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

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

[1]Jomo Kenyatta University of Agriculture and Technology, P.o Box 62000–00200, Nairobi, Kenya;

[2]Wildlife Research and Training Institute, P.o Box 842–20117, Naivasha, Kenya;

[3]Bioline Agrosciences Africa Limited, 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 for 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. Tel: +254 722 923008; Email: priscillahm89@gmail.com

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 8 isolates grew at 1.5M 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% percent, whereas seedlings showed increased chlorophyll and biomass content and decreased hydrogen peroxide content under salinity stress, as compared to control. 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 has 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; Tnay, 2019). 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 semiarid 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 (Rho et al., 2018; Zhou et al., 2015; Berg et al., 2014; Kaul et al., 2016; Verma et al., 2021; Berg et al., 2016; Andreote et al., 2014). These microorganisms, especially fungi, form symbiotic relationships that confer fitness benefits to plants, such as biotic and abiotic stress tolerance and improved nutrient acquisition (Rodriguez & Redman, 2008). However, the ecological roles of endophytic fungi are not fully understood (Reis et al., 2021).

Some benefits conferred by microorganisms are hypothesized to be related to habitat adaptation (Rodriguez & Redman, 2008). For example, inoculation of *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 (Morsy et al., 2020; Redman et al., 2011; Moghaddam et al., 2022; Mutungi 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 & Renaut, 2016). Studies on fungal populations in these unique ecosystems in Kenya are scanty, 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

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 approximately 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 &Renault

2016). The region is semiarid with temperatures ranging between 18°C and 35°C. Shrubs growing along the shores of Lake Magadi were collected in March 2016 and GPS coordinates recorded. One set of plant samples were kept in plastic resealable bags in a cool box. Fungal endophytes were isolated from these plants within 48 hours of sample collection. A second set of plants were 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 minutes followed by 70% ethanol for 1 minute, 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 of about 1cm long with a sterile surgical blade and placed on a sterile filter paper. The sections were air dried under a clean bench for about 5min 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°C ± 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 hour with occasional shaking. The contents were centrifuged for 10 minutes at room temperature (25-27^{0}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 minutes at room temperature, and the supernatant was carefully collected in a fresh tube, 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 hours. The DNA was pelleted by centrifugation for 15 minutes 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 seconds s, annealing at 55°C for 1 minute, elongation at 72°C for 1 minute, and a final elongation at 72°C for 10 minutes. 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 & 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^{o}C for 3 days. Amylase production was detected by flooding the plates with Lugol's iodine solution (1 g iodine crystals and 2 g potassium iodide dissolved in 100 ml 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^{o}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.

196 ***Protease activity***

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

202 ***Phosphate solubilization***

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

208 ***Fungal growth at increasing sodium chloride concentrations***

209 The ability of fungal isolates to grow at increasing concentrations of sodium chloride was tested
210 by growing them in plates of fresh PDA medium supplemented with 0 mM, 0.5 mM, 1 M, and
211 1.5 M sodium chloride. An agar plug from a sporulating plate was placed at the center of plates
212 containing the different sodium chloride concentrations. Three replicate plates per NaCl
213 concentration were incubated for 14 days, and radial growth of each culture was measured.

214 ***Seed inoculation and assessment of endophytic competence of the isolates***

215 ***Fungal cultures***

216 Four fungal isolates were selected for further in vitro experiments. Seeds were inoculated
217 following the procedure described by Jaber (2018). Fungal cultures were grown on PDA
218 supplemented with 50 µg/ml streptomycin sulfate and incubated until sporulation (18–20 days).

Each sporulating fungal culture was flooded with approximately 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 >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-minute wash in 3% sodium hypochlorite, followed by 2 minutes in 70% ethanol, and 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 minutes 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 minutes 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°C ± 2 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 approximately 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°C ± 2 for 14 days. The growing fungal cultures were stained with lactophenol cotton blue stain. The morphological characteristics of the seedling-derived cultures were compared to 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 the 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 in a 10^8 conidia /ml of each fungal isolate. Inoculated seeds were air dried on sterile filter paper under sterile conditions for two hours 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°C ± 2 for four days, and then the germinated seeds were transferred to plastic pots (15 × 17cm) 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, and then autoclaved again. Approximately 1 kg of sterile soil was distributed in each pot, and two germinated seedlings per isolate were transplanted approximately 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 25^0C -28^0C, 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 6 treatments at 20 seedlings per treatment were compared: (1) F04 + 125 mM NaCl; (2) F05 + 125 mM NaCl; (3) F18 + 125 mM NaCl; (4) F21 + 125 mM NaCl; (5) Non-inoculated seedlings (C + 125 mM NaCl); and (6) 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^oC for 48 hrs (Balliu et al., 2015).

Chlorophyll and carotenoid contents were measured using the procedure described by Lichtenthaler & Buschmann 2001). Briefly, 1.5 g of fresh leaves were ground in the dark in 100% acetone and centrifuged at 10000 × 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:

Chlorophyll A: $12.25A_{662}–2.79A_{647}$

Chlorophyll B: $21.50A_{647}–5.10A_{662}$

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 12000 × 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). 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 National Centre for Biotechnology Information (NCBI) genebank database revealed that the genus *Fusarium* was isolated at the highest frequency and was represented by 8 morphogroups; and these isolates represent 7 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 4 morphogroups, to which 17 of the 60 isolates belonged. One species within the genus *Cephalotricum* (*C. cylindricum)* was in two morphogroups representing 7 of the 60 isolates. The other identified genera (*Schizophyllum, Saracladium*, *Daratomyces*, and Fungal species) were each represented by one morphogroup (Fig. 1). Ninety-five percent of the isolates belonged to phylum Ascomycota and the remaining belonged to phylum Basidiomycota, both of which are in the subkingdom Dikarya. Isolates (3 of the 60) classified under the Phylum 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 three 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 to those of the control with salt stress. Seed inoculation with

isolate F04 resulted in the highest increases in both wet and dry weights compared to those of the control 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 to 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 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 contents of chlorophyll a ($p < 0.0001$), chlorophyll b ($p < 0.001$) and total chlorophyll ($p < 0.0001$) as compared to the control. 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 to those of the control (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 macro 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 & Ferraro (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; Etesami & Beattie, 2018). 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 (Khalil et al., 2021; Zhou et al., 2018; Maciá-vicente & Ferraro, 2012; Hamzah et al., 2018; Sahoo et al., 2021). The ubiquity of Ascomycetes in soil can probably explain their abundance as endophytes.

In the current study, 46% (28 isolates) 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 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

447 industrial applications (El-Hawary et al., 2020). They have been implicated in the production of
448 endogenous plant hormones, amino acids, and other soluble organic acids that help the plant
449 mitigate stress and enhance growth (Waqas et al., 2015).

450 Establishing endophytism in nonhost plants is especially important for beneficial endophytes,
451 because they offer the possibility of conferring similar benefits to crop plants. In this study, we
452 tested the ability of four selected endophytic fungi to competently colonize tomato plants
453 growing in sterile vermiculite by seed inoculation using two different fungal spore
454 concentrations. All isolates colonized tomato at both concentrations but differed in individual
455 fungal performance and plant part. Similar results were obtained by Akutse et al., 2017; Jaber &
456 Enkerli (2016) who reported differences in colonization rates for different plant parts. Other
457 studies have also inoculated seeds with a conidial concentration of 10^8 conidia/ml, resulting in
458 successful post-inoculation recovery of the endophytes from all plant parts and effective
459 performance on the test variable (Jaber, 2018; Mutune et al., 2016). Several factors contribute to
460 successful endophyte establishment in nonhost plants, including the concentration of inoculum
461 used, medium used (sterile or nonsterile), and method of inoculation (Bamisile et al., 2018).
462 Using seed soaking and sterile vermiculite in our study, the concentration of conidia used
463 correlated with the recovery rates , which is consistent with the results of other studies (Ownley
464 et al., 2008).

465 Salinity stress in plants can be a lethal factor that limits the normal functioning of plants and
466 eventually affects growth and productivity. At elevated salinity levels, all growth stages (seed
467 germination, seedling, vegetative growth, and maturity) as well as the quality of the seeds/fruits
468 are negatively affected (Jafarzadeh & Aliasgharzad 2007; Yao et al., 2022). Germination and
469 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, resulting in oxidative stress that tends to prolong the seed germination period and lower the germination rate (Zhang & 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 (Kumar & Verma 2018; Verma et al., 2021; Jogawat et al., 2016; Molina-Montenegro et al., 2020). 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 reactive oxygen species. 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 & 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

493 negatively affected at 125 mM NaCl, which markedly reduced leaf chlorophyll content and
494 increased levels of hydrogen peroxide compared to those of control seedlings without NaCl. This
495 reduction in chlorophyll could be associated with the plants' inability to manage ion toxicity
496 caused by increased Na^+ and Cl^- ions in the plant tissues.(Zhang & Mu, 2009). Hydrogen
497 peroxide is a reactive oxygen species (ROS) and a signaling molecule generated by plants in
498 response to stress conditions (Zhu et al., 2016). Elevated levels of hydrogen peroxide damage
499 cellular metabolites oxidatively, which affects plant growth (Hossain et al., 2015). Symbiotic
500 association with endophytic fungi significantly ($p < 0.001$) reduced the amount of hydrogen
501 peroxide produced by the plants, increased their chlorophyll content, and increased their dry
502 weight as compared to uninoculated control plants exposed to sodium chloride. These results
503 indicate the endophytes enhanced sodium chloride tolerance of the seedlings. We speculate the
504 endophytic fungi in the tomato seeds and seedlings helped maintain the ionic balance in the plant
505 cytosol, thereby preventing accumulation of toxic Na^+ ions while enhancing photosynthesis in
506 the seedlings under sodium chloride stress. Ionic homeostasis in plants, reduced ROS production,
507 and concomitant increases in shoot and root weight have been reported in several studies as
508 mechanisms by which fungal endophytes alleviate salt stress (Ali et al., 2022; Bouzouina et al.,
509 2021) .

510 Fungal endophytes are prolific producers of extracellular enzymes and secondary metabolites
511 (Debbab et al., 2013), which are important in the selection of beneficial microorganisms for use
512 in agricultural production. Our experiment used four fungal endophytes that were selected based
513 on their abilities to produce the exoenzymes amylase, protease, and cellulases and to solubilize
514 inorganic phosphorus. Of the 60 fungal isolates obtained, 62% solubilized inorganic phosphate, a
515 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 & 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 developmentt 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.

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Author Contributions

Mutungi PM, Wekesa VW, Onguso J, and Boga HI conceived and designed the experiments. Mutungi PM performed the experiments with guidance from Wekesa VW, Onguso J, Boga HI, and Kanga E. Mutungi PM and Baleba SBS analyzed the data. Mutungi PM wrote the manuscript with guidance and input from Wekesa VW, Onguso J, Kanga E, Baleba SBS, and Boga HI. All authors have read, edited, and approved the final manuscript.

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

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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.

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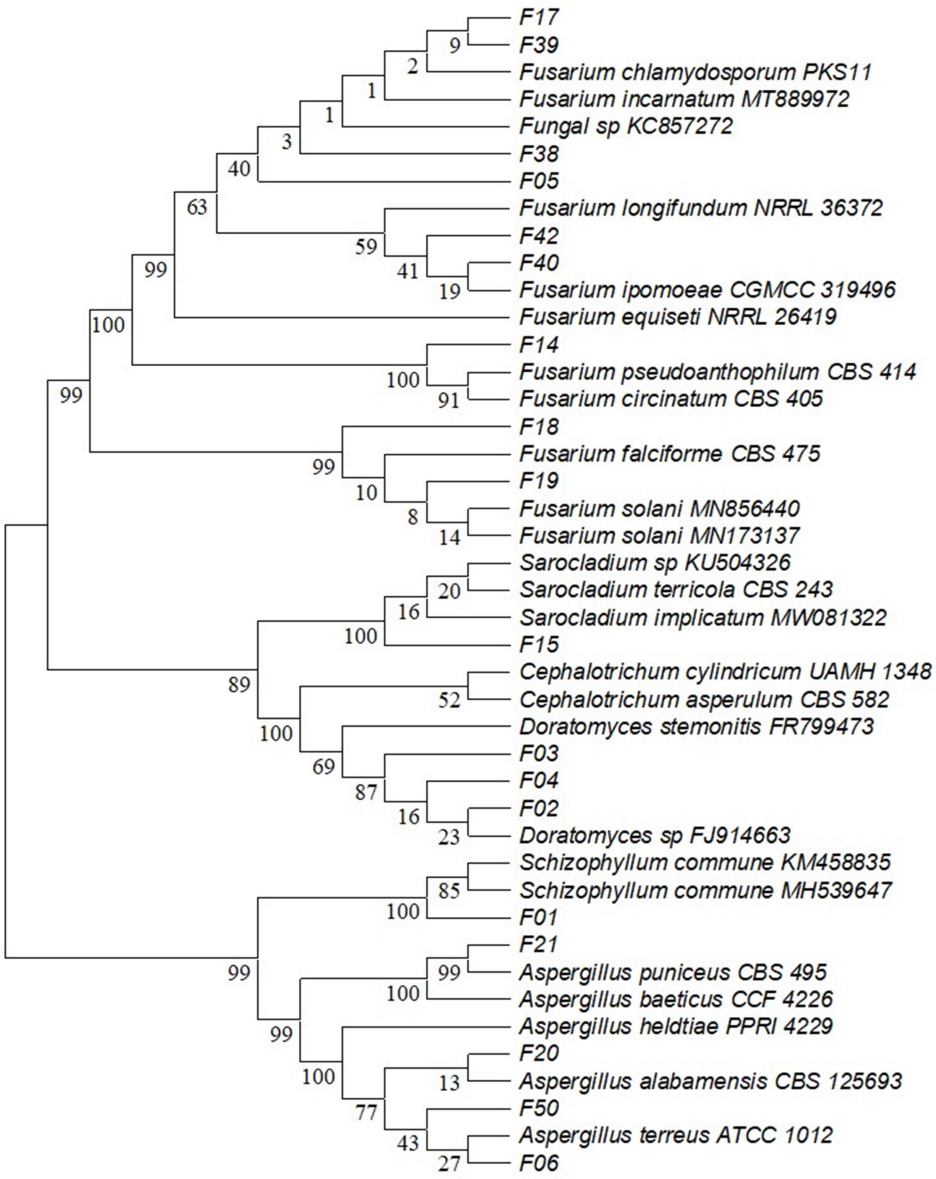

Figure1. Unrooted phylogenetic tree of fungal endophytes depicting the evolutionary history of the isolates using the maximum likelihood method with 1000 bootstrap replicates and complete elimination of gaps and missing data. Phylogenetic analysis was performed in MEGA 11. The percentage of trees in which the associated taxa clustered together is shown below the branches.

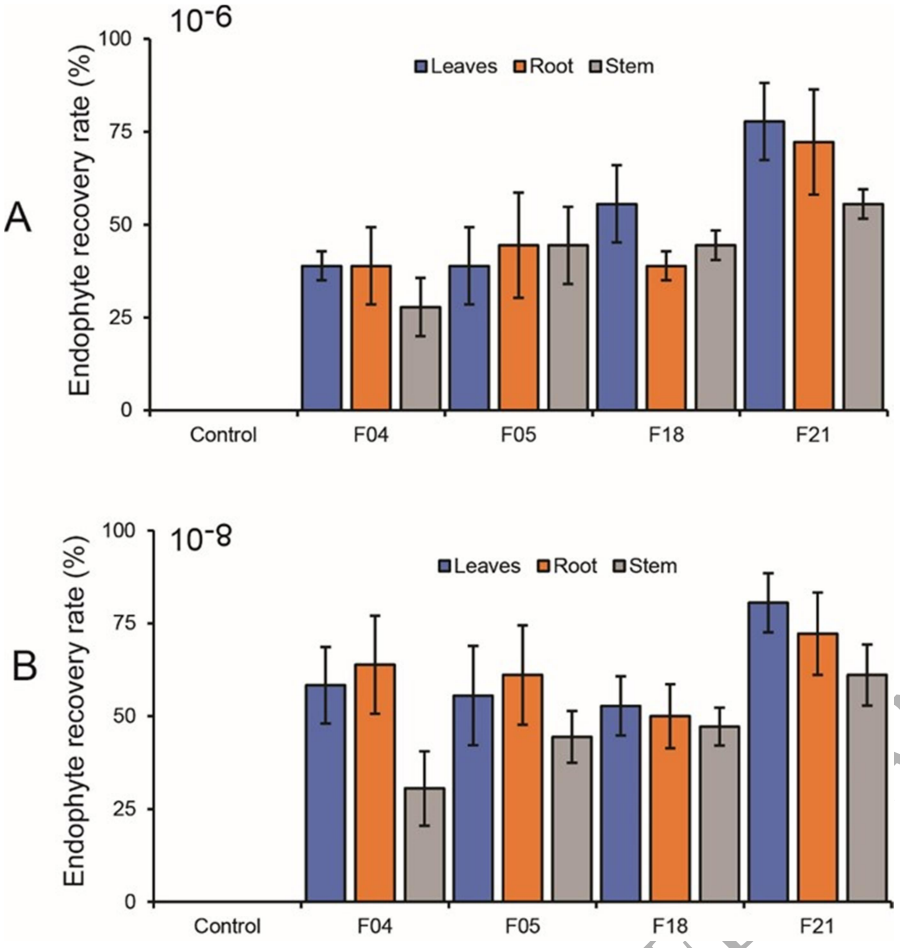

Figure 2. Fungal endophyte recovery rate from leaves, stems, and roots of tomato seedlings inoculated via the seed soaking method. A and B show endophyte recovery rates using conidial concentrations of 106 and 108 Conidia/ml, respectively. Bars represent standard error of the mean (SEM).

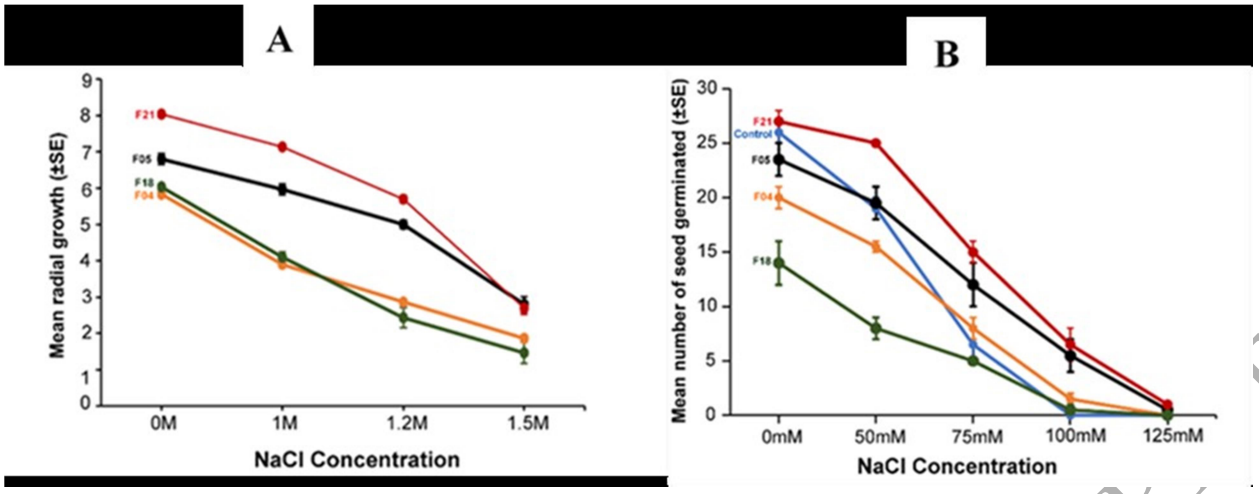

Figure 3. (A) Mean (±SE) radial growth of endophytes on PDA plates (n = 3) supplemented with different concentrations of sodium chloride. (B) Effect of fungal endophytes on seed germination following seed soaking with 108 conidia/ml and incubation on PDA plates supplemented with 0 to 125mM NaCl concentrations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

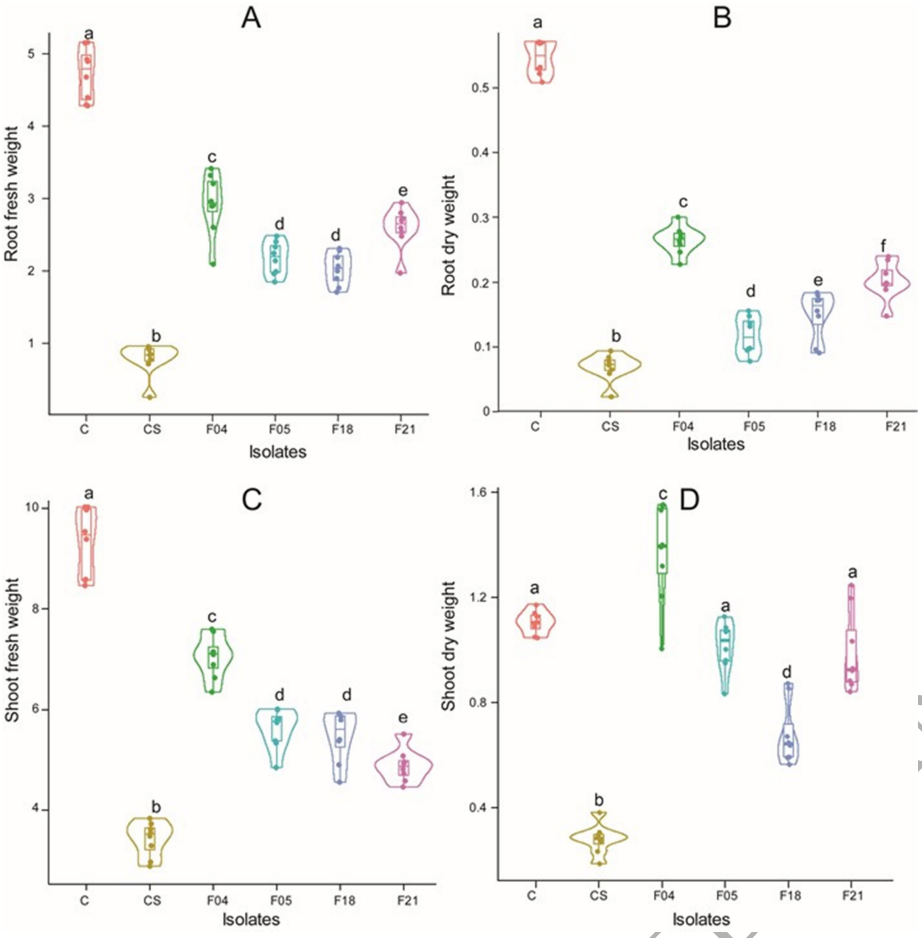

Figure 4. Violin plot representing the effect of different fungal endophytes on seedling growth in terms of root fresh weight (A),root dry weight (B), shoot fresh weight (c),and shoot dry weight (D) after exposure of seedlings to salinity stress for 21 days. CS, uninoculated control seedlings exposed 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)

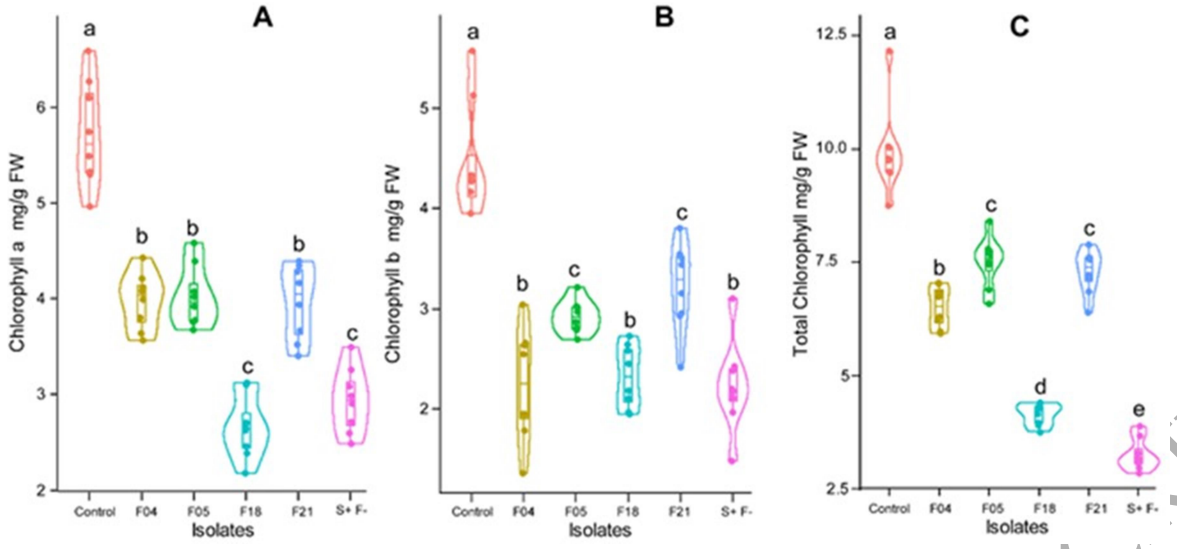

Figure 5. Effect of endophytic fungi on chlorophyll a (A), chlorophyll b (B), and total chlorophyll (C) contents of the leaves of tomato seedlings exposed to 125 mM NaCl for 28 days. The same letter indicates no significant difference in chlorophyll concentration. S+F-, uninoculated control seedlings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

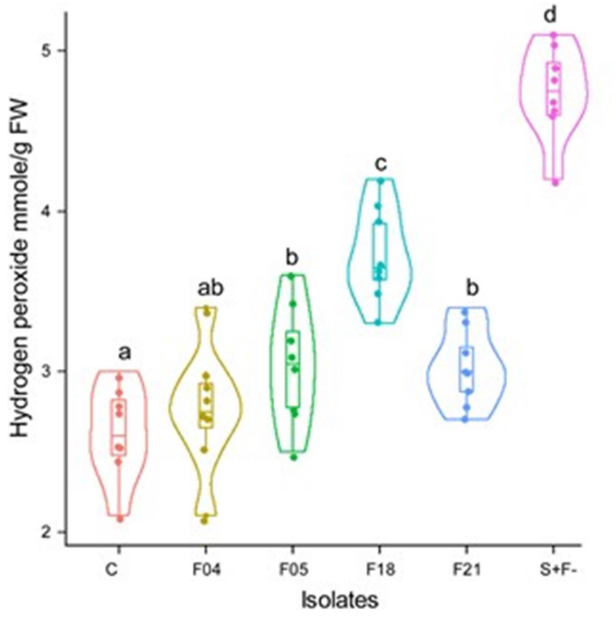

Figure 6. Effect of fungal endophytes on hydrogen peroxide production by tomato seedlings exposed to 125 mM NaCl for 28 days. Isolates followed by the same letter indicate no significant difference in hydrogen peroxide production. S + F-, uninoculated control seedlings exposed to salinity stress. C,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

820

821 **Table 1: Endophytic fungal isolates, their respective source plants, and their physiological characteristics**

Isolate No.	Source plant name	Plant part	GPS coordinatess	Growth on NaCl[a]				Enzymatic activities[b]			Phosphate solubilization[b]
				0M	0.5 M	1.0 M	1.5 M	Amylase	Protease	Cellulase	
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	+	+	+
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***	**Root**	**1°52′02″S**	+++	++	+	+	+	+	+	+

	spinosa Forssk		**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	++	++	-	-	-	-	-	+
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	+++	-	-	-	-	+	+	-
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	$2^0$00′04″S $36^0$13′56″E 606M	++++	++	+	-	+	-	-	-
F60	*Lactuca inermis Forssk*	Leaves	$2^0$00′04″S $36^0$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