FEMS Microbes, 2024, 5, xtae012
DOI: 10.1093/femsmc/xtae012
Advance access publication date: 29 April 2024
Research Article – Microbes & Environment

Fungal endophytes from saline-adapted shrubs induce salinity stress tolerance in tomato seedlings

Priscillar Mumo Mutungi[1,2,*], Vitalis Wafula Wekesa[3], Justus Onguso[1], Erustus Kanga[4], Steve B. S. Baleba[5], Hamadi Iddi Boga[1]

[1]Jomo Kenyatta University of Agriculture and Technology, Institute for Biotechnology Research, P.O. Box 62000–00200, Nairobi, Kenya
[2]Wildlife Research and Training Institute, Research, Development and Coordination, P.O. Box 842–20117, Naivasha, Kenya
[3]Bioline Agrosciences Africa Limited, Production, P.O. Box 1927–20117, Naivasha, Kenya
[4]Kenya Wildlife Service, P.O. Box 40241–00100, Nairobi, Kenya
[5]Department of Evolutionary Neuroethology, Max Planck Institute of Chemical Ecology, Hans-Knöll-Straße 8, D-07745 Jena, Germany
*Corresponding author. Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000–00200, Nairobi, Kenya. E-mail: priscillahm89@gmail.com
Editor: [Jana Jass]

Abstract

To meet the food and feed demands of the growing population, global food production needs to double by 2050. Climate change-induced challenges to food crops, especially soil salinization, remain a major threat to food production. We hypothesize that endophytic fungi isolated from salt-adapted host plants can confer salinity stress tolerance to salt-sensitive crops. Therefore, we isolated fungal endophytes from shrubs along the shores of saline alkaline Lake Magadi and evaluated their ability to induce salinity stress tolerance in tomato seeds and seedlings. Of 60 endophytic fungal isolates, 95% and 5% were from *Ascomycetes* and *Basidiomycetes* phyla, respectively. The highest number of isolates (48.3%) were from the roots. Amylase, protease and cellulase were produced by 25, 30 and 27 isolates, respectively; and 32 isolates solubilized phosphate. Only eight isolates grew at 1.5 M NaCl. Four fungal endophytes (*Cephalotrichum cylindricum*, *Fusarium equiseti*, *Fusarium falciforme* and *Aspergilus puniceus*) were tested under greenhouse conditions for their ability to induce salinity tolerance in tomato seedlings. All four endophytes successfully colonized tomato seedlings and grew in 1.5 M NaCl. The germination of endophyte-inoculated seeds was enhanced by 23%, whereas seedlings showed increased chlorophyll and biomass content and decreased hydrogen peroxide content under salinity stress, compared with controls. The results suggest that the the four isolates can potentially be used to mitigate salinity stress in tomato plants in salt-affected soils.

Keywords: biotechnology; endophyte; fungi; Lake Magadi; salinity stress; tomato

Introduction

Soil salinity is a major abiotic stress that affects individual plant growth and development and influences the diversity of plant species in affected soils, except those in salt-tolerant plant communities (Bandel et al. 2022). Soil salinity is caused either by natural processes, such as rock weathering and high evapotranspiration, or man-made processes such as irrigation using brackish water in farmlands (Jones et al. 2012) and continuous growth of shallow-rooted crops that raise the water table. The effects of salinity on plants are exacerbated by climate change that can seriously change water cycles through changing patterns of rainfall and prolonged droughts (FAO 2021).

Lands available for agriculture have declined by 22% over the last decade, while land under irrigation has almost doubled within the same period (FAO 2021). However, the expanded irrigated lands face challenges as more than one-third of the global irrigated land is already degraded by induced salinity, while most staple crops consumed by humans are sensitive to moderately tolerant to salt (Cheeseman 2015). The Food and Agriculture Organization has estimated the need to increase agricultural productivity by 50% by 2050 to meet the demands of the growing population (FAO 2021).

Irrigated agriculture continues to play an important role in meeting the food needs of the world's population. Soil salinization, particularly resulting from irrigation and extreme weather conditions, is expected to increase and thereby continue to threaten food security in the future, especially in lands with arid and semi-arid climates, where there is a rising demand for irrigation water to support agricultural production (Tnay 2019).

Efforts have been put in place in the last three decades to understand the mechanisms of salt stress tolerance in plants, especially in halophytes (Zhao et al. 2020). Several physiological, metabolic and molecular mechanisms are used by plants to mitigate salinity stress, and these can be used to engineer crops with enhanced salinity tolerance. However, crop engineering for salinity tolerance has been slow, expensive and challenging due to the many knowledge gaps regarding plant responses to salinity stress, especially at the organelle, transcriptional and expression levels (Zhao et al. 2020).

In addition to efforts to understand the mechanisms of plant salinity stress tolerance, dedicated and rigorous efforts have been made to mine the plant microbiome communities and study their interactions. Various studies on plant–microbe interactions have revealed the functions of endophytes in different plants growing in different environments, including saline, neutral, geothermal, desert and marine ecosystems (Andreote et al. 2014, Berg et al. 2014, Zhou et al. 2015, Berg et al. 2016, Kaul et al. 2016, Rho et al. 2018, Verma et al. 2021). These microorganisms, especially fungi,

Received 4 August 2023; **revised** 15 March 2024; **accepted** 27 April 2024

form symbiotic relationships that confer fitness benefits to plants, such as biotic and abiotic stress tolerance and improved nutrient acquisition (Rodriguez and Redman 2008). However, the ecological roles of endophytic fungi are not fully understood (Gonçalves et al. 2021).

Some benefits conferred by microorganisms are hypothesized to be related to habitat adaptation (Rodriguez and Redman 2008). For example, inoculation of an *Ampelomyces* sp. isolated from a plant growing under drought and poor nutrient conditions into tomato seedlings and grown for 8 days without water resulted in plant survival in the absence of water. Similarly, inoculation of *Penicillium chrysogenum* isolated from a plant growing in a salt-stressed environment into tomato seedlings and exposed to 300 mM NaCl resulted in plants that were healthier than uninoculated plants throughout the salinity exposure period (Morsy et al. 2020).

Therefore, collecting novel fungal endophytes from plants growing in extreme environments is of great biotechnological value for economically important crop plants because of the changing climatic conditions, especially in arid and semiarid regions.

Endophytes from extreme environments can confer tolerance to biotic and abiotic stresses on crop plants (Redman et al. 2011, Morsy et al. 2020, Mutungi et al. 2021, Moghaddam et al. 2022). Kenya is home to the East African Rift Valley System, which harbors several saline alkaline lakes (soda lakes) that are characterized by saline and alkaline conditions (Schagerl and Renaut 2016). Studies on fungal populations in these unique ecosystems in Kenya are sparse, and the few that have been conducted have mainly focused on the diversity of fungi in soil sediments and water (Orwa et al. 2020). Therefore, the current study focused on the isolation of fungal endophytes from five shrubs collected along the shores of the soda Lake Magadi in Kenya. The isolated fungal endophytes were assessed for their potential to enhance tomato seed germination and improve tomato growth under salinity stress in a greenhouse.

Materials and methods

Study area and sample collection

The plant samples used in this study were collected from Lake Magadi, an internally drained saline alkaline lake (Deocampo et al. 2022) in the southern part of the Kenyan rift valley (2°S and 36°E), with an elevation of ∼600 m. The lake is the most hypersaline of the East African Rift Valley lakes that were formed through tectonic and volcanic activities. It is situated in a hydrologically closed basin and is characterized by a thick trona deposit (Schagerl and Renault 2016). The region is semiarid with temperatures ranging from 18 to 35°C. Shrubs growing along the shores of Lake Magadi were collected in March 2016 and GPS coordinates recorded. One set of plant samples was kept in plastic resealable bags in a cool box. Fungal endophytes were isolated from these plants within 48 h of sample collection. A second set of plants was wrapped in newspapers, labeled and pressed in pieces of carton. These plants were submitted for identification by a botanist at the National Museums of Kenya.

Isolation of endophytic fungi

Isolation of endophytic fungi followed the procedure described by Fouda et al. (2015), with some modifications. Briefly, the plant samples were separated into roots, stems and leaves and washed in running tap water to remove adhering soil and dust particles. Plants were then surface sterilized using 3% sodium hypochlorite for 3 min followed by 70% ethanol for 1 min, followed by several rinses of sterile distilled water. The last rinse water was plated out to confirm the sterilization process. Sterilized sections were asceptically cut into small pieces ∼1-cm long with a sterile surgical blade and placed onto sterile filter paper. The sections were air dried under a clean bench for ∼5 min and then they were placed onto freshly prepared Potato Dextrose Agar (PDA) medium (HiMedia, India) containing 50 µg/ml streptomycin sulfate and 0.25 M NaCl. The plates with plant pieces were then incubated at 28 ± 2°C for 7–20 days with regular monitoring. Emerging fungal colonies were isolated onto fresh PDA media and incubated under the same conditions.

Preservation of fungal cultures

Fungal cultures were preserved via agar slants and fungal spores for short- and long-term preservation, respectively. Short-term preservation followed the procedure described by Paul et al. (2015), with slight modifications. Slant cultures of pure isolates grown in PDA and incubated at 28°C for 4 days were overlaid with 15% v/v sterile glycerol and stored at 4°C. Fungal spores for long-term preservation were collected from cultures grown in PDA for 2 weeks and then harvested in sterile 15% dimethyl sulfoxide (DMSO). One milliliter of the spore-DMSO mixture was transferred to a −80°C freezer, where the temperature was decreased slowly and at a controlled rate from room temperature to −80°C (Dahmen et al. 1983). The percentage of spore germination was calculated for each fungal culture before preservation, and only those with more than 90% spore germination were preserved.

Characterization of fungal endophytes

Sixty fungal endophytes were grouped into 18 groups based on morphological characteristics of the growing cultures as displayed on PDA. These characteristics included growth rate, colony morphology and pigmentation. Representative isolates from each morphological group were further characterized.

DNA extraction, amplification and sequence analysis

Fungal DNA was extracted using the manual Cetyltrimethylammonium bromide (CTAB) extraction method, as described by Umesha et al. (2016). Pure fungal cultures were inoculated in PDA and incubated for 3 to 5 days. Growing mycelia were harvested using a sterile surgical blade and transferred into a sterile 1.5-ml Eppendorf microcentrifuge tube. The mycelia were ground with liquid nitrogen using a micropestle. Lysis buffer (800 µl of 0.1 M Tris-HCL, 50 mM EDTA, 2.5 M NaCl, 3% SDS and 3.5% CTAB) was added to the ground mycelia and the mixture was vortexed and incubated in a water bath at 65°C for 1 h with occasional shaking. The contents were centrifuged for 10 min at room temperature (25–27°C). An equal volume of phenol-chloroform-isoamyl alcohol (25:24:1) was added to 500 µl of the supernatant and mixed well. The mixture was centrifuged 10 000 × g for 10 min at room temperature, then the supernatant was carefully collected in a fresh tube and mixed with an equal volume of chloroform-isoamyl alcohol (24:1) and 30 µl of sodium acetate. The mixture was then centrifuged. An equal volume of ice-cold isopropanol was added, and the sample was kept at −20°C for 2 h. The DNA was pelleted by centrifugation for 15 min at 13 000 × g at room temperature. Pelleted DNA was washed with 800 µl of 70% ethanol and air dried before dissolving in TE buffer (10 mM Tris-HCl PH 8, 1 mM EDTA). The purity of the DNA was checked by 0.8%

agarose gel electrophoresis. The internal transcribed region (ITS1, 5.8S ITS2) of the ribosomal DNA was amplified by PCR using the primer set ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATGATGC-3′) (White et al. 1990). PCR was performed in a 50-µl reaction volume under the following conditions: 95°C for 5 min for the initial denaturation and enzyme activation followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 1 min, elongation at 72°C for 1 min, then a final elongation at 72°C for 10 min. The PCR product was visualized under UV light on a 1.5% agarose gel stained with ethidium bromide. Thirty microliters of amplicons were submitted to macrogen-Europe for bidirectional sequencing.

Sequence assembly and phylogenetic analysis

The resulting sequences were trimmed and edited using Chromas version 2.6.6 (www.technelysium.com.au/wp/chromas). Chromatogram viewing and editing, sequence assembly, ambiguity correction and double-pick mutation detection were performed using DNABaser version 4 (www. DNABaser.com). The resulting consensus sequences were matched to highly similar sequences in the National Institute for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLASTn) to infer evolutionary relationships. The MEGA11 (Molecular Evolutionary Genetic Analysis) program was used for phylogenetic analysis (Tamura et al. 2021). Sequences were aligned according to inferred evolutionary history using the maximum likelihood method with a bootstrap consensus of 1000 replicates. Evolutionary distance was inferred using the Tamura–Nei method (Tamura and Nei 1993), which considers the number of base substitutions per site and eliminates all positions with gaps and missing data.

Determination of the enzyme activities

Amylase activity

Amylase production of the isolates was screened using the plate culture technique as described by Sunitha et al. (2012), with slight modifications. Glucose yeast extract peptone agar (1 g/l glucose, 0.1 g/l yeast extract, 0.5 g/l peptone, 16 g/l agar) supplemented with 2% soluble starch and 50 µg/ml streptomycin was used to screen the isolates. First, an agar plug from a sporulating fungal plate was placed at the center of a glucose yeast extract peptone agar plate, and then the plate was incubated at 28°C for 3 days. Amylase production was detected by flooding the plates with Lugol's iodine solution (1 g of iodine crystals and 2 g of potassium iodide dissolved in 100 ml of distilled water). A clear zone around a fungal colony indicates amylase production.

Cellulase activity

Celluase production was tested by growing the isolates on yeast extract peptone agar supplemented with 0.5% carboxymethyl cellulose, as described by Carrasco et al. (2016). An agar plug from a sporulating fungal plate was placed at the center of a freshly prepared plate containing yeast extract peptone agar. The plate was incubated for 3 days at 28°C, and then it was flooded with 0.2% Congo red and destained with 1 M NaCl. The development of a yellow ring around a fungal colony indicated the production of cellulases.

Protease activity

Protease production was tested on fungal cultures inoculated on yeast extract peptone agar supplemented with 0.4% gelatin at pH 6 (Sharma et al. 2015). The plate was incubated at 28°C for 3 days and then flooded with saturated aqueous ammonium sulfate, which was prepared by dissolving 541.8 g in one liter of distilled water (4.1 M) at 25°C. Clear zones around the colonies indicated protease activity.

Phosphate solubilization

The ability of pure cultures of fungi to solubilize phosphate was tested in Pikovskaya agar (Hi Media, India) supplemented with 0.3% tricalcium phosphate. The sterilized medium was poured into 9-mm plastic Petri dishes and left to cool. Fungal mycelial plugs from actively growing cultures were placed onto the agar medium and incubated for 5–8 days. Clear zones around the fungal colonies indicate phosphate solubilization (Bilal et al. 2018).

Fungal growth at increasing sodium chloride concentrations

The ability of fungal isolates to grow at increasing concentrations of sodium chloride was tested by growing them in plates of fresh PDA medium supplemented with 0 mM, 0.5 mM, 1 M and 1.5 M sodium chloride. An agar plug from a sporulating plate was placed at the center of plates containing the different sodium chloride concentrations. Three replicate plates per NaCl concentration were incubated for 14 days, and the radial growth of each culture was measured.

Seed inoculation and assessment of endophytic competence of the isolates

Fungal cultures

Four fungal isolates were selected for further *in vitro* experiments. Seeds were inoculated following the procedure described by Jaber (2018). Fungal cultures were grown on PDA supplemented with 50 µg/ml streptomycin sulfate and incubated until sporulation (18–20 days). Each sporulating fungal culture was flooded with ∼3 ml of sterile distilled water containing 1% Tween 80, and the conidia were harvested by gently scraping the surface using a sterile glass rod. The conidial suspension was then gently vortexed, and the conidial concentration was determined using a Neubauer hemocytometer (Electron Microscopy Sciences). Conidial viability was tested by plating 100 µl of spore suspension on a fresh PDA plate. A sterile coverslip was placed on top of the media and the plate was incubated for 24 h. Conidia with germ tubes longer than the length of the conidia were considered germinated. Only suspensions with more than 90% spore germination were considered for the experiment (Jaber 2018). Two concentrations of 10^6 and 10^8 conidia/ml were used to inoculate seeds to determine the endophytic competence of the isolates in tomato seedlings.

Determination of the endophytic competence of fungal isolates

Seed inoculation

Solanum lycopersicum variety Cal J seeds were surface sterilized by washing them first in tap water followed by a 2-min wash in 3% sodium hypochlorite, followed by 2 min in 70% ethanol, then three rinses in sterile distilled water. The final rinse water was plated on PDA to confirm the effectiveness of surface sterilization (Muvea et al. 2014). Sterilized seeds were then air dried for 30 min on sterile filter paper and soaked in either 10^6 or 10^8 conidia/ml of each isolate overnight. Control seeds were soaked in sterile distilled water containing 1% Tween 80. Inoculated seeds were air dried for 30 min before being transferred to plastic pots containing sterile vermiculite moistened with half-strength Hoagland's solution. Three seeds were sown in each pot, and the pots were transferred to a

growth chamber set at 27 ± 2°C at a 12 h: 12 h light–dark cycle. The pots were arranged in a completely randomized block design with four replicates per treatment. Sterilized half-strength Hoagland's solution was added as necessary.

Assessment of endophyte colonization

Twenty-one days after germination, the seedlings were gently uprooted from the pots. Seedlings were washed in running tap water to remove any vermiculite adhering to the roots, and then each seedling was divided into roots, stems and leaves. Each of these plant parts was surface sterilized as described above. Six pieces of each plant part per conidial concentration per seedling were cut into ∼1-cm-long pieces using a sterile surgical blade under a laminar flow hood. The pieces were plated onto PDA plates supplemented with 50 µg/ml streptomycin sulfate and incubated in the dark at 25 ± 2°C for 14 days. The growing fungal cultures were stained with lactophenol cotton blue stain. The morphological characteristics of the seedling-derived cultures were compared with those of the original isolates (Muvea et al. 2014).

Effect of endophytic fungi on germination of tomato seeds under salinity stress

The conidial concentration of 10^8 conidia/ml gave a higher endophyte recovery rate than 10^6 conia/ml and was therefore chosen for use in greenhouse experiments. Procedures for seed sterilization and colonization were performed as described above. Colonized seeds were transferred to 9-mm diameter Petri dishes containing sterile water agar supplemented with 0, 50, 75, 100 and 125 mM sodium chloride. The seeds were incubated in the dark for up to 10 days while checking daily for germination. Two plates were set per isolate per salinity concentration and non-inoculated seeds served as controls.

Effect of endophytic fungi on tomato seedlings under salinity stress in a greenhouse

Solanum lycopersicum variety Cal J seeds were surface sterilized as described above and soaked overnight in 10^8 conidia/ml of each fungal isolate. Inoculated seeds were air dried on sterile filter paper under sterile conditions for 2 h before transferring them to 1% sterile water agar plates to avoid additional handling of seedlings in the course of the experiment. The seeds were germinated by incubating them in the dark at 25 ± 2°C for 4 days, and then the germinated seeds were transferred to plastic pots (15 × 17 cm) containing a 5:1 mixture of sterilized forest soil and cattle manure, respectively. Before potting, the soil and cattle manure mixture was sterilized by autoclaving for 40 min at 121°C, left to cool overnight, then autoclaved again. Approximately 1 kg of sterile soil was distributed in each pot, and two germinated seedlings per isolate were transplanted ∼2-cm deep into the soil. Uninoculated seedlings grown under salinity stress and no salinity stress served as controls. The seedlings were grown in a greenhouse and maintained under ambient conditions at 25–28°C, arranged in a completely randomized design. The seedlings were watered with sterile tap water as required for 30 days with no additional fertilization, followed by watering with sterile tap water supplemented with 125 mM NaCl for 28 days. The following six treatments at 20 seedlings per treatment were compared: (i) F04 + 125 mM NaCl; (ii) F05 + 125 mM NaCl; (iii) F18 + 125 mM NaCl; (iv) F21 + 125 mM NaCl; (v) non-inoculated seedlings (C + 125 mM NaCl); and (vi) non-inoculated (C with no NaCl). After the treatments, the seedlings were flooded with tap water overnight, uprooted and washed under running tap water to remove any adhering soil particles. The seedlings were then wrapped in a paper towel to remove excess water. Ten seedlings (one from each replicate) were selected per treatment for measurements of root and shoot wet and dry weights. Dry weight was measured by drying the seedlings in an oven at 68°C for 48 h (Balliu et al. 2015).

Chlorophyll and carotenoid content were measured using the procedure described by Lichtenthaler and Buschmann (2001). Briefly, 1.5 g of fresh leaves were ground in the dark in 100% acetone and centrifuged at 10 000 × g for 10 min. The supernatant was collected for absorbance measurements using a microplate spectrophotometer (Versamax). The quantities of the pigments were calculated as follows:

$$\text{Chlorophyll A: } 12.25A_{662}–2.79A_{647}$$
$$\text{Chlorophyll B: } 21.50A_{647}–5.10A_{662}$$
$$\text{Total chlorophyll: } 20.2A_{647}–8.02A_{662},$$

where A_{662} is the absorbance of the solution at 662 nm and A_{647} is the absorbance of the solution at 647 nm.

Hydrogen peroxide levels in the leaves were measured using the method of Junglee et al. (2014), with slight modifications. Leaves were harvested from tomato seedlings and 500 mg were ground in liquid nitrogen using a mortar and pestle. Five milliliters of 1% TCA (w/v) was added to the ground powder and mixed well. The homogenate was then centrifuged at 12 000 × g for 15 min at 4°C. The supernatant was mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7) and 1 ml of 1 M potassium iodide. The absorbance of the mixture was measured at 390 nm. The mixture without the supernatant served as the control. A standard curve of hydrogen peroxide was developed by diluting 57 µl of 30% hydrogen peroxide to 100 µl with distilled water. Additional 10 x dilutions were prepared and the absorbances of the various dilutions and measured at 390 nm.

Statistical analysis

The salinity tolerance of the isolates and seed germination rates were analyzed using one-way ANOVA ($P < 0.05$) and means compared using the Student's Newman–Keuls test. The effects of the endophytes on seedling biomass, chlorophyll content and hydrogen peroxide production were determined using the Kruskal–Wallis chi-square test. Post hoc analysis was performed using Dunn's test. Data on endophyte colonization and recovery rates were fitted to a generalized linear model with a Poisson distribution. The analysis was performed using R statistical software version 2.15.4.

Results

Isolation and characterization of fungal endophytes

Five different shrubs were collected from the shores of Lake Magadi and used for the isolation of endophytic fungi. All sampled plants harbored fungal endophytes. Sixty fungal isolates were purified from the leaves, stems and roots of collected shrubs (Table 1). Grouping of the isolates based on the morphological characteristics of their growth on PDA resulted in 18 different groups. *Indigofera spinosa* Forssk generated the highest number of isolates, whereas *Commicarpus grandifloras* and *Lactuca inermis* Forssk generated the least number of isolates. Most of the fungal isolates were isolated from roots (48.3%), whereas stems and leaves produced 30% and 21.7%, respectively. Of the 60 isolates, 25, 30 and 27 were positive for amylase, protease and cellulase production, respectively. Thirty-two isolates solubilized phosphate (Table 1).

Table 1. Endophytic fungal isolates, their respective source plants, and their physiological characteristics.

				Growth on NaCl[a]				Enzymatic activities[b]			
Isolate no.	Source plant name	Plant part	GPS coordinates	0 M	0.5 M	1.0 M	1.5 M	Amylase	Protease	Cellulase	Phosphate solubilization[b]
F01	*Commicarpus grandiflorus*	Stem	1°59′00″S 36°14′25″E 651M	++	−	−	−	+	+	−	+
F02	*Commicarpus grandifloras*	Root	1°59′00″S 36°14′25″E 651M	+++	+	−	−	+	−	+	+
F03	*Commicarpus grandifloras*	Stem	1°59′00″S 36°14′25″E 651M	++++	+	−	−	+	−	−	−
F04	***Commicarpus grandifloras***	**Stem**	**1°59′00″S 36°14′25″E 651M**	**++**	**+++**	**++**	**+**	**+**	**+**	**+**	**+**
F05	***Commicarpus grandifloras***	**Stem**	**1°59′00″S 36°14′25″E 651M**	**++**	**+++**	**++**	**+**	**+**	**+**	**+**	**+**
F06	*Commicarpus grandifloras*	Root	1°59′00″S 36°14′25″E 651M	++++	−	−	−	+	+	−	ND
F07	*Commicarpus grandifloras*	Root	1°59′00″S 36°14′25″E 651M	++++	+	−	−	−	−	−	−
F08	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++++	+	−	−	ND	−	−	+
F09	*Indigofera spinosa* Forssk	Stem	1°52′02″S 36°14′46″E 587M	++++	++	+	−	−	+	−	ND
F10	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	+++	+	−	−	−	−	−	−
F11	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	+++	−	−	−	−	−	−	+
F12	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++++	+	−	−	−	−	ND	+
F13	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	++++	+	+	−	−	+	−	−
F14	*Indigofera spinosa* Forssk	Stem	1°52′02″S 36°14′46″E 587M	+++	++	−	−	+	−	−	+
F15	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++++	++	−	−	ND	+	+	+

Table 1. Continued

Isolate no.	Source plant name	Plant part	GPS coordinates	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0 M	0.5 M	1.0 M	1.5 M	Amylase	Protease	Cellulase	
F16	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	+++	++	−	−	−	−	−	+
F17	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	++++	++	+	−	−	−	−	−
F18	***Indigofera spinosa* Forssk**	**Root**	**1°52′02″S 36°14′46″E 587M**	**+++**	**++**	**+**	**+**	**+**	**+**	**+**	**+**
F19	*Indigofera spinosa* Forssk	Stem	1°52′02″S 36°14′46″E 587M	++++	−	−	−	−	+	−	+
F20	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	+++	+	−	−	−	−	+	−
F21	***Indigofera spinosa* Forssk**	**Root**	**1°52′02″S 36°14′46″E 587M**	**+++**	**++**	**+**	**+**	**+**	**+**	**+**	**+**
F22	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	++++	++	+	−	+	+	ND	+
F23	*Indigofera spinosa* Forssk	Leaves	1°52′02″S 36°14′46″E 587M	+++	+	+	−	−	+	+	ND
F24	*Indigofera spinosa* Forssk	Stem	1°52′02″S 36°14′46″E 587M	++++	++	−	−	−	+	+	+
F25	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++	−	−	−	+	−	+	−
F26	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++++	+	++	+	+	+	−	ND
F27	*Indigofera spinosa* Forssk	Root	1°52′02″S 36°14′46″E 587M	++++	++	+	−	+	−	+	+
F28	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	++	++	+	+	+	−	+	−
F29	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	++	++	−	−	−	−	−	+

Table 1. Continued

Isolate no.	Source plant name	Plant part	GPS coordinates	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0 M	0.5 M	1.0 M	1.5 M	Amylase	Protease	Cellulase	
F30	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	+++	+	−	−	−	−	−	+
F31	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	++	+	−	−	+	−	−	−
F32	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	+++	++	+	−	−	−	+	+
F33	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	++++	+	−	−	−	+	−	−
F34	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	++++	−	−	−	ND	−	−	ND
F35	*Tarchonanthus camphoratus*	Root	1°53′41″S 36°15′12″E 616M	+++	++	+	−	+	−	+	ND
F36	*Tarchonanthus camphoratus*	Stem	1°53′41″S 36°15′12″E 616M	+++	+	−	−	−	+	−	+
F37	*Tarchonanthus camphoratus*	Stem	1°53′41″S 36°15′12″E 616M	++	−	−	−	−	+	−	−
F38	*Tarchonanthus camphoratus*	Leaves	1°53′41″S 36°15′12″E 616M	++++	−	−	−	+	+	−	+
F39	*Tarchonanthus camphoratus*	Leaves	1°53′41″S 36°15′12″E 616M	++++	+	+	−	−	ND	−	−
F40	*Tarchonanthus camphoratus*	Leaves	1°53′41″S 36°15′12″E 616M	++++	++	+	+	−	+	+	+
F41	*Tarchonanthus camphoratus*	Leaves	1°53′41″S 36°15′12″E 616M	++++	+	−	−	+	−	+	−
F42	*Prosopis juliflora*	Stem	1°56′52″S 36°14′25″E 654M	++++	+	−	−	+	+	+	+
F43	*Prosopis juliflora*	Stem	1°56′52″S 36°14′25″E 654M	+++	−	−	−	−	−	−	−
F44	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	++	−	−	−	+	ND	−	ND
F45	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	+++	−	−	−	−	+	+	−

Table 1. Continued

Isolate no.	Source plant name	Plant part	GPS coordinates	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0 M	0.5 M	1.0 M	1.5 M	Amylase	Protease	Cellulase	
F46	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	++++	++	+	−	−	+	−	+
F47	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	+++	−	−	−	−	+	+	+
F48	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	+++	−	−	−	ND	+	−	−
F49	*Prosopis juliflora*	Stem	1°56′52″S 36°14′25″E 654M	++++	−	−	−	+	−	−	+
F50	*Prosopis juliflora*	Stem	1°56′52″S 36°14′25″E 654M	+++	++	+	+	−	−	+	−
F51	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	++	−	−	−	+	+	+	+
F52	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	+++	+	−−	−	−	−	+	+
F53	*Prosopis juliflora*	Root	1°56′52″S 36°14′25″E 654M	++++	++	+	−	−	+	−	+
F54	*Lactuca inermis* Forssk	Root	2°00′04″S 36°13′56″E 606M	++++	+	+	−	−	−	+	−
F55	*Lactuca inermis* Forssk	Root	2°00′04″S 36°13′56″E 606M	++	+	−	−	+	+	−	ND
F56	*Lactuca inermis* Forssk	Leaves	2°00′04″S 36°13′56″E 606M	+++	−	−		−	−	+	−
F57	*Lactuca inermis* Forssk	Stem	2°00′04″S 36°13′56″E 606M	++	+	−	−	+	+	+	+
F58	*Lactuca inermis* Forssk	Stem	2°00′04″S 36°13′56″E 606M	+++	−	−	−	−	+	+	+
F59	*Lactuca inermis* Forssk	Leaves	2°00′04″S 36°13′56″E 606M	++++	++	+	−	+	−	−	−
F60	*Lactuca inermis* Forssk	Leaves	2°00′04″S 36°13′56″E 606M	++	+	−	−	−	+	+	+

Key: [a]Growth response to salt concentrations: −, no growth; +, slight growth: ++, low growth: +++, moderate growth; ++++, full growth. [b]Exo-enzyme production: −, no production: +, production. ND, not tested. Lines in bold indicate the isolates and source plants that were used for further experiments.

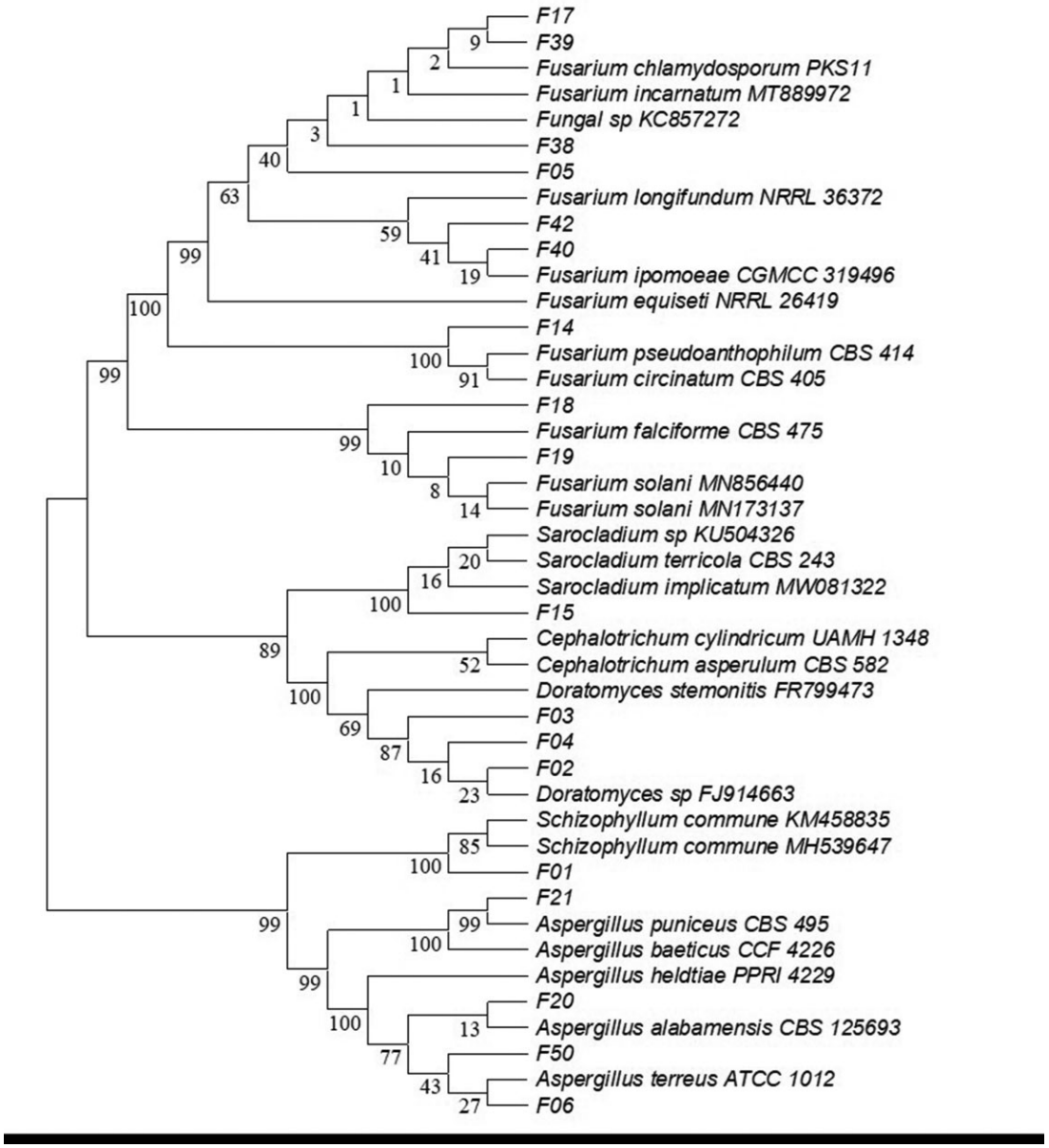

Figure 1. Unrooted phylogenetic tree of fungal endophytes depicting the evolutionary history of the isolates using the maximum likelihood method with 1000 bootstrap replicates and complete elimination of gaps and missing data. Phylogenetic analysis was performed in MEGA 11. The percentage of trees in which the associated taxa clustered together is shown below the branches.

Four isolates were selected for further experiments on the basis of the rate of growth, sporulation and production of exoenzymes (data not provided). Two of these isolates, *Cephalotrichum cylindricum* (F04) and *Fusarium equiseti* (F05), were from the stem of *Commicarpus grandifloras*; and the other two, *Fusarium falciforme* (F18) and *Aspergilus puniceus* (F21), were from the roots of *Indigofera spinosa* Forssk (Fig. 1). All four isolates were able to grow on all tested NaCl concentrations; they were all positive for the production of amylase, cellulase and protease enzymes; and they all solubilized phosphate (Table 1).

Molecular identification

DNA was extracted from a representative of each of the 18 morphological groups, and the ITS rRNA gene of each was sequenced for species identification. Analysis of the resulting concensus sequences and comparison with homologous sequences in the NCBI genebank database revealed that the genus *Fusarium* was isolated at the highest frequency and was represented by eight morphogroups; and these isolates represent seven different *Fusarium* species (*F. equiseti*, *F. pseudoathophilum*, *F. longifundum*, *F. falciforme*, *F. clamidosporum*, *F. solani* and *F. ipomea*). These morphogroups represented 28 of the 60 isolates. Species within the genus *Aspergillus* were the second most frequently isolated (*A. puniceus* and *A. terreus*), and these were represented by four morphogroups, to which 17 of the 60 isolates belonged. One species within the genus *Cephalotricum* (*C. cylindricum*) was in two morphogroups representing seven of the 60 isolates. The other identified genera (*Schizophyllum, Saracladium*, *Daratomyces* and Fungal species) were each represented by one morphogroup (Fig. 1). Ninety-five per cent of the isolates belonged to phylum *Ascomycota* and the remaining belonged to phylum *Basidiomycota*, both of which are in the subkingdom Dikarya. Isolates (three of the 60) classified under the phylum

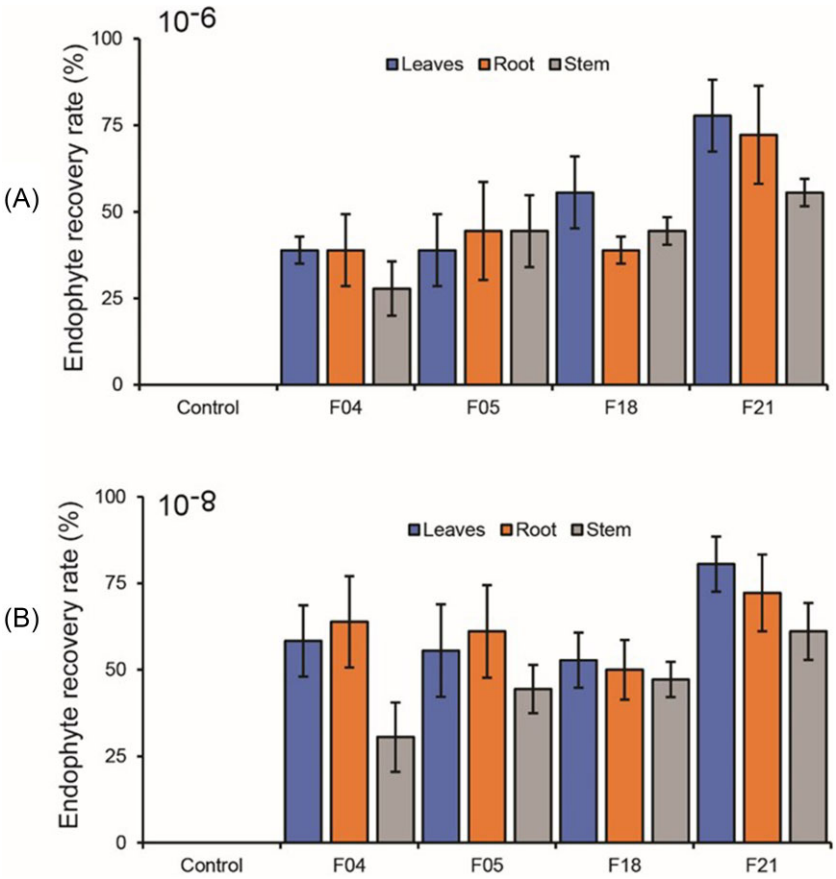

Figure 2. Fungal endophyte recovery rate from leaves, stems and roots of tomato seedlings inoculated via the seed-soaking method. (A) and (B) show endophyte recovery rates using conidial concentrations of 106 and 108 Conidia/ml, respectively. Bars represent standard error of the mean (SEM).

Basidiomycota all belonged to one morphogroup and to the genus *Schizophyllum*. The rest of the isolates belonged to the phylum *Ascomycota*. BLAST search results generated similarity matches ranging from 97% to 100% identity with known species. The distribution of the genus in isolation did not show any tissue or plant specificity.

Endophytic competence of the fungal isolates

Two different concentrations (10^6 and 10^8 conidia/ml) of the isolates were tested for their endophytic competence in tomato seedlings grown on sterile vermiculite. The two conidial concentrations were recovered at significantly different rates ($P = 0.0018$). However, both concentrations of the four isolates were able to colonize all the tomato seedling parts (leaves, stems and roots) within 3 weeks (Fig. 2). We detected a significant difference in endophytic performance ($P < 0.001$). Specifically, isolate F21 was re-isolated at the highest rate at both concentrations. Although the four isolates were derived from the stem and root, we found no significant difference in fungal colonization for the different plant parts ($P = 0.2492$). No isolates were recovered from the control seedlings that were mock inoculated.

Effect of salinity on fungal endophytes and seed germination

Salinity significantly affected the radial growth of the isolates ($F_{15–32} = 169.2$, $P < 0.001$). The isolates significantly differed in their levels of salinity tolerance across the various sodium chloride concentrations tested ($P < 0.0001$) (Fig. 3A), with isolate F21 showing the largest radial growth across all concentrations. For example, at 1.5 M NaCl, the mean radial growth values of isolates F18 and F21 were 1.8 ± 0.1 and 2.7 ± 0.2 cm, respectively. Increasing concentrations of sodium chloride significantly reduced the germination of seeds ($F_{24–25} = 80.53$, $P < 0.0001$) (Fig. 3B). In the controls, NaCl concentrations of 75 mM and above resulted in no germination. Inoculation of seeds with fungal endophytes significantly affected germination under salinity stress ($P < 0.0001$). In the presence of endophytes, seeds germinated at 100 mM NaCl, with isolate F21 showing the highest number of germinated seeds at all NaCl concentrations (Fig. 3B).

Effect of fungal endophytes on tomato seedlings under NaCl stress

The effect of fungal endophytes on tomato was tested in seedlings grown in a greenhouse with sterile soil and a fungal spore concentration of 10^8 conidia/ml. Inoculation of tomato seedlings with the fungal isolates significantly affected both wet and dry weights ($\chi^2 = 21.193$, df = 5, $P = 0.00074$) of the seedlings compared with those of the controls with salt stress. Seed inoculation with isolate F04 resulted in the highest increases in both wet and dry weights compared with those of the controls with salt stress (Fig. 4). Growth of the seedlings symbiotically with fungal endophytes significantly ($P \leq 0.01$) increased the wet and dry weights of both roots and shoots compared with those of the control plants deprived of the endophytes and exposed to salinity stress (Fig. 4). Isolates F05 and F18 had similar impacts on both root and shoot fresh weights. On average, the fresh weights of the roots and shoots of inoculated

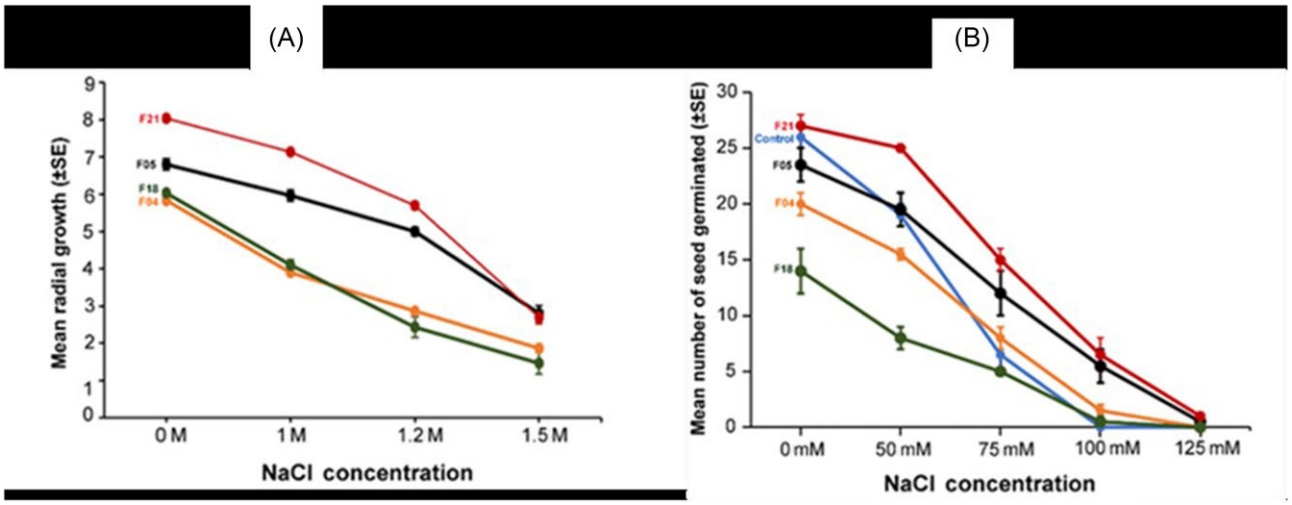

Figure 3. (A) Mean (±SE) radial growth of endophytes on PDA plates (n = 3) supplemented with different concentrations of sodium chloride. (B) Effect of fungal endophytes on seed germination following seed soaking with 108 conidia/ml and incubation on PDA plates supplemented with 0 to 125 mM NaCl concentrations (n = 30).

seedlings were 34% and 56% higher, respectively, than those of the control plants.

Except for isolate F18, the symbiotic association of the isolates with tomato seedlings positively affected the biosynthesis of photosynthetic pigments (Fig. 5). Specifically, inoculation of the seedlings with fungal endophytes enhanced the content of chlorophyll a ($P < 0.0001$), chlorophyll b ($P < 0.001$) and total chlorophyll ($P < 0.0001$) compared with the controls. Plants inoculated with isolates F05 and F21 showed a higher percentage increase of chlorophyll b than chlorophyll a under salinity stress.

The endophytes significantly ($\chi^2 = 35.364$, df = 5, $P = 0.0001$) reduced the quantity of hydrogen peroxide produced by the seedlings under salinity stress compared with that of the controls (Fig. 6). Seedlings inoculated with isolate F18 exhibited the lowest tolerance to salinity stress in terms of hydrogen peroxide production, whereas those inoculated with isolate F04 showed the best performance of the four isolates in reducing hydrogen peroxide. Isolates F05 and F21 did not differ significantly ($P \leq 0.05$) in their performance. Salinity increased the amount of hydrogen peroxide produced in the uninoculated control plants.

Discussion

Lake Magadi is an alkaline saline lake situated in a semiarid region in the southern part of the Kenyan rift valley. It is fed by ephemeral streams and has no outlet. Human activities, climate, geology, altitude and soil type shape and control the vegetation, soil macrocommunities and microcommunities and habitats along the Magadi Natron basin (Muiruri et al. 2021). The region's climate is changing from dry to even greater aridity, which, coupled with high evapotranspiration rates, creates high pH and alkalinity (Owen et al. 2019). These changes in environmental conditions have shaped the plant and microbial communities along the lake ecosystem to those more adapted to saline and alkaline conditions. This phenomenon has been noted by Maciá-Vicente et al. (2012), who reported a variable shift in endophytic and rhizosphere fungal communities along a spatially short salinity gradient in which halophytes harbor an endophytic assemblage of saline-adapted fungi.

Habitat-adapted microorganisms have been used to enable plants to adapt to biotic and abiotic stresses, enhance growth and increase reproductive success; some plants are unable to survive in their habitats without fungal symbiosis (Redman et al. 2002, Bouzouina et al. 2021, Moghaddam et al. 2022). Our results are consistent with these findings. Specifically, we showed that selected endophytic isolates can tolerate and grow in salinity concentrations of up to 1.5 M NaCl. Moreover, our results complement the growing body of knowledge on the importance of microorganisms symbiotic to plants in stress environments and their applications in crop plants. Isolation and utilization of these habitat-adapted microorganisms in agricultural systems offer an important, cheaper and more reliable solution than plant breeding, especially in saline soils.

We were able to isolate representatives of only two fungal phyla, *Ascomycetes* and *Basidiomycetes*, with a bias toward the former. Fungal endophyte communities are shaped by various factors, including host genotype, nutrient status around the plant and other environmental factors, although the plant is largely responsible for shaping the association (Bulgarelli et al. 2012, Wehner et al. 2014, Cheng et al. 2019). Certain fungal phyla have been more frequently found as endophytes and in the soil rhizosphere than others, especially in abiotically stressed environments (Maciá-Vicente et al. 2012, Hamzah et al. 2018, Zhou et al. 2018, Khalil et al. 2021, Sahoo et al. 2021). The ubiquity of *Ascomycetes* in soil can probably explain their abundance as endophytes.

In the current study, 46% (28) of the isolates were classified as *Fusarium*, based on the DNA internal transcribed spacer gene region. *Fusarium* species include both pathogenic and beneficial plant endophytes, and they are ubiquitous and economically important fungi that can cause diseases in plants. They can also produce mycotoxins that are passed on to animals when they feed on contaminated plants; and they can act as pathogens to humans (Ji et al. 2019, Srinivas et al. 2019). However, *Fusarium* endophytes in plants have been shown to lose their pathogenicity under stress conditions, and thus they become beneficial to the plant by inducing resistance to stress and enhancing growth (Pappas et al. 2018, Ogbe et al. 2023). This characteristic can be a key reason why plants symbiotically associate with *Fusarium* species.

Aspergillus was the second most frequently isolated genus of fungal endophytes in our study (17 isolates classified as either *A. terreus* or *A. puniceus*). The genus *Aspergillus* is a frequently isolated

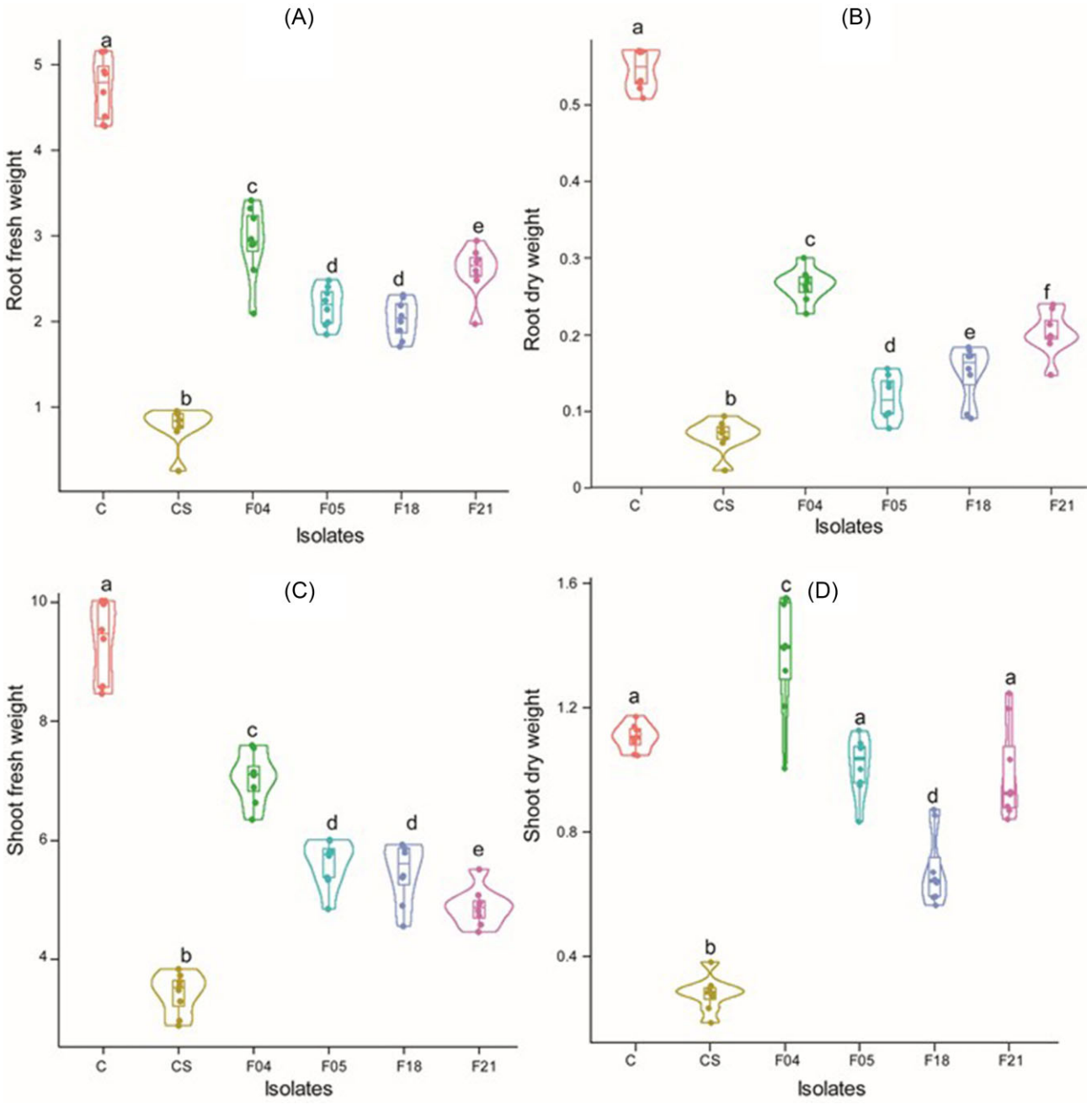

Figure 4. Violin plot representing the effect of different fungal endophytes on seedling growth in terms of root fresh weight (A), root dry weight (B), shoot fresh weight (C) and shoot dry weight (D) after exposure of seedlings to salinity stress for 21 days. CS, uninoculated control seedlings exposed to salinity stress. C, uninoculated seedlings without exposure to salinity stress. Treatments with different letters are significantly different from each other (ANOVA test followed by Student Newman–Keuls test, $P = 0.05$).

endophyte, as it is capable of growing in vital nutrient-depleted environments, including within plants growing in extreme environments (Kim et al. 2014, Sahoo et al. 2021). They have also been found to produce highly diverse secondary metabolites with various potential industrial applications (El-Hawary et al. 2020). They have been implicated in the production of endogenous plant hormones, amino acids and other soluble organic acids that help the plant mitigate stress and enhance growth (Waqas et al. 2015).

Establishing endophytism in non-host plants is especially important for beneficial endophytes, because they offer the possibility of conferring similar benefits to crop plants. In this study, we tested the ability of four selected endophytic fungi to competently colonize tomato plants growing in sterile vermiculite by seed inoculation using two different fungal spore concentrations. All isolates colonized tomato at both concentrations but differed in individual fungal performance and plant part. Similar results were obtained by Akutse et al. (2013), as well as Jaber and Enkerli (2016), who reported differences in colonization rates for different plant parts. Other studies have also inoculated seeds with a conidial concentration of 10^8 conidia/ml, resulting in successful post-inoculation recovery of the endophytes from all plant parts and effective performance on the test variable (Mutune et al. 2016, Jaber 2018). Several factors contribute to successful endophyte establishment in non-host plants, including the concentration of in-

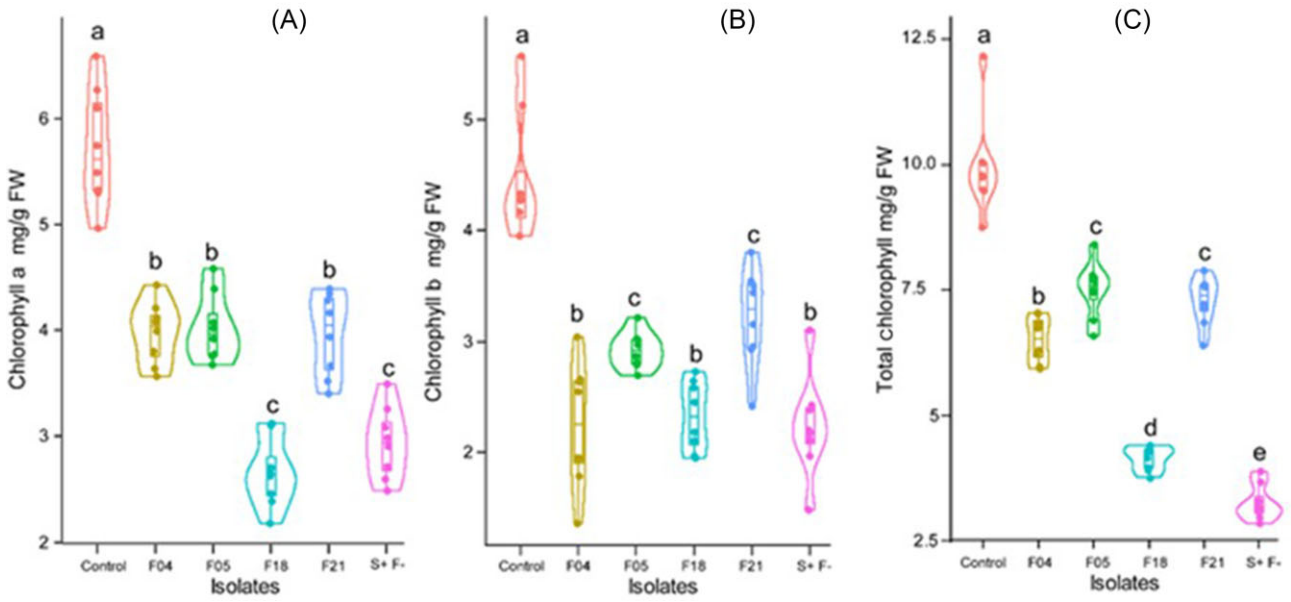

Figure 5. Effect of endophytic fungi on chlorophyll a (A), chlorophyll b (B) and total chlorophyll (C) content measured from fresh weight (FW) of the leaves of tomato seedlings exposed to 125 mM NaCl for 28 days. The same letter indicates no significant difference in chlorophyll concentration. S+F-, uninoculated control seedlings exposed to salinity stress. Control refers to uninoculated seedlings with no exposure to salinity stress.

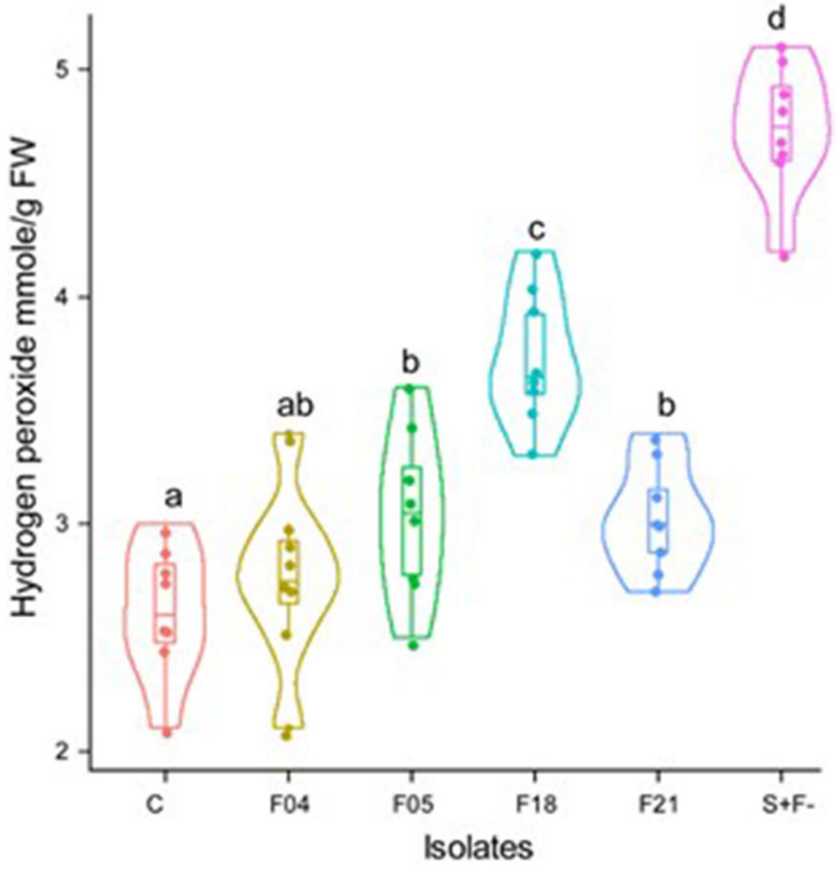

Figure 6. Effect of fungal endophytes on hydrogen peroxide production (mmole/g Fresh weight (FW)) by tomato seedlings exposed to 125 mM NaCl for 28 days. Isolates followed by the same letter indicate no significant difference in hydrogen peroxide production. S + F-, uninoculated control seedlings exposed to salinity stress. C, uninoculated seedlings without exposure to salinity stress.

oculum used, medium used (sterile or non-sterile) and method of inoculation (Bamisile et al. 2018). Using seed soaking and sterile vermiculite in our study, the concentration of conidia used correlated with the recovery rates, which is consistent with the results of other studies (Ownley et al. 2008).

Salinity stress in plants can be a lethal factor that limits the normal functioning of plants and eventually affects growth and productivity. At elevated salinity levels, all growth stages (seed germination, seedling, vegetative growth and maturity), as well as the quality of the seeds/fruits, are negatively affected (Jafarzadeh and Aliasgharzad 2007, Yao et al. 2022). Germination and seedling establishment are the most crucial stages in the plant life cycle. High salinity stress negatively affects the germination of seeds as it creates low water potential that disrupts cellular homeostasis and increases the production of reactive oxygen species (ROS), resulting in oxidative stress that tends to prolong the seed germination period and lower the germination rate (Zhang and Mu 2009, Dehnavi et al. 2020).

Under salinity stress conditions, fungal endophytes produce osmolytes and other stress response mechanisms that ameliorate the effects of salinity (Niu et al. 2022). We speculate that such mechanisms were responsible for the enhanced germination of inoculated seeds growing at sodium chloride concentrations of 50, 75 and 100 mM. Although few studies have focused on the use of endophytes to improve seed germination under salinity stress, several studies have indicated that endophytes can be beneficial to seedling growth under salinity stress (Jogawat et al. 2016, Kumar and Verma 2018, Molina-Montenegro et al. 2020, Verma et al. 2021). More studies on these positive effects of endophytes on seed germination, at the molecular and physiological levels, are essential to aid in the development of strategies to mitigate the impacts of climate change on food crops.

Salinity stress inhibits plant growth and development by decreasing chlorophyll production and accumulating ROS. The development of osmotic stress resulting from the accumulation of Na^+ in the cytosol under saline conditions leads to stomatal closure and the suppression of enzymes involved in chlorophyll synthesis, which reduces photosynthesis and nutrient absorption (Zhao et al. 2020). Tomato is moderately sensitive to salinity; thus, under highly saline conditions, the amount of chlorophyll in leaves decreases, eventually leading to decreases in fruit yield, weight and quality (Ebrahim and Saleem 2017). In our experiment, we used a commercial cultivar, Cal J variety, locally known as Kamongo, which is popular in Kenya due to its high market value and long shelf life (Geoffrey et al. 2014). This variety was negatively affected at 125 mM NaCl, which markedly reduced leaf chlorophyll content and increased levels of hydrogen peroxide compared with those of control seedlings without NaCl. This reduction in chlorophyll could be associated with the plants' inability to manage ion toxicity caused by increased Na^+ and Cl^- ions in the plant tissues.(Zhang and Mu 2009). Hydrogen peroxide is a ROS and a signaling molecule generated by plants in response to stress conditions (Zhu et al. 2016). Elevated levels of hydrogen peroxide damage cellular metabolites oxidatively, which affects plant growth (Hossain et al. 2015). Symbiotic association with endophytic fungi significantly ($P < 0.001$) reduced the amount of hydrogen peroxide produced by the plants, increased their chlorophyll content and increased their dry weight compared with uninoculated control plants exposed to sodium chloride. These results indicate the endophytes-enhanced sodium chloride tolerance of the seedlings. We speculate the endophytic fungi in the tomato seeds and seedlings helped maintain the ionic balance in the plant cytosol, thereby preventing accumulation of toxic Na^+ ions while enhancing photosynthesis in the seedlings under sodium chloride stress. Ionic homeostasis in plants, reduced ROS production and concomitant increases in shoot and root weight have been reported in several studies as mechanisms by which fungal endophytes alleviate salt stress (Bouzouina et al. 2021, Ali et al. 2022).

Fungal endophytes are prolific producers of extracellular enzymes and secondary metabolites (Debbab et al. 2013), which are important in the selection of beneficial microorganisms for use in agricultural production. Our experiment used four fungal endophytes that were selected based on their abilities to produce the exoenzymes amylase, protease and cellulases and to solubilize inorganic phosphorus. Of the 60 fungal isolates obtained, 62% solubilized inorganic phosphate, a finding that agrees with those of Ogbe et al. (2023). Phosphorus is the second most important plant nutrient after nitrogen (Radhakrishnan et al. 2015). Although it is present at high concentrations in soil, plants are often starved for phosphorus because it occurs in a form that they cannot absorb (Castrillo et al. 2017). In soils with high salinity and pH, phosphorus forms stable complexes with other ions and becomes unavailable to plants (Penn and Camberato 2019, Xie et al. 2022). It has been suggested that to increase plant productivity, plants growing in such soils can select and symbiotically associate with microorganisms that help them alleviate environmental challenges such as nutrient deficiency (Bulgarelli et al. 2013). Therefore, the ability of a large number of our isolates to solubilize phosphate may be the result of the plant's natural selection during plant–microbiome evolution.

All endophytic isolates were able to colonize tomato seedlings that germinated from seeds inoculated via the seed-soaking method. Seed germination of inoculated seedlings was positively affected. Moreover, seedling shoot and root weight, and chlorophyll content increased, while hydrogen peroxide production decreased under salinity stress in the presence of the endophytes. Of the four isolates tested, isolate F21 (*A. puniceus*) exerted the greatest effect, and therefore, this isolate has the most potential for use in the development of a less expensive approach to climate resilient agriculture, especially in arid and semiarid regions where crops are exposed to several biotic and abiotic stresses. Further studies are, however, necessary to understand the interactions between these endophytes and crop plants in the presence of other naturally existing soil microbiota under salinity stress. The applicability of seed inoculation under field conditions should also be studied. We conclude that endophytic fungi from shrubs along the shores of saline alkaline lakes are potentially beneficial microorganisms that can be harnessed for sustainable agricultural production.

Acknowledgements

The authors would like to acknowledge the National Research Fund (NRF) in Kenya for the financial support to undertake the research activities. The authors declare that they have no conflict of interest in the funding of the activities

Author contributions

Priscillar Mumo Mutungi (conceived and designed the experiments, performed the experiments, analyzed the data, wrote the manuscript, read, edited and approved the final manuscript), Vitalis Wafula Wekesa (conceived and designed the experiments, provided guidance with the performing of the experiments, provided guidance and input into the writing of the manuscript, read, edited and approved the final manuscript), Justus Onguso (con-

ceived and designed the experiments, provided guidance with the performing of the experiments, provided guidance and input into the writing of the manuscript, read, edited and approved the final manuscript), Erustus Kanga (provided guidance with the performing of the experiments, provided guidance and input into the writing of the manuscript, read, edited and approved the final manuscript), Steve B. S. Baleba (analyzed the data, provided guidance and input into the writing of the manuscript, read, edited and approved the final manuscript), and Hamadi Iddi Boga (conceived and designed the experiments, provided guidance with the performing of the experiments, provided guidance and input into the writing of the manuscript, read, edited and approved the final manuscript)

Supplementary data

Supplementary data is available at FEMSMC *Journal* online.

Conflict of interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The research activities were approved by the Kenya Wildlife Service under research Authorization ref. KWS/BRM/5001 and NACOSTI research permit number NACOSTI/P/17/22929/14802.

Funding

This study was financially supported through funding from the National Research Fund to undertake the research activities. The funders played no role in the experimental design, data analysis authorship, ownership or publication of the results.

Data availability

All relevant data are within the manuscript, and supporting information for sequences used in green house trials is available for download from https://submit.ncbi.nlm.nih.gov/subs/?search=SUB13605466.

References

Akutse KS, Maniania NK, Fiaboe KKM *et al.* Endophytic colonization of *Vicia faba* and *Phaseolus vulgaris* (Fabaceae) by fungal pathogens and their effects on the life-history parameters of *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Fungal Ecol* 2013;**6**:293–301.

Ali R, Gul H, Rauf M *et al.* Growth-promoting endophytic fungus (*Stemphylium lycopersici*) ameliorates salt stress tolerance in maize by balancing ionic and metabolic status. *Front Plant Sci* 2022;**13**:890565.

Andreote FD, Gumiere T, Durrer A. Exploring interactions of plant microbiomes. *Sci Agric* 2014;**71**:528–39.

Balliu A, Sallaku G, Rewald B. AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability* 2015;**7**:15967–81.

Bamisile BS, Dash CK, Akutse KS *et al.* Fungal endophytes: beyond herbivore management. *Front Microbiol* 2018;**9**:544.

Bandel K, Salameh EM, Al-Gharaibeh M *et al.* Plants of saline coastal and inland sabkha areas as indicators of environmental conditions in Southern Jordan. *JJEES* 2022;**13**:190–8.

Berg G, Grube M, Schloter M *et al.* Unraveling the plant microbiome: looking back and future perspectives. *Front Microbiol* 2014;**5**:148.

Berg G, Rybakova D, Grube M *et al.* The plant microbiome explored: implications for experimental botany. *J Exp Bot* 2016;**67**:995–1002.

Bilal L, Asaf S, Hamayun M *et al.* Plant growth promoting endophytic fungi Asprgillus fumigatus TS1 and Fusarium proliferatum BRL1 produce gibberellins and regulates plant endogenous hormones. *Symbiosis* 2018;**76**:117–27.

Bouzouina M, Kouadria R, Lotmani B. Fungal endophytes alleviate salt stress in wheat in terms of growth, ion homeostasis and osmoregulation. *J Appl Microbiol* 2021;**130**:913–25.

Bulgarelli D, Rott M, Schlaeppi K *et al.* Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature* 2012;**488**:91–5.

Bulgarelli D, Schlaeppi K, Spaepen S *et al.* Structure and functions of the bacterial microbiota of plants. *Annu Rev Plant Biol* 2013;**64**:807–38.

Carrasco M, Villarreal P, Barahona S *et al.* Screening and characterization of amylaseand cellulase activities in psychrotolerant yeasts. *BMC Microbiol* 2016;**16**:21

Castrillo G, Teixeira PJPL, Paredes SH *et al.* Root microbiota drive direct integration of phosphate stress and immunity. *Nature* 2017;**543**:513–8.

Cheeseman JM. The evolution of halophytes, glycophytes and crops, and its implications for food security under saline conditions. *New Phytol* 2015;**206**:557–70.

Cheng YT, Zhang L, He SY. Plant-microbe interactions facing environmental challenge. *Cell Host Microbe* 2019;**26**:183–92.

Dahmen H, Staub T, Schwinn FJ. Technique for long-term preservation of phytopathogenic fungi in liquid nitrogen. *Phytopathology* 1983;**73**:241–6.

Debbab A, Aly AH, Proksch P. Mangrove derived fungal endophytes—A chemical and biological perception. *Fungal Divers* 2013;**61**:1–27.

Dehnavi AR, Zahedi M, Ludwiczak A *et al.* Effect of salinity on seed germination and seedling development of sorghum (*Sorghum bicolor* (L.) Moench) genotypes. *Agronomy* 2020;**10**:859.

Deocampo DM, Owen RB, Lowenstein TK *et al.* Orbital control of Pleistocene euxinia in Lake Magadi, Kenya. *Geology* 2022;**50**:42–7.

Ebrahim MKH, Saleem AR. Alleviating salt stress in tomato inoculated with mycorrhizae: photosynthetic performance and enzymatic antioxidants. *J Taibah Univ Med Sci* 2017;**11**:850–60.

El-Hawary SS, Moawad AS, Bahr HS *et al.* Natural product diversity from the endophytic fungi of the genus Aspergillus. *RSC Adv* 2020;**10**:22058–79.

Food and Agriculture Organization. The State of the World's Land and water Resources for Food and Agriculture—systems at breaking point (SOLAW 2021). In: *The State of the World's Land and Water Resources for Food and Agriculture—Systems at Breaking Point (SOLAW 2021)*. Rome: FAO, 2021. https://doi.org/10.4060/cb7654en.

Fouda AH, Hassan SED, Eid AM *et al.* Biotechnological applications of fungal endophytes associated with medicinal plant *Asclepias sinaica* (Bioss.). *Ann Agric Sci* 2015;**60**:95–104.

Geoffrey SK, Hillary NK, Antony KM *et al.* Challenges and strategies to improve tomato competitiveness along the tomato value chain in Kenya. *IJBM* 2014;**9**:205–12.

Gonçalves DR, Pena R, Zotz G *et al.* Effects of fungal inoculation on the growth of *Salicornia* (Amaranthaceae) under different salinity conditions. *Symbiosis* 2021;**84**:195–208.

Hamzah TNT, Lee SY, Hidayat A *et al.* Diversity and characterization of endophytic fungi isolated from the tropical mangrove species, *Rhizophora mucronata*, and identification of potential antagonists against the soil-borne fungus, *Fusarium solani*. *Front Microbiol* 2018;**9**:1707.

Hossain MA, Bhattacharjee S, Armin SM *et al.* Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. *Front Plant Sci* 2015;**6**:420.

Jaber LR, Enkerli J. Effect of seed treatment duration on growth and colonization of *Vicia faba* by endophytic *Beauveria bassiana* and *Metarhizium brunneum*. *Biol Control* 2016;**103**:187–95.

Jaber LR. Seed inoculation with endophytic fungal entomopathogens promotes plant growth and reduces crown and root rot (CRR) caused by Fusarium culmorum in wheat. *Planta* 2018;**248**:1525–35.

Jafarzadeh AA, Aliasgharzad N. Salinity and salt composition effects on seed germination and root length of four sugar beet cultivars. *Biologia* 2007;**62**:562–4.

Ji F, He D, Olaniran AO *et al.* Occurrence, toxicity, production and detection of Fusarium mycotoxin: a review. *Food Prod Process Nutr* 2019;**1**:6.

Jogawat A, Vadassery J, Verma N *et al.* PiHOG1, a stress regulator MAP kinase from the root endophyte fungus Piriformospora indica, confers salinity stress tolerance in rice plants. *Sci Rep* 2016;**6**:36765.

Jones A, Panagos P, Barcelo S *et al.* The state of soil in Europe: a contribution of the JRC to the European Environment Agency's environment state and outlook report – SOER 2010. *European Environment Agency* 2012. http://www.eea.europa.eu/soer/2010 (7 October 2022, date last accessed).

Junglee S, Urban L, Sallanon H *et al.* Optimized assay for hydrogen peroxide determination in plant tissue using potassium iodide. *Am J Anal Chem* 2014;**05**:730–6.

Kaul S, Sharma T, K Dhar MK. "Omics" tools for better understanding the plant–endophyte interactions. *Front Plant Sci* 2016;**7**:955.

Khalil AMA, Hassan SED, Alsharif SM *et al.* Isolation and characterization of fungal endophytes isolated from medicinal plant *Ephedra pachyclada* as plant growth-promoting. *Biomolecules* 2021;**11**:140.

Kim H, You YH, Yoon H *et al.* Culturable fungal endophytes isolated from the roots of coastal plants inhabiting Korean east coast. *Mycobiology* 2014;**42**:100–8.

Kumar A, Verma JP. Does plant-microbe interaction confer stress tolerance in plants: a review? *Microbiol Res* 2018;**207**:41–52.

Lichtenthaler HK, Buschmann C. Chlorophylls and carotenoids: measurement and characterization by UV-vis spectroscopy. *Curr Protoc Food Anal Chem* 2001;**1**:F4.3.1–8.

Maciá-Vicente JG, Ferraro V, Burruano S *et al.* Fungal assemblages associated with roots of halophytic and non-halophytic plant species vary differentially along a salinity gradient. *Microb Ecol* 2012;**64**:668–79.

Moghaddam MSH, Safaie N, Rahimlou S *et al.* Inducing tolerance to abiotic stress in *Hordeum vulgare* L. by halotolerant endophytic fungi associated with salt lake plants. *Front Microbiol* 2022;**13**:906365.

Molina-Montenegro MA, IS A-R, Torres-Díaz C *et al.* Antarctic root endophytes improve physiological performance and yield in crops under salt stress by enhanced energy production and Na+ sequestration. *Sci Rep* 2020;**10**:5819.

Morsy M, Cleckler B, Armuelles-Millican H. Fungal endophytes promote tomato growth and enhance drought and salt tolerance. *Plants* 2020;**9**:1–18.

Muiruri VM, Owen RB, Lowenstein TK *et al.* A million year vegetation history and palaeoenvironmental record from the Lake Magadi Basin, Kenya Rift Valley. *Palaeogeogr Palaeoclimatol Palaeoecol* 2021;**567**:110247.

Mutune B, Ekesi S, Niassy S *et al.* Fungal endophytes as promising tools for the management of bean stem maggot *Ophiomyia phaseoli* on beans *Phaseolus vulgaris*. *J Pest Sci* 2016;**89**:993–1001.

Mutungi PM, Wekesa VW, Onguso J *et al.* Culturable bacterial endophytes associated with shrubs growing along the draw-down zone of Lake Bogoria, Kenya: assessment of antifungal potential against *Fusarium solani* and induction of bean root rot protection. *Front Plant Sci* 2021;**12**:796847.

Muvea AM, Meyhöfer R, Subramanian S *et al.* Colonization of onions by endophytic fungi and their impacts on the biology of *thrips tabaci*. *PLoS One* 2014;**9**:e108242.

Niu S, Gao Y, Zi H *et al.* The osmolyte-producing endophyte Streptomyces albidoflavus OsiLf–2 induces drought and salt tolerance in rice via a multi-level mechanism. *Crop J* 2022;**10**:375–86.

Ogbe AA, Gupta S, Stirk WA *et al.* Growth-promoting characteristics of fungal and bacterial endophytes isolated from a drought-tolerant mint species *Endostemon obtusifolius* (E. Mey. ex Benth.) N.E.Br. *Plants* 2023;**12**:12030638.

Orwa P, Mugambi G, Wekesa V *et al.* Isolation of haloalkaliphilic fungi from Lake Magadi in Kenya. *Heliyon* 2020;**6**:e02823.

Owen RB, Renaut RW, Muiruri VM *et al.* Quaternary history of the Lake Magadi Basin, southern Kenya Rift: tectonic and climatic controls. *Palaeogeogr Palaeoclimatol Palaeoecol* 2019;**518**:97–118. https://doi.org/10.1016/j.palaeo.2019.01.017.

Ownley BH, Griffin MR, Klingeman WE *et al.* *Beauveria bassiana*: endophytic colonization and plant disease control. *J Invertebr Pathol* 2008;**98**:267–70.

Pappas ML, Liapoura M, Papantoniou D *et al.* The beneficial endophytic fungus *fusarium solani* strain K alters tomato responses against spider mites to the benefit of the plant. *Front Plant Sci* 2018;**9**:1603.

Paul JS, Tiwari KL, Jadhav SK. Long term preservation of commercial important fungi in glycerol at 4°C. *Int J Biol Chem* 2015;**9**:79–85.

Penn CJ, Camberato JJ. A critical review on soil chemical processes that control how soil PH affects phosphorus availability to plants. *Agriculture* 2019;**9**:9060120.

Radhakrishnan R, Khan AL, Kang SM *et al.* A comparative study of phosphate solubilization and the host plant growth promotion ability of *Fusarium verticillioides* RK01 and Humicola sp. KNU01 under salt stress. *Ann Microbiol* 2015;**65**:585–93.

Redman RS, Kim YO, Woodward CJDA *et al.* Increased fitness of rice plants to abiotic stress via habitat adapted symbiosis: a strategy for mitigating impacts of climate change. *PLoS One* 2011;**6**:e14823.

Redman RS, Sheehan KB, Stout RG *et al.* Thermotolerance generated by plant/fungal symbiosis. *Science* 2002;**298**:1581.

Rho H, Hsieh M, Kandel SL *et al.* Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. *Microb Ecol* 2018;**75**:407–18.

Rodriguez R, Redman R. More than 400 million years of evolution and some plants still can't make it on their own: plant stress tolerance via fungal symbiosis. *J Exp Bot* 2008;**59**:1109–14.

Sahoo S, Subban K, Chelliah J. Diversity of marine macro-Algicolous endophytic fungi and cytotoxic potential of *Biscogniauxia petrensis* metabolites against cancer cell lines. *Front Microbiol* 2021;**12**:650177.

Schagerl M, Renaut RW. Dipping into the soda lakes of East Africa. In: Schagerl M (ed.), *Soda Lakes of East Africa*. Berlin: Springer International Publishing, 2016, 3–24.

Sharma AK, Sharma V, Saxena J *et al.* Isolation and screening of extracellular protease enzyme from bacterial and fungal isolates of soil. *IJSRES* 2015;**3**:334–40.

Srinivas C, Nirmala Devi D, Narasimha Murthy K *et al.* *Fusarium oxysporum f. sp. lycopersici* causal agent of vascular wilt disease of tomato: biology to diversity–A review. *Saudi J Biol Sci* 2019;**26**:1315–24.

Sunitha VH, Ramesha A, Savitha J *et al.* Amylase production by endophytic fungi Cylindrocephalumsp. isolated from medicinal plant Alpinia calcarata (Haw.) Roscoe. *Braz J Microbiol* 2012;**43**:1213–21.

Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Biol Evol* 1993;**10**:512–26.

Tamura K, Stecher G, Mega KS. Molecular evolutionary genetics analysis, version 11. *Mol Biol Evol* 2021;**38**:3022–7.

Umesha S, Manukumar HM, Raghava S. A rapid method for isolation of genomic DNA from food-borne fungal pathogens. 3 *Biotech* 2016;**6**:123.

Verma H, Kumar D, Kumar V *et al.* The potential application of endophytes in management of stress from drought and salinity in crop plants. *Microorganisms* 2021;**9**:9081729.

Waqas M, Khan AL, Hamayun M *et al.* Endophytic infection alleviates biotic stress in sunflower through regulation of defence hormones, antioxidants and functional amino acids. *Eur J Plant Pathol* 2015;**141**:803–24.

Wehner J, Powell JR, Muller LAH *et al.* Determinants of root-associated fungal communities within A steraceae in a semi-arid grassland. *J Ecol* 2014;**102**:425–36.

White TJ, Bruns TD, Lee SB *et al.* Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ *et al.* (eds.), *PCR Protocols: A Guide to Methods and Applications*. New York: Academic Press, 1990, 315–22. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1.

Xie W, Yang J, Gao S *et al.* The effect and influence mechanism of soil salinity on phosphorus availability in coastal salt-affected soils. *Water* 2022;**14**:2804.

Yao D, Wu J, Luo Q *et al.* Effects of salinity stress at reproductive growth stage on rice (Oryza sativa L.) composition, starch structure, and physicochemical properties. *Front Nutr* 2022;**9**:926217.

Zhang JT, Mu CS. Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. *Soil Sci Plant Nutr* 2009;**55**:685–97.

Zhao C, Zhang H, Song C *et al.* Mechanisms of plant responses and adaptation to soil salinity. *Innovation* 2020;**1**:100017.

Zhou J, Diao X, Wang T *et al.* Phylogenetic diversity and antioxidant activities of culturable fungal endophytes associated with the mangrove species *Rhizophora stylosa* and *R. mucronata* in the South China Sea. *PLoS One* 2018;**13**:e019735.

Zhou W, White JF, Soares MA *et al.* Diversity of fungi associated with plants growing in geothermal ecosystems and evaluation of their capacities to enhance thermotolerance of host plants. *J Plant Interact* 2015;**10**:305–14.

Zhu T, Deng X, Zhou X *et al.* Ethylene and hydrogen peroxide are involved in brassinosteroid–induced salt tolerance in tomato. *Sci Rep* 2016;**6**:35392.

Received 4 August 2023; **revised** 15 March 2024; **accepted** 27 April 2024