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Induction of abiotic stress tolerance in plants by endophytic fungi hosted wild plants

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ABSTRACT

The production of economically valuable plants, such as cucurbits and tomatoes, has been negatively impacted by a variety of living organisms and environmental factors. Endophytic fungi can trigger systemic resistance in their host plant, which enhances the plant's ability to withstand biotic stress and also improves its tolerance to abiotic challenges. In our continuing search for biologically active native fungi from Egypt with special reference to endophytic fungi, our teamwork screened endophytic fungi hosted wild plants from different ecological habitats. This investigation employed wild plant-hosted *Trichoderma atroviride* (PP055997.1), *Fusarium acutatum* (PP038127.1), and *Aspergillus terreus* (PP038155.1). The target plant was inoculated with these endophytes via seed, root dipping, and leaf spray. Colonisation with systemic fungal endophytes was tested on plant development at 12 weeks under 150 and 300 mM NaCl salt stress and 10 and 20% polyethylene glycol 6000 drought stress for 14 days. After 16 weeks following stress, the plants were taken to analyse growth and physiological data. *A. terreus*-colonized plants had higher biomass output and photosynthetic efficiency. The endophyte increased height biomass and fresh weight biomass, dry weight biomass at 150 and 300 mM NaCl and 10 and 20 % PEG 6000 respectively. On the other hand, oxidative activity of plants colonized with *A. terreus* was always lower in comparison to non-colonized control plants in response to salt and drought stress. The endophyte increased total chlorophyll, carotenoid, proline, and SOD content. Additionally, decreased malondialdehyde (MDA) content and electrolyte leakage (EL) by 50.39, 85.64, 48.23, and 75.10 % at 150 and 300 mM NaCl and 10 and 20 % PEG 6000 respectively. We conclude that PP038155.1 has the potential to improve agriculture and horticulture on salinized and dry soils which are common phenomenon in semi-arid environments.

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Introduction

Plants could alleviate environmental stress by different physiological and biochemical processes. Plant growth, and crop yield are negatively affected by biotic and abiotic stresses (Bohnert et al. 1995). Abiotic factors include all the environmental conditions (temperature, humidity, light intensity, as well as water, mineral and CO_2 availability) that could affect plant growth and yield. soil

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salinity and drought are considered vital factors that reduce crop production worldwide (Chaves et al. 2009; Azad & Kaminskyj 2016). Plants need optimum condition of abiotic and biotic factors for achievement the maximum crop yield. Tomato (*Solanum lycopersicum* L.) is the most important crop which enriched with considerable amounts of minerals and vitamins (Khan et al. 2014).

Tomatoes are the most widely farmed fresh market vegetable in the world, according to (Desneux et al. 2011), reported that 180 million tons of tomatoes are estimated as the total world production of crop which are cultivated in areas of approximately 4 M ha. In arid and semi-arid conditions water is considered as limited resource for agronomic practices Tomato crop is highly sensitive to drought stress, furthermore water deficit by 15% and 30% resulting in net profits reduction by 15% and 22%, respectively (Obreza et al. 1996). Traditional human agronomic activities are considered the main cause secondary salinization of soil and water supplies (Shrivastava & Kumar 2015). Saline soil has an electrical conductivity (EC) (ECe) in the root zone that surpasses 4 ds/m (about 40 mM NaCl) (Munns 2005, Jamil et al. 2011). The tomato crop (*S. lycopersicum* L.) is highly salt sensitive to salt stress.

Agriculture is one of the most vulnerable sectors to climate change. Agricultural crops exhibit a spectrum of responses under abiotic stress. A plant's first line of defence against abiotic stress is in its roots (Abo Nouh & Abdel-Azeem 2020). Salinity affects almost all aspects of plant development including germination, vegetative growth, and reproductive development. Soil salinity imposes ion toxicity, osmotic stress, nutrient deficiency(Bano & Fatima 2009); and increasing reactive oxygen species (ROS) production in chloroplasts (Talaat & Shawky 2013). Farooq et al. (2009) also mentioned that drought stress suppresses plant growth by affecting various physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrate metabolism, and nutrient uptake. It interferes with photosynthesis and protein synthesis, increases photorespiration, changes plant hormone balance, impairs cell homeostasis, and causes high levels of ROS in plant cells (Cohen et al. 2015). To mitigate salt and drought stress, plants use two strategies: stress adaptation or stress avoidance. These mechanisms used vary on differences in stress perception, signal transduction, and appropriate gene expression programs, or metabolic pathways of stress tolerant plants (Bartels & Sunkar 2005).

The fungal endophytes provide an immune system to the host plant, allowing it to defend against phytopathogenic organisms (Jain & Pundir 2017); and assisting plants in adapting to new habitats by modifying its host plants genetically, physiologically, and ecologically (Lugtenberg et al. 2016; Abdel-Azeem et al. 2021; Abo Nouh et al. 2021). Tolerance of plants to salt stress is associated with the alleviation of antioxidant enzymes, i.e., ROS scavengers including glutathione, ascorbate, and tocopherol, and the enzymes superoxide dismutases (SOD), catalases (CAT), ascorbate- or thiol-dependent peroxidases (APX), glutathione reductases (GR), dehydroascorbate reductases (DHAR) and mono-dehydroascorbate reductases (MDHAR) (Verma et al. 2022).

Different fungal and plant species, as well as various environmental pressures, were used to confirm the idea that fungal endophytes adapt to stress in a habitat-specific manner. This phenomenon is known as habitat-adapted symbiosis, and scientists believe that fungal endophytes play a role in plant adaptation to environmental challenges (Rodriguez et al. 2008; Abo Nouh 2019; El Mansy et al. 2020). Salinity and drought conferred by mutualistic fungi, which may also enhance growth and nutrient uptake. In the absence of fungal endophytes, it has become obvious that at least some plants are unable to withstand habitat-imposed abiotic and biotic pressures (Rodriguez & Redman 2008). These findings suggest that incorporating fungal symbionts into crops could be a viable method for both minimizing abiotic stress impacts on major crops and expanding agricultural production onto marginal lands (Redman et al. 2011).

Scientists in continuous search for new techniques and technologies to minimize the negative impacts of stress on plants. Endophytic fungi are the new hope for reducing the negative impacts of drought and salinity stresses, as it could be used as bio-inoculants for enhancement plant growth (Abo Nouh et al. 2022). Despite of the harsh environmental conditions, fungal endophytes could enhance plant growth (Rodriguez et al. 2009). As fungal endophytes have ability for colonization of a variety of plant species, so it could be used as biofertilizers for many crops (Rodriguez et al. 2004, 2008).

Abiotic stressors such as drought, salt, and high temperatures have a negative impact on agricultural productivity, yet growing human populations require higher crop yields for food security. Plants use the development of stress tolerance as a coping mechanism for the harmful impacts of unfavorable environmental conditions. This study may help in the development of biotechnological applications of native endophytic fungi in plant growth promotion and crop improvement under abiotic stress conditions.

Materials and Methods

Fungal isolates

Three endophytic fungal strains were *T. atroviride* (PP055997.1), *F. acutatum* (PP038127.1) and *A. terreus*

(PP038155.1) isolated from wild plants *Zygophyllum album*, *Datura metel* and *Artemisia monosperma* respectively. In our previous study by Abo Nouh et al. (2023) surveyed those taxa for extracellular enzyme production and plant-growth-promoting activity, revealing the highest levels under drought and salinity stress tolerance in comparison with other taxa.

Spore suspension preparation

Fungal spores of 10 days culture of potato dextrose agar medium. The conidia concentration was then adjusted to the desired concentration of 1 × 10^8 conidia ml^{-1} after cell counting in a Neubauer haemocytometer (Tefera & Vidal 2009).

Germination of tomato seeds

As an economic plant in Egypt, tomato (*S. lycopersicum)* seeds of variety (GS-12), were cultivated in pot-tray filled with (2:1) peat moss and vermiculite according to manufacturer recommendations (Pourtaghi et al. 2020). Seedlings growing for 8 weeks in greenhouse at 25°C, with 70% relative humidity and under 12: 12 h (L: D) photoperiod.

Inoculation techniques

Tomato seeds were surface sterilized according to (Abdel-Azeem & Salem 2012) and dried on sterile filter paper for 30 min. For seeds inoculation, seeds (5 g) were immersed in 10 ml of a conidial suspension for 24 h according to (Brownbridge et al. 2012). For root dipping application, surface sterilized seeds (5 g) were germinated in sterile potting medium at greenhouse with above-mentioned condition for 21 days. Each seedling was removed from the pot and rinsed in test tubes with 2 ml of a conidial suspension for 24 h according to Allegrucci et al. (2018). For leave spray application, surface sterilized seeds (5 g) were cultivated in sterile potting medium and maintained in a greenhouse condition for two weeks. leaves of tomato plants were sprayed with 3 ml of a conidial using a sterile glass hand sprayer according to Pourtaghi et al. (2020).

Evaluation of endophytic fungi development

For all inoculation methods, the colonization efficiency of tomato plants with inoculated endophytic fungi was evaluated 14 days after inoculation. Tomato plants were uprooted from the soil and plant parts were surface sterilized according to Abdel-Azeem & Salem (2014). The surface sterilized plant parts plated onto potato dextrose agar (PDA) amended with 300 mg/ml streptomycin. Twenty plant pieces per plant part (4 segments / plate) for each of five plants. The presence or absence of endophyte will record after 10 days at 25°C. The colonization frequency (CF) was calculated as follows: CF= [Number of colonized plant part pieces / Total number of plant pieces] x 100

Pot experiment

The experiment was designed in a completely random manner using 3 individual fungal isolates inoculated into tomato plants with seedling spray method (five replicates for each treatment). These fungal strains were selected based on their high activities in plant-growth-promoting activities and growth under abiotic stress. *S. lycopersicum* plants were cultivated from February (2022) to June (2022). The seedlings were maintained in portray in a greenhouse at 25°C, with 70% relative humidity and under 12: 12 h (L: D) photoperiod for one months, and then transplanted to pots approximately 2 kg of non-sterile soil and treated with NPK (2 g/L).

Three-months seedling treated with salinity and drought stress for 14 days. The experiment was divided into two sets of stress, as follow: **Salinity stress (**normal, 150 mM of NaCl and 300 mM of NaCl) tomato plants inoculated with 3 fungal isolates and control. **Drought stress,** (normal, 10% (PEG 6000), and 20% (PEG 6000) on tomato plants treated with 3 fungal isolates and control.

Morphological and physiological parameters were estimated during plant growth and at the end of experiment including shoot and root lengths, shoot and root fresh weight, shoot and root dry weight.

Biochemical measurements

Photosynthetic pigments

Chlorophyll a, chlorophyll b, total chlorophyll [Chl (a+b)], and total carotenoids were determined according to the method by recommended by Lichtenthaler (1987). The absorbance was read at 666, 653, and 470 nm by spectrophotometer, respectively. The pigments content was calculated according to the following formula:

Chlorophyll a = 15.65 A666 − 7.340 A653

Chlorophyll b = 27.05 A653 − 11.21 A666

Total Carotenoids = 1000 A470 − 2.860 Ca− 129.2 Cb/245

Total chlorophyll = Chl (a+b)

Estimation of proline

Proline content was measured according to ninhydrin-based colorimetric assay (Bates et al. 1973). The absorbance was recorded by UV spectrophotometer at 520 nm against a toluene blank. Proline content in sample was estimated by referring to a standard curve of proline according to following formula: Proline concentration μg/g FW = (μg proline/mL× mL toluene) / 115.5/ (g sample/5).

Lipid peroxidation as malondialdehyde (MDA) accumulation

For the determination of MDA, the thiobarbituric acid (TBA) assay is one of the widely used assays (Hagège et al. 1995). Measure the optical density at 532 and 600 nm and calculate the concentration of MDA-TBA concentration based on the Ɛ value by comparing the standard curve. Where, Ɛ is the coefficient of absorbance (1.53 mM^{-1} cm^{-1}).

MDA protein = (A532 - A600) ×Vr × (V/Vt)/Ɛ×1000/Cp

Electrolyte leakage

The technique used to determine membrane stability was followed Sullivan (2015) with some modifications. The following formula was used to define electrolyte leakage: EL (%) = (EC1/EC2) × 100

Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD) activity was determined by measuring the inhibition in photoreduction of nitroblue tetrazolium (NBT) by SOD enzyme (Kumar et al. 2012). The absorbance was recorded at 560 nm using a spectrophotometer. One unit (U) of SOD activity was defined as the amount of enzyme causing 50% inhibition of photochemical reduction of NBT.

% Inhibition of NBT reduction by SOD = Control A560 - Treatment A560/Control × 100. As 50% inhibition is equal to 1 unit of enzyme.

Statistical Analysis

The data obtained were analysed by one-way analysis of variance (ANOVA), T-test (independent test) with SPSS version 25 (IBM). The significance of differences within treatments was separated using Duncan's multiple range tests at a probability level of 0.05, Standard deviation represents ±SD of three replicates.

Results

The effect of inoculation method on percent of tomato seedling germination

Method of inoculation is considered crucial step in colonization process of endophytes to plant. this experiment showed that leave spray method is most effective treatment with high seedling percent (Fig. 1).

Effect of different inoculation methods on tomato plant growth

Morphological and physiological parameters of tomato plant colonized with fungal endophytes by different methods after harvested at 8 weeks was presented as Mean and SD in table (1). Plants inoculated with *F. acutatum* (PP038127.1) by leave spray, and root dipping methods showed highest plant shoot length (18.2 and 17 cm), respectively, which significantly different from un-inoculated control.

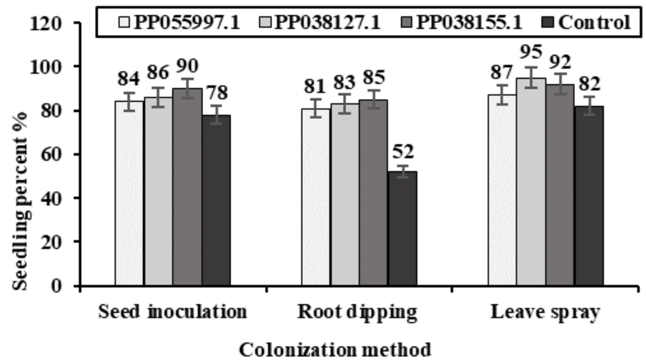

Fig. 1 Growth seedling percent of colonized tomato plant with fungal endophytes by different methods.

While seed inoculation method by *A. terreus* (PP038155.1) showed highest plant shoot length (16.4 cm) which significantly different from un-inoculated control (12.6 cm). As revealed, that leave spray method is the most suitable method for enhancement root length, as highest root length (6.2 and 6.1 cm) obtained by inoculation with *T. atroviride* (PP055997.1), *F. acutatum* (PP038127.1), respectively which significantly different than un-inoculated control.

As indicated in table (1) the highest the shoot fresh weight (1.43 and 1.40 g) was recorded with seed inoculation technique and leave spray by *A. terreus* (PP038155.1) and *F. acutatum* (PP038127.1), respectively which both significantly different than their un-inoculated controls. That result to the seed inoculation and leave spray treatment as the most effective colonization method for shoot fresh weight improvement in tomato plant. Results indicated that highest root fresh weight (0.38 and 0.29 g) were obtained by seed inoculation and leaf spray methods, respectively in plants inoculated with *T. atroviride* (PP055997.1) in both methods compared to un-inoculated control.

Data of table (1) showed that highest shoot dry weight (0.35 and 0.34 g) were recorded by leave spray methods in plants inoculated with *F. acutatum* (PP038127.1) and *A. terreus* (PP038155.1), respectively. As leave spray treatment was the most effective colonization method for shoot dry weight improvement in tomato plant. The results showed that highest root dry weight (0.028 and 0.026 g) was obtained with leave spray method inoculation by *F. acutatum* (PP038127.1) and *A. terreus* (PP038155.1), respectively. As leave spray treatment was the most effective colonization method for root dry weight improvement in tomato plant.

Table 1 Morphological and physiological parameters of tomato plant colonized by different methods after 8 weeks

Method	Fungal isolate	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Seed inoculation	PP055997.1	16.0 ± 0.79 ^{a}	5.9 ± 0.87 ^{a}	1.02 ± 0.25 ^{b}	0.29 ± 0.05 ^{a}	0.18 ± 0.05 ^{b}	0.014 ± 0.005 ^{a}
	PP038127.1	15.9 ± 1.14 ^{ab}	5.7 ± 0.87 ^{a}	1.29 ± 0.19 ^{b}	0.24 ± 0.07 ^{ab}	0.27 ± 0.07 ^{a}	0.017 ± 0.009 ^{a}
	PP038155.1	16.4 ± 1.34 ^{a}	5.1 ± 1.21 ^{a}	1.43 ± 0.25 ^{a}	0.25 ± 0.11 ^{a}	0.20 ± 0.09 ^{a}	0.021 ± 0.006 ^{a}
	Control	12.6 ± 1.52 ^{b}	4.6 ± 0.79 ^{b}	0.95 ± 0.20 ^{b}	0.20 ± 0.06 ^{b}	0.15 ± 0.04 ^{b}	0.013 ± 0.004 ^{a}
Root dipping	PP055997.1	14.6 ± 1.29 ^{b}	5.4 ± 0.27 ^{a}	1.11 ± 0.23 ^{ab}	0.22 ± 0.03 ^{a}	0.19 ± 0.15 ^{ab}	0.015 ± 0.002 ^{b}
	PP038127.1	17.0 ± 1.41 ^{a}	5.7 ± 0.89 ^{a}	1.37 ± 0.43 ^{a}	0.24 ± 0.10 ^{a}	0.25 ± 0.12 ^{a}	0.025 ± 0.010 ^{a}
	PP038155.1	14.6 ± 1.14 ^{b}	5.1 ± 0.79 ^{a}	1.03 ± 0.18 ^{b}	0.20 ± 0.06 ^{a}	0.16 ± 0.07 ^{b}	0.014 ± 0.004 ^{b}
	Control	11.9 ± 1.43 ^{c}	4.4 ± 0.84 ^{b}	0.88 ± 0.29 ^{b}	0.17 ± 0.08 ^{b}	0.12 ± 0.08 ^{c}	0.009 ± 0.002 ^{b}
Leave spray	PP055997.1	17.1± 1.02 ^{b}	6.2 ± 0.72 ^{a}	1.21 ± 0.16 ^{ab}	0.38 ± 0.90 ^{a}	0.28 ± 0.14 ^{b}	0.022 ± 0.002 ^{b}
	PP038127.1	18.2 ± 0.84 ^{a}	6.1 ± 0.69 ^{a}	1.40 ± 0.33 ^{a}	0.28 ± 0.08 ^{b}	0.35 ± 0.14 ^{a}	0.028 ± 0.012 ^{a}
	PP038155.1	16.8 ± 0. 84 ^{b}	5.9 ± 0.74 ^{a}	1.29 ± 0.24 ^{ab}	0.27 ± 0.04 ^{b}	0.34 ± 0.12 ^{a}	0.026 ± 0.004 ^{b}
	Control	12.8 ± 0. 84 ^{c}	4.9 ± 0.67 ^{b}	0.98 ± 0.22 ^{b}	0.21 ± 0.08 ^{c}	0.16 ± 0.08 ^{c}	0.015 ± 0.007 ^{b}

Mean values followed by the same letters (a, b, c, etc.) are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Evaluation of endophytic establishment

The method of fungal inoculation into plants is avital step for determining the ability of fungal isolates to be colonized in the plants. Results of fig. (2) revealed that highest colonization frequency (60 %) were obtained in plant leaves by *A. terreus* (PP038155.1), on the other hand, the lowest colonization frequency (35%) was recorded in root by *T. atroviride* (PP055997.1) using root dipping inoculation techniques.

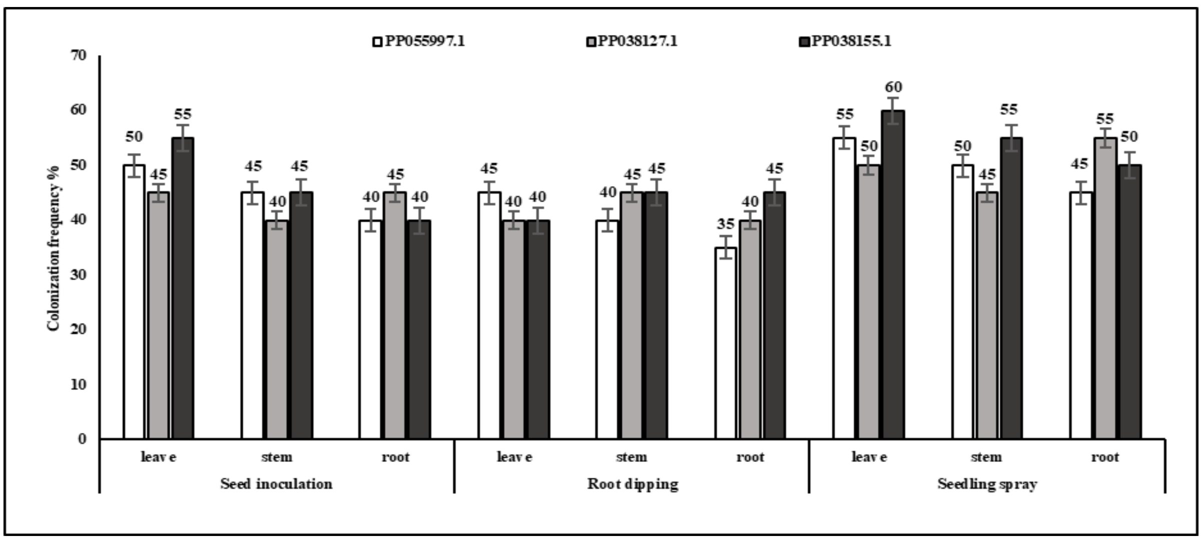

Fig. 2 Colonization frequency of tomato colonized plant by fungal endophytes with inculation methods.

Pot Experiment

All fungal isolates were tested for their ability to enhance tomato plant growth under a pot experiment of salinity and drought stress treatment. Many plant growth parameters were investigated after 16 weeks (i.e., plant height, fresh biomass, dry biomass of shoots and roots). The effects of the salinity and fungal inoculation treatments on tomato plant height and weight showed in table (2), which all results were significantly different than for the control. As showed in table (2) in case of normal condition plants inoculated with *T. atroviride* (PP055997.1) showed highest shoot length (40.67 cm) which significantly different than un-inoculated control (34.67 cm). On the other hand, the isolate *A. terreus* (PP038155.1) enhances shoot length by 43 and 36.33 (cm) which was significantly different than un-inoculated control under saline condition (150 and 300 mM NaCl). As observed from pot experiment that root length of control un-inoculated plants is severely affected by salinity stress from 26.33 to 14.23 cm in normal and saline stress (300 mM NaCl), respectively. While results indicated that *T. atroviride* (PP055997.1) enhance root length by 32.33 (cm). On the other hand, plants inoculated with *A. terreus* (PP038155.1) showed highest root length by 32.83 and 28.33 (cm) under saline conditions (150 and 300 mM NaCl), respectively. Salinity stress negatively affects shoot fresh weight of un-inoculated plants from 17.76 g to 7.02 g in normal and salinity stress (300 mM NaCl), respectively. While shoot fresh weight of plants inoculated with *A. terreus*

(PP038155.1) wasn't affected by salinity stress at (150 mM NaCl) and showed highest fresh weight (26.7 g) compared by un-inoculated control. Data showed that salinity stress could decrease root fresh weight of un-inoculated plants from 9.29 to 2.83 in normal condition and salinity stress 300 mM NaCl, respectively. While plants inoculated with *A. terreus* (PP038155.1) recorded highest root fresh weight (12.72 and 6.11 g) under salinity stress (150 and 300 mM NaCl), respectively compared to their respective un-inoculated controls. Plant shoot dry weight is considered as important parameter in plant growth, as salinity adversely decreased control- un-inoculated from 2.79 to 1.32 g in normal and salinity stress (300 mM NaCl). Inoculation with *A. terreus* (PP038155.1) showed highest shoot dry weight (3.5 and 2.64 g) under salinity stress 150 and 300 mM NaCl), respectively compared to their respective controls. Also root dry weight affected by salinity stress, while plants inoculated with *A. terreus* (PP038155.1) showed high root dry weight (1.93 and 0.96 g) under saline conditions (150 and 300 mM NaCl), respectively, compared to their respective controls.

The effects of the drought and fungal inoculation treatments on tomato plant height and weight (Table 3), which all results were significantly different than for the control. As observed from results, drought stress (10 and 20 % PEG) could suppress shoot and root length of un-inoculated plants to lowest levels compared to their respective inoculated one. Plants inoculated with *A. terreus* (PP038155.1) showed high shoot and root length (42.5, 36.6 and 32.5, 25.3 cm) under drought stress (10 and 20 % PEG 6000), respectively compared to their respective un-inoculated controls. Drought stress (10 and 20 % PEG) decreased shoot and root fresh weight of un-inoculated controls to (12.31, 7.02 and 5.6, 3.3 g), respectively. While plants inoculated with *A. terreus* (PP038155.1) showed high shoot and root fresh weight (23.5, 13.5 and 10.1, 7.6 g) under drought stress (10 and 20 % PEG), respectively, compared to their respective un-inoculated controls. Drought stress at (10 and 20 % PEG) has negative effect on shoot and root dry weight of control un-inoculated controls. Plants inoculated with isolate *T. atroviride* (PP055997.1) showed high shoot dry weight 2.51 g at drought stress (20% PEG) compared to un-inoculated control. While plants inoculated with *A. terreus* (PP038155.1) recorded high root dry weight (1.08 g) at drought stress 20 % PEG compared to its control.

Table 2 Plant growth parameters investigated under salinity stress after 16 weeks

Stress	Fungal taxa	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
No stress	PP055997.1	40.67 ± 2.08 ^{a}	32.33 ± 1.15 ^{a}	27.05 ± 2.08 ^{a}	13.88 ± 2.48 ^{a}	3.63 ± 0.79 ^{a}	2.00 ± 0.15 ^{a}
	PP038127.1	36.67 ± 2.08 ^{ab}	30.20 ± 2.65 ^{a}	24.94 ± 3.21 ^{a}	12.64 ± 0.91 ^{a}	3.47 ± 0.72 ^{b}	1.95 ± 0.03 ^{a}
	PP038155.1	39.67 ± 2.08 ^{a}	28.67 ± 5.69 ^{ab}	23.87 ± 3.14 ^{a}	11.05 ± 2.32 ^{ab}	3.43 ± 0.80 ^{b}	1.52 ± 0.54 ^{b}
	Control	34.67 ± 1.52 ^{b}	26.33 ± 1.53 ^{b}	17.76 ± 1.42 ^{b}	9.29 ± 1.32^{b}	2.79 ± 0.25 ^{c}	0.92 ± 0.09 ^{c}
150 mM NaCl	PP055997.1	35.17 ± 0.29 ^{a}	27.83 ± 3.33 ^{ab}	17.45 ± 2.86 ^{b}	10.27 ± 2.30 ^{b}	2.84 ± 0.21 ^{ab}	1.61 ± 0.15 ^{a}
	PP038127.1	31.67 ± 0.58 ^{ab}	27.00 ± 0.02 ^{ab}	19.54 ± 3.03 ^{ab}	8.68 ± 2.75 ^{b}	2.53 ± 0.69 ^{ab}	1.43 ± 0.01 ^{a}
	PP038155.1	43.00 ± 2.65 ^{a}	32.83 ± 3.25 ^{a}	26.76 ± 0.71 ^{a}	12.72 ± 2.36 ^{a}	3.50 ± 0.34 ^{a}	1.93 ± 0.09 ^{a}
	Control	31.00 ± 1.00 ^{ab}	21.00 ± 3.46 ^{b}	14.56 ± 1.68 ^{b}	4.52 ± 3.88 ^{c}	2.12 ± 0.14 ^{b}	0.72 ± 0.39 ^{b}
300 mM NaCl	PP055997.1	35.00 ± 1.00 ^{a}	25.67 ± 1.53 ^{b}	15.11 ± 2.82 ^{a}	5.66 ± 0.25 ^{a}	2.33 ± 0.35 ^{a}	0.78 ± 0.06 ^{a}
	PP038127.1	31.67 ± 1.53 ^{b}	23.83 ± 1.61 ^{b}	10.83 ± 2.34 ^{b}	3.79 ± 0.26 ^{b}	1.74 ± 0.12 ^{b}	0.52 ± 0.13 ^{b}
	PP038155.1	36.33 ± 2. 84 ^{a}	28.33 ± 1.53 ^{a}	17.51 ± 1.49 ^{a}	6.11 ± 0.71 ^{a}	2.64 ± 0.23 ^{a}	0.96 ± 0.04 ^{a}
	Control	22.33 ± 0. 58 ^{c}	14.67 ± 0.31 ^{c}	7.02 ± 0.22 ^{c}	2.83 ± 0.62 ^{b}	1.32 ± 0.10 ^{b}	0.49 ± 0.09 ^{b}

Mean values followed by the same letters (a, b, c, etc.) are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 3 Plant growth parameters investigated under drought stress after 16 weeks

Stress	Fungal taxa	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
No stress	PP055997.1	42.67 ± 2.08 ^{a}	31.33 ± 1.15 ^{a}	23.05 ± 2.08 ^{a}	13.88 ± 2.48 ^{a}	4.23 ± 0.79 ^{a}	1.9 ± 0.15 ^{a}
	PP038127.1	36.67 ± 2.08 ^{b}	28.00 ± 2.65 ^{ab}	17.94 ± 3.21 ^{b}	9.64 ± 0.91 ^{b}	3.47 ± 0.72 ^{a}	1.57 ± 0.03 ^{b}
	PP038155.1	41.67 ± 2.08 ^{a}	28.67 ± 3.69 ^{ab}	16.87 ± 3.14 ^{b}	8.05 ± 2.32 ^{b}	2.93 ± 0.8 ^{b}	1.32 ± 0.54 ^{b}
	Control	36.67 ± 2.31 ^{b}	27.33 ± 1.53 ^{b}	17.76 ± 1.42 ^{b}	7.29 ± 1.32 ^{b}	2.59 ± 0.25 ^{b}	1.02 ± 0.09 ^{c}
10% PEG 6000	PP055997.1	37.67 ± 1.53 ^{ab}	27.33 ± 0.27 ^{b}	20.93 ± 3.09 ^{a}	9.13 ± 1.13 ^{a}	3.17 ± 0.46 ^{a}	1.27 ± 0.08 ^{a}
	PP038127.1	33.67 ± 2.73 ^{b}	23.88 ± 2.89 ^{b}	19.17 ± 2.6 ^{b}	7.19 ± 2.2 ^{b}	2.31 ± 0.29 ^{b}	1.02 ± 0.09 ^{b}
	PP038155.1	42.56 ± 3.14 ^{a}	32.53 ± 2.79 ^{a}	23.56 ± 1.75 ^{a}	10.16 ± 2.1^{a}	3.89 ± 0.25 ^{a}	1.43 ± 0.16 ^{a}
	Control	30.00 ± 1.43 ^{b}	23.67 ± 1.84 ^{b}	12.31 ± 2.31 ^{b}	5.68 ± 2.52 ^{b}	2.16 ± 0.3 ^{b}	0.96 ± 0.22 ^{c}
20% PEG 6000	PP055997.1	31.33 ± 0.58 ^{ab}	23.50 ± 3.04 ^{a}	11.11 ± 0.64 ^{b}	7.06 ± 1.94 ^{a}	2.51 ± 0.16 ^{a}	0.96 ± 0.24 ^{ab}
	PP038127.1	29.67 ± 0.29 ^{b}	19.50 ± 2.78 ^{b}	10.83 ± 1.92 ^{b}	5.68 ± 1.25 ^{b}	2.17 ± 0.23 ^{a}	0.77 ± 0.17 ^{b}
	PP038155.1	36.67 ± 2.31 ^{a}	25.33 ± 2.08 ^{a}	13.51 ± 3.42 ^{a}	7.69 ± 2.05 ^{a}	2.48 ± 0.4 ^{a}	1.08 ± 0.34 ^{a}
	Control	24.67 ± 2.08 ^{c}	15.67 ± 3.75 ^{b}	7.02 ± 0.93 ^{c}	3.32 ± 0.8 ^{b}	1.24 ± 0.54 ^{b}	0.83 ± 0.15 ^{b}

Mean values followed by the same letters (a, b, c, etc.) are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

After 16 weeks of inoculation by fungal endophytes, different physiological and biochemical changes related to the host defensive system were estimated under salinity and drought stress., Photosynthetic pigments, proline, MDA, electrode leakage, SOD) in plant inoculated and un-inoculated tomato plants were estimated under salinity and drought stress conditions. After observation of the photosynthetic pigments of growing tomato plants under salinity stress, the results expressed in table (4) showed that there were significant differences in chlorophyll a and chlorophyll b between isolates and controls. And was a significant increase in the total chlorophyll recorded for inoculated plants with endophytic fungal isolates showed high value (2.92 mg/g) under normal conditions in plants inoculated by *T. atroviride* (PP055997.1) , on the other hand, plants inoculated with *A. terreus* (PP038155.1) showed high chlorophyll content (3.19 and 1.65 mg/g) under saline conditions (150 and 300 mM NaCl), respectively compared to their corresponding un-inoculated controls. Total carotenoids of un-inoculated plants were negatively affected by increasing salinity stress at (300 mM NaCl), while plants inoculated with *A. terreus* (PP038155.1) showed high carotenoids content 0.89 and 0.63 mg/g under saline stress (150 and 300 mM NaCl) compared to their corresponding un-inoculated controls.

As shown in table (4) Drought stress using different PEG 6000 concentration (10 and 20 %) has negative impact on photosynthetic pigments of un-inoculated plants. Total chlorophyll content decreased from 1.44 to 0.69 mg/g in normal and drought stress at (20 % PEG), respectively. On the other hand, plants inoculated with *A. terreus* (PP038155.1) recorded high total chlorophyll contents 3.02 and 1.77 mg/g under drought conditions (10 and 20 % PEG), respectively as compared to their un-inoculated controls. Also, total carotenoids of un-inoculated control tomato plants affected by drought stress, but inoculation with *A. terreus* (PP038155.1) resulted in high levels of carotenoids under drought conditions (10 and 20 % PEG), respectively in comparison with their controls. As observed, there was a significant increase in the carotenoids obtained by endophytic fungal isolates expresses as high value in unstressed plants in *T. atroviride* (PP055997.1) by 0.97 mg/g, in stressed plants at (10% PEG 6000) express as high value in *A. terreus* (PP038155.1) by 0.99 mg/g and at (20% PEG 6000) in *A. terreus* (PP038155.1) by 0.77 mg/g, compared with both controls.

Table 4 Effect of fungal inoculation on photosynthetic pigments of tomato plants under salinity stress

Stress	Fungal taxa	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll (a+b) (mg/g FW)	Carotenoid (mg/g FW)
No stress	PP055997.1	2.03± 0.03 [a]	0.84 ± 0.26 [a]	2.92 ± 0.25 [a]	0.98 ± 0.16 [a]
	PP038127.1	1.67 ± 0.10 [a]	0.79 ± 0.09 [a]	2.56 ± 0.16 [a]	0.79 ± 0.12 [b]
	PP038155.1	1.74 ± 0.02 [a]	0.72 ± 0.24 [ab]	2.39 ± 0.25 [a]	0.60 ± 0.05 [b]
	Control	0.94 ± 0.24 [b]	0.50 ± 0.35 [b]	1.43 ± 0.18 [b]	0.30 ± 0.09 [c]
150 mM NaCl	PP055997.1	1.23 ± 0.15 [ab]	0.73 ± 0.26 [ab]	1.96 ± 0.11 [ab]	0.73 ± 0.07 [ab]
	PP038127.1	0.99 ± 0.06 [b]	0.52 ± 0.09 [b]	1.50 ± 0.16 [ab]	0.53 ± 0.10 [b]
	PP038155.1	2.19 ± 0.12 [a]	0.99 ± 0.24 [a]	3.19 ± 0.08 [a]	0.89 ± 0.05 [a]
	Control	0.81 ± 0.55 [b]	0.31 ± 0.35 [b]	1.12 ± 0.14 [b]	0.21 ± 0.06 [c]
300 mM NaCl	PP055997.1	0.87 ± 0.06 [ab]	0.47 ± 0.26 [ab]	1.34 ± 0.25 [ab]	0.57 ± 0.07 [ab]
	PP038127.1	0.69 ± 0.06 [ab]	0.39± 0.09 [b]	1.09 ± 0.16 [ab]	0.49 ± 0.12 [b]
	PP038155.1	1.03 ± 0.16 [a]	0.63 ± 0.24 [a]	1.65 ± 0.20 [a]	0.63 ± 0.05 [a]
	Control	0.59 ± 0.18 [b]	0.23 ± 0.35 [b]	0.81 ± 0.18 [b]	0.12 ± 0.09 [c]

Mean values followed by the same letters (a, b, c, etc.) are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

Table 5 Effect of fungal inoculation on photosynthetic pigments of tomato plants under drought stress

Stress	Fungal taxa	Chlorophyll a (mg/g FW)	Chlorophyll b (mg/g FW)	Chlorophyll (a+b) (mg/g FW)	Carotenoid (mg/g FW)
No stress	PP055997.1	2.10 ± 0.03 [a]	0.82 ± 0.26 [a]	2.87 ± 0.25 [a]	0.97 ± 0.16 [a]
	PP038127.1	1.85 ± 0.10 [a]	0.71 ± 0.09 [a]	2.46 ± 0.16 [a]	0.75 ± 0.12 [b]
	PP038155.1	1.71 ± 0.02 [a]	0.69 ± 0.24 [ab]	2.49 ± 0.25 [a]	0.64 ± 0.05 [b]
	Control	0.98 ± 0.24 [b]	0.46 ± 0.35 [b]	1.44 ± 0.18 [b]	0.31 ± 0.09 [c]
10% PEG 6000	PP055997.1	1.18 ± 0.05 [a]	0.71 ± 0.07 [ab]	1.89 ± 0.10 [b]	0.83 ± 0.06 [b]
	PP038127.1	0.75 ± 0.07 [b]	0.61 ± 0.14 [ab]	1.36 ± 0.09 [b]	0.58 ± 0.04 [b]
	PP038155.1	2.05 ± 0.06 [a]	0.97 ± 0.18 [a]	3.02 ± 0.23 [a]	0.99 ± 0.10 [a]
	Control	0.50 ± 0.06 [b]	0.34 ± 0.03 [b]	0.84 ± 0.10 [c]	0.23 ± 0.07 [c]
20% PEG 6000	PP055997.1	0.90 ± 0.14 [a]	0.65 ± 0.30 [ab]	1.55 ± 0.22 [a]	0.65 ± 0.08 [a]
	PP038127.1	0.71 ± 0.10 [b]	0.41 ± 0.16 [b]	1.12 ± 0.10 [b]	0.47 ± 0.06 [b]
	PP038155.1	1.02 ± 0.14 [a]	0.75 ± 0.17 [a]	1.77 ± 0.08 [a]	0.77 ± 0.06 [a]
	Control	0.44 ± 0.12 [c]	0.26 ± 0.31 [c]	0.69 ± 0.26 [c]	0.15 ± 0.08 [c]

Mean values followed by the same letters (a, b, c, etc.) are significantly different according to Duncan's multiple range test at $p \leq 0.05$.

The biochemical parameters changes related to the host defensive system were estimated under salinity and drought stress (Figs. 4 and 5). Proline content increases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 4a). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their proline contents of tomato plants under salinity stress. Proline content of plants inoculated with *A. terreus* (PP038155.1) showed high levels (25.56 and 39.20 µg/g) at salinity stress (150 and 300mM NaCl), respectively in comparison with their controls.

MDA content decreases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 4b). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their MDA contents of tomato plants under salinity stress. MDA content of plants inoculated with *A. terreus* (PP038155.1) showed low levels (1.48 and 2.44 nmol/mg) at salinity stress (150 and 300mM NaCl), respectively in comparison with their controls.

Electrolyte leakage % decreases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 4c). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their Electrolyte leakage % of tomato plants under salinity stress. Electrolyte leakage % of plants inoculated with *A. terreus* (PP038155.1) showed low levels (50.39 and 85.64 %) at salinity stress (150 and 300mM NaCl), respectively in comparison with their controls.

SOD activity increases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 4d). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their SOD activity of tomato plants under salinity stress. SOD activity of plants inoculated with *A. terreus* (PP038155.1) showed high levels (0.99 and 0.56 U/mg) at salinity stress (150 and 300mM NaCl), respectively in comparison with their controls.

Proline content increases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 5a). As observed from results, there is a significant difference between un-inoculated plants control and inoculated plants in their proline contents of tomato plants under drought stress. Proline content of plants inoculated with *A. terreus* (PP038155.1) showed high levels (28.20 and 42.30 µg/g) at drought stress (10 and 20% PEG 6000), respectively in comparison with their controls.

MDA content decreases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 5b). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their MDA contents of tomato plants under drought stress. MDA content of plants inoculated with *A. terreus* (PP038155.1) showed low levels (1.36 and 2.32 nmol/mg) at drought stress (10 and 20% PEG 6000), respectively in comparison with their controls.

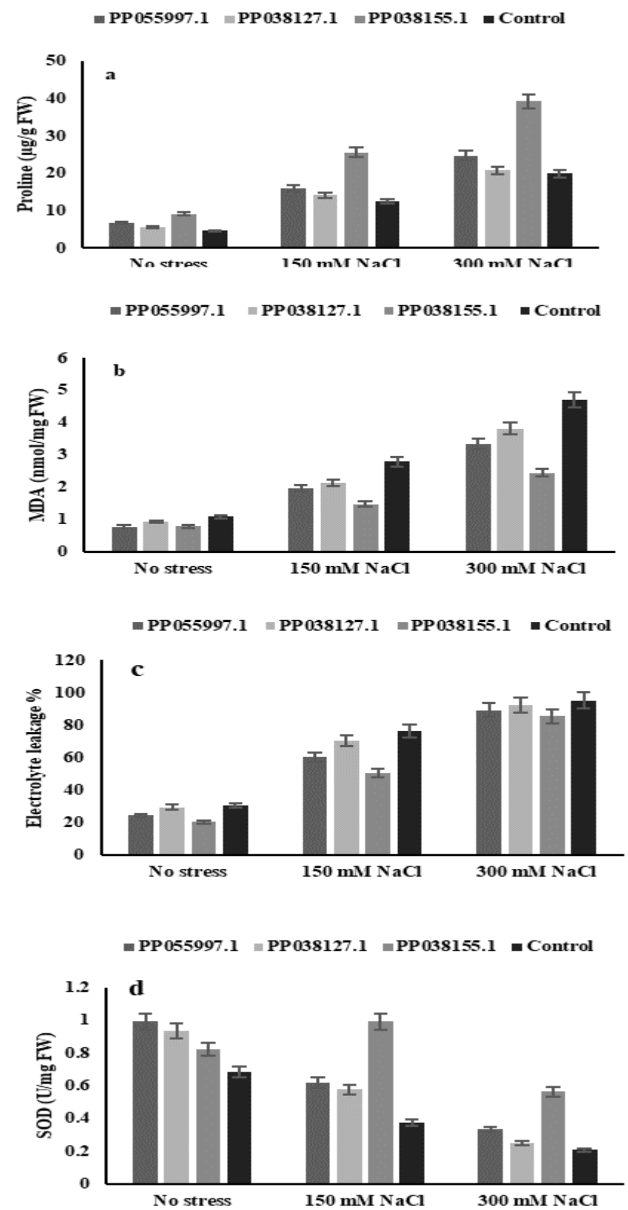

Fig. 4 Contents of (A) proline, (B) MDA, (C) electrolyte leakage %, and (D) superoxide dismutase (SOD), in leaves of tomato plants inoculated with fungal isolates and control at 16 weeks at salt stress conditions.

Electrolyte leakage % decreases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 5c). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their Electrolyte leakage % of tomato plants under drought stress. Electrolyte leakage % of plants inoculated with *A. terreus* (PP038155.1) showed low levels (48. 23 and 75.10 %) at drought stress (10 and 20% PEG 6000), respectively in comparison with their controls.

SOD activity increases significantly with increasing stress for mitigate stress in plants inoculated with endophytes at variance in control plants (Fig. 5d). As observed from results, there is significant difference between un-inoculated plants control and inoculated plants in their SOD activity of tomato plants under drought stress. SOD activity of plants inoculated with *A. terreus* (PP038155.1) showed high levels (1.18 and 0.51 U/mg) at drought stress (10 and 20% PEG 6000), respectively in comparison with their controls.

Discussion

Fungal endophytic colonization induces physiological changes and modifies gene expression in the plants, thereby uplifting plant productivity via higher photosynthesis rate, promoting the shoots and roots growth, enhancing uptake and nutrient use efficiency conferring abiotic and biotic stress tolerance (Harman & Uphoff 2019, Grabka et al. 2022). In the last decades, it has been demonstrated that several beneficial EF can be artificially introduced on tomato using different inoculation methods and numerous protocols have been developed to successfully achieve this colonization, as well as to detect the fungi within the plant tissues (Sinno et al. 2020). In this study used three inoculation methods (seed inoculation, root dipping, leave spray), and tomato plant colonized with three fungal isolates *T. atroviride* (PP055997.1), *F. acutatum* (PP038127.1) and *A. terreus* (PP038155.1) respectively, selected as the most potent taxa from screening tests. As leave spray method is the most effective colonization method compared to other methods. This result with agreement with other studies by (Allegrucci et al. 2018, Pourtaghi et al. 2020) that show leave spray method is the most effective colonization method in tomato plant. To ensure that the inoculation of the fungal species is followed by actual endophytic colonization of the plant, it is mandatory to include an experimental stage to detect the EF within the plant. The re-isolation of the fungal colony from the host plant tissue is the most used method to assess the endophytic colonization according to other studies by (Klieber & Reineke 2016).

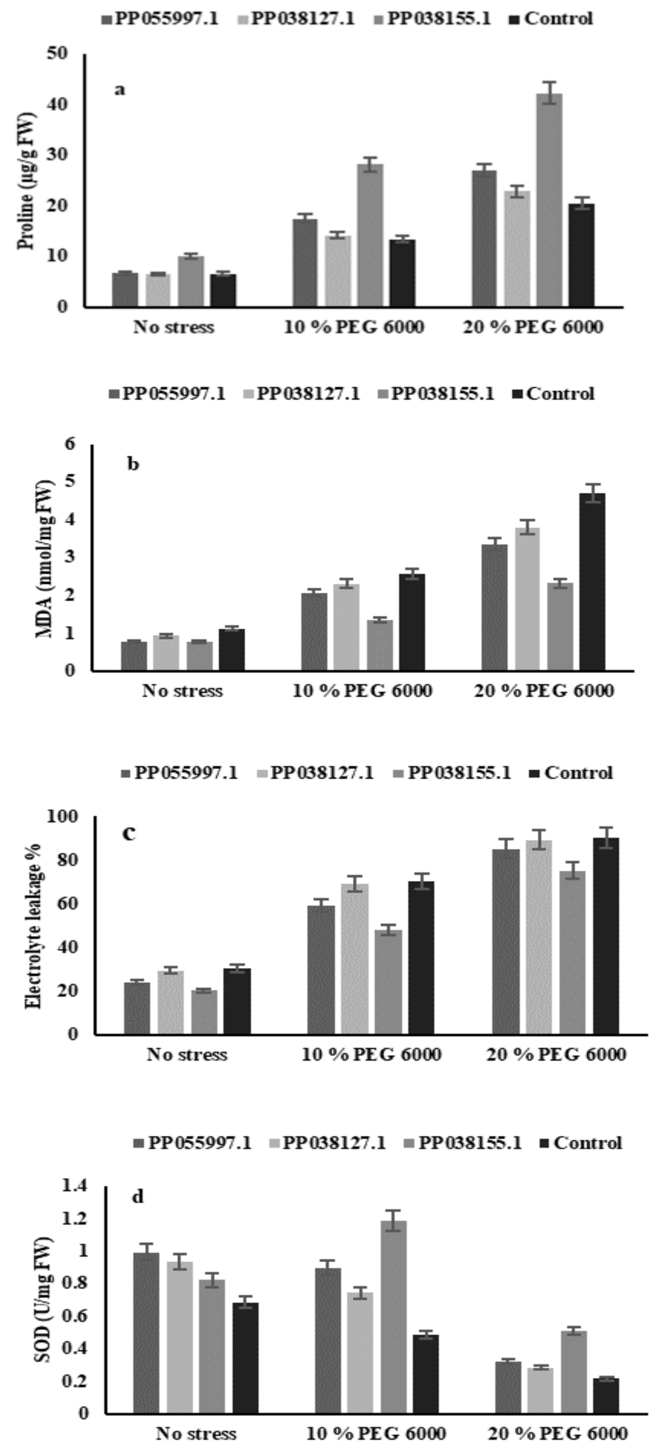

Fig. 5 Contents of (A) proline, (B) MDA, (C) electrolyte leakage %, and (D) superoxide dismutase (SOD), in leaves of tomato plants inoculated with fungal isolates and control at 16 weeks at drought stress conditions.

Tomato colonized with three fungal isolates *T. atroviride* (PP055997.1), *F. acutatum* (PP038127.1) and *A. terreus* (PP038155.1) respectively, with leave spray method treated with salinity stress (150 and 300 mM NaCl) and drought stress (10 and 20% PEG 6000) at 12 weeks old for 14 days, after stress plants watered by water without stress for another 14 days and harvested at 16 weeks old. In these studies, tomato plants showed a trend of increased tolerance to salinity and drought when colonized by systemic fungal endophytes isolated from saline habitats. Salinity tolerance conferred by some systemic fungi induced increased shoot and root biomass and yield in tomato plants colonized by *A. terreus* (PP038155.1) by 43, 36.33, 32.83, and 28.33 (cm) which was significantly different than un-inoculated control under saline condition (150 and 300 mM NaCl), respectively. Also, drought tolerance conferred by *A. terreus* (PP038155.1) induced increased shoot and root biomass and yield in tomato plants by (42.5, 36.6 and 32.5, 25.3 cm) under drought stress (10 and 20 % PEG 6000), respectively compared to their respective un-inoculated controls compared to control. These results agree with reports in the literature (Rodriguez et al. 2008, Redman et al. 2011). My hypothesis was that when plants were severely stressed, the protective effects of the endophytes would be more apparent. Photosynthetic efficiency often used as a sensitive indicator of abiotic stress in plants (Azad & Kaminskyj 2016). Salinity and drought have negative effect on chlorophyll content which may be due to the suppression of chlorophyll biosynthesis enzymes, activation of chlorophyllase and excessive production of ROS. The isolate *A. terreus* (PP038155.1) colonized tomato plants showed high photosynthetic efficiency value (3.19 and 1.65 mg/g) under saline conditions (150 and 300 mM NaCl), 3.02 and 1.77 mg/g under drought conditions (10 and 20 % PEG), respectively compared to their corresponding un-inoculated controls. This result is an agreement with previous results where endophyte-colonized plants had significantly increased photosynthetic efficiency during intermittent stress (Azad & Kaminskyj 2016). Positive effects of fungal endophytes on chlorophyll have been reported in some plants, especially under environmental stress (Khalid et al. 2018, Ghabooli et al. 2020). While *T. atroviride* (PP055997.1) endophyte-colonized plants were observed indicating higher light absorbance by the chlorophyll pigments by (2.92 mg/g) under normal conditions in plants inoculated by *T. atroviride* (PP055997.1).

Abiotic stresses cause a change in plant-water relation resulting in accumulation of osmolytes or compatible solutes (Bohnert et al. 1995). Endophyte *A. terreus* (PP038155.1) colonized tomato plants showed increased activity of proline production by (25.56 and 39.20 µg/g) at salinity stress (150 and 300mM NaCl), and (28.20 and 42.30 µg/g) at drought stress (10 and 20% PEG 6000) respectively in comparison with their controls. As agreement with previous reports Endophytes may alleviate oxidative stress in extreme salinity and drought stress (Redman et al. 2011). Stress increased endogenous MDA and EL contents in the current study, which is in harmony with previous findings (Abdelaziz et al., 2019). Our results revealed that *A. terreus* (PP038155.1) inoculation decreased the content of MDA and EL during salinity (150 and 300 mM) by (1.48 and 2.44 nmol/mg), and (50.39 and 85.64 %), respectively. Also *A. terreus* (PP038155.1) inoculation decreased the content of MDA and EL during drought (10 and 20 % PEG 6000) stress by (1.36 and 2.32 nmol/mg), and (48.23 and 75.10 %), respectively in comparison with their controls.

These results may confirm the role of this fungus in stabilizing membrane integrity and combating ROS-induced oxidative damage. This can be supported by the result of (Khalid et al. 2018). Endophyte *A. terreus* (PP038155.1) colonized tomato plants showed increased activity of antioxidants such as superoxide dismutase (SOD) upon salinity stress (150 and 300 mM) levels by (0.99 and 0.56 U/mg) respectively, and (1.18 and 0.51 U/mg) at (10 and 20% PEG 6000) drought stress respectively in comparison with their controls. Higher antioxidant activities superoxide dismutase (SOD) was observed in plants colonized with *A. terreus* (PP038155.1), which was also correlated with improved biomass and root length. These results support the hypothesis that the significant decrease in the EL and MDA contents of treated inoculated plants may be associated with the relatively higher SOD and proline content under stress (Ghabooli et al. 2020). Thus, while we cannot tell from our results whether the endophyte plants are experiencing reduced stress levels, or if they are better able to cope with the increased stress, the result is an overall reduction in ROS accumulation. One can certainly use this as general evidence of greater stress tolerance.

Conclusion

Finally, systemic fungal endophytic isolate *A. terreus* (PP038155.1) shields tomatoes from salt and drought stress through impact on physiological and biochemical parameters. Enhanced osmolytes and photosynthetic efficiency by *A. terreus* boosted plant stress resistance and lowered ROS and plant oxidation. Increased antioxidant activity under salt and drought stress may protect endophyte-colonized plants from oxidative damage. This could be a much faster, more efficient, and more effective way to protect plants from stress brought on by climate change or salinization caused by over irrigation.

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Conflict of interest

The authors declare that they have no conflict of interest.

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