, rice ranks second to maize (Awika 2011). Approximately 34 million metric tons of rice were consumed in 2002–2004 in Bangladesh and 44.9 million metric tons are anticipated to be needed

\*Correspondence:

Zeba Islam Seraj  
zebai@du.ac.bd

<sup>1</sup> Plant Biotechnology Lab, Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka 1000, Bangladesh



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

in 2024, which is an increase of 32% from the consumption rate in 2004. The estimated consumption of rice in developed countries was 17 Mt in 2002–2004 and is projected to reach 18.2 Mt by 2024 (OECD 2015). Rice production therefore needs to be increased to fulfill the demand of a very rapidly growing population (2 million people/year) (Streatfield and Karar 2008).

Along with the increasing food demand, the reduction in cultivable land is a matter of big concern. The increase in the construction of industries and dwellings is dynamically reducing croplands by > 1% each year (Boston 2016). Climate change has added a new dimension in reducing cultivable land availability due to an increasing amount of coastal land being affected by salinity and a consequential reduction in crop fertility and production in these lands. Soil salinity has progressively increased in terms of intensity, and 0.22 million hectares were newly affected from 1973 to 2009 in the coastal region in Bangladesh (Salehin et al. 2018) causing a detrimental effect on agricultural yield. According to Dasgupta et al. (2018), rice production will fall by 15.6% in nine sub-districts of Bangladesh where soil salinity will exceed 4 dS/m by 2050.

Crop loss due to salinity is projected to get worse by 2050 worldwide (Pitman and Läuchli 2002). Almost 1128 million hectares of agricultural land have already been affected by salinity. It is estimated that at least 50% of agricultural land will be affected by saline stress by 2050 and it will continue to worsen if global climate change remains unchecked (Jamil et al. 2011). Plants are sensitive to arid and saline environments throughout all developmental phases leading to decreased growth. Long-term saltwater irrigation impairs plant physiology and metabolite biosynthesis, affecting total biomass and grain yield. Rice is one of the most sensitive crops to salinity and cannot grow in saline soil.

Plants respond in a multifaceted way when stressed by salinity. In the initial phase, salt stress increases osmotic pressure, which triggers the activation of the osmotic stress pathway and inhibition of stomatal conductance and photosynthetic activity. Ionic stress generated by  $\text{Na}^+$  and  $\text{Cl}^-$  ion accumulation enhances reactive oxygen species (ROS) generation and oxidative stress in the later phase of salt stress (Majeed et al. 2010). Very few novel approaches have been explored until now to reduce crop damage brought about by salinity stress (Zhang et al. 2019). One approach is to discover any endophytic fungi in a mutually beneficial existence with a halophytic host plant. Endophytes provide protection from plant pathogens and produce useful metabolites that enable the host plants to counter salt stress (Zhang et al. 2019). They can enhance the growth and development of the host plant by increased nutrient absorption and production of siderophore, ACC-deaminase,

and phytohormone (Sarkar et al. 2018). Natural plant growth-promoting endophytic fungi (PGPEF) can induce multiple stress tolerance in their host plants at the molecular level and bestow the plant with remarkable genetic flexibility (Zhang et al. 2016; Jan et al. 2021). Previous studies have demonstrated that endophytic fungi can develop naturally occurring substances for plant defense mechanisms that are physiologically active and structurally diverse against environmental stresses (Schulz et al. 2002; Zhang et al. 2019). For instance, several plant growth-promoting endophytes have been reported to reduce damage caused by saline stress in a range of plants such as rice, wheat, rye, and *Arabidopsis* (Bal et al. 2013; Kang et al. 2014; Afridi et al. 2019; Lanza et al. 2019). Despite their versatile roles, very little is known about the fungal endophyte's ecological, physiological, and growth responses to salt stress in the majority of vital agricultural crops, such as rice (Tisarum et al. 2020).

Endophytic fungi are organisms that reside for all or a portion of their lives intracellularly or in the intercellular spaces of host plants without causing disease or damaging their hosts (Arnold 2007). Previous studies have shown that some fungi are essential for improving a plant's ability to withstand abiotic stress (Waqas et al. 2012; Tisarum et al. 2020). The symbiotic relationship between halophytes and their respective endophytic fungi is an extremely important area for investigation specially for finding solutions for mitigation of salinity stress on crops. Halophytes grow naturally in saline environments and respond to salinity in diverse manners. The ability to control cellular  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{K}^+$  concentrations to modify the external water potential is a trait shared by all halophytes. It has been reported that an endophyte from a halophyte developed its own mechanism of salt stress tolerance and positively contributed to provide tolerance to salt-sensitive crops upon inoculation (Nanjundappa et al. 2021). Endophytic fungi of coastal habitats may promote salt tolerance due to their symbiotic relationship with halophytic host plants, which optimizes their defense against water salinity and enhances physiological response under stress.

*Oryza coarctata*, a halophytic wild rice, can be found all along Bangladesh's coastline. It exhibits strong tolerance to high salinity (20–40 dS/m) while submerged entirely for extended periods of time (Garg et al. 2014). Endophytic fungal communities of *O. coarctata* may promote plant growth and saline stress tolerance. However, information about the plant growth-promoting role of the fungal endophyte community of *O. coarctata* plant is still unknown. This study isolated and identified fungal endophytes from the roots and rhizomes of the *O. coarctata* from the coastal region of Bangladesh, investigated their

plant growth-promoting activities in vitro, examined their effects on salt stress mitigation, and estimated the effect of fungal inoculation on rice plant growth at different developmental stages and their yields under saline stress.

## Materials and methods

### Collection of the host plant material

Healthy, disease-free, and mature *Oryza coarctata* plants along with the rhizospheric soil were collected from the coastal region of Ashashuni (22.549342 N, 89.175143 E), Satkhira, Bangladesh, and freshly transported in sterile poly bags, then immediately processed for the isolation of endophytic fungi. Another set of *O. coarctata* was collected from the same place but grown and acclimated in locally acquired soil for at least 4 years in a net house.

### Endophytic fungi isolation and maintenance

Endophytic fungi were isolated following the method of Kusari et al. (2008) with some optimizations. Surface sterilization of fragmented plant parts (roots and rhizomes) was done by immersion in 70% ethanol for 1 min, then in 13% sodium hypochlorite for 4 min, again in 70% ethanol for 30 s, rinsing several times with autoclaved distilled water, and blot drying with sterile filter paper. Then 3–4 segments (3–4 cm in length) were placed separately on water agar (HiMedia) medium supplemented with 100 mg l<sup>-1</sup> streptomycin and incubated at 28 °C ± 2 °C for 4–6 weeks without light. When growth was observed, hyphal tips were transferred to fresh potato dextrose agar (PDA, HiMedia, India) plates to obtain a pure culture. Endophytes were isolated from different parts of the *Oryza coarctata* (*Porteresia coarctata*) plant. Those from mature plant (grown in a net house) rhizomes were designated as OPCRh-1, OPCRh-2, etc. Isolates from mature plant roots were named OPCRE-1, OPCRE-2, etc. Additionally, isolates from freshly transported plant roots were labeled as NPCRE-1, NPCRE-2, etc.

### Identification of fungal endophytes

Endophytic fungi were identified by observation of morphological characteristics, microscopic examination, and molecular sequencing. Fungal colony characters (PDA culture as mentioned in 2.2) such as color, growth pattern, surface and marginal appearance, hyphae, and mycelium were examined for up to 14 days (Sadananda et al. 2014). Sporulation was microscopically observed by staining a small portion of mycelia with Lactophenol-Cotton Blue using a bright-field (Olympus, Japan) microscope (Devi and Prabakaran 2014). For molecular identification, genomic DNA was extracted by the CTAB method (Baker and Mullin 1994) and the entire internal transcribed spacer region (ITS1, 5.8S, ITS2) was sequenced (Martin and Rygiewicz 2005)

(Fig. S1 and S2). For OPCRh1 and OPCRE-2 isolates, each sequence was identified via individual BLAST search, aligned (ClustalW function) with twenty highly similar sequences using MEGA 11.0, and a phylogenetic tree was constructed using the neighbor-joining (N-J) method with a bootstrap value of 1000. The whole genome sequencing (WGS) method was used for confirmation of the novel NPCRE2 strain (separate manuscript in preparation; BioProject ID PRJNA985106). The sequences of NPCRE2 were assembled de novo using the SPAdes tool and annotated using AUGUSTUS. Average nucleotide identity (ANI) calculation and phylogenetic analysis were performed using dREb tools.

### In vitro characterization of PGP traits of fungal isolates

#### Qualitative screening

Fungal hyphae (6 mm) were inoculated in the center of the plates containing Pikovskaya's Agar (HiMedia) and zinc-solubilizing agar media (HiMedia) for qualitative screening of phosphate and zinc solubilization, respectively. The plates were incubated for 14 days at 28 °C, and visualization for phosphate and zinc solubilization by the fungi was identified by clearing the opaque media (Bapiri et al. 2012; Gupta et al. 2012).

The fungi were inoculated on nitrogen-free Burk's medium (HiMedia) for nitrogen fixation ability test and on Dworkin and Foster (DF) minimal salt agar media (Dworkin and Foster 1958) supplemented with 3 mm ACC as the sole nitrogen source for ACC deaminase activity. Growth was observed for 2 weeks at 28 °C (Maheshwari et al. 2020).

For the IAA production test, the methodology of Deshwal and Kumar (2013) was followed and the siderophore test was done through the modifications of the CAS (Chrome Azurol S) assay solution method (Alexander and Zuberer 1991). As HDTMA (hexadecyltrimethylammonium) is harmful to the growth of most fungi, the modified CAS agar universal test plating method was used as described by Machuca and Milagres (2003). After becoming solid, the medium was cut into half aseptically so that half was replaced by 15-mL PDA media for culturing fungi. Fungal hyphae (6 mm) were inoculated as far as possible from the borderline between the two media. The plates were allowed to incubate for 14 days at 28 °C in the dark. The blue color of the media will change as the siderophore chelates iron from the dye complex.

#### Quantitative assay

The quantitation of solubilized phosphate and IAA that was produced was done following the methodology described by Suebrasri et al. (2020) and Gordon and Weber (1951), respectively.

Siderophore was assessed in liquid culture following the methodology described by Schwyn and Neilands (1987), and the modified faster-reacting CAS assay solution (Shuttle solution) was prepared according to Alexander and Zuberer (1991).

#### **PGP activity test of fungal isolates in the presence of 900-mM salt stress**

Qualitative phosphate and zinc solubilization, siderophore production, nitrogen fixation, and IAA production abilities were tested in the presence of high salt concentration to determine the PGP activity of fungal isolates under salt stress. For this purpose, 900-mM salt was added to each respective media and further procedures for each test were followed as described earlier.

#### **Screening for the effects of endophytic fungal inoculum on rice seedlings**

The efficacy of all selected PGPEF isolates as bioinoculants was investigated on salt-sensitive rice variety BRRI dhan28 (*Oryza sativa*). Seeds of BRRI dhan28 (from Bangladesh Rice Research Institute, BRRI) were kept at 50 °C for 48 h to break dormancy, then surface sterilized by soaking in 99% ethanol for 1 min followed by washing with 30% sodium hypochlorite along with 30 µl Tween 20 for 1 min, finally washed five times with sterilized distilled water. For fungal treatment, spores were counted using the Hemocytometer Improved Neubauer Counting Chamber (German) and a suspension of 10<sup>6</sup> spores/ml in distilled water was prepared. Sterile seeds were germinated at 37 °C and 24 h after germination, and seeds were incubated with 10 ml of each fungal spore suspension for 72 h with shaking at 30 rpm. Sterile water without fungal inoculation was used as a control treatment. Seeds were then planted in clay pots (24 cm width) filled with heat-sterilized (at 70 °C for 3 h and sun-dried) ready-mix commercial garden soil (soil, vermicompost, coco dust, bone meal powder, sand, CaCO<sub>3</sub>, zinc, charcoal, boric powder, cow manure). Finally, 1 l of fungal spore suspension (10<sup>6</sup> spores/ml) was added to each pot and only sterilized water was used for control pots. Later, pots were irrigated with sterilized tap water every day. After 14 days of planting, a 100-mM salt solution was applied and after 21 days, plants were collected and analyzed for root length, shoot length, root weight, shoot weight, chlorophyll content, and root Na<sup>+</sup>/K<sup>+</sup>.

From this preliminary test, the most efficient PGPEF isolate, NPCRE2, was chosen for further study. This isolate was tested for its effect on the yield of the rice plant at maturity under both no salt and salt stress. During the reproductive stage, a different soil was used to see effects in normal soil mixed with cocopeat in a 2:1 ratio. Physiological screening, plant metabolite assay, and relative

expression study of one salt stress-related gene, “SOS1,” at the seedling stage were performed.

#### **Plant metabolite assays**

The total chlorophyll content of endophyte-treated and untreated (control) fresh rice leaf extracts was estimated according to Inskeep and Bloom (1985). Total flavonoids and phenolics from the fungus-treated and control rice seedlings were extracted in methanol (Cui et al. 2019). Then, the total flavonoid content and phenolic content were estimated spectrophotometrically according to the Dowd method (Arvouet-Grand et al. 1994) and the Folin-Ciocalteu method (Ainsworth and Gillespie 2007), respectively.

For protein extraction, 0.25 g of fresh leaves was homogenized in 5 ml of ice-cold 50-mM phosphate buffer (pH 7.8), the homogenate was centrifuged at 15,000 rpm for 15 min at 4 °C, and protein content was determined from the supernatant (Lowry et al. 1951). Total sugar content was determined by the methodology described by Lubna et al. (2018).

#### **Screening of stress-related parameter**

The amount of H<sub>2</sub>O<sub>2</sub>, Na<sup>+</sup> and K<sup>+</sup> content, and electrolyte leakage was calculated using the method of Velikova et al. (2000), Yamane et al. (2009), and Parvin et al. (2015), respectively.

#### **Determination of relative expression of the salt stress-related gene SOS1**

Salt stress response gene *SOS1* was studied by quantitative real-time PCR (qRT-PCR) analysis. The total RNA was extracted from shoots of both unstressed and salt-stressed samples (48 h of 100-mM salt treatment) using TRIZOL reagent (Ambion, Invitrogen) following the manufacturer's protocol. Three replicates of each sample were taken for the experiment.

Synthesis of cDNA was done using ProtoScript<sup>®</sup> II First-Strand cDNA Synthesis Kit (NEB). Then, qRT-PCR was performed using iTaq Universal SYBR Green Supermix (Bio-Rad). The expression of target genes at 48-h samples of both endophyte-treated and control plants was normalized with the expression of the respective housekeeping (Sno-RNA) gene and was fixed on a scale of 1.0. The fold changes of target genes in consecutive samples were quantified by the relative quantification (2<sup>-ΔΔCT</sup>) method.

#### **Screening at the reproductive stage**

For the reproductive stage experiment, break of seed dormancy, seed sterilization and germination, fungal inoculation, and sowing of plants into pots were done as described in the section [Screening for the effects of](#)

**endophytic fungal inoculum on rice seedlings.** For control plants and inoculated plants, four replicates were used. Nearly 2 weeks before the appearance of the booting stage, plants were treated with 45-mm salt stress. After 140 days of planting, plants were harvested and yield parameters were taken. Plant height, total tillering, effective tillering, panicle length, filled grain number, filled grain weight, % of fertility, and yield were measured.

#### Root colonization assay for confirmation of fungal colonization inside plant root

Root colonization assay of endophytic fungus was done according to the methods described earlier in the section **Endophytic fungi isolation and maintenance**. The newly isolated fungi were identified according to their morphology and by microscopic analysis (Potshangbam et al. 2017) (Fig. S9).

#### Measurement of salinity of the soil

The electrical conductivity (EC) of the soil was measured to determine the salt content after inducing the salinity stress. The soil was collected from the pots after 7 days of salinization and dried. Then, 200 g of dry soil was mixed with deionized water to make a saturated paste. Using a vacuum pump, approximately 20–25 ml of water was extracted from the saturation paste and the EC of the water was measured using a Hanna Portable EC Meter (Mandal et al. 2015).

#### Statistical analysis

Statistical analysis was performed, and graphs were prepared using GraphPad Prism version 9 and Microsoft Excel for Windows 2016 add-in with XLSTAT Version 2020.4.1 (XLSTAT, 2020). Statistical analyses involved a comparison of the growth and yield parameters of the uninoculated plants and the endophyte-inoculants as control and treatment, respectively, under both non-stress and salt stress. The percent changes in these parameters were calculated to evaluate the impact of the endophyte treatment by the following formula:

$$\text{Percent changes} = (\text{Value}_{\text{endophyte-treated}} - \text{Value}_{\text{control}} / \text{Value}_{\text{control}}) \times 100\%$$

One-way analysis of variance (ANOVA) followed by Šídák multiple comparison test was performed for describing the variation between control (uninoculated) and inoculated plants in both saline stress and non-stressed conditions at the seedling and reproductive stages using the condition and individual plants as factors. Values are expressed as mean  $\pm$  SD (standard deviation). Values of  $p < 0.05$  and  $p < 0.01$  were deemed significant and highly significant, respectively.

## Results

### Endophytic fungi isolation

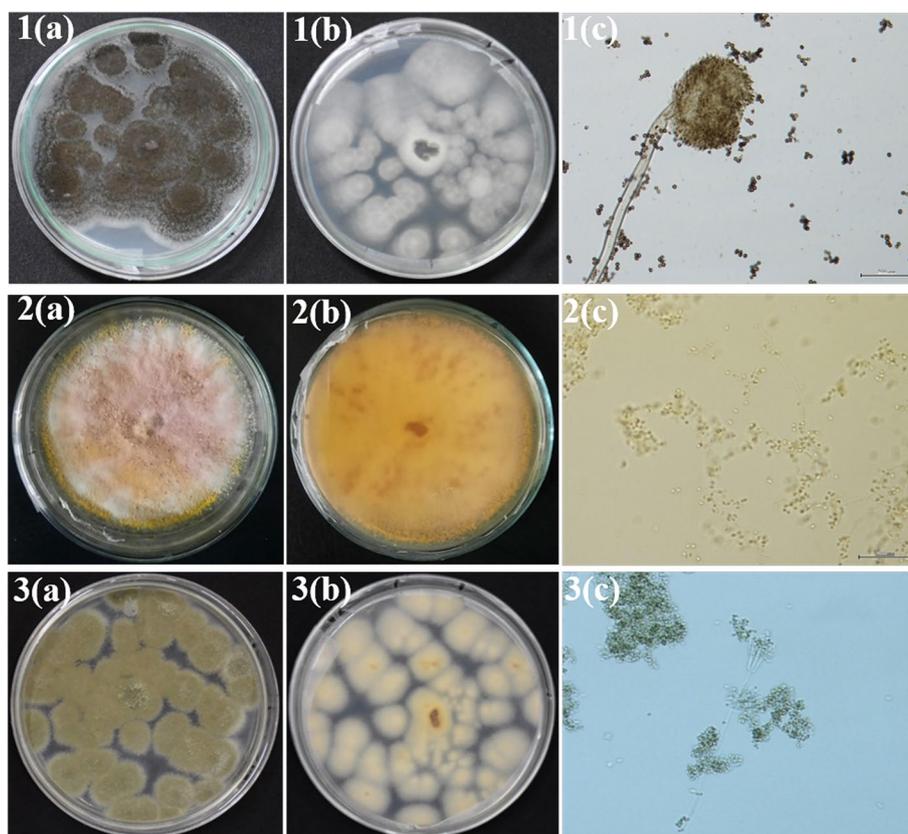
Among the three endophytic fungal isolates, one endophyte was isolated from the root interior of an *O. coarctata* plant collected directly from Ashashuni (Shatkhira, Khulna), named as NPCRE2. The other two endophytes, OPCRE2 and OPCRh1, were isolated from the interior of the root and rhizome of *O. coarctata*, respectively, which were previously brought from the same place but had been inhabiting the net house for 4 years. Each endophytic fungus exhibited distinct colonies and microscopic features.

### Identification

Growth observation followed by microscopic characterization confirmed that the isolated root fungal endophyte NPCRE2 was a species of the genus *Aspergillus* section *nigri* (black aspergilli) (Fig. 1) (Silva et al. 2011). On the PDA medium, the surface appeared as dark greenish black with white leading edge. This fungus showed branched mycelium, and the surface texture was granular. Dark brown to black, globular, rough conidia with radiated conidial head shape and ampulliform phialides were observed. The stipe of conidiophore was smooth and colorless. Whole genome sequencing was done for the molecular identification of NPCRE2 (it will be reported in a separate article), and phylogenetic analysis based on average nucleotide identity (ANI) was performed using several bioinformatics tools. NPCRE2 was placed in the same clade with more than 98% ANI value with *Aspergillus wetwitschiae* (Fig. 2). Based on morphological and molecular analysis, the endophytic fungus isolate NPCRE2 has been identified as a novel strain of *Aspergillus wetwitschiae* and named as *Aspergillus wetwitschiae* Ocstreb1 henceforth called AwOcstreb1.

Isolate OPCRh1 (Fig. 1) showed funiculus colonies on PDA, white-to-yellow mycelium with occasional pale pinkish vinaceous shades, poor sporulation, exudate-less, with the reverse being yellowish-orange and identified as *Talaromyces* sp. (Peterson and Jurjević 2019).

Isolate OPCRE2 (Fig. 1) showed nearly circular green colonies, planar but slightly protuberant at the center, texture velutinous to powdery; margins entire with slightly filiform; white mycelium; dense sporulation, grayish green conidia, and the reverse side to be yellowish-white. Smooth-walled stripes and terverticillate conidiophores were observed with the conidia spheroidal to globose, smooth to finely rough greenish conidia, phialides ampulliform, 3 per metula was observed under



**Fig. 1** Cultural morphology and microscopic picture of fungal endophyte (1) NPCRE2\_ *AwOcstreb1*, (2) OPCRh1\_ *Talaromyces argentinensis*, and (3) OPCRE2\_ *Talaromyces adpressus* grown on PDA. Keys: (a) surface view; (b) reverse view, and (c) microscopic picture

microscope and identified the genus as *Talaromyces* (Chen et al. 2016).

For species identification, nucleotide sequences of the ITS region of OPCRh1 and OPCRE2 were first subjected to blast search and highly similar sequences were obtained to construct a phylogenetic tree (neighbor-joining method with 1000 bootstrap value). In the tree, OPCRh1 was placed in the same clade with *Talaromyces argentinensis* (Fig. 3) and OPCRE2 was placed in the same clade with *Talaromyces adpressus* (Fig. 3). On the basis of morphological and phylogenetic analysis, OPCRh1 and OPCRE2 were identified as *Talaromyces argentinensis* and *Talaromyces adpressus*, respectively.

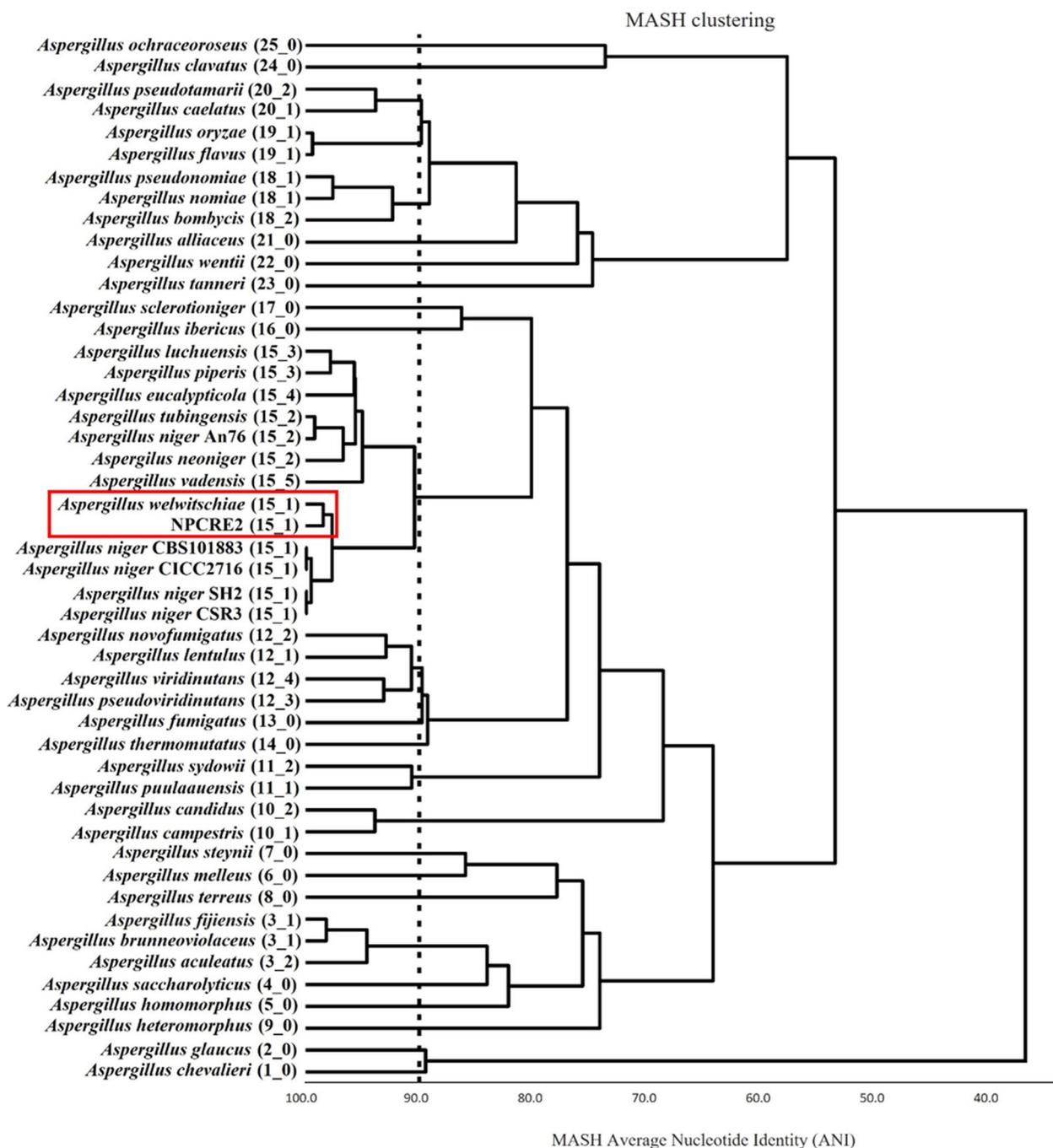
#### **In vitro screening of plant growth-promoting (PGP) traits of fungal isolates**

In vitro PGP traits of all the isolated endophytic fungi are described in Table 1. The results showed that *AwOcstreb1* tested positive for all the tested PGP abilities and these activities were very rapidly expressed even under 900-mM stress (Fig. 4). The other two fungal isolates also showed prominent zinc and phosphate solubilization in both normal and salt stress (Fig. S3). The highest phosphate

solubilization from the insoluble tricalcium phosphate was measured for *AwOcstreb1* ( $67.32 \text{ mg ml}^{-1}$ ) followed by OPCRh1 ( $61.63 \text{ mg ml}^{-1}$ ) and OPCRE2 ( $55.59 \text{ mg ml}^{-1}$ ). All three fungal isolates produced IAA and siderophore in significant amounts. Again, the highest amount of siderophore ( $0.122 \text{ } \mu\text{M ml}^{-1}$ ) was produced by *AwOcstreb1*. This endophyte produced  $7.33 \text{ } \mu\text{g ml}^{-1}$  IAA, but the highest amount was produced by OPCRE2 ( $8.97 \text{ } \mu\text{g ml}^{-1}$ ) (Fig. 5).

#### **Seedling stage screening of plant growth promotion in rice**

Based on the PGP performance, the influence of endophytic fungi with multiple PGP activities on rice growth was screened in a pot experiment. All the tested fungi-treated plants showed an increase in shoot length, root length, shoot weight, root weight, and chlorophyll content compared to the uninoculated control in both non-stressed and 100-mM salt-stressed conditions (Fig. S4; Table S1). The rice plants inoculated with *AwOcstreb1* exhibited the highest increase in all tested parameters compared to plants inoculated with the other two endophytes and to uninoculated control plants. A remarkable change in root architecture was observed for

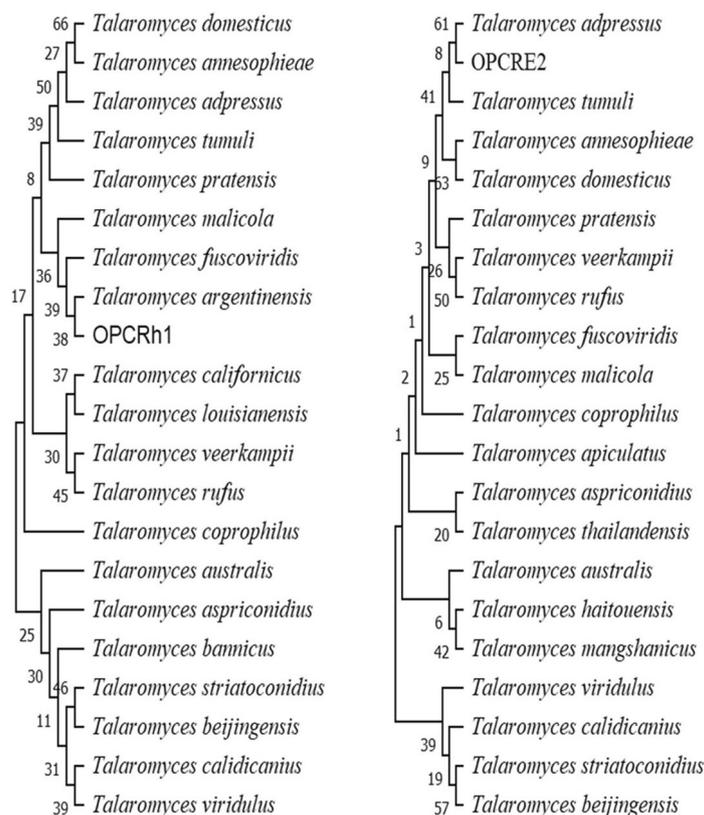


**Fig. 2** MASH Average Nucleotide Identity (ANI). NPCRE2 was placed with *Aspergillus welwitschiae* with > 98 ANI value

*AwOcstreb1*-inoculated plants compared to control and other fungus-inoculated plants both in the absence and presence of salt (Fig. S5). They showed very thick, highly branched, and very long roots, as well as increased root hair, which is a significant indication of plant growth promotion and defense. They also showed comparatively increased tiller numbers, which is an indication of high

yield. Thus, the noticeable effect of *AwOcstreb1* on the growth of the tested rice variety led us to further investigate its effects on the yield of the selected rice plants.

The same experiment was repeated to observe the effect of *AwOcstreb1* on the growth of the same commercial rice by measuring different physiological and biochemical changes related to the salt stress such as Na<sup>+</sup>/



**Fig. 3** NJ phylogenetic tree based on the ITS sequences. OPCRh1 was placed with *Talaromyces argentinensis* (left) and OPCRE2 was placed with *Talaromyces adpressus* (right)

$K^+$  ratio and host defensive system parameters, like  $H_2O_2$  content and electrolyte leakage. Relative expression of one stress-related gene, “SOS1” was also determined.

The presence of inoculated *AwOcstreb1* resulted in a significant increase in root length, shoot length, root weight, and shoot weight of seedlings under both non-stress and saline stress (Fig. 6; Table 2). Without stress, the difference in shoot weight of *AwOcstreb1*-inoculated plants (729.2 mg) was 155.59% ( $p < 0.05$ ) compared to uninoculated control plants (285.3 mg). Significant differences ( $p < 0.05$ ) in root length (by 35.13%), shoot length (by 25.83%), and root weight (by 189.55%) were also exhibited in non-stressed conditions. Similar trends were also observed under saline stress such as 88.10% and 121.54% increase in root weight and shoot weight, respectively.

#### Rice metabolites assessment

We speculated that notable changes in the metabolite content of rice (*O. sativa*) plants might support the rice plant’s survival capacity. The assessment of metabolites of the endophyte-inoculated and uninoculated plants (Fig. 7; Table 2) indicated significant increases ( $p < 0.05$ )

in chlorophyll content in *AwOcstreb1* inoculated plants both in non-stressed and saline-stressed conditions by 37.10% and 47.51%, respectively, over uninoculated control plants. Flavonoid content was also significantly higher in *AwOcstreb1*-treated plants without stress (11.90%) and saline stress (69.73%) compared to uninoculated control plants. Total sugar and phenolic content also increased significantly ( $p < 0.05$ ) by 683.94% and 85.32%, respectively, in *AwOcstreb1* inoculated plants in non-stressed conditions. However, total protein content also increased in *AwOcstreb1*-treated plants without or with salt compared to the uninoculated control. In this study, the endophytic fungus *AwOcstreb1* not only promoted rice plant growth but also enhanced the level of its metabolites, chlorophyll, and flavonoids both without salt and under salt conditions.

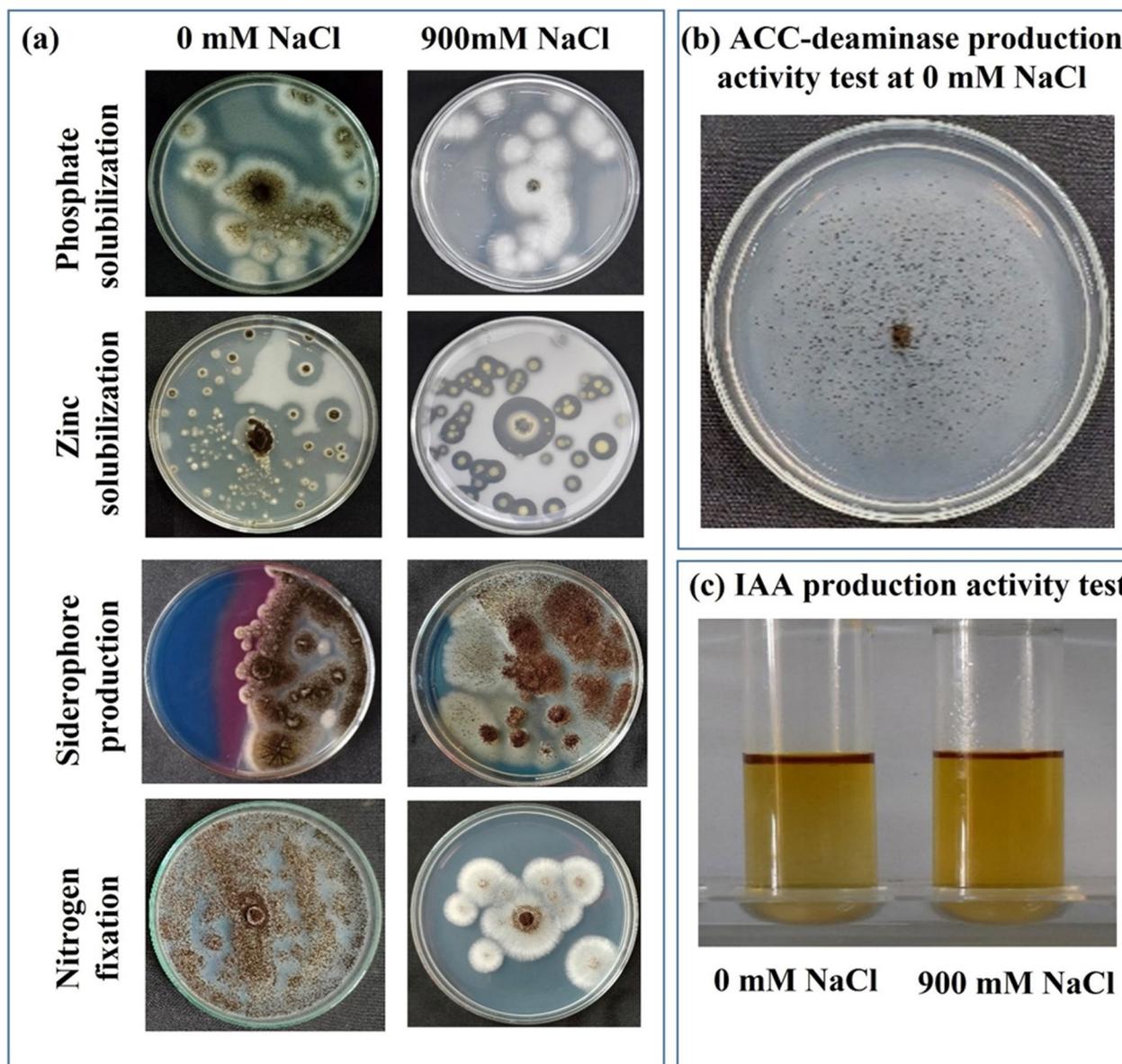
#### Screening of stress-related parameters

For screening defensive responses of host plants conferred by the endophyte *AwOcstreb1*,  $Na^+/K^+$  ratio,  $H_2O_2$  content, and electrolyte leakage were estimated (Fig. 8; Table 2). Relative expression of salt stress-related gene *SOS1* was also determined. Results showed

**Table 1** List of endophytic fungal isolates of *O. coarctata* and their PGP characteristics in without salt and 900-mM salt stress

Isolate	Nitrogen fixation		Phosphate solubilization		Zinc solubilization		IAA production		Siderophore production		ACC deaminase production	
	Without salt	In salt stress	without salt	In salt stress	without salt	In salt stress	without salt	In salt stress	Without salt	In salt stress	Without salt	In salt stress
NPCRE2	+++	++	+++	+++	+++	+++	+	+++	+++	+++	+	+
OPCRh1	+	+	+	+	+++	+	+	+	+	-	-	-
OPCRE2	+++	++	+++	+++	+++	+++	+	+	+	+	+	-

Note: +++ very excellent activity, ++ excellent activity, + good activity, -- negative activity

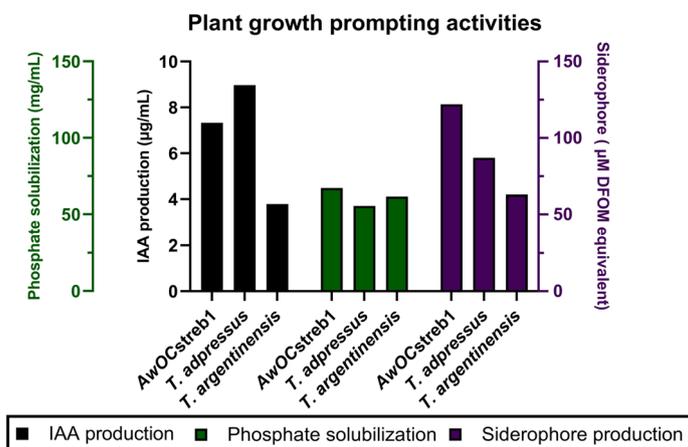


**Fig. 4** Some plant growth promoting (PGP) activities of *AwOcstrebl1* (NPCRE2) tested in vitro. **a** Phosphate solubilization, zinc solubilization, siderophore production, and nitrogen fixation activity at 0 mM and 900 mM NaCl. **b** ACC,- deaminase production activity at 0 mM NaCl. **c** IAA,- production activity at 0- mM and 900- mM NaCl

that  $H_2O_2$  was significantly ( $p < 0.05$ ) lower by 34.70% under salinity stress in *AwOcstrebl1*-inoculated plants which is indicative of better plant health. Electrolyte leakage was also significantly ( $p < 0.05$ ) lower by 17.42% under saline stress in fungus-inoculated plants.  $Na^+/K^+$  ratio in root and shoot was also significantly lower in *AwOcstrebl1*-inoculated plants by 84.25% and 55.44%, respectively, in normal and saline stress. This indicates that the presence of *AwOcstrebl1* in the plant relieved salinity stress.

**Relative expression of SOS1 gene**

The expression of target gene *SOS1* which is essential for plant salt tolerance (Shi et al. 2000) was determined at the 48th hour of salt (100 mm) treatment in both inoculated and uninoculated plants. The expression level of the *SOS1* gene was significantly ( $p < 0.05$ ) higher in *AwOcstrebl1*-inoculated rice plants than in uninoculated plants under saline stress (Fig. 9). These results further confirm that *AwOcstrebl1* not only promotes plant growth but also helps the plant host to fight salinity stress.



**Fig. 5** In vitro quantitative assay phosphate solubilization, siderophore production, and IAA production by fungal isolates at 0- mM NaCl condition

### Reproductive stage screening

The effect of the endophytic fungus *AwOcstrebl* on yield demonstrated that inoculated plants were visually much better than uninoculated plants under both control and saline stress ns (Fig. 10). For example, the roots and panicles of *AwOcstrebl*-inoculated plants looked healthier (Fig. S7 and S8).

Agronomic parameters such as plant height, total tiller number, panicle number, filled grain number, filled grain weight, and yield were determined. The *AwOcstrebl* treatments had a significant effect on the growth and yield of plants under salt-stress conditions. Compared to the control group, the *AwOcstrebl*-treated plants showed a significant ( $p < 0.05$ ) increase in plant height, total tillers, panicle number, filled grain number, % fertility, and yield (Fig. 10; Table 2).

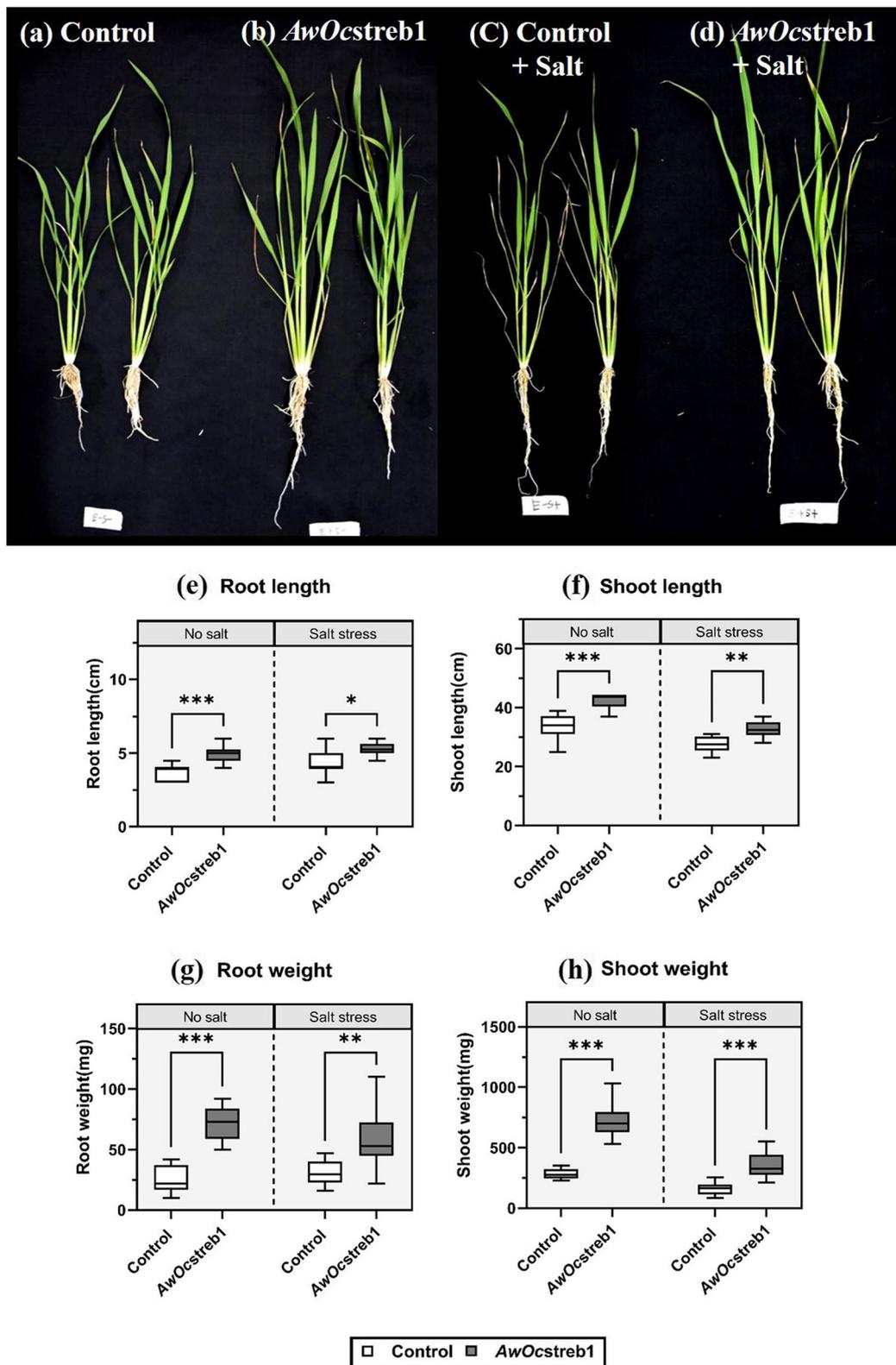
The yield of the uninoculated plants and *AwOcstrebl*-inoculated plants was 1.28 g/plant and 2.9 g/plant without stress, and 0.91 g/plant and 2.76 g/plant under salinity, respectively. The *AwOcstrebl*-treated plants showed a significantly ( $p < 0.05$ ) higher amount of yield compared to the uninoculated plants. The difference in yield was by 125.16% and 203.96% without stress and saline stress conditions, respectively. Overall, the yield data suggest that the *AwOcstrebl* inoculation, despite being under salt stress, produced a significantly higher yield compared to the uninoculated (control) plants without salt stress.

### Discussion

The use of endophytic microorganisms to minimize the impact of abiotic stress is widely acknowledged as a successful strategy for restoring agricultural productivity in challenging environments. Plant growth-promoting fungi (PGPF) benefit their hosts in many ways, causing increased growth, biomass accumulation, and nutrient uptake as well as providing resistance to various stresses (Chitnis et al. 2020). Hence, plants like rice having associated microorganisms may show better growth and higher yields despite the presence of several biotic and abiotic stresses. Ultimately, this reduces the strain on the economy and the environment by producing more food at a lower cost and reducing the need for breeding enhancement and the use of agrochemicals. PGPF can be endophytic, whereby they live inside plant cells and exchange metabolites directly or can be epiphytic, when living freely outside plant cells on the root surface, i.e., in the rhizosphere. We aimed to look for microbial fungi associated with halophytes, in the presumption that they may help the latter survive under high salt stress. Halophytes have evolved diverse physiological and biochemical strategies to survive in saline environments (Khan et al. 2009), establishing symbiotic relationships with halotolerant fungi that aid in their growth and development (Yasmeen et al. 2019). The objective of the study was to discover endophytic fungal strains from the halophyte *O. coarctata* with diverse

(See figure on next page.)

**Fig. 6** Representation of the growth of a rice seedling under normal and salt-stressed conditions upon treatment with NPCRE2 (*AwOcstrebl*) comparing with uninoculated control plants (a-d). Root length (e), shoot length (f), root weight (g), and shoot weight (h) are depicted in the box plots. The boxes on all box plots represent the first and third quartiles, while the lower and upper lines, respectively, denote the minimum and maximum values. The middle black line in each box plot denotes the median. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$



**Fig. 6** (See legend on previous page.)

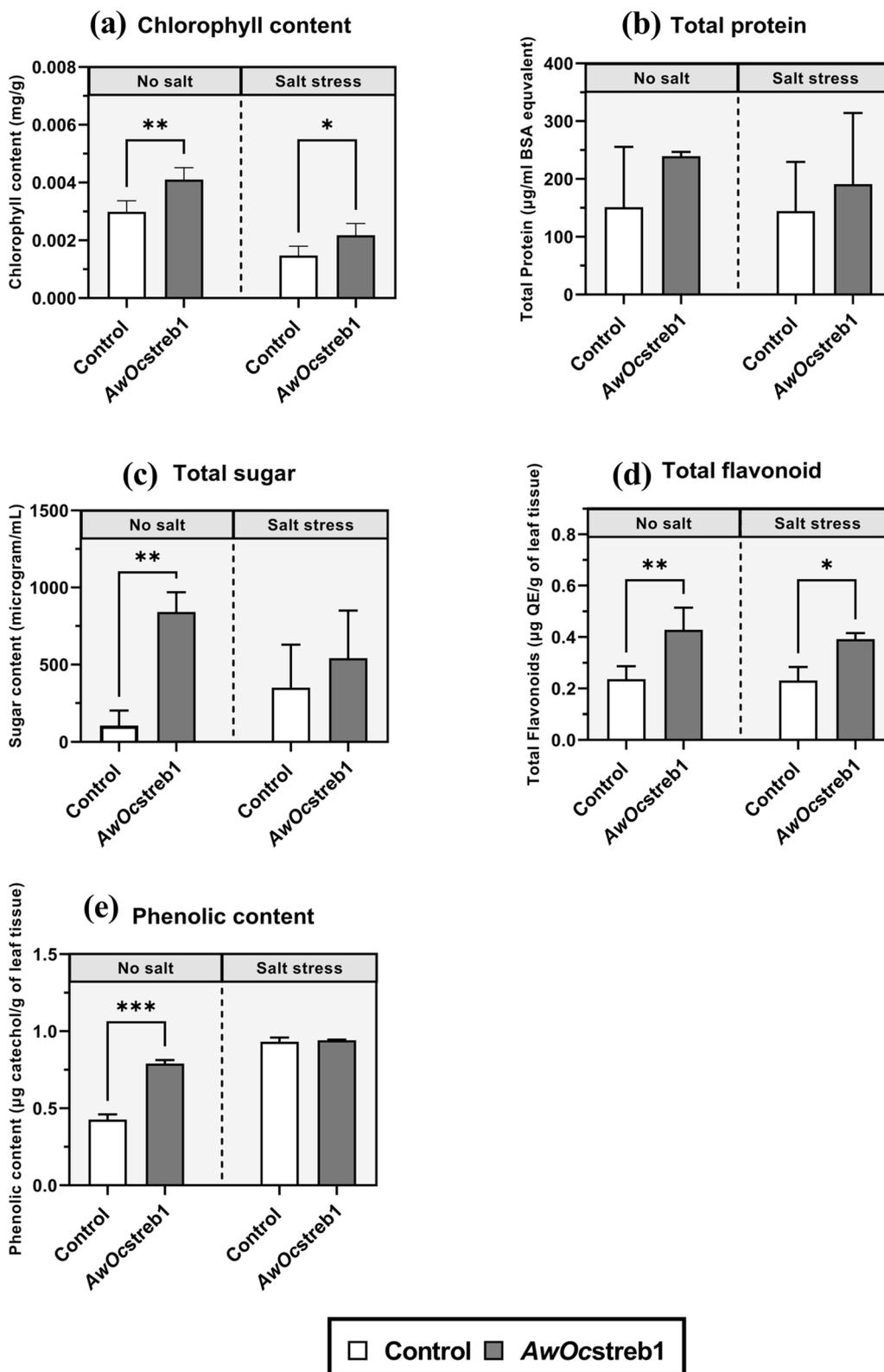
**Table 2** Effect of *AwOcstreb1* inoculation on growth-related parameters, metabolite content, salinity tolerance, and yield parameters under normal and saline stress conditions (100 mM and 45 mM at seedling and reproductive stages, respectively)

	Treatments	Control	<i>AwOcstreb1</i>	Percent change (vs control)	P value
Growth related parameters					
Root length (cm)	No salt	3.7	5	35.13%	<0.001
	Salt stress	4.4	5.3	20.45%	0.01
Shoot length (cm)	No salt	33.6	42.3	25.83%	<0.001
	Salt stress	27.6	32.7	18.47%	0.004
Root weight (mg)	No salt	24.9	72.1	189.55%	<0.001
	Salt stress	31.1	58.5	88.10%	0.002
Shoot weight (mg)	No salt	285.3	729.2	155.59%	<0.001
	Salt stress	162	358.9	121.54%	<0.001
Plant metabolites content					
Chlorophyll content (mg/g)	No salt	0.0029	0.0041	37.10%	0.001
	Salt stress	0.0014	0.0021	47.51%	0.004
Total protein (µg/ml)	No salt	151	239.33	58.49%	NS
	Salt stress	144.33	191	32.33%	NS
Total sugar (µg/ml)	No salt	107.33	841.43	683.94%	0.008
	Salt stress	350.41	541.94	54.66%	NS
Total flavonoids (µg/g)	No salt	0.236	0.427	80.99%	0.007
	Salt stress	0.23	0.391	69.73%	0.02
Phenolic content(µg/g)	No salt	0.426	0.79	85.32%	<0.001
	Salt stress	0.931	0.942	1.16%	NS
Salt stress related parameters					
H <sub>2</sub> O <sub>2</sub> content	No salt	523.57	459.6	(-13.91%)	NS
	Salt stress	623.12	462.56	(-34.7%)	<0.001
Electrolyte leakage	No salt	0.4	0.4	0	NS
	Salt stress	0.86	0.73	(-17.42%)	0.02
Na <sup>+</sup> /K <sup>+</sup> ratio in root	No salt	0.9	0.13	(-547.78%)	NS
	Salt stress	2.89	1.57	(-84.25%)	0.02
Na <sup>+</sup> /K <sup>+</sup> ratio in shoot	No salt	0.038	0.03	(-7.76%)	NS
	Salt stress	1.26	0.81	(-55.44%)	<0.001
Reproductive stage parameters					
Plant height (cm)	No salt	87.33	102.66	17.55%	0.008
	Salt stress	81	93	14.81%	0.02
Total tillering	No salt	4	9.5	137.50%	<0.001
	Salt stress	3.5	7.25	107.14%	<0.001
Panicle number	No salt	3	7	133.33%	0.002
	Salt stress	3.33	6.66	100%	0.02
Filled grain number	No salt	81.33	228.66	181.14%	0.02
	Salt stress	61.66	207.33	236.21%	0.02
% fertility	No salt	47.12	59.02	25.23%	0.01
	Salt stress	41.29	53.89	30.51%	0.001
Yield	No salt	1.28	2.9	125.16%	0.04
	Salt stress	0.91	2.76	203.96%	0.03

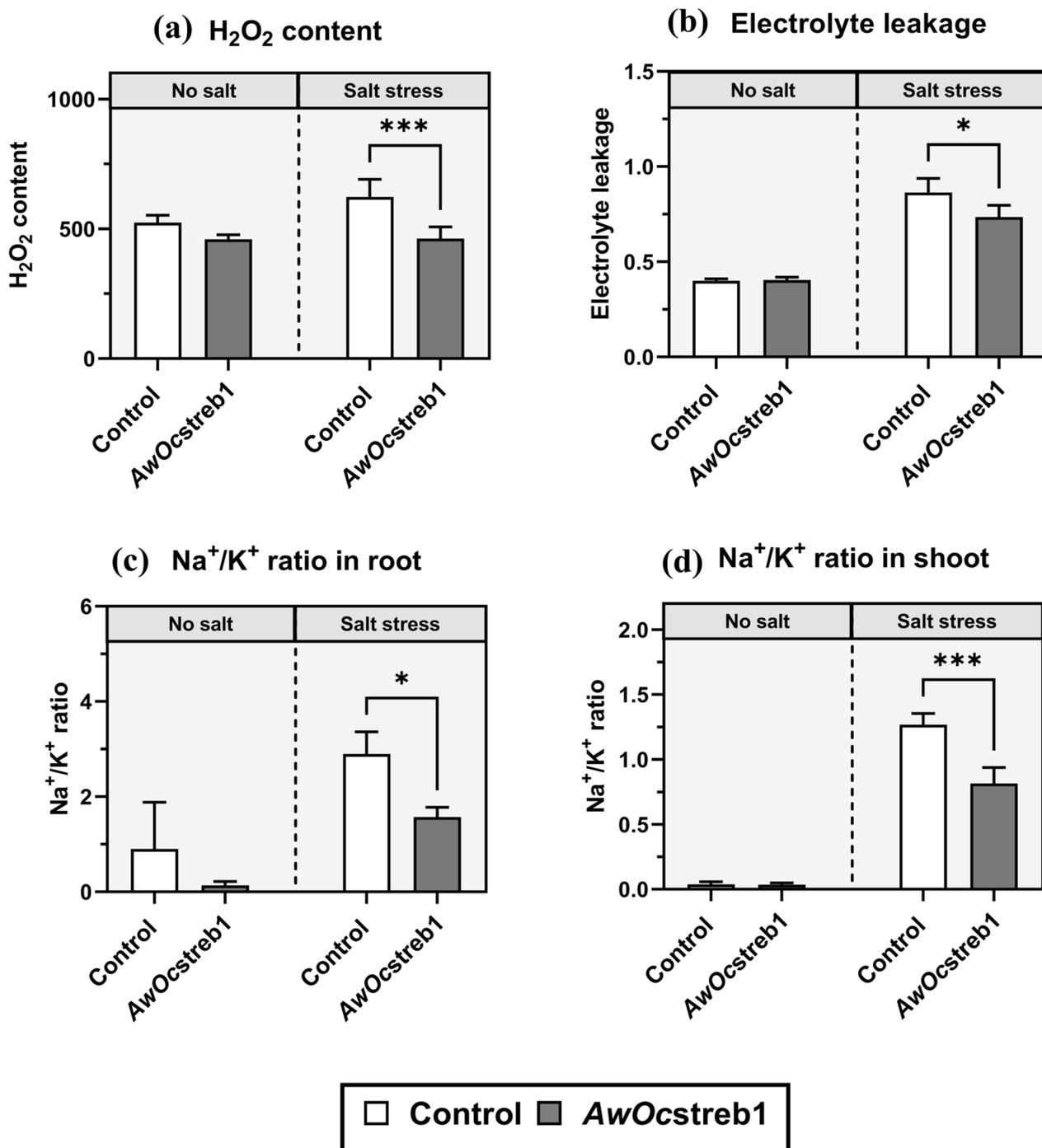
(-) indicates reduction

PGP attributes, such as phosphate and zinc solubilization, phytohormone production, nitrogen fixation, bio-control, and abiotic stress tolerance properties, which

would be crucial to produce a bio-inoculant for improving rice yield under saline stress conditions (Jorquera et al. 2008).



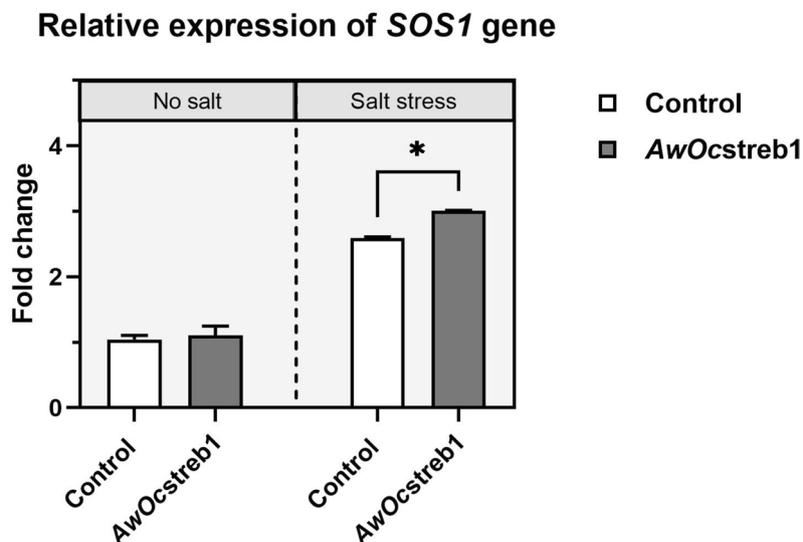
**Fig. 7** Effect of AwOcstre1 treatment on the metabolite content of rice plants under normal and saline stress conditions. **a** Chlorophyll content, **(b)** total protein, **(c)** total sugar, **(d)** total flavonoids, and **(e)** phenolic content. Bars represent mean ± SD. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$



**Fig. 8** Effect of *AwOcstreb1* treatment on the salt-stress- related parameters of rice plants under normal and saline stress conditions. **a** H<sub>2</sub>O<sub>2</sub> content, **b** electrolyte leakage, **c** Na<sup>+</sup>/K<sup>+</sup> ratio in the root, and **d** Na<sup>+</sup>/K<sup>+</sup> ratio in the shoot. Bars represent mean ± SD. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$

We could successfully isolate, characterize, and identify three endophytic fungi from *Oryza coarctata*. The first one, *AwOcstreb1*, was confirmed by whole genome sequencing and phylogenetic analysis based on average

nucleotide identity (ANI). Two more fungi, *Talaromyces adpressus* and *Talaromyces argentinensis*, were verified based on morphological features, sequence of their ITS regions, and phylogenetic analyses.



**Fig. 9** Relative expression of *SOS1* gene in control and *AwOcstreb1* inoculated plants under normal and saline stress conditions. Bars represent mean  $\pm$  SD. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$

Endophytic fungi not only increase plant growth, yield, and stress resistance but can produce phytohormones and secondary metabolites as well. In addition, they can also alter the structure of roots, boost photosynthesis, and accumulate osmo-protectants. These attributes make fungi assist plants in growth and survival under both normal and high-salinity environments (Sheng et al. 2008). Previously, researchers have identified a diverse array of fungal endophytes isolated from plants, all of which exhibit distinct plant growth-promoting (PGP) traits such as nitrogen fixation, zinc and phosphate solubilization, siderophore synthesis, the production of Indole-3-acetic acid (IAA), and ACC deaminase, all of which have been shown to enhance plant growth in both normal and stressful environments (Chitnis et al. 2020; Rehman et al. 2022). All three isolates of this study showed significant nitrogen fixation ability and solubilization of phosphates and zinc even under high salt (900 mM) stress. *AwOcstreb1* showed all of the PGP activities itemized above, which is indicative of its strong effect on the improvement of plant fertility growth and restoration under salt stress.

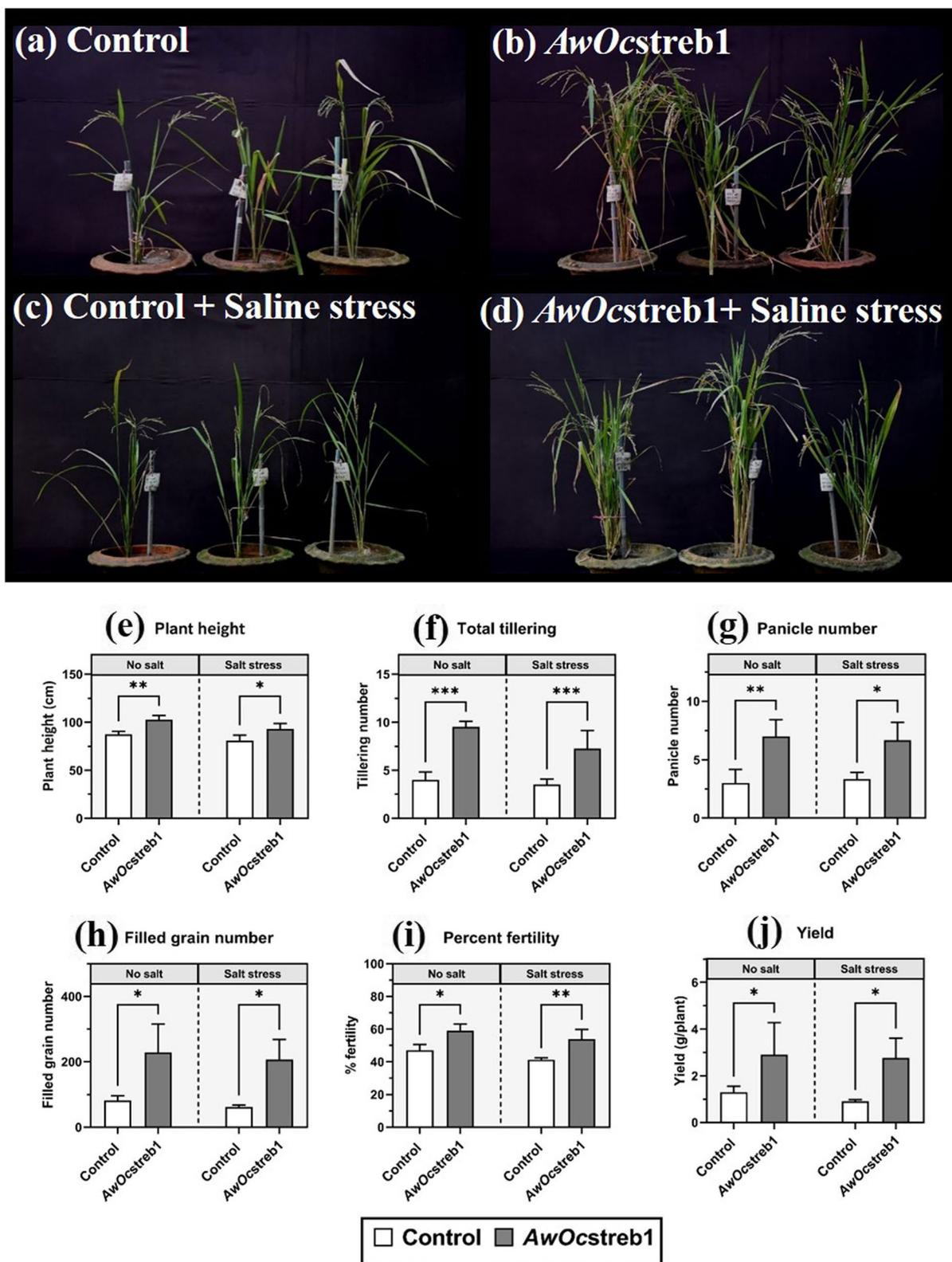
This study found a significant increase in shoot length, root length, shoot weight, root weight, and chlorophyll content at the seedling stage of all three fungi-inoculated

plants. Among them, *AwOcstreb1* inoculated plants showed the best result. Similarly, in a prior study, other species of *Taloromyces* genus have demonstrated plant growth promotion (Halo et al. 2020; Sun et al. 2022). In other studies, *Aspergillus welwitschiae* has been reported to improve the growth of *Glycine max* under heavy metal toxicity (Hussain et al. 2021). Previous reports have shown that inoculation of maize plants with the endophytic fungus *Aspergillus welwitschiae* BK, improved plant growth, and influenced metabolite production under saline stress conditions at the seedling stage (Gul et al. 2023). In this study, we observed growth parameters not only at the seedling but at the reproductive stage as well. At the latter stage, we have reported a higher yield of *AwOcstreb1* inoculated plants in both stressed and non-stressed conditions. In accordance with these results, our findings suggested that inoculation of rice plants with *AwOcstreb1* can enhance plant growth at all major developmental phases under both normal and saline stress conditions.

The primary mechanism driving plant growth promotion (PGP) involves the production of phytohormones, with auxin (IAA) being particularly crucial. Low concentrations of IAA stimulate primary root elongation,

(See figure on next page.)

**Fig. 10** Effect of *AwOcstreb1* on the reproductive stage of rice plant under normal and saline stress conditions. (a-d) The comparison of *AwOcstreb1*-treated plants with control in pots. The bar plots showing the changes in (d) plant height, (e) total tillering, (f) panicle number, (g) filled grain number, (h) percent fertility, and (i) yield of control and *AwOcstreb1*-inoculated plants under without and with salt-stress conditions. Bars represent mean  $\pm$  SD. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$



**Fig. 10** (See legend on previous page.)

while higher concentrations promote lateral and adventitious root development (Duca et al. 2014). Studies have demonstrated that IAA-producing fungi, like *A. welwitschiae*, can significantly increase endogenous IAA levels in plants (Hussain et al. 2021). Additionally, microbes producing IAA play a vital role in regulating root architecture parameters, promoting plant growth, and maintaining biomass, especially during stress conditions (Ambreetha et al. 2018).

In our study, *AwOcstreb1* stood out for its comparatively higher IAA production, leading to a remarkable increase in lateral roots and root hairs in treated rice plants. This resulted in enhanced root weight and length. Manipulating plant growth hormones, therefore, proved to be valuable in protecting plants from environmental stressors.

Furthermore, *AwOcstreb1* was found to produce ACC deaminase, a crucial enzyme for PGsP under salt stress. This enzyme hydrolyzes ACC, a precursor to ethylene production, into useful components, preventing ethylene-induced harm to root growth (Honma and Shimomura 1978). Studies have consistently shown that PGP microbes producing ACC deaminase enhance plant growth, particularly under stressful conditions (Bal et al. 2013). Reduction in ethylene has also been shown to increase yields and drought tolerance in maize plants (Shi et al. 2017). Our findings align with this, indicating that *AwOcstreb1* treatment leads to improved root growth, yield, and salinity stress resistance in rice plants.

Overall, plants inoculated with *AwOcstreb1*, producing both IAA and ACC deaminase, not only shield plants from stress but also promote robust root growth, facilitating enhanced nutrient and mineral uptake.

Saline conditions result in changes in the net photosynthesis and stomatal conductance as a result of damage to the photosynthetic machinery (Yasmeen et al. 2019). Salt stress induces an increase in the production of ROS, such as  $H_2O_2$  in plants, resulting in oxidative damage, and disrupting the balance within cells leading to membrane leakage (Tuna et al. 2008). In rice cultivated in high salt conditions, membrane deterioration due to ROS has been reported, leading to cellular toxicity (Kim et al. 2005). The synthesis of flavonoids and phenolics, which are important antioxidants, reduction of ROS in plant cells, and protection of plants from stress, is heavily reliant on chlorophyll molecules (Ghasemzadeh et al. 2010). The results of this study demonstrated a noteworthy rise in the levels of antioxidants, i.e., phenolic and flavonoid compounds, as well as a considerable reduction in  $H_2O_2$  content and electrolyte leakage in rice seedlings that were inoculated with PGPEF. This study also showed increased sugar content in PGPEF-inoculated plants, which is important for regulating genes related to photosynthesis,

metabolism, and osmotic stress (Rosa et al. 2009). Similarly, Gul et al. (2023) also observed higher levels of total sugar, total proteins, total flavonoids, and phenolic content in maize plants treated with *Aspergillus welwitschiae* BK. Their finding corroborates the results of the current study.

Salinity-induced elevation in  $Na^+$  concentrations leads to an increase in the  $Na^+/K^+$  ratio, which can inhibit cytosolic activities, including photosynthesis and respiration (Bhat et al. 2020). Root and shoot  $Na^+/K^+$  ratio significantly decreased in *AwOcstreb1* inoculated plants under salinity stress in our study. A low  $Na^+/K^+$  ratio was also observed in *Aspergillus welwitschiae* BK inoculated maize plants under salinity stress (Gul et al. 2023). Furthermore, in agreement with our results, Kord et al. (2019) and Abdelaziz et al. (2019) reported that the application of *P. indica* reduced the concentration of  $Na^+$  and increased the level of  $K^+$  in rice, tomato, and barley plants experiencing salt stress. The current study showed that *OsSOS1* gene expression was significantly higher in fungus-inoculated plants compared to uninoculated control under salt stress conditions. It has been proposed that *SOS1* facilitates cellular signaling to maintain ion ( $Na^+$ ) homeostasis under conditions of salt stress and its increased expression has already been reported to enhance salt tolerance in *Arabidopsis* and rice plants (Shi et al. 2003; Yasmin et al. 2015). In their study, Jaemsaeng et al. (2018) reported that ACCD-producing endophytic *Streptomyces* sp. GMKU 336 promoted growth and protected salt-sensitive *Oryza sativa* L. cv. KDML105 from salt stress damage by inducing overexpression of several genes including *OsSOS1*, which aligns with our results.

## Conclusion

Saline conditions can have a negative impact on various agronomic and biochemical parameters in rice plants, which is caused to a large extent by an increase in the concentration of sodium ( $Na^+$ ) ions. Our research has shown that rice plants associated with PGPEF, *Aspergillus welwitschiae* *Ocstreb1* (*AwOcstreb1*), *Talaromyces adpressus*, and *Talaromyces argentinensis*, are less affected by salinity stress. This study also marks *AwOcstreb1* as a novel endophyte strain from *O. coarctata* with its remarkable growth promotion abilities. It is noteworthy that this fungus was found only in the roots of freshly collected *O. coarctata* from the coast and not from samples growing in the net house for over a year. Furthermore, *AwOcstreb1*-inoculated plants exhibit a significant improvement in physiological traits, stress-mitigating metabolites, antioxidant potential, lateral root, root hair, and yield than the other two isolated endophytic fungi-treated plants, both in the absence and presence of salinity stress. The increased expression

of the *OsSOS1* gene in PGPEF-associated plants may be another reason for salt stress tolerance and regulation of  $\text{Na}^+$  ions homeostasis under salt stress conditions. Therefore, the halotolerant PGPEF from *O. coarctata*, and in particular *AwOdstreb1*, can be used as a biofertilizer to increase rice crop growth, particularly in soils affected by high salinity such as in coastal regions.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13213-023-01738-3>.

**Additional file 1: Table S1.** Effect of NPCRE2, OPCRE2 and OPCRh1 treatments on the growth of rice seedlings.

**Additional file 2: Fig. S1.** Gel electrophoresis of genomic DNA extracted from OPCRh1, OPCRE2, and NPCRE2. **Fig. S2.** Gel electrophoresis of amplified PCR product of NPCRE2, OPCRh1, and OPCRE2 using 1kb plus DNA ladder. **Fig. S3.** Plant growth promoting activities of endophytic fungus *Talaromyces adpressus* (OPCRE2) (left) and *Talaromyces argentinensis* (OPCRh1) (right) at 0 mM and 900 mM NaCl stress. **Fig. S4.** Representation of the growth of a rice seedling under normal and salt-stressed conditions upon treatment with (b) NPCRE2 (*AwOdstreb1*), (c) OPCRE2 (*Talaromyces argentinensis*), (d) OPCRh1 (*Talaromyces adpressus*) comparing with (a) Control (un-inoculated plants). Root length (e), Shoot length (f), Root weight (g), and Shoot weight (h) are depicted in the box plots. The boxes on all box plots represent the first and third quartiles, while the lower and upper lines, respectively, denote the minimum and maximum values. The middle black line in each box plot denotes the median. The column bar graphs represent total chlorophyll content (i). Bars represent mean  $\pm$  SD. One-way ANOVA was done for all parameters separately. Significant differences between control and treated plants were indicated by asterisks (\*, \*\*, \*\*\*) at  $p \leq 0.05$ . **Fig. S5.** Comparison of root of 28 days old *AwOdstreb1* treated rice plants with uninoculated control plants. **Fig. S6.** Comparison of root of 140 days old *AwOdstreb1* treated rice plants with uninoculated control plants. **Fig. S7.** Comparison of 35 days old *AwOdstreb1* treated rice plants with uninoculated control plants at normal condition. **Fig. S8.** Comparison of the panicles of *AwOdstreb1* treated rice plants with uninoculated control plants at normal and saline stress condition. **Fig. S9.** Confirmation of colonization of *Talaromyces adpressus* (up), *AwOdstreb1* (middle), and *Talaromyces argentinensis* (down) in the roots of BR-28 rice by root colonization assay.

## Acknowledgements

We extend our gratitude to ADA Shahinuzzaman and Mst Nadira Begum for their help in endophyte isolation and to Md. Hossain Sohrab for providing laboratory facilities that were specifically tailored for fungal studies at the Pharmaceutical Science Research Division (PSRD) Laboratory of the Bangladesh Council of Scientific and Industrial Research. We also express immense thanks to Ms. Tomalika Azim for her kind assistance during the whole study.

## Authors' contributions

AAA and MIA did the isolation, identification, characterization of growth-promoting traits, physiological screening, statistical analysis, and manuscript writing. MRI and RAB helped in the study design and edited the manuscript. ZIS supervised and designed the study, arranged funds, and helped in writing and editing the manuscript. All authors read and approved the final manuscript.

## Funding

High appreciation to The Bangladesh Climate Change Trust (BCCT), Government of the People's Republic of Bangladesh, for funding this work.

## Availability of data and materials

The ITS sequences of OPCRE2 and OPCRh1 can be found in the GenBank repository with accession numbers OQ785901 and OQ785903, respectively.

The WGS data of *AwOdstreb1* will soon be shared in another manuscript containing the genomic study of *AwOdstreb1* which is under preparation. The data has been deposited on the GeneBank repository with BioProject accession number PRJNA985106.

## Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

Received: 13 June 2023 Accepted: 26 September 2023

Published online: 14 October 2023

## References

- Abdelaziz ME, Abdelsattar M, Abdeldaym EA et al (2019) Piriformospora indica alters  $\text{Na}^+/\text{K}^+$  homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Sci Hortic (amsterdam)* 256:108532
- Afridi MS, Mahmood T, Salam A et al (2019) Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: involvement of ACC deaminase and antioxidant enzymes. *Plant Physiol Biochem* 139:569–577
- Ainsworth EA, Gillespie KM (2007) Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin-Ciocalteu reagent. *Nat Protoc* 2:875–877
- Alexander DB, Zuberer DA (1991) Use of chrome azurol S reagents to evaluate siderophore production by rhizosphere bacteria. *Biol Fertil Soils* 12:39–45
- Ambreetha S, Chinnadurai C, Marimuthu P, Balachandrar D (2018) Plant-associated *Bacillus* modulates the expression of auxin-responsive genes of rice and modifies the root architecture. *Rhizosphere* 5:57–66
- Arnold AE (2007) Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol Rev* 21:51–66
- Arvouet-Grand A, Vennat B, Pourrat A, Legret P (1994) Standardization of propolis extract and identification of principal constituents. *J Pharm Belg* 49:462–468
- Awika JM (2011) Major cereal grains production and use around the world. In: *Advances in cereal science: implications to food processing and health promotion*. ACS Publications, pp 1–13
- Baker DD, Mullin BC (1994) Diversity of Frankia nodule endophytes of the actinorhizal shrub *Ceanothus* as assessed by RFLP patterns from single nodule lobes. *Soil Biol Biochem* 26:547–552
- Bal HB, Das S, Dangar TK, Adhya TK (2013) ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. *J Basic Microbiol* 53:972–984
- Bapiri A, Asgharzadeh A, Mujallali H, et al (2012) Evaluation of Zinc solubilization potential by different strains of Fluorescent Pseudomonads. *J Appl Sci Environ Manag* 16:
- Bhat MA, Kumar V, Bhat MA et al (2020) Mechanistic insights of the interaction of plant growth-promoting rhizobacteria (PGPR) with plant roots toward enhancing plant productivity by alleviating salinity stress. *Front Microbiol* 11:1952
- Boston K (2016) The potential effects of forest roads on the environment and mitigating their impacts. *Curr for Reports* 2:215–222
- Chen AJ, Sun BD, Houbraken J et al (2016) New *Talaromyces* species from indoor environments in China. *Stud Mycol* 84:119–144
- Chitnis VR, Suryanarayanan TS, Nataraja KN et al (2020) Fungal endophyte-mediated crop improvement: the way ahead. *Front Plant Sci* 11:561007
- Cui M, Wu D, Bao K et al (2019) Dynamic changes of phenolic compounds during artificial aging of soybean seeds identified by high-performance liquid chromatography coupled with transcript analysis. *Anal Bioanal Chem* 411:3091–3101

- Dasgupta S, Hossain MM, Huq M, Wheeler D (2018) Climate change, salinization and high-yield rice production in coastal Bangladesh. *Agric Resour Econ Rev* 47:66–89
- Deshwal VK, Kumar P (2013) Production of plant growth promoting substance by Pseudomonads. *J Acad Ind Res* 2:221–225
- Devi NN, Prabakaran JJ (2014) Bioactive metabolites from an endophytic fungus *Penicillium* sp. isolated from *Centella asiatica*. *Curr Res Env Appl Mycol* 4:34–43
- Duca D, Lorv J, Patten CL et al (2014) Indole-3-acetic acid in plant-microbe interactions. *Antonie Van Leeuwenhoek* 106:85–125
- Dworkin M, Foster J (1958) Experiments with some microorganisms which utilize ethane and hydrogen. *J Bacteriol* 75:592–603
- Garg R, Verma M, Agrawal S et al (2014) Deep transcriptome sequencing of wild halophyte rice, *Porteresia coarctata*, provides novel insights into the salinity and submergence tolerance factors. *DNA Res* 21:69–84
- Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Synthesis of phenolics and flavonoids in ginger (*Zingiber officinale* Roscoe) and their effects on photosynthesis rate. *Int J Mol Sci* 11:4539–4555
- Gordon SA, Weber RP (1951) Colorimetric estimation of indoleacetic acid. *Plant Physiol* 26:192
- Gul H, Ali R, Rauf M, et al (2023) *Aspergillus welwitschiae* BK isolate ameliorates the physicochemical characteristics and mineral profile of maize under salt stress. *Plants* 12: <https://doi.org/10.3390/plants12081703>
- Gupta M, Kiran S, Gulati A et al (2012) Isolation and identification of phosphate solubilizing bacteria able to enhance the growth and aloin-a biosynthesis of *Aloe barbadensis* Miller. *Microbiol Res* 167:358–363
- Halo BA, Al-Yahyai RA, Al-Sadi AM (2020) An endophytic *Talaromyces omanensis* enhances reproductive, physiological and anatomical characteristics of drought-stressed tomato. *J Plant Physiol* 249:153163. <https://doi.org/10.1016/j.jplph.2020.153163>
- Honma M, Shimomura T (1978) Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agric Biol Chem* 42:1825–1831
- Hussain A, Shah M, Hamayun M, et al (2021) Heavy metal tolerant endophytic fungi *Aspergillus welwitschiae* improves growth, ceasing metal uptake and strengthening antioxidant system in *Glycine max* L. *Environ Sci Pollut Res* 1–15
- Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll a and b in N, N-dimethylformamide and 80% acetone. *Plant Physiol* 77:483–485
- Jaemsaeng R, Jantasuriyarat C, Thamchaipenet A (2018) Molecular interaction of 1-aminocyclopropane-1-carboxylate deaminase (ACCD)-producing endophytic *Streptomyces* sp. GMKU 336 towards salt-stress resistance of *Oryza sativa* L. cv. KDML105. *Sci Rep* 8:1950
- Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. *CRC Crit Rev Plant Sci* 30:435–458
- Jan B, Reshi ZA, Mohiddin FA (2021) Role of fungal endophytes in improving abiotic stress tolerance in plants. In: *Plant-microbe dynamics: recent advances for sustainable agriculture*. CRC Press, pp 151–164
- Jorquera MA, Hernández MT, Rengel Z et al (2008) Isolation of culturable phosphobacteria with both phytate-mineralization and phosphate-solubilization activity from the rhizosphere of plants grown in a volcanic soil. *Biol Fertil Soils* 44:1025–1034
- Kang S-M, Khan AL, Waqas M et al (2014) Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *J Plant Interact* 9:673–682
- Khan MA, Ansari R, Ali H et al (2009) *Panicum turgidum*, a potentially sustainable cattle feed alternative to maize for saline areas. *Agric Ecosyst Environ* 129:542–546
- Kim S-Y, Lim J-H, Park M-R et al (2005) Enhanced antioxidant enzymes are associated with reduced hydrogen peroxide in barley roots under saline stress. *BMB Rep* 38:218–224
- Kord H, Fakheri B, Ghabooli M et al (2019) Salinity-associated microRNAs and their potential roles in mediating salt tolerance in rice colonized by the endophytic root fungus *Piriformospora indica*. *Funct Integr Genomics* 19:659–672
- Kusari S, Lamshöft M, Zühlke S, Spiteller M (2008) An endophytic fungus from *Hypericum perforatum* that produces hypericin. *J Nat Prod* 71:159–162
- Lanza M, Haro R, Conchillo LB, Benito B (2019) The endophyte *Serendipita indica* reduces the sodium content of *Arabidopsis* plants exposed to salt stress: fungal ENA ATPases are expressed and regulated at high pH and during plant co-cultivation in salinity. *Environ Microbiol* 21:3364–3378
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Lubna AS, Hamayun M et al (2018) *Aspergillus niger* CSR3 regulates plant endogenous hormones and secondary metabolites by producing gibberellins and indoleacetic acid. *J Plant Interact* 13:100–111
- Machuca A, Milagres AMF (2003) Use of CAS-agar plate modified to study the effect of different variables on the siderophore production by *Aspergillus*. *Lett Appl Microbiol* 36:177–181
- Maheshwari R, Bhutani N, Suneja P (2020) Isolation and characterization of ACC deaminase producing endophytic *Bacillus mojavensis* PRN2 from *Pisum sativum*. *Iran J Biotechnol* 18:e2308
- Majeed A, Nisar MF, Hussain K (2010) Effect of saline culture on the concentration of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in *Agrostis tolonifera*. *Curr Res J Biol Sci* 2:76–82
- Mandal UK, Burman D, Mahanta KK et al (2015) Bulk soil electrical conductivity for coastal salt affected soils of West Bengal. *J Indian Soc Coast Agric Res* 33:11–18
- Martin KJ, Rygielwicz PT (2005) Fungal-specific PCR primers developed for analysis of the ITS region of environmental DNA extracts. *BMC Microbiol* 5:1–11
- Mia S, Ahmed NU, Islam MZ et al (2022) Genetic diversity and yield performance among T. Aman rice (*Oryza sativa* L.) landraces in Barishal region of Bangladesh. *J Crop Sci Biotechnol* 25:123–132. <https://doi.org/10.1007/s12892-021-00117-1>
- Nanjundappa M, Manjunatha N, Li H, et al (2021) Fungal endophytes from salt-adapted plants confer salt tolerance and promote growth in Wheat (*Triticum aestivum* L.) at early seedling stage. *bioRxiv* 2012–2021
- OECD P (2015) OECD-FAO agricultural outlook 2015–2024
- Parvin S, Biswas S, Razzaque S et al (2015) Salinity and drought tolerance conferred by in planta transformation of SNAC1 transcription factor into a high-yielding rice variety of Bangladesh. *Acta Physiol Plant* 37:1–12
- Peterson SW, Jurjević Ž (2019) The *Talaromyces pinophilus* species complex. *Fungal Biol* 123:745–762
- Pitman MG, Läuchli A (2002) Global impact of salinity and agricultural ecosystems. *Salin Environ* 3–20
- Potshangbam M, Devi SI, Sahoo D, Strobel GA (2017) Functional characterization of endophytic fungal community associated with *Oryza sativa* L. and *Zea mays* L. *Front Microbiol* 8:325
- Rehman B, Javed J, Rauf M et al (2022) ACC deaminase-producing endophytic fungal consortia promotes drought stress tolerance in *M. oleifera* by mitigating ethylene and H<sub>2</sub>O<sub>2</sub>. *Front Plant Sci* 13:1–22. <https://doi.org/10.3389/fpls.2022.967672>
- Rosa M, Prado C, Podazza G et al (2009) Soluble sugars: metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal Behav* 4:388–393
- Sadananda TS, Govindappa M, Ramachandra YL (2014) In vitro antioxidant activity of lectin from different endophytic fungi of *Viscum album* L. *Br J Pharm Res* 4:626
- Salehin M, Chowdhury MMA, Clarke D, et al (2018) Mechanisms and drivers of soil salinity in coastal Bangladesh. *Ecosyst Serv Well-Being Deltas Integr Assess Policy Anal* 333–347
- Sarkar A, Ghosh PK, Pramanik K et al (2018) A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Res Microbiol* 169:20–32
- Schulz B, Boyle C, Draeger S et al (2002) Endophytic fungi: a source of novel biologically active secondary metabolites. *Mycol Res* 106:996–1004
- Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. *Anal Biochem* 160:47–56
- Sheng M, Tang M, Chen H et al (2008) Influence of arbuscular mycorrhizae on photosynthesis and water status of maize plants under salt stress. *Mycorrhiza* 18:287–296
- Shi H, Ishitani M, Kim C, Zhu JK (2000) The *Arabidopsis thaliana* salt tolerance gene SOS1 encodes a putative Na<sup>+</sup>/H<sup>+</sup> antiporter. *Proc Natl Acad Sci U S A* 97:6896–6901. <https://doi.org/10.1073/pnas.120170197>
- Shi H, Lee B, Wu S-J, Zhu J-K (2003) Overexpression of a plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter gene improves salt tolerance in *Arabidopsis thaliana*. *Nat Biotechnol* 21:81–85
- Shi J, Gao H, Wang H et al (2017) ARGOS8 variants generated by CRISPR-Cas9 improve maize grain yield under field drought stress conditions. *Plant Biotechnol J* 15:207–216. <https://doi.org/10.1111/pbi.12603>
- Silva DM, Batista LR, Rezende EF et al (2011) Identification of fungi of the genus *Aspergillus* section *nigri* using polyphasic taxonomy. *Brazilian J Microbiol* 42:761–773

- Streatfield PK, Karar ZA (2008) Population challenges for Bangladesh in the coming decades. *J Health Popul Nutr* 26:261
- Suebrasri T, Harada H, Jogloy S et al (2020) Auxin-producing fungal endophytes promote growth of sunchoke. *Rhizosphere* 16:100271
- Sun XR, Xu MY, Kong WL, et al (2022) Fine identification and classification of a novel beneficial *talaromyces* fungal species from masson pine rhizosphere soil. *J Fungi* 8. <https://doi.org/10.3390/jof8020155>
- Tisarum R, Theerawitaya C, Samphumphuang T, et al (2020) Alleviation of salt stress in upland rice (*Oryza sativa* L. ssp. *indica* cv. Leum Pua) using arbuscular mycorrhizal fungi inoculation. *Front Plant Sci* 11:348
- Tuna AL, Kaya C, Dikilitas M, Higgs D (2008) The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environ Exp Bot* 62:1–9
- Velikova V, Yordanov I, Edreva A (2000) Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. *Plant Sci* 151:59–66
- Waqas M, Khan AL, Kamran M et al (2012) Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules* 17:10754–10773
- Yamane K, Mitsuya S, Kawasaki M et al (2009) Antioxidant capacity and damages caused by salinity stress in apical and basal regions of rice leaf. *Plant Prod Sci* 12:319–326
- Yasmeen T, Tariq M, Iqbal S, et al (2019) Ameliorative capability of plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) against salt stress in plant. *Plant Abiotic Stress Toler Agron Mol Biotechnol Approaches* 409–448
- Yasmin F, Biswas S, Jewel GMNA et al (2015) Constitutive overexpression of the plasma membrane Na<sup>+</sup>/H<sup>+</sup> antiporter for conferring salinity tolerance in rice. *Plant Tissue Cult Biotechnol* 25:257–272
- Zhang S, Gan Y, Xu B (2016) Application of plant-growth-promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front Plant Sci* 7:1405
- Zhang Y, Yu X, Zhang W et al (2019) Interactions between endophytes and plants: beneficial effect of endophytes to ameliorate biotic and abiotic stresses in plants. *J Plant Biol* 62:1–13

### Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Ready to submit your research? Choose BMC and benefit from:

- fast, convenient online submission
- thorough peer review by experienced researchers in your field
- rapid publication on acceptance
- support for research data, including large and complex data types
- gold Open Access which fosters wider collaboration and increased citations
- maximum visibility for your research: over 100M website views per year

At BMC, research is always in progress.

Learn more [biomedcentral.com/submissions](https://biomedcentral.com/submissions)

