Desert endophytic fungi improve reproductive, morphological, biochemical, yield and fruit quality characteristics of tomato under drought stress

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Abstract

Purpose – Crops are increasingly affected by drought; hence, the current study explored the potential role of three desert endophytic fungi, Aspergillus fumigatus, Aspergillus terreus and Talaromyces variabilis, in conferring drought tolerance in tomato plants.

Design/methodology/approach – Preserved endophytic fungi from a Rhazya stricta desert plant were adopted to obtain the required fungal treatment; tomatoes received fungal treatments directly in plastic trays and subsequently in pots. Drought was applied using 15% of PEG-6000 at two stages: flowering and fruiting. The following parameters were measured: pollen sterility, growth characteristics, morphological analysis and biochemical analysis, including proline, gibberellic acid (GA3) and chlorophyll measurements; thus, the data were analyzed statistically using SPSS software.

Findings – All applied endophytes significantly promoted pollen viability and tomato yield under stressed and nonstressed conditions. Interestingly, these endophytes significantly enhanced the number of trichomes under drought stress and promoted tomato fruit quality. The colonized tomato plants accumulated a high proline level under drought stress but lower than un-inoculated stressed plants. Also, a significant rise in growth characteristics was observed by A. fumigatus and A. terreus under normal conditions. Moreover, both raised GA3 levels under drought-stressed and nonstressed conditions. Also these two endophytes enhanced chlorophyll and carotenoid contents under drought stress. Fruit characteristics were enhanced by nonstressed T. variabilis and stressed A. fumigatus.

Originality/value – The present endophytic fungi provide impressive benefits to their host in normal and drought-stressed conditions. Consequently, they represent valuable sources as sustainable and environmentally friendly alternatives to mitigate drought stress.

Keywords – Abiotic stress, Aspergillus fumigatus, Aspergillus terreus, Endophytes, Talaromyces variabilis, Trichomes

Paper type – Research paper

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1. Introduction
Climate change is accelerating, negatively affecting agriculture, reducing yield productivity and putting food security and people’s lives at risk. Hence, crops and agricultural adaptation are strongly required (Anderson, Bayer, & Edwards, 2020; Parry, 2019; Westengen & Brysting, 2014). Drought is a disastrous phenomenon that increased in the last century and is most likely to rise due to climate change (Jehanzaib & Kim, 2020). Several reports proved the considerable reduction in crop yield in many countries under drought conditions (Chen, Liang, Liu, Jiang, & Xie, 2018; Ray, Fares, & Risch, 2018). Other essential features of plants were also affected by drought stress, including growth parameters, physiological and biochemical attributes, morphological characteristics, reproductive development and seed germination (Halo, Al-Yahyai, & Al-Sadi, 2020; Muscolo, Sidari, Anastasi, Santonoceto, & Maggio, 2014; Yaseen et al, 2020).

The previous destructive effects of drought on plants and the agriculture sector encourage farmers, scientists and researchers to mitigate this mounting problem. Improving drought tolerance is a constructive attempt to overcome drought impact; this include traditional and molecular breeding (Ali, Rai, Jan, & Raina, 2022; Cuc et al., 2021), exogenous substances application (Nazar et al., 2020), metabolic engineering (Ilyas et al., 2020) and microbial inoculation (Azeem, Haider, Javed, Saleem, & Alatawi, 2022; Yasmin et al., 2022). Recently, there has been growing interest to enhance drought tolerance in crops using endophytic inoculation (Morsy, Cleckler, & Armuelles-Millican, 2020; Sadeghi, Samsampour, Seyahooei, Bagheri, & Soltani, 2020).

Endophytic fungi provide multiple benefits to their hosts in normal conditions (Khan et al., 2015). Also, they enhance the ability of their hosts to survive under extreme conditions such as biotic stress (Halo, Al-Yahyai, & Al-Sadi, 2018; Halo, Al-Yahyai, Maharachchikumbura, & Al-Sadi, 2019) and abiotic stress, including drought, salinity and heat (Bilal et al., 2020; Hubbard, Germida, & Vujanovic, 2014; Khan et al., 2011). Regarding their role in water use efficiency, it was found the inoculation with root-endophytes maintained higher water use efficiency and enhanced net photosynthesis in drought conditions; moreover, these are correlated with greater fresh and dry biomass production and better root system development (Molina-Montenegro et al., 2016).

The previous reports attempted to clarify this remarkable role of endophytes, but the mechanisms were not fully clear, principally related to the activation of the host response system (Khare, Mishra, & Arora, 2018); however, some mechanisms were evident such as the production of phytohormones and other efficacious secondary metabolites, induction expression of the stress-responsive genes and activation of silent gene clusters resulting in production metabolites (Dinesh, Srinivasan, TE, Anandaraj, & Srambikkal, 2017; Nataraja, Dhanyalakshmi, Govind, & Oelmüller, 2022).

Several plant features have got attention in studying the effect of endophytic treatments under drought stress, such as growth, biochemical, physiological and molecular characteristics; however, some features received little attention despite their great importance, such as reproductive and morphological features; hence, pollen viability and trichomes and stomata density were investigated in our study.

Tomato (Lycopersicon esculentum Mill.) is a herbaceous and dicotyledonous plant, highly branched and spreading between 60–180cm with phyllotaxy leaf arrangement; a terminal bud provides the actual growing at the apex of the stem; the flowers are pendant and clustered. Fruits are valuable sources of folate, vitamin C and minerals; also, high carotenoid content in tomato fruits increases their antioxidant activity (Beecher, 1998). However, drought caused harmful impacts on tomato growth and yield (Cui, Shao, Lu, Keabetswe, & Hoogenboom, 2020; Zhou et al., 2017).

A. fumigatus, A. terreus and T. variabilis endophytes were reported for their efficient role as plant enhancing survival and growth under abiotic stress conditions. Also, they serve as
effective antagonistic agents against plant pathogens (Halo et al., 2018, 2019; Khan et al., 2011; Khushdil et al., 2019); furthermore, both A. terreus and A. fumigatus endophytes are valuable sources of bioactive metabolisms, including antioxidants, antitumors, biopharmaceuticals and phytohormones; consequently, they are utilized widely for medical, agricultural and industrial purposes (El-Sayed et al., 2021; Hussein et al., 2022; Xiao-Feng et al., 2021); however, the bio-role of T. variabilis endophyte was not well investigated.

To our knowledge, few studies have investigated the role of endophytic fungi in improving crop yield under drought conditions; also, the current endophytes have not yet been investigated in promoting drought tolerance in tomatoes. Furthermore, applying excess-habitat-adapted symbiotic microbes is a well-known effective mechanism to mitigate the devastating impacts of abiotic stresses (Moghaddam, Safaie, Soltani, & Hagh-Doust, 2021). Accordingly, our selected desert endophytic fungi are most likely adapted to drought stress conditions. Thus, the present study investigated whether these endophytes (independent variables) can enhance plant features (dependent variables: growth, yield, morphological and biochemical characteristics) to provide drought tolerance to their hosts (the primary purpose).

2. Materials and methods

2.1 Experimental design and treatment applications

The treatments were the fungal treatments with and without drought application: nonstressed A. fumigatus (10P), stressed A. fumigatus (D10P), nonstressed A. terreus (65P), stressed A. terreus (D65P), nonstressed T. variabilis (48P), stressed T. variabilis (D48P), the control (W) and the drought stress (D) treatments. The endophytes were obtained from preserved collections of the Plant Sciences Department at Agricultural and Marin Sciences College, Sultan Qaboos University (SQU). All of them were isolated from Rhazya stricta desert plant in an arid location in Oman.

The steps provided in our previous paper, Halo et al. (2020), were followed to carry out the experiment, that confirmed the advantageous role of an endophytic Talaromyces omanensis in mitigating drought stress in tomatoes; accordingly, the seeds were surface sterilized, fungi were grown in potato dextrose broth for 10 days to get a fungal suspension and the compost soil was autoclaved twice, which has the following composition: sphagnum peat (0.3 m dark and 0.6 m light (layer thickness)), nutrient added in peat per m³ include 1.1 Kg base fertilizer NPK 14-16-18. 4.5 kg LIME/Dolodust, 0.4 Liter wetting agent with a pH value of 4.5–5.5, organic matter 91.4% (w/w), C/N ratio: 36.6, sodium chloride: 850 mg/kg, moisture content 38.5% (w/w) (BULRUSH, Bellaghy, UK), the seeds were sown in plastic trays at the first step and some received a fungal suspension; the tiny seedlings were transferred to their appropriate pots (30-cm diameter) under glasshouse conditions at SQU (approx. 75% RH, 28°C, latitude: 23°36’N and altitude: 51 m above sea level), these include pots with pure compost soil for control and nontreated stressed groups, pots with compost soil mixed with fungal suspension for fungal treatment groups, each pot was mixed with one liter of fungal suspension. Drought was applied using 15% of PEG-6000 at two stages: flowering and fruiting for nine days. During both stages, 0.5 liters of PEG was applied daily for each plant (Halo et al., 2020). Three pots were set for each treatment, with three seedlings in each. All parameters were measured after the second drought application (fruiting stage) except pollen sterility which was detected after the first one.

2.2 Pollen sterility and growth characteristics

The acetocarmine staining test was used to calculate pollen sterility (Fernandez-Munoz, Gonzalez-Fernandez, & Cuartero, 1994). Pollen was taken from flowers on the lowest three inflorescences (Abdul-Baki, 1992). The experiment was repeated three times.
The features of the shoot and root (length and fresh and dry weight), in addition to the leaf area, were measured. For dry weight, samples were dried in an oven until they reached a constant weight. The fourth branch from the top was selected to measure the leaf area. All nine replicates were used for shoot features; however, seven were selected randomly for root features.

2.3 Fruit characteristics
The total number of fruits and number of ripe and mature fruits were counted for all replicates; however, fruit weight and size were measured for 10 random samples.

The following biochemical characteristics of tomato fruit juice were detected: pH, titratable acidity and total soluble solids (°Brix). Titration to pH 8.3 was obtained using 0.1 NaOH, the values of titratable acidity were expressed as anhydrous citric acid, and the applied formula was: % acid = ([mls NaOH used] × [0.1 N NaOH] × [millequivalent factor] × [100])/grams of sample (Garner, Crisosto, Wiley, & Crisosto, 2005). A digital Brix pocket refractometer measured total soluble solids in °Brix. Each parameter has five replicates.

2.4 Morphological analysis
A scanning electron microscope (JEOL JSM-7600F Field Emission Scanning Electron Microscope (FESEM), Japan) was used to count trichomes and stomata in tomato leaves. A method by Karcz (2009) was applied to prepare the samples. Accordingly, the leaf samples were fixed in 3% glutaraldehyde for 2 hours; then, they were washed with 0.1 M phosphate buffer (pH 7.2); after that, the samples were fixed using 1–2% osmium tetroxide for two hours and rinsed in 0.1 M phosphate buffer, and then, the dehydration was conducted using consecutive concentrations of ethanol (25/75/95/100/100 %). After that, critical point drying was carried out. Lastly, the specimens were set on metal stubs and coated with gold. The photos were obtained using 20 kV voltages. There were four replicates for each treatment.

2.5 Biochemical analysis
2.5.1 Proline. Proline plays a beneficial role in plants exposed to diverse stress conditions. Besides acting as an excellent osmolyte, it provides three main advantages during stress: an antioxidative defense molecule, a metal chelator and a signaling molecule (Hayat et al., 2012). A protocol of Bates, Waldren and Teare (1973) was applied to determine proline content in tomato leaves. Accordingly, 0.5 g of leaf samples were weighed and extracted using sulphasalicylic acid (3%), the extract (2 mL) was transferred to a test tube, ninhydrin reagent (2 mL) and glacial acetic acid (2 mL) was added. The mixture was boiled for 30 min in a water bath; after cooling the mixture, toluene (6 mL) was added, mixed and separated. The reading was observed at 520 nm, a standard curve was created, and the readings were compared to it; finally, the formula provided by Bates et al. (1973) was applied. Four replicates were set for this test.

2.5.2 Gibberellic acid (GA3). GA3 is among plants’ most critical growth regulators which promotes their tolerance to drought stress under optimum concentration (Sarwar et al., 2017). The extraction of leaf samples was performed in methanol solvent (70% v/v) following Kelen, Demiralay, Şen, and Alsancak (2004) method. Accordingly, the leaf materials were homogenized with the solvent and stirred overnight at 4°C, then the matrix was filtered, and the methanol was evaporated under vacuum; the obtained aqueous phase was adjusted to pH 8.5; after that, it partitioned with ethyl acetate three times, the obtained aqueous phase was adjusted to pH 2.5, then the solution was partitioned using diethyl ether three times, and passed through sodium sulfate; the diethyl ether phase was evaporated, and the obtained dry material was dissolved in 2 mL of methanol and stored at 4°C. GA3 content was determined.
using liquid chromatography-tandem mass spectrometry (LC–MS/MS: AB Sciex). Several standards of GA3 (0.001, 0.01, 0.1, 1 mg/L) were prepared, and acetonitrile water (26:74; 30.70%; v/v) was selected as the mobile phase. Three replicates were set for this test.

2.5.3 Chlorophyll measurements. Chlorophyll fluorescence: the ratio of $F_v$ (variable fluorescence) to $F_m$ (maximum fluorescence) represents the quantum efficiency of open photosystem II centers (Maxwell & Johnson, 2000). It was measured in dark-adapted tomato leaves using a plant efficiency analyzer. The adaptation continued for 30 min before measurement (Morales, Abadía, & Abadia, 1991). The microelectrode was positioned vertically during the reading recording. A total of 16 random leaves from each treatment were selected to perform this test; the measurement of chlorophyll fluorescence was a valid and reliable tool for rapid screening drought stress tolerance in other plants (Faraloni et al., 2011; Li, Guo, Michael, Stefania, & Salvatore, 2006).

Chlorophyll contents: chlorophyll $a$, chlorophyll $b$ and carotenoids were measured following the protocol of Sumanta, Haque, Nishika, and Suprakash (2014). Briefly, the extraction of tomato leaves was carried out using acetone (80%), the absorption was read by spectrophotometer at 663.2, 646.8 and 470 nm, and the formulas provided by Sumanta et al. (2014) were applied to obtain the values of Chl$a$, Chl$b$ and carotenoids. Four replicates were set for this test.

2.6 Statistical analysis
Statistical analysis was performed using SPSS software. One-way analysis of variance (ANOVA) with POSTHOC test: least significant difference (LSD) was selected to compare the means of treatments. The significance level was adjusted at 0.05.

3. Results
3.1 Pollen viability and growth characteristics
Pollen viability and growth characteristics of tomato plants are displayed in Table 1. All endophytic fungi treatments (stressed and nonstressed $A. fumigatus$ (18.90) (14.18), stressed and non–stressed $A. terreus$ (16.36) (16.75), stressed and non–stressed $T. variabilis$ (16.54) (15.07) consecutively) and control (17.72%) significantly decreased the pollen sterility of tomatoes compared to drought treatment (25.78%) (Table 1). Also, non–stressed $A. fumigatus$ (98.86 cm) significantly increased the root length of the tomato compared to all other treatments, including the control (57.62 cm) and drought (58 cm) (Table 1). Shoot fresh weight was significantly enhanced under the effect of nonstressed $A. fumigatus$ (154.4 g) and nonstressed $A. terreus$ (139.84 g) compared to the control (77.5 g) and drought stress treatment (78.01 g). Shoot dry weight was significantly increased under nonstressed $A. fumigatus$ (24.19 g), $A. terreus$ (stressed (23.23 g) and nonstressed (24.59 g)) and stressed $T. variabilis$ (22.98 g) compared to the control (14.35 g). Root fresh weight was significantly enhanced by nonstressed $A. terreus$ (36.53 g) compared to the control (18.27 g) and drought stress (18.06 g) (Table 1). Similarly, nonstressed $A. terreus$ significantly increased root dry weight (4.84 g) compared to the control (2.58 g) and drought stress (2.52 g). The leaf area was significantly enhanced by stressed $T. variabilis$ (26.34 cm$^2$) followed by nonstressed $A. fumigatus$ (22.69 cm$^2$) compared to the control (17.51 cm$^2$) and drought stress (14.59 cm$^2$) (Table 1).

3.2 Fruit characteristics
As illustrated in Table 2, the total numbers of fruits were significantly increased by all endophytic fungi treatments under favorable and drought stress conditions compared to drought treatment; the best treatment among them was the stressed $A. terreus$. Specifically,
Table 1. Pollen viability and growth characteristics of tomato plants under the effect of endophytic fungi.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Pollen sterility (%)</th>
<th>Shoot length (cm)</th>
<th>Root length (cm)</th>
<th>Shoot fresh weight (g)</th>
<th>Root fresh weight (g)</th>
<th>Shoot dry weight (g)</th>
<th>Root dry weight (g)</th>
<th>Leaf area (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10P</td>
<td>14.18 ± 2.96b</td>
<td>77 ± 10.13a</td>
<td>98.86 ± 25.29a</td>
<td>154.4 ± 32.72a</td>
<td>25.76 ± 4.25ab</td>
<td>24.19 ± 5.94a</td>
<td>3.63 ± 0.86ab</td>
<td>22.69 ± 4.22ab</td>
</tr>
<tr>
<td>D10P</td>
<td>18.90 ± 2.42b</td>
<td>60.7 ± 8.60b</td>
<td>68.57 ± 7.74b</td>
<td>98.94 ± 35.32c</td>
<td>23.23 ± 9.57b</td>
<td>19.79 ± 3.85ab</td>
<td>4.03 ± 1.21ab</td>
<td>19.66 ± 3.75bc</td>
</tr>
<tr>
<td>65P</td>
<td>16.75 ± 2.9b</td>
<td>63.6 ± 6.43b</td>
<td>76 ± 30.47b</td>
<td>139.84 ± 40.55ab</td>
<td>36.53 ± 19.28a</td>
<td>24.59 ± 6.66a</td>
<td>4.84 ± 2.97a</td>
<td>21.41 ± 3.39bc</td>
</tr>
<tr>
<td>D65P</td>
<td>16.36 ± 2.94b</td>
<td>72.8 ± 20.80ab</td>
<td>75.29 ± 7.28b</td>
<td>84.97 ± 23.49c</td>
<td>26.04 ± 10.13ab</td>
<td>23.23 ± 6.52a</td>
<td>4.29 ± 1.43ab</td>
<td>12.91 ± 1.65d</td>
</tr>
<tr>
<td>48P</td>
<td>15.07 ± 2.50b</td>
<td>72.8 ± 6.27ab</td>
<td>73.86 ± 17.79b</td>
<td>106.39 ± 32.58bc</td>
<td>23.78 ± 8.46b</td>
<td>19.68 ± 7.14ab</td>
<td>3.39 ± 1.37ab</td>
<td>20.86 ± 5.66bc</td>
</tr>
<tr>
<td>D48P</td>
<td>16.54 ± 0.21b</td>
<td>65.45 ± 7.33ab</td>
<td>74.88 ± 11.69b</td>
<td>102.45 ± 41.73bc</td>
<td>29.32 ± 11.55ab</td>
<td>22.98 ± 9.41a</td>
<td>4.26 ± 2.05ab</td>
<td>26.34 ± 3.09a</td>
</tr>
<tr>
<td>W</td>
<td>17.72 ± 1.63b</td>
<td>72.45 ± 10.63ab</td>
<td>57.62 ± 8.76b</td>
<td>77.5 ± 19.90c</td>
<td>18.27 ± 4.78b</td>
<td>14.35 ± 3.76b</td>
<td>2.58 ± 0.80b</td>
<td>17.51 ± 2.43cd</td>
</tr>
<tr>
<td>D</td>
<td>25.78 ± 5.45a</td>
<td>71.63 ± 6.39ab</td>
<td>58 ± 14.48b</td>
<td>78.01 ± 19.58c</td>
<td>18.06 ± 4.51b</td>
<td>16.92 ± 5.97ab</td>
<td>2.52 ± 0.94b</td>
<td>14.59 ± 1.62d</td>
</tr>
</tbody>
</table>

**Note(s):** Values of pollen viability represent the means ± SD, other values represent the means ± 95% confidence limits. Different letters in the same column refers to significant differences according to LSD test (α = 0.05).
Treatments of tomato plants under the effect of endophytic fungi provide drought tolerance.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Total number of fruits</th>
<th>Number of ripe fruits</th>
<th>Number of mature fruits</th>
<th>Fruit weight (g)</th>
<th>Fruit width (cm)</th>
<th>Fruit length (cm)</th>
<th>pH</th>
<th>Titratable acidity (%)</th>
<th>Total soluble solids (Brix)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10P</td>
<td>5.11 ± 2.38bc</td>
<td>1 ± 0.86d</td>
<td>4.11 ± 1.98ab</td>
<td>33.37 ± 4.77b</td>
<td>3.19 ± 0.36d</td>
<td>3.49 ± 0.32c</td>
<td>4.66 ± 0.14b</td>
<td>0.39 ± 0.06a</td>
<td>6.28 ± 0.76b</td>
</tr>
<tr>
<td>D10P</td>
<td>5 ± 1.47bc</td>
<td>1.9 ± 1.37bcd</td>
<td>3.1 ± 1.04bcd</td>
<td>47.53 ± 14.58a</td>
<td>4.06 ± 0.96abc</td>
<td>3.78 ± 0.38abc</td>
<td>4.7 ± 0.23b</td>
<td>0.41 ± 0.12a</td>
<td>6.6 ± 0.77ab</td>
</tr>
<tr>
<td>65P</td>
<td>5.3 ± 2.52bc</td>
<td>3.5 ± 1.76a</td>
<td>1.8 ± 1.00cde</td>
<td>45.7 ± 7.59ab</td>
<td>4.58 ± 0.14a</td>
<td>4.1 ± 0.22ab</td>
<td>4.72 ± 0.24b</td>
<td>0.41 ± 0.09a</td>
<td>6.54 ± 0.33ab</td>
</tr>
<tr>
<td>D65P</td>
<td>9.1 ± 2.14a</td>
<td>3.2 ± 1.00ab</td>
<td>5.9 ± 1.49a</td>
<td>44.64 ± 8ab</td>
<td>3.68 ± 0.46bcd</td>
<td>4.22 ± 0.43a</td>
<td>4.68 ± 0.24b</td>
<td>0.4 ± 0.11a</td>
<td>6.18 ± 0.97b</td>
</tr>
<tr>
<td>48P</td>
<td>6.2 ± 2.63b</td>
<td>2.7 ± 0.89ab</td>
<td>3.5 ± 2.44bc</td>
<td>50.51 ± 10.39a</td>
<td>4.51 ± 0.41a</td>
<td>4.05 ± 0.30ab</td>
<td>4.72 ± 0.20b</td>
<td>0.4 ± 0.04a</td>
<td>6.54 ± 0.50ab</td>
</tr>
<tr>
<td>D48P</td>
<td>5.11 ± 1.18bc</td>
<td>3.56 ± 1.16a</td>
<td>1.56 ± 0.56de</td>
<td>40.25 ± 8.75ab</td>
<td>4.33 ± 0.38abc</td>
<td>3.78 ± 0.33abc</td>
<td>4.72 ± 0.10b</td>
<td>0.42 ± 0.05a</td>
<td>6.74 ± 0.64ab</td>
</tr>
<tr>
<td>W</td>
<td>3.5 ± 0.84cd</td>
<td>2.6 ± 0.84abc</td>
<td>0.9 ± 0.86e</td>
<td>40.91 ± 12.93ab</td>
<td>3.53 ± 0.41cd</td>
<td>3.86 ± 0.66abc</td>
<td>5.12 ± 0.16a</td>
<td>0.31 ± 0.04b</td>
<td>5.07 ± 0.10c</td>
</tr>
<tr>
<td>D</td>
<td>2.44 ± 1.64d</td>
<td>1.11 ± 0.81d</td>
<td>1.33 ± 1.2de</td>
<td>33.71 ± 16.53b</td>
<td>3.14 ± 0.82d</td>
<td>3.6 ± 0.66bc</td>
<td>5.16 ± 0.17a</td>
<td>0.44 ± 0.02a</td>
<td>7.16 ± 1.87a</td>
</tr>
</tbody>
</table>

**Note(s):** The values represent the means ± 95% confidence limits. Different letters in the same column refers to significant differences according to LSD test (α = 0.05)
A. terreus and T. variabilis fungal treatments (stressed and nonstressed) and the control significantly increased the number of ripe fruits compared to drought stress treatment. The numbers of mature fruits were significantly enhanced by stressed A. terreus, nonstressed A. fumigatus and nonstressed T. variabilis treatments compared to the control and drought stress treatment (Table 2). Fruit weight was significantly increased through the inoculation by stressed A. fumigatus (47.53 g) and nonstressed T. variabilis (50.51 g) compared to drought stress treatment (33.71 g). Also, fruit width was significantly increased through the inoculation by nonstressed A. terreus (4.58 cm), stressed A. fumigatus (4.06 cm), and T. variabilis (stressed (4.33 cm) and nonstressed (4.51 cm)) compared to the drought treatment (3.14 cm). Moreover, stressed A. terreus significantly increased the length of tomato fruits (4.22 cm) compared to the drought treatment (3.6 cm) (Table 2). pH values were significantly decreased under the fungal treatments normally and under stress conditions compared to the control and drought stress treatment. However, titratable acidity was significantly increased by all fungal treatments (stressed and nonstressed) and drought treatment compared to the control. Total soluble solids were significantly increased under the effects of all fungal treatments (stressed and nonstressed) and drought treatment compared to the control (Table 2).

### 3.3 Morphological analysis

The morphological characteristics of tomato leaves are shown in Table 3 and Figure 1. Trichomes’ numbers (per mm²) were significantly increased under all fungal treatments (stressed and nonstressed) compared to the control. Also, most of the fungal treatments: (A. fumigatus (stressed (195.25) and nonstressed (117.75)), A. terreus (stressed (184.5) and nonstressed (207)), and stressed T. variabilis (337)) significantly increased the numbers of trichomes compared to drought treatment (147.6). Moreover, under drought stress, A. fumigatus and T. variabilis significantly increased number of trichomes compared to nonstress treatments. Similarly, drought treatment significantly increased number of trichomes (147.6) compared to the control (77) (Table 3 and Figure 1). The number of stomata (per mm²) was significantly increased under all the fungal treatments (stressed and nonstressed) and drought treatment compared to the control. Moreover, the maximum number of stomata was observed under nonstressed A. terreus treatment (45.4) (Table 3).

### 3.4 Biochemical analysis

The biochemical characteristics of tomato plants are displayed in Table 4. The highest proline concentration was found under drought stress treatment; proline concentrations significantly increased under all stressed fungal treatments compared to the control.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Trichomes number (per mm²)</th>
<th>Stomata number (per mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10P</td>
<td>117.75 ± 4.11d</td>
<td>28.5 ± 8.7b</td>
</tr>
<tr>
<td>D10P</td>
<td>195.25 ± 20.76b</td>
<td>26.5 ± 3.21b</td>
</tr>
<tr>
<td>65P</td>
<td>207 ± 8b</td>
<td>45.4 ± 5.22a</td>
</tr>
<tr>
<td>D65P</td>
<td>184.5 ± 14.36b</td>
<td>24.8 ± 4.32b</td>
</tr>
<tr>
<td>48P</td>
<td>124.67 ± 12.1cd</td>
<td>29 ± 6.23b</td>
</tr>
<tr>
<td>D48P</td>
<td>337 ± 19.52a</td>
<td>26.6 ± 4.77b</td>
</tr>
<tr>
<td>W</td>
<td>77 ± 10.12e</td>
<td>16.83 ± 3.97c</td>
</tr>
<tr>
<td>D</td>
<td>147.6 ± 35.9c</td>
<td>30.75 ± 7.93b</td>
</tr>
</tbody>
</table>

**Note(s):** The values represent the means ± SD. Different letters in the same column refers to significant differences according to LSD test (α = 0.05).
nonstressed fungal treatments. However, stressed *A. fumigatus* and stressed *A. terreus* treatments significantly had a higher amount of proline than stressed *T. variabilis* treatment (Table 4). GA3 concentrations were significantly enhanced under *A. fumigatus* (stressed (0.6 ppm) and nonstressed (0.6 ppm)) and *A. terreus* (stressed (0.6 ppm) and nonstressed (0.45 ppm)) treatments compared to the control (0.12 ppm) and drought treatment (0.12 ppm) (Table 4). Chlorophyll fluorescence significantly increased under the nonstressed *T. variabilis* treatment (0.83) and the control (0.83) compared to the drought treatment (0.79) (Table 4).
<table>
<thead>
<tr>
<th>Treatments</th>
<th>Proline content (ppm)</th>
<th>Gibberellic acid content (ppm)</th>
<th>Chlorophyll fluorescence</th>
<th>Chlorophyll $a$ content (µg/mL)</th>
<th>Chlorophyll $b$ content (µg/mL)</th>
<th>Carotenoids content (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10P</td>
<td>0.22 ± 0.15d</td>
<td>0.6 ± 0a</td>
<td>0.81 ± 0.04abc</td>
<td>3.93 ± 0.83ab</td>
<td>1.79 ± 0.43b</td>
<td>288.58 ± 48.93c</td>
</tr>
<tr>
<td>D10P</td>
<td>33.4 ± 14.95b</td>
<td>0.6 ± 0a</td>
<td>0.8 ± 0.04abc</td>
<td>5.27 ± 1.41a</td>
<td>2.48 ± 0.67a</td>
<td>415.31 ± 89.12a</td>
</tr>
<tr>
<td>65P</td>
<td>0.76 ± 0.24d</td>
<td>0.45 ± 0.3ab</td>
<td>0.81 ± 0.05abc</td>
<td>4.24 ± 0.12ab</td>
<td>1.85 ± 0.16ab</td>
<td>311.27 ± 14.68bc</td>
</tr>
<tr>
<td>D65P</td>
<td>36.32 ± 14.70b</td>
<td>0.6 ± 0a</td>
<td>0.81 ± 0.07abc</td>
<td>5.19 ± 1.25a</td>
<td>2.43 ± 0.52a</td>
<td>400.53 ± 91.44ab</td>
</tr>
<tr>
<td>48P</td>
<td>0.12 ± 0.00d</td>
<td>0 ± 0c</td>
<td>0.83 ± 0.02a</td>
<td>4.21 ± 1.03ab</td>
<td>1.87 ± 0.50ab</td>
<td>312.74 ± 76.06bc</td>
</tr>
<tr>
<td>D48P</td>
<td>22.82 ± 8.50c</td>
<td>0.2 ± 0.35bc</td>
<td>0.79 ± 0.06bc</td>
<td>4.33 ± 0.28ab</td>
<td>1.9 ± 0.21ab</td>
<td>319.16 ± 63.00abc</td>
</tr>
<tr>
<td>W</td>
<td>1.3 ± 0.84d</td>
<td>0.12 ± 0.27c</td>
<td>0.83 ± 0.01ab</td>
<td>4.38 ± 0.92ab</td>
<td>2.08 ± 0.45ab</td>
<td>342.06 ± 65.57abc</td>
</tr>
<tr>
<td>D</td>
<td>48.47 ± 9.35a</td>
<td>0.12 ± 0.27c</td>
<td>0.79 ± 0.04c</td>
<td>3.75 ± 0.75b</td>
<td>1.64 ± 0.47b</td>
<td>272.7 ± 75.93c</td>
</tr>
</tbody>
</table>

**Note(s):** The values represent the means ± SD. Different letters in the same column refers to significant differences according to LSD test ($\alpha = 0.05$).
Chlorophyll $a$, chlorophyll $b$, and carotenoids contents were significantly increased under stressed $A. \textit{fumigatus}$ and stressed $A. \textit{terreus}$ treatments (Table 4).

4. Discussion
The current study found that the inoculation with endophytic fungi induced multiple responses in tomato plants; they enhanced several characteristics under stressed and unstressed conditions.

The expansion of drought in many parts of the world is a catastrophic problem that has destructive impacts at several levels, the most important of which is its effect on plant growth, reproduction and production. Hence, finding cheap and eco-friendly strategies that improve plant tolerance to drought stress is an essential destination. Currently, we investigated the role of desert endophytic fungi, $A. \textit{fumigatus}$, $A. \textit{terreus}$ and $T. \textit{variabilis}$, in enhancing the drought tolerance of tomato plants. These endophytes can achieve that purpose by enhancing several characteristics under drought stress.

Pollen viability is an effective indicator that measures a plant’s ability to develop into seed and fruit; drought stress decreased pollen viability of tomatoes and other crops according to our findings and previous studies (Hu et al., 2020); nevertheless, this was promoted by our fungal treatments under stressed and nonstressed conditions. Similarly, $\textit{Talaromyces omanensis}$ enhanced the pollen viability of tomatoes under natural and drought-stress conditions (Halo et al., 2020). Also, the inoculation by $\textit{Piriformospora indica}$ fungus enhanced the pollen viability of $\textit{Cyclamen persicum}$ (Ghanem, Ewald, Zerche, & Hennig, 2014). Most of our endophytic fungi increased the levels of GA3 in tomato leaves, which elucidates the valuable role in improving pollen viability (Li, Tian, Guo, Luo, & Li, 2021).

The current fungal treatments partly enhanced growth characteristics. The best treatment was nonstressed $A. \textit{fumigatus}$ which increased shoot length, root length, shoot fresh weight, shoot dry weight and leaf area. The next one was nonstressed $A. \textit{terreus}$ which enhanced the shoot fresh weight, root fresh weight and shoot dry weight of tomatoes. Several endophytic fungi had the ability to improve growth features, including a desert-adapted endophytic fungus, $\textit{Serendipita indica}$, which promoted shoot and root growth of stressed and non-stressed tomato plants (Ghabooli & Kaboosi, 2022). Similarly, $\textit{Aspergillus flavus}$ and $A. \textit{terreus}$ fungi promoted the growth features of tomatoes (Abdel-Motaal, Kamel, El-Zayat, & Abou-Ellail, 2020; Yoo, Shin, Won, Song, & Sang, 2018).

Drought stress decreased the yields of tomatoes and other essential crops; the current results proved this trend. Various mechanisms were conducted to mitigate that expanded disaster. In particular, inoculation with endophytic microbes provided a safe and sustainable approach (Harman & Uphoff, 2019). As proof, an endophytic fungus $\textit{Ampelomyces sp.}$ promoted the yield of tomatoes under drought stress (Morsy et al., 2020); the yield enhancement was also detected under nonstressed status, as clarified in a Flores et al. (2020) study that applied $\textit{Bacillus thuringiensis}$ endophyte to enhance the growth and yield of $\textit{Cucumis sativus}$.

Similarly, the current study clarified that all endophytic fungi treatments improved the total yield of tomatoes under normal and stressful conditions. Moreover, the number of ripe fruits was enhanced by the inoculation with $A. \textit{terreus}$ and $T. \textit{variabilis}$ in both stressed and nonstressed conditions; however, $A. \textit{fumigatus}$ and $T. \textit{variabilis}$ enhanced the number of mature fruits under normal conditions.

Endophytic fungi improve fruit characteristics, such as $\textit{Piriformospora indica}$, which enhanced tomato fruit weight and $\textit{Trichoderma koningiopsis}$, which enhanced the fruit weight of $\textit{Ananas comosus}$ (Trocoli, Monteiro, Santos, & De Souza, 2017). Moreover, an endophytic $\textit{Talaromyces omanensis}$ increased the width of tomato fruits under favorable and drought-stress conditions (Halo et al., 2020).
Similarly, our endophytic fungi enhanced some fruit features, including fruit weight and size. Nonstressed *T. variabilis* and stressed *A. fumigatus* enhanced the weight of tomato fruits. Also, most fungal treatments showed increased tomato width (*T. variabilis*, nonstressed *A. terreus* and stressed *A. fumigatus*). However, only one treatment (stressed *A. terreus*) increased the length of tomato fruits.

In the current study, titratable acidity and total soluble solids were enhanced under all fungal and drought treatments. Similarly, drought stress increased these fruit qualities according to Rad, Asghari, and Hasan’s (2015) study. Moreover, the inoculation by endophytes enhanced fruit soluble sugar of apple (Rho, Van Epps, Kim, & Doty, 2020), as well, *Diversispora versiformis* fungus improved orange fruit quality, including total soluble solids and mineral element contents of their hosts (Cheng et al., 2022).

The applied endophytic fungi reduced pH values under stressed and unstressed treatments; similarly, the pH values of strawberry were decreased under the bacterial inoculation of *Pseudomonas* sp. (Todeschini et al., 2018). Malic and citric acids contents mainly affect pH values; numerous factors control the accumulation of these two acids in fruit cells, including the interactions between metabolism and vacuolar storage, sink ratio, water supply, mineral nutrition and temperature (Etienne, Génard, Lobit, Mbeguié-A-Mbéguié, & Bugaud, 2013), consequently, the applied microbes likely control one or more of these influential factors which resulted in pH reduction.

Numerous mechanisms are utilized by endophytes to enhance their host growth, yield and fruit quality under favorable and drought stress conditions, involving promoting photosynthetic activity, phytohormones production, enhancing nutrient absorption, synthesis of osmolytes, scavenging of reactive oxygen species and altering gene expression (Lu, Wei, Lou, Shu, & Chen, 2021); currently, proline, GA3, chlorophyll measurements and trichome density clarified some aspects of the provided role as follows:

According to the present study, all fungal treatments and drought treatment increased the number of trichomes. Similarly, studies of Muthukumaran and Anusuya (2018) and del Rosario Cappellari, Santoro, Schmidt, Gershenzon, and Banchio (2019) found that the microbial inoculation increased trichome density of *Solanum lycopersicum* and *Mentha piperita* plants. In addition, the present study showed that the stressed fungal treatments significantly had greater trichomes than the drought treatment. This finding represents a real benefit for the plants under drought conditions because the trichomes have an influential role in protecting drought-stressed plants through preserving higher levels of water, absorbing dew deposits, reflecting solar radiations and enhancing photosynthetic performance (Ning et al., 2016). Thus, our endophytic fungi efficiently adapted tomato plants to drought stress through the significant increase of trichomes density; previous studies illustrated that gibberellins and jasmonates stimulate trichomes formation in multiple stressed plants (Castro-Camba, Sánchez, Vidal, & Vielba, 2022; Hua et al., 2022); hence, it is probably because endophytes elevated the density of trichomes through GA3 secretion.

The current study found that stomata numbers increased in the fungal-treated and drought-stressed plants; nonstressed *A. terreus* recorded the highest number of stomata. However, the inoculation by an endophytic fungus *Trichoderma asperellum* reduced the stomata density of *Theobroma cacao* (Rosmana, Nasaruddin, Hendarto, Hakkar, & Agriansyah, 2016).

Although the reduction in stomata number feature is more tolerant to drought stress (Caine et al., 2019), the remarkable rise of trichomes density by our endophytes maintains a high level of water even though the number of stomata was more.

The current study showed that the highest concentration of proline was observed under the drought treatment, followed by drought-stressed treatments of endophytic fungi compared to the control and nonstressed treatments. Similarly, tomato plants that were inoculated with an endophytic fungus *Talaromyces omanensis* accumulated lower
concentrations of proline under drought stress than non-inoculated stressed plants (Halo et al., 2020); also, Trichoderma harzianum endophyte caused the same impact on their rice host (Shukla, Awasthi, Rawat, & Kumar, 2012). The fungal-inoculated plants were less affected by drought stress than noninoculated stressed plants; this most likely explains the decrease in proline content in their leaves (Zou, Wu, Huang, Ni, & He, 2013). In the present study, the plants inoculated with A. fumigatus and A. terreus concentrated higher amounts of GA3 under stressed and non-stressed conditions. Some studies reported that the endophytic fungi could increase the levels of GA3 and other phytohormones in their unstressed host plants such as A. fumigatus and Fusarium proliferatum (Bilal et al., 2018) and under drought stress conditions such as Trichoderma harzianum (Mona et al., 2017).

The improvement of growth characteristics of crops by endophytes was associated with improving phytohormones content (Verma et al., 2021). Similarly, the current study found that A. fumigatus and A. terreus accumulated a higher amount of GA3, which stimulated cell elongation and division; thus, yield characteristics and several growth features of tomato plants were enhanced. According to the current study, nonstressed T. variabilis and the control increased the Fv/Fm ratio and enhanced chlorophyll fluorescence of tomato leaves; chlorophyll contents and carotenoids were enhanced under stressed A. fumigatus and stressed A. terreus, this impact probably explained through enhancing chlorophyll synthesis by promoting its components absorption via the applied endophytes. Several endophytes were reported to enhance chlorophyll fluorescence and chlorophyll contents in their hosts, such as Piriformospora indica (Shahabivand, Parvaneh, & Aliloo, 2017). Also, an endophytic Exophiala pisciphila enhanced the chlorophyll fluorescence of Sorghum bicolor under drought-stress conditions (Zhang et al., 2017).

5. Conclusion
Abiotic stress management using endophytes is a promising and sustainable field mitigating mounting climate change risks; therefore, they have been increasingly studied in the last two decades. Distinctively, stress-adapted endophytes attracted growing research attention as they are more efficient in achieving the desired aims. Currently, the drought-adapted endophytes, A. fumigatus, A. terreus and T. variabilis provide several benefits to their tomato hosts under drought stress. They significantly promoted pollen viability, trichome and stomata density and the yield of tomatoes under stressed and nonstressed conditions. Moreover, all of them enhanced tomato fruit quality involving total soluble solids and titratable acidity compared to the control; also, they reduced the pH values of tomato fruits; individually, the best endophytic fungus that enhanced growth characteristics under normal conditions was A. fumigatus followed by A. terreus; however, fruit characteristics were enhanced by T. variabilis.

Overall, adding the present endophytic fungi to crop plants provides impressive benefits in normal and stressed agricultural systems, particularly enhancing yield and fruit quality.

These results were partly interpreted through the present investigated measurements: GA3 concentrations, chlorophyll and carotenoids contents, chlorophyll fluorescence and trichomes formations. Since our study introduced some elucidated mechanisms provided by drought-adapted endophytic fungi, it is recommended to investigate other mechanisms of stress-adapted endophytes on the molecular, biochemical and anatomical levels, such as phytohormone synthesis, nutrient absorption, phosphate solubilization and antioxidant enzymes activity, inducing systemic resistance and modulating anatomical composition.

References


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