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The role of the endophytic fungus, *Thermomyces lanuginosus*, on mitigation of heat stress to its host desert plant *Cullen plicata*

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Introduction: Endophytic fungi associated with desert plants have a crucial role to enable these plants to tolerate abiotic stress, such as heat and drought. *Methods:* In this study, a thermophilic fungal endophyte was isolated from a hot desert-adapted plant, *Cullen plicata* Delile. The endophytic fungus was (molecularly) identified as *Thermomyces lanuginosus*, and inoculated plants were coded as E+ and the control as E−. *Results:* This fungus had an effective growth-promoting activity on its host plant and increased the plant resistance to heat stress as well. *Discussion:* Our findings demonstrate that thermophilic fungal endophytes can enhance drought and heat stress tolerance in desert plants by ecophysiological mechanisms and improve growth of its host plants.

Original Article

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INTRODUCTION

The global climate is predicted to change rapidly over the next century and many environmental changes will happen (Giannopolitis & Ries, 1977). Climate change has been affecting Egypt for at least 4,000 years, resulting in a profound impact on agricultural production. In the forthcoming years, it is expected to extend the effect on water management and agricultural production (Copetta et al., 2006). The potential impact of climate change on field crops is expected to decrease national production of about 11% for rice to 28% for soybean (El-Marsafawy, 2007; El-Ramady et al., 2013). The climate-changing parameters also affect terrestrial microorganisms as well as plants (Compant et al., 2010). Endophytic fungi that live in desert plants are considered to be a vital source for many natural products (Strobel et al., 2004). The genetic recombination of the endophytic fungi and its host might be the reason that endophytes are able to provide plant with natural compounds (Tan & Zou, 2001). For example, *Curvularia* sp. confers heat tolerance to its host plant *Dichanthelium lanuginosum* (Redman et al., 2002). *Thermomyces* sp. is a thermophilic fungus that can survive at 62 °C (Mchunu et al., 2013). *Thermomyces lanuginosus* was first isolated from soil and involved in compost degradation until its first isolation as plant endophyte (Novas & Carmarán, 2008). The plants under extreme temperatures exhibit various symptoms like chlorosis and necrosis leading to death (Sanghera et al., 2011). Recently, there is a worldwide trend to minimize the negative effects of the abiotic stress by the applications of genetic approaches (Fahad et al., 2017). *Cullen plicata* (Delile) C. H. Stirt is one of the extreme hot desert plants, member of tribe *Psoralaea* (Kumar & Chandan, 2015). Only a few studies are concerned with desert plants–endophytic fungal interaction, so the responses of endophytic fungus to its host *C. plicata* in arid environment under drought and heat stress were examined.

MATERIALS AND METHODS

Isolation of the endophytic fungus from its hot desert soil-adapted plant C. plicata

Fresh roots of hot desert-adapted plant, *C. plicata*, were collected from three sites at Aswan University campus (39.59 °N 32.82 °E). Roots were initially rinsed in tap water

and then surface-sterilized by successive immersion in 95% ethanol (10 s), 2% sodium hypochlorite (3 min), and 70% ethanol (2 min). Surface-sterilized roots were cut into approximately ~5 mm pieces under sterile conditions. Five pieces of roots were placed on potato dextrose agar plate and incubated at 45 °C for 1 week (Shivanna et al., 1996).

Morphological and molecular characterization of the endophyte isolate

The isolate fungus was examined under light microscope to identify morphologically according to keys of Moubasher (1993).

Inoculation of fungal endophyte to C. plicata

C. plicata seeds were surface-sterilized with 0.5% sodium hypochlorite for 3 min and then rinsed in sterile-distilled water (SDW) for 10 min. Sterilized seeds were soaked in 50 ml conidial spore suspension (1×10^6) of *T. lanuginosus* isolate or SDW as control for 6 hr. The primed seeds were sown in plastic pots containing mixture (2:1, v/v) of clay and sand infested with the fungus spores; control pots E− received SDW. Seedlings were grown in the growth chamber at 25 ± 2 °C for 3 weeks before transferring into field station under normal climatic conditions.

Measurement of photosynthetic parameters

Measurements of photosynthesis (Pn) rate, transpiration (Tr) rate, leaf intercellular CO_2 concentration, and stomatal conductance (Gs) were performed using infrared gas analyzer (IRGA, CI 340) Pn system (CID Bio-Science, Inc., USA).

Growth characteristic measurements

Leaf area was measured by leaf area meter, the shoot and root lengths of the plants were measured using a meter scale, root and shoot fresh weight were measured by harvesting the plant and weighed using electrical balance, after which they were air-dried and reweighed to get the dry weight. Stomata number and size were measured in lower epidermis peel. The soil water content (SWC) was determined by oven-drying method and calculating as percentage (%).

Determination of phenylalanine ammonia-lyase (PAL), superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) activities

Frozen plant tissue (200 mg) was ground to a fine powder in a pre-cooled mortar, and 2 ml of extraction buffer [0.2 M phosphate buffer (pH 7.2), 0.1 mM ethylenediaminetetraacetic acid, 1 mM dithiothreitol, and 2 U protease inhibitor cocktail] was added. The macerated suspension was centrifuged at 10,000 rpm for 5 min at 4 °C. The supernatant was collected and used as the source of enzymatic analyses. *PAL* activity was assayed by measuring the trans-cinnamic acid formation at 290 nm, according to Nagarathna et al. (1993). *SOD* activity was determined according to Giannopolitis and Ries (1977). *POX* activity was estimated according to Kim and Yoo (1996). *CAT* activity was determined spectrophotometrically at 240 nm as described by Aebi (1984).

Determination of total saponin, carbohydrate, flavonoids, proteins, and ascorbic acid

The dry materials were extracted according to the procedure described by Mostafa et al. (2013). Total saponin content was determined spectrophotometrically at 473 nm (Ebrahimzadeh & Niknam, 1998). The water-soluble carbohydrates and total carbohydrates were quantified by anthrone-sulfuric acid method, which was carried out according to Fales (1951) and Trevelyan et al. (1952). The total flavonoid content of the plant extract was determined by the aluminum chloride colorimetric method using absorbance wavelength at 510 nm (Chen et al., 2005). Total soluble and insoluble proteins were determined according to the method adopted by Lowry et al. (1951). Ascorbic acid was determined by the modified method of Roe and Kuether (1943).

Statistical analysis

Data obtained were subjected to Student's *t*-test using Minitab 18 software (www.minitab.com). Values shown in the figures are the means ± standard errors of four independent replicates.

RESULTS

Morphological and molecular identification of the endophytic fungus

A thermophilic fungus was isolated from the roots of an extreme hot desert plant *C. plicata* Delile (Aswan Governorate, Egypt). The climatic environments in this region ranged between moderately cold dry winters to very hot dry summer (Fig. 1). The isolated fungus was identified using the morphology of the fungal culture (Moubasher, 1993). The fungus was confirmed as *T. lanuginosus* according to Ali et al. (2017).

Photosynthetic responses to heat stress

The photosynthetic responses, including Pn, Tr, Gs, and Ci (internal CO_2), were determined at 20, 30, and 45 °C in the 2-month-old *C. plicata* plants treated with endophytic fungus E+ isolated from the same plant and untreated control plants E−. The Pn response to heat stress increased over time and with temperature rising in E+ treated where the Pn response was threefold higher at 45 °C in E+ plants, whereas the heat stress accelerated the Pn decline process in untreated plants (Fig. 2a). In contrast, heat stress had very highly significantly increasing effect ($p \leq .000$) on Gs and Tr in untreated E− plants, whereas the E+ plants exhibited low rates of Gs and Tr (Fig. 2b and c). In addition, there was no significant difference in Ci level between E+ and E− plants but steadily decreasing was observed with temperatures rising from 20 to 45 °C (Fig. 2d).

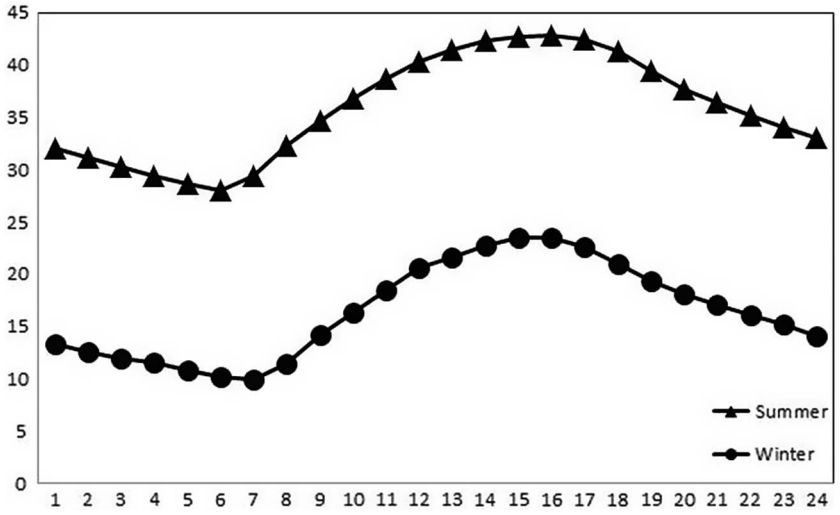

Fig. 1. Seasonal diurnal air temperature (°C) averages at field meteorological station RUSPAL Station (Research Unit for Study Plants of Arid Lands), South-Eastern Desert, Egypt (2015–2017)

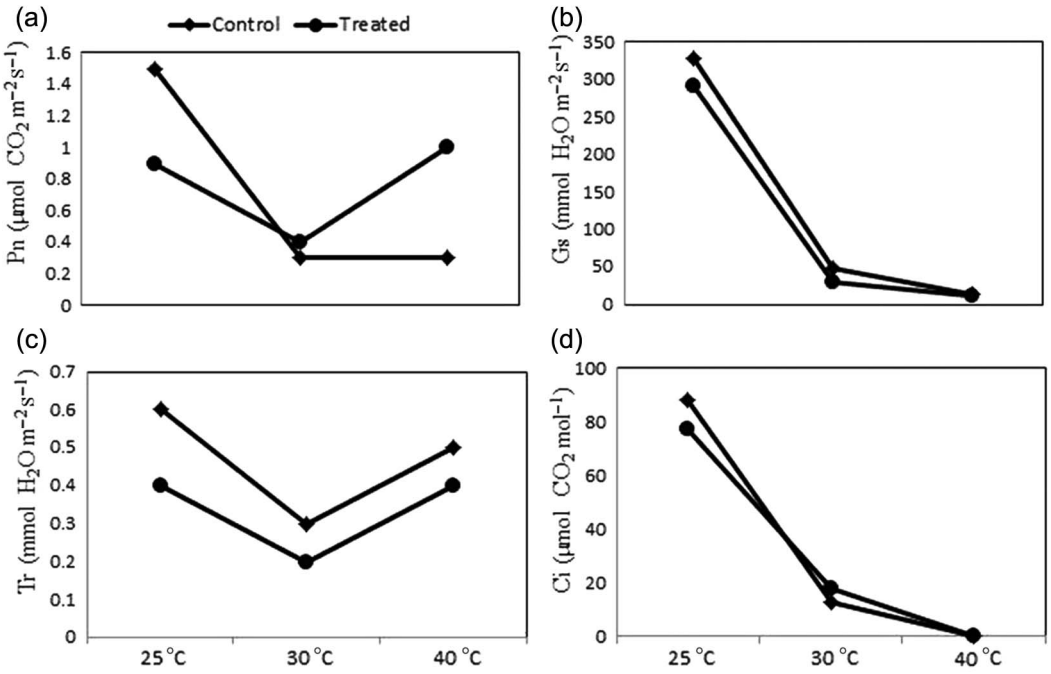

Fig. 2. Changes of photosynthetic parameters in *Cullen plicata* plants treated with fungal endophyte E+ and untreated control plants E− at different temperatures. Values are means ± standard errors (*SE*s) of four independent biological replicates ($n = 4$). (a) Photosynthetic rate, Pn; (b) stomatal conductance, Gs; (c) transpiration rate, Tr; and (d) leaf intercellular CO_2 concentration, Ci

Phenological traits of C. plicata *in response to endophytic fungus inoculation*

The impacts of endophyte inoculation showed significant differences in SWC, leaves number, fruits number, fruits weight, root length, and root fresh and dry weight. Figure 3a shows the effect of the endophytic fungus on the root length of our studied plant where it is significantly higher ($p \leq .006$) in E+ group than E− group (50 ± 0.8 and 26.6 ± 2.3 cm/plant, respectively). The root/shoot ratio is given as the ratio of the dry weight of the roots to the dry weight of the shoots; in this experiment, E+ plants showed a significant increase in root/shoot ratio where $p \leq .01$ (Fig. 3a). Figure 3b shows the SWC. The E+ group was highly significant ($p \leq .008$) as compared to the non-inoculated group (2.7 ± 2.6 and 0.6 ± 0.9). Similar findings were found with root fresh and dry weight ($p \leq .009$ and $p \leq .01$, respectively). Moreover, the E+ group showed highly significant increase in leaves number ($p \leq .005$; Fig. 3c). The fruits (yield) number of E+ group significantly increased ($p \leq .01$) compared to the E− group (0.4 ± 0.06 and 0.07 ± 0.01); subsequently, the fruits' weight was higher in E+ group ($p \leq .01$) than E− (0.58 ± 0.08 and 0.12 ± 0.02; Fig. 3d). In response to inoculation with the endophytic fungus stomata, number and size (Fig. 5) were affected, where E+ group

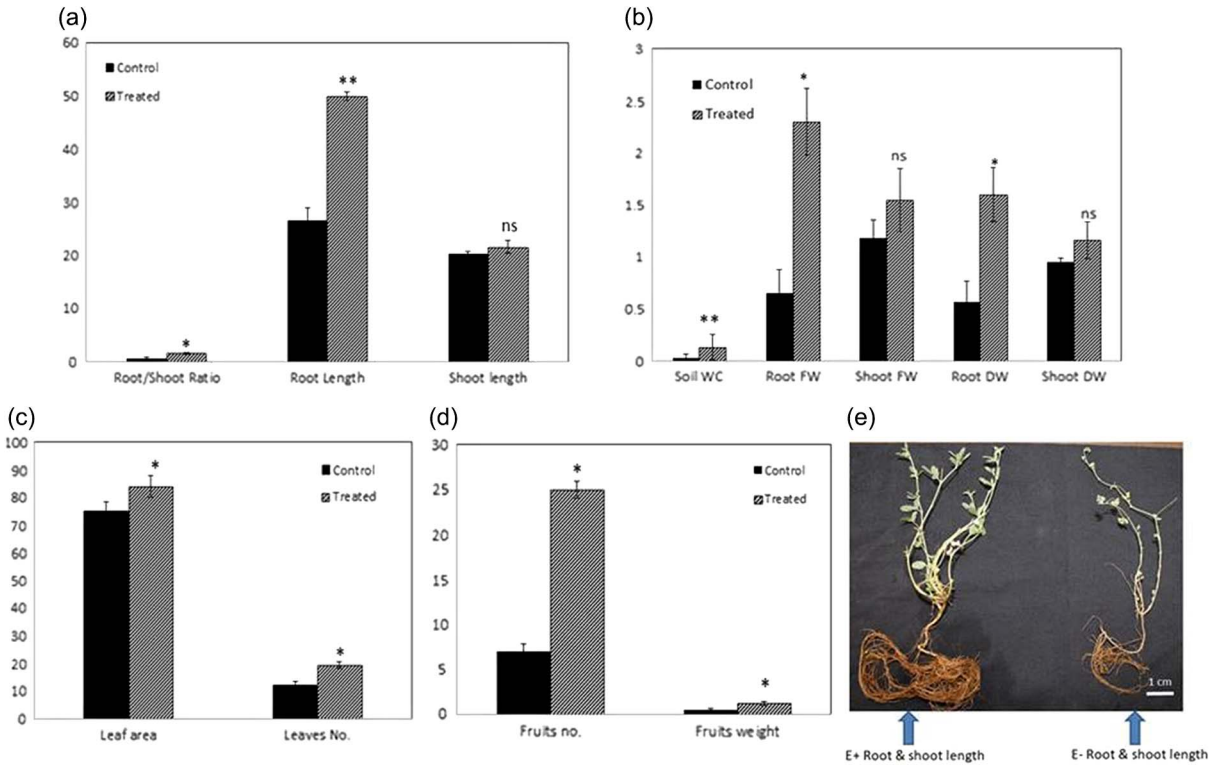

Fig. 3. (a) Root and shoot length (cm) and root/shoot ratio (g) of *Cullen plicata* plants treated with fungal endophyte E+ and untreated control plants E−; (b) Soil water content (SWC), root fresh and dry weight, and shoot fresh and dry weight (gm); (c) Leaf area and leaves number in *C. plicata*-treated and -untreated plants; (d) Fruits number and fruits weight (gm) of *C. plicata*-treated and -untreated plants; (e) The effect of endophytic fungus treatment on the root and shoot length in comparison with untreated control plant. Values are means ± standard errors (*SE*s) of four independent biological replicates ($n = 4$) (significant $*p < .05$, highly significant $**p < .01$, and ns = non-significant)

stomata number was significantly higher than in the E− group ($p \leq .02$); on the contrary, the stomata size of E+ group was significantly lower than E− group ($p \leq .03$; Fig. 5e).

Changes in metabolite contents and antioxidant activities

We hypothesized that significant changes in the metabolite pool of E+-treated *C. plicata* plants may occur, and these changes enhanced the *C. plicata* plant survival capacity under heat stress condition. There were significant increases ($p < .05$) in the total carbohydrates (310 mg/g DW), flavonoid (4.7 mg/g DW), and highly significant increase was found in ascorbic acid content (44.2 mg/g FW; Fig. 4a). Antioxidant enzyme levels of E+ plants showed highly significant increases in PAL, CAT, and POX activities (70.6, 80.9, and 51.8 U/g FW, respectively) relative to E− plants (Fig. 4b).

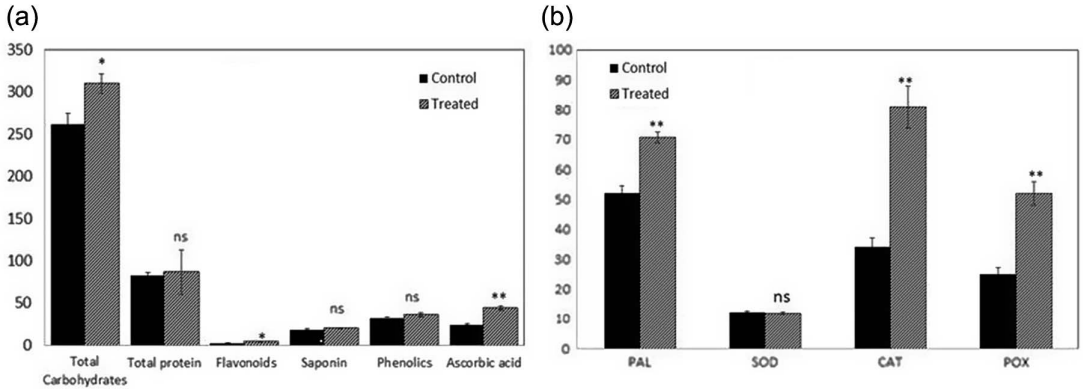

Fig. 4. (a) Changes in total carbohydrates, total protein, flavonoid, saponin, phenolics, and ascorbic acid contents (mg/g DW) in E+-treated and -untreated plants; (b) Changes in total PAL, SOD, CAT, and POX activities (U/g FW) in E+-treated and -untreated plants. Values are the mean of four independent replicates ($n = 4$). Asterisks indicate significant difference as determined by a Student's *t*-test ($*p < .05$ and $**p < .01$)

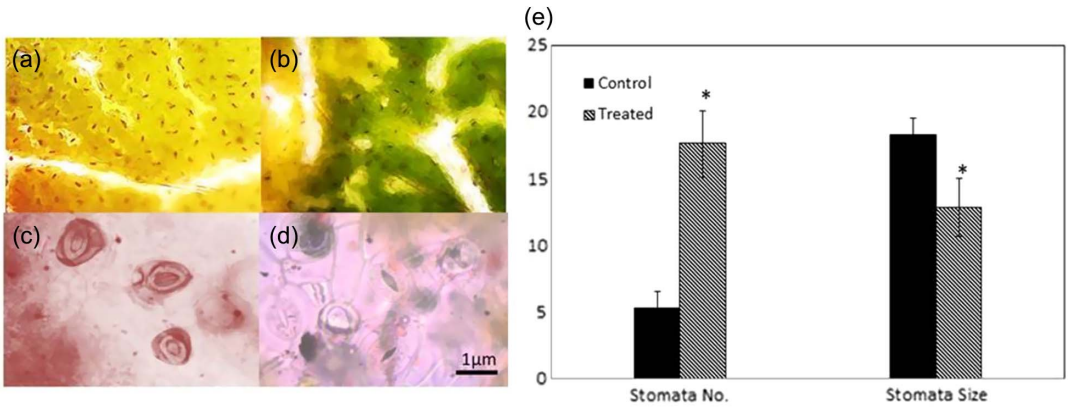

Fig. 5. Microscopic images of intact leaves mounted in sufranin solution and glycerol. (a)Stomatal density response to endophytic fungus inoculation; (b) Stomatal density of control plants; (c and d) The reduction of stomata size of E+-inoculated plant compared to control ones (d); (e) Stomata number (mm^2) and size (µm) in *Cullen plicata* plants treated with fungal endophyte E+ and untreated control plants E−. Values are means ± standard errors (*SE*s) of four independent biological replicates ($n = 4$). Asterisks indicate significant difference as determined by a Student's *t*-test ($*p < .05$ and $**p < .01$)

DISCUSSION

Endophyte–plant relationships may influence how plant species respond to environmental change. For example, endophytic fungi have been demonstrated to improve drought stress for a few species of agronomically important forage and turf grasses, namely *Lolium* and *Festuca* spp. (Kannadan & Rudgers, 2008). Egypt climate is described as arid and semi-arid, has a hot, dry summers, moderate winters, and erratic rainfall, and most of the regions of Egypt are occupied by the Sahara desert, which has been classified as the most extensive area of severe aridity on the earth (Domroes & EL-Tantawi, 2005). Examples of studies on the association process of endophytic fungi with host plants were performed in the United States by Khidir et al. (2010), who studied the community of endophytic fungi associated with the roots of grasses. In Venezuela, Loro et al. (2012) have analyzed the diversity of endophytic fungi from dominant grasses and sedges. Previous studies have contributed to the understanding how endophytic fungi benefit their hosts in extreme regions to resist abiotic factors (Bezerra et al., 2017). In this study, we investigated the role of *T. lanuginosus* to its host hot desert plant *C. plicata* (Delile). *T. lanuginosus* is a thermophilic fungus that grows between a maximum temperature of 60 °C and a minimum of 20 °C with an optimum growth temperature of 50 °C. *T. lanuginosus* is an ascomycete that grows rapidly on various media whose colonies reaching 2.5 to over 5 cm in diameter at 45–50 °C within 2 days (Singh et al., 2003). *T. lanuginosus* thermally adapted by the presence of several DNA-related pathways, including ubiquitin degradation, histone acetylation/deacetylation, and poly adenosine diphosphate-ribosylation, plays major role in cell signaling and DNA repair under heat stress conditions (Mchunu et al., 2013). Heat stress may disturb Pn by affecting carbon metabolism, which is considered as the primary limiting factor of Pn at high temperature (Kurek et al., 2007). Our data showed obvious fluctuation in Pn rate in response to heat stress in E + and E− plants (Fig. 2a). Some desert C3 shrubs have a high photosynthetic yield at temperature greater than 40 °C (Mooney et al., 1978). High temperature directly affects the activity of several Calvin cycle enzymes, especially Rubisco activase (Galmés et al., 2013; Parry et al., 2003). The heat stress effect on Gs and transpiration rate in this study showed different trend in E+ and E− plants where both were lower in E+ plants, which is in agreement with Ali et al. (2017). Endophytic fungi secrete favorable secondary metabolites that facilitate their host plants to survive under stress condition (Khan et al., 2008). This study provides a good evidence of the vital role of endophytic fungi to its host desert plants through improvement of secondary metabolite accumulations. Endophytic fungi isolated from sand dune plants produce metabolites and help in growth and development of their host plants (Khan et al., 2008). Antioxidants are highly effective agents for the protection from damage caused by abiotic stress (Abdelrahman et al., 2015). Some endophytic fungi activate certain enzymes, which could promote the growth and fitness of their host plants (Chen et al., 2005). In this study, E+-treated plants showed a significant increase in PAL, CAT, and POX activities relative to untreated E− plants. Hariri et al. (2013) demonstrated the role of endophytic fungus *Piriformospora indica* in increasing rice growth efficiency under salt stress via enhancement of the levels of antioxidant enzymes (Hariri et al., 2013). Our result is congruous with the hypothesis that the water conservation strategies in the plant are promoted using endophyte symbiosis interaction (Malinowski & Belesky, 2000). Our findings of the fresh and dry weight (Fig. 3b) and shoot and root length of E+ plants (Fig. 3a) were higher than E− plants, which is in agreement with Choudhary and Varma (2016). In the current research, root/shoot ratio of E+ plants was significantly increased compared to E− ones. Similarly, the fruits yield of our investigated [non-arbuscular mycorrhizal (AM) fungus] E+ plants was significantly higher than E− plants. AM fungi have a direct effect on the plant physiology with detectable beneficial effects on the root/shoot ratios and fruits (Al-Karaki et al., 2004; Berta et al., 2014; Bona et al., 2015; Domroes & EL-Tantawi, 2005; Guerrieri et al., 2004; Lingua et al., 2013). Stomatal density responses to various environmental factors, such as heat stress (Beerling & Chaloner, 1993) and drought (Lecoeur et al., 1995; Galmés et al., 2007).

The stomata number of E+ was significantly higher than the E− plant but there was reduction in stomatal size of E+ (Fig. 5e). As described by Morse et al. (2002), *Neotyphodium* sp. promoted stomatal density in its host plant *Festuca arizonica* under stress conditions. According to our data, *C. plicata* plant displayed a better adaptation to heat stress depending on its beneficial endophytic fungus (*T. lanuginosus*).

CONCLUSION FOR FUTURE BIOLOGY

Fungal endophytes are one of the important future microbial researches as they have unique adaptations according to their environments. Thermophilic fungal endophytes isolated from desert plants could be exploited in crop productivity improvement due to their role in the protection of biotic and abiotic stresses. Further future studies are needed for revealing the mitigation mechanisms made by fungal endophytes of plant heat and drought tolerance.

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Competing Interests: The authors declare no competing interests.

Authors' Contributions: AHA and MAE-S suggested the research point, carried out experiments, performed statistical analysis, and wrote the main manuscript. SEZ critically edited the manuscript. MAE-S, AHA, UR, and SE-Z approved the final version of the manuscript to be published.

REFERENCES

Abdelrahman, M., Sawada, Y., Nakabayashi, R., Sato, S., Hirakawa, H., El-Sayed, M., Hirai, M. Y., Saito, K., Yamauchi, N., Shigyo, M. (2015) Integrating transcriptome and target metabolome variability in doubled haploids of *Allium cepa* for abiotic stress protection. *Mol. Breed. 35,* 195.

Aebi, H. (1984) Catalase *in vitro*. *Method. Enzym. 105,* 121–126.

Ali, A. H., Abdelrahman, M., Radwan, U., El-Zayat, S., El-Sayed, M. (2017) Effect of *Thermomyces* fungal endophyte isolated from extreme hot desert-adapted plant on heat stress tolerance of cucumber. *Appl. Soil. Ecol. 124,* 155–162.

Al-Karaki, G., McMichael, B., Zak, J. (2004) Field response of wheat to arbuscular mycorrhizal fungi and drought stress. *Mycorrhiza 14,* 263–269.

Beerling, D. J., Chaloner, W. G. (1993) The impact of atmospheric CO_2 and temperature change on stomatal density: observations from *Quercus robur* Lammad leaves. *Ann. Bot. 71,* 231–235.

Berta, G., Copetta, A., Gamalero, E., Bona, E., Cesaro, P., Scarafoni, A., D'Agostino, G. (2014) Maize development and grain quality are differentially affected by mycorrhizal fungi and a growth-promoting pseudomonad in the field. *Mycorrhiza 24,* 161–170.

Bezerra, J. D. P., de Azevedo, J. L., Souza-Motta, C. M. (2017) Why study endophytic fungal community associated with cacti species? In: de Azevedo, J. L., Quecine, M. C. (eds.) *Diversity and Benefits of Microorganisms from the Tropics* (ebook), Chapter 2. Springer, Cham, Switzerland, pp. 21–25.

Bona, E., Lingua, G., Manassero, P., Cantamessa, S., Marsano, F., Todeschini, V., Copetta, A., D'Agostino, G., Massa, N., Avidano, L., Gamalero, E., Berta, G. (2015) AM fungi and PGP pseudomonads increase flowering, fruit production, and vitamin content in strawberry grown at low nitrogen and phosphorus levels. *Mycorrhiza 25,* 181–193.

Chen, X., Guo, S., Wang, C. (2005) Effects of four endophytic fungi on the growth and polysaccharide content of *Anoectochilus roxburghii* (Wall.) Lindl. *Chin. Pharm. J. 40,* 13–16.

Choudhary, D. K., Varma, A. (2016) *Microbial-Mediated Induced Systemic Resistance in Plants* (eBook). Springer, New York.

Compant, S., van der Heijden, M. G., Sessitsch, A. (2010) Climate change effects on beneficial plant-microorganism interactions. *FEMS Micro. Ecol. 73,* 197–214.

Copetta, A., Lingua, G., Berta, G. (2006) Effect of three AM fungi on growth, distribution of glandular hairs, and essential oil production in *Ocimum basilicum* L. var. Genovese. *Mycorrhiza 16,* 485–494.

Domroes, M., EL-Tantawi, A. (2005) Recent temporal and spatial temperature changes in Egypt. *Int. J. Climatol. 25,* 51–63.

Ebrahimzadeh, H., Niknam, V. (1998) A revised spectrophotometric method for determination of triterpenoid saponins. *Indian Drugs 35,* 379–382.

El-Marsafawy, S. M. (2007) Impact of climate change on sun flower crop production in Egypt. *Egypt J. Agric. Res, Porto, Portugal 85*(5), 1547–1563.

El-Ramady, H. R., El-Marsafawy, S. M., Lewis, L. N. (2013) Sustainable agriculture and climate changes in Egypt. *Sustainable Agric. Rev. 12,* 41–95.

Fahad, S., Bajwa, A. A., Nazir, U., Anjum, S. A., Farooq, A., Zohaib, A., Sadia, S., Nasim, W., Adkins, S., Saud, S., Ihsan, M. Z., Alharby, H., Wu, C., Wang, D., Huang, J. (2017) Crop production under drought and heat stress: plant responses and management options. *Front. Plant Sci. 8,* 1147.

Fales, F. W. (1951) The assimilation and degradation of carbohydrates by yeast cells. *J. Biol. Chem. 193,* 113.

Galmés, J., Flexas, J., Savé, R., Medrano, H. (2007) Water relations and stomatal characteristics of Mediterranean plants with different growth forms and leaf habits: responses to water stress and recovery. *Plant Soil 290,* 139–155.

Galmés, J., Perdomo, J. A., Flexas, J., Whitney, S. M. (2013) Photosynthetic characterization of Rubisco transplantomic lines reveals alterations on photochemistry and mesophyll conductance. *Photosyn. Res. 115,* 153–166.

Giannopolitis, C. N., Ries, S. K. (1977) Superoxide dismutases occurrence in higher plants. *Plant Physiol. 59*(2), 309–314.

Guerrieri, E., Lingua, G., Digilo, M. C., Massa, N., Berta, G. (2004) Do interactions between plant roots and the rhizosphere affect parasitoid behavior? *Ecol Entomol 29,* 753–756.

Hariri, B. B., Calvanese, D., De Giacomo, G., Deutsch, A., Montali, M. (2013) *Verification of Relational Data-Centric Dynamic Systems with External Services*. In: Proceedings of the 32nd ACM SIGACT SIGMOD SIGART Symposium on Principles of Database Systems, New York, NY.

Kannadan, S., Rudgers, J. A. (2008) Endophyte symbiosis benefits a rare grass under low water availability. *Funct. Ecol. 22,* 706–713.

Khan, S. A., Hamayun, M., Rim, S., Lee, I., Seu, J., Choo, Y., Jin, I., Kim, S., Lee, I., Kim, J. (2008) Isolation of endophytic fungi capable of plant growth promotion from monocots inhabited in the coastal sand dunes of Korea. *J. Life Sci. 18*(10), 1355–1359.

Khidir, H. H., Eudy, D. M., Porras-Alfaro, A., Herrera, J., Natvig, D. O., Sinsabaugh, R. L. (2010) A general suite of fungal endophytes dominate the roots of two dominant grasses in a semiarid grassland. *J. Arid Environ. 74,* 35–42.

Kim, Y., Yoo, J. Y. (1996) Peroxidase production from carrot hairy root cell culture. *Enzyme Mircob. Techn. 18,* 531–535.

Kumar, S., Chandan, S. P. (eds.) (2015) *Cullen plicata* (*Fabaceae*). In: *Conservation of Desert Plants*. Scientific Publishers, Jodhpur, p. 87.

Kurek, I., Change, T. K., Bertain, S. M., Madrigal, A., Liu, L., Lassner, M. W., Zhu, G. (2007) Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate stress. *Plant Cell 19,* 3230–3241.

Lecoeur, J., Wery, J., Turc, O., Tardieu, F. (1995) Expansion of pea leaves subjected to short water-deficit: cell number and cell-size are sensitive to stress at different periods of leaf development. *J. Exp. Bot. 46,* 1093–1101.

Lingua, G., Bona, E., Manassero, P., Marsano, F., Todeschini, V., Cantamessa, S., Copetta, A., D'Agostino, G., Gamalero, E., Berta, G. (2013) Arbuscular mycorrhizal fungi and plant growth-promoting pseudomonads increases anthocyanin concentration in strawberry fruits (*Fragaria x ananassa* var. Selva) in conditions of reduced fertilization. *Int. J. Mol. Sci. 14,* 16207–16225.

Loro, M., Valero-Jiménez, C. A., Nozawac, S., Márquez, L. M. (2012) Diversity and composition of fungal endophytes in semiarid Northwest Venezuela. *J Arid Environ. 85,* 46–55.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem. 193,* 265–275.

Malinowski, D. P., Belesky, D. P. (2000) Adaptations of endophyte-infected cool-season grasses to environmental stresses: mechanisms of drought and mineral stress tolerance. *Crop Sci 40,* 923–940.

Mchunu, N. P., Permaul, K., Abdul Rahman, A. Y., Saito, J. A., Singh, S., Alam, M. (2013) Xylanase super producer: genome sequence of a compost-loving thermophilic fungus, *Thermomyces lanuginosus* strain SSBP. *Genome Announcement 1,* e00388-13.

Mooney, H. A., Bjorkman, O., Collatz, G. J. (1978) Photosynthetic acclimation to temperature in the desert shrub *Larrea divaricata*. *Plant Physiol. 61,* 406–410.

Morse, L. J., Day, T. A., Faeth, S. H. (2002) Effect of *Neotyphodium* endophyte infection on growth and leaf gas exchange of Arizona fescue under contrasting water availability regimes. *Environ. Exp. Bot. 48,* 257–268.

Mostafa, S., Abou El Kheir, A. W., Shehata, H. S. (2013) Effect of plant growth promoting substances from rhizo- and cyanobacteria on sugar beet growth, yield and yield quality in saline soil. *Int. J. Acad. Res. 5*(6), 58–65.

Moubasher, A. H. (1993) *Soil Fungi in Qatar and Other Arab Countries*. The Scientific and Applied Research Centre, University of Qatar, Doha, p. 566.

Nagarathna, K. C., Shetty, S. A., Shetty, H. S. (1993) Phenylalanine ammonia lyase activity in pearl millet seedlings and its relation to downy mildew disease resistance. *J. Exp. Bot. 265,* 1291–1296.

Novas, M., Carmarán, C. C. (2008) Studies on diversity of foliar fungal endophytes of naturalised trees from Argentina, with a description of *Haplotrichum minutissimum* sp. nov. *Flora Morphol. Distrib. Funct. Ecol. Plant 203*(7), 610–616.

Parry, M. A., Andralojc, P. J., Mitchell, R. A., Madwick, P. J., Keys, A. I. (2003) Manipulation of Rubisco: the amount, activity, function and regulation. *J. Exp. Bot. 54,* 1321–1333.

Redman, R. S., Sheehan, K. B., Stout, R. G., Rodriguez, R. J., Henson, J. M. (2002) Thermotolerance conferred to plant host and fungal endophyte during mutualistic symbiosis. *Science 298,* 1581.

Roe, J. H., Kuether, C. A. (1943) The determination of ascorbic acid in whole blood and urine through the 2, 4-dinitrophenylhydrazine derivative of dehydroascorbic acid. *J. Biol. Chem. 147,* 399–407.

Sanghera, G. S., Wani, S. H., Hussain, W., Singh, N. B. (2011) Engineering cold stress tolerance in crop plants. *Cur Genomics 12,* 30–43.

Shivanna, N., Siddalingamurthy, G. S., Ramesh, S. R. (1996) Larval pupation site preference and its relationship to the glue proteins in a few species of *Drosophila*. *Genome 39,* 105–111.

Singh, S., Andreas, M. M., Bernard, A. P. (2003) *Thermomyces lanuginosus*: properties of strains and their hemicellulases. *FEMS Microb. Rev. 27,* 3–16.

Strobel, G., Daisy, B., Castillo, U., Harper, J. (2004) Natural products from endophytic microorganisms. *J. Nat. Prod. 67,* 257–268.

Tan, R. X., Zou, W. X. (2001) Endophytes: a rich source of functional metabolites. *Nat. Prod. Rep. 18,* 448–459.

Trevelyan, W. E., Forrest, R. S., Harrison, J. S. (1952) Determination of yeast carbohydrates with the anthrone reagent. *Nature 170,* 626–627.