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Research Article

Diversity and effectiveness of arbuscular mycorrhizal fungi species in alleviating drought stress in tomato

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Abstract

Water scarcity poses significant challenges to sustainable agriculture, and the anticipated increase in the frequency and severity of droughts due to climate change intensifies these constraints. Therefore, there is an urgent need to identify eco-friendly approaches to enhance drought tolerance, especially in arid regions, to ensure global food security, considering the growing world population. This study investigated the morphological diversity of Arbuscular Mycorrhizal Fungi (AMF) species associated with the rhizosphere of cultivated tomato (*Solanum lycopersicum* L.) plants across diverse agroecosystems in twenty locations in Egypt. The results reveal the presence of five AMF species from the genus *Glomus*: *G. invarmaium, G. xanthium, G. intraradices, G. mosseae, and G. macrocarpum.* The study further explored the impact of mixed inoculation with the identified *G.* species on the physiological and morphological performance of tomato plants exposed to drought stress. The results showed that AMF inoculation significantly improved root colonization under drought stress. Inoculated plants showed significantly higher shoot and root fresh and dry weights than non-inoculated controls. AMF-inoculated plants also exhibited higher leaf chlorophyll concentrations and increased accumulation of stress-related metabolites such as proline, total soluble sugars, and glycine betaine compared to non-inoculated plants under drought stress. Leaf phosphorus concentration was enhanced in inoculated plants, whereas lipid peroxidation, indicated by malondialdehyde (MDA) levels, was reduced. Thermal imaging revealed that AMF inoculation led to lower canopy temperatures, indicating better stress tolerance. Therefore, AMF inoculation, particularly *Glomus* species, mitigated the adverse effects of drought stress on tomato plants, which indicates its potential for improving crop performance under water-limited conditions. These findings documented the importance of AMF in enhancing drought tolerance in tomato plants, suggesting that their application could be a viable strategy for sustainable agricultural practices in arid regions.

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Keywords: climate resilience; mycorrhizal inoculation; osmolyte accumulation; physiological parameters; plant-microbe interactions; plant stress mitigation; sustainable agriculture

Introduction

Arbuscular mycorrhizal fungi (AMF) are obligate plant symbionts that have significant potential in producing biostimulants for their host plants (Sun and Shahrajabian, 2023). Currently, almost 300 species belonging to the AMF are morphologically described worldwide (Madouh and Quoreshi, 2023). AMF are physically and chemically influenced by soil texture, organic matter, pH, and phosphorus (Bhardwaj and Chandra, 2018). Several published reports elucidated the impacts of tillage, monocultures, rotations, and host plants on cultivated crops (Gagliardi *et al*., 2023). However, little is known about the diversity of AMF in these various soil types where monoculture or association crops such as cowpea and tomato are produced. Accordingly, exploring the morphological diversity of AMF species associated with the rhizosphere of cultivated crops in diverse agroecosystems is important.

Tomato (*Solanum lycopersicum* L.) is among the most frequently cultivated and economically significant crops in the world. Due to its sensitivity to drought stress, the plant requires adequate water to grow and produce an acceptable yield and quality (Zannat *et al*., 2023). Drought considerably limits agricultural sustainability and crop productivity (Ali *et al*., 2021; Galal *et al*., 2023; Sedhom *et al*., 2024). Particularly under the abrupt climate change in arid regions, drought is a major factor limiting crop productivity (Carvajal *et al.* 2025). Water scarcity has destructive effects on all plant development processes, from seed germination to physiological maturity (Desoky *et al*., 2023; Mousa *et al*., 2024). Plants have adapted physiological and biochemical mechanisms to cope with drought stress (Abd-El-Aty *et al*., 2024). Accumulation of suitable osmolytes like proline and soluble carbohydrates and synthesis of free radical scavenging chemicals such as ascorbate and glutathione have been intensively researched (Kamara *et al*., 2022; Selem *et al*., 2022). AMF indigenous to arid areas displays a long-term adaptation to dry soils (Wahab *et al*., 2023). The AMF *G.* species are prevalent in semi-arid environments and thrive under situations of low water supply (Omirou *et al*., 2013). Through various strategies, AMF can attenuate drought stress and deleterious effects on plant growth (Pavithra and Yapa, 2018). It is generally recognized that AMF increases plant nutrient uptake, protects plants from infections, and buffers against harsh environmental conditions, including drought (Zhang *et al*., 2019). AMF improves seedling survival (Wu *et al*., 2017), promotes water uptake and transport in the host plant (Ortas *et al*., 2021), and changes root morphology to protect host plants from drought stress (Quiroga *et al*., 2019). AMF also produces glomalin-related soil protein (GRSP), which acts as a glue to construct water-stable aggregates by entangling extraradical hyphae, enhancing soil water-holding capacity, and stabilizing soil structure (Gupta, 2020). AMF reduces membrane lipid peroxidation and membrane permeability and increases osmotic adjustment chemicals and antioxidant enzyme activity (Bahadur *et al*., 2019). This occurs due to the involvement of extraradical hyphae in the process of water absorption by the mycorrhizal roots and concurrent improvements in the rate of photosynthesis (Li *et al*., 2016). Higher osmolyte accumulation, such as sugars, proline, and glycine betaine, lowers the cell osmotic potential in AMF-inoculated plants under water stress, allowing higher water retention (Yooyongwech *et al*., 2016).

The current study aimed to explore the diversity of *G.* species in tomato rhizospheres across a range of agroecosystems, providing new insights into the potential of these species to alleviate drought stress in tomato plants. Unlike previous studies that focus on a single *G.* species or limited environmental conditions, this study investigates the impact of mixed *G.* inoculation on plant growth, physiological parameters, and stress-related metabolites under drought stress. This research not only focuses on the effectiveness of AMF in mitigating water scarcity effects but also emphasizes the practical application of *G.* species for enhancing crop resilience in the context of climate change and water-limited environments. Accordingly, the research was performed to determine the morphotypes of arbuscular mycorrhizal fungi connected with tomato (*S. lycopersicum* L.) in the Ismailia Governorate, Egypt. This work was prompted by the necessity of accomplishing a long-term goal, which is to enhance the production of tomato by making use of the wide variety of native arbuscular mycorrhizal fungi that are connected with tomato rhizospheres.

Materials and Methods

Classification of detected arbuscular mycorrhizal fungi (AMF) species

Field sample collection

Twenty locations were randomly chosen in the governorate of Ismailia, Egypt, to collect soil cores, which were combined to provide representative bulk samples. Tomato fields that had been conventionally managed for several seasons (at least five) were chosen. The soil was obtained from the rhizosphere of growing plants by excavating to a depth of 10 to 30 centimeters. The samples were taken to the laboratory and stored at 4 °C to ensure the viability of collected mycorrhizal spores.

Extraction and morphological identification of collected AMF

In a glass container, 200 g of the soil sample were dissolved in 1000 ml of water; allowing heavier particles to settle. Then the liquid was purred through sieves (mesh size of 500, 250, 45 mm) to remove the organic matter. After washing in the last sieve, it was transfer it to a Petri dish. At least 15-25 intact spores were mounted in water or lactic acid on a microscope slide to determine shape and size. A digital computer program and a light microscope were used for measurements (LIECA model MD502). Spores freshly isolated and crushed in PVLG or PVLG + Melzer's reagent were measured for spore wall and germination wall thickness. Spores crushed in water or PVLG were analyzed for spore wall layers or germinal wall layers. The colors were determined according to Koske (1989).

Mass production of AMF on the host plant

Onion (*Alium cepa* L.) seedlings were utilized for mass production of AMF spores. Due to their rapid growth, onions are an ideal host plant for AMF; they develop numerous fine, hairy roots for extensive sporulation. The ratio of sand to soil utilized as a growing medium was 1:2. To eliminate all microorganisms, including AMF, the soil was air-dried, passed through a 2-mm screen, and then autoclaved. Mature, viable AMF spores were collected manually and identified by depositing them on seedling roots in 15 cm × 20 cm plastic pots containing 3 kg of the growth substrate. Moreover, 10 mL of AMF inoculum per seedling (containing 400 ± 20 propagules per cell) was administered. Hoagland's nutrient solution (Hoagland and Arnon, 1938) was used to feed plants in a greenhouse for 3 months. Greenhouse day/night cycles averaged 32/25 °C with natural light. Tap water was used to water plants.

Measuring AMF root colonization

Roots were soaked in a 10% w/v KOH solution at 90 °C for 10-30 min, then rinsed in tap water and soaked in 1% HCl overnight. Samples were stained in acidic glycerol with 0.5% trypan blue for 24 hours at room temperature. The roots were de-stained at room temperature in acidic glycerol (Koske, 1989) .

Greenhouse trial to assess the impact of AMF on tomato plants under drought stress

A greenhouse trial was conducted at Suez Canal University in Ismailia, Egypt (30°37'10.91"N and 32°16'1.33"E). All pots were distributed at random in the greenhouse under natural temperature conditions. Physiochemical properties of soil and compost used in the present study are presented in Table S1. The AMF inoculum was applied at a concentration of 5 mL per seedling (containing 250 ± 20 propagules per cell) and 20 grams of mycorrhizal onion roots. The tomato plants were evaluated under two irrigation treatments. The well-watered (WW) treatment was irrigated once every two days, while the plants were irrigated once every four days under drought stress (DS) conditions. In the control pots, 0.85% salt solution replaced the AMF suspension. After the 45-day experiment, the root systems were carefully cleansed with water to remove clinging soil particles to determine mycorrhizal root colonization percentage, shoot dry weight (g), root dry weight (g), and physiological parameters of the plants.

Measured parameters of the greenhouse trial

A SPAD-502 chlorophyll meter (Minolta Co., Ltd.; Japan) was used to assess chlorophyll concentration by measuring leaf absorbance in the red and near-infrared areas. Two LEDs emit light at 650 and 940 nm. When the measuring head is closed, these LEDs light a photodiode detector in sequence. When light travels through the measuring head of the leaf sample, some is transmitted and transformed into electrical impulses. SPAD-502 derives a company-defined SPAD value by dividing light transmission intensities at 650 nm by 942 nm. SPAD value indicates leaf chlorophyll concentration. The phenol sulfuric acid technique was utilized to determine leaf total soluble sugars (Dubious, 1956). This approach used ethanol to homogenize 0.5 g of fresh leaves. The extract was filtered and treated with five percent phenol and ninety-eight percent sulfuric acid. This mixture was allowed for one hour before its absorbance at 485 nm was measured using a spectrophotometer. Proline interaction with ninhydrin was utilized for the colorimetric determination of leaf proline .In a mortar, leaf tissue (0.5 g) was pulverized and homogenized. The extract was dissolved in 10 ml of 3% sulfosalicylic acid and centrifuged to remove the leaf tissue. For proline colorimetric determinations, a 1:1:1 solution of proline, ninhydrin acid, and water is used (Bates *et al.*, 1973). Glycine betaine (GB) was estimated according to Grieve and Grattan (1983). Dry plant material (0.5 g) was mechanically shaken for 48 h at 25 °C before filtration. 2 N sulfuric acid was used to dilute thawed extracts. Aliquots were chilled in ice water for 1 hour. Add 0.2 mL of cold potassium iodide-iodine reagent. The materials were frozen for 16 hours and centrifuged at 10,000g for 15 minutes. The supernatant was aspirated, and 9 ml of 1,2-dichloroethane was used to dissolve periodic crystals. After 2 h, 365 nm absorbance was recorded. Thermal image acquisition of the plots was captured using an infrared thermal camera Ti-32 (Fluke Thermography; Germany) with a 320 × 240-pixel microbolometer sensor sensitive in the spectral region of 7.5-13 µm. The height of the canopy was around 1 m. Images were analyzed using the software Ti-32 Pro (Infrared Solutions; United States); emissivity for measurements of leaves and plant canopies was set to 0.96, and transmission correction was set at 85%. For increased precision, the range of the auto-adjusted thermal picture was manually set to the level of the shown image to determine the maximum and minimum temperature of the entire display (Wilcox et Makowski, 2014).

Statistical analysis

The data obtained were subjected to variance analysis. Tukey's HSD test was applied to compare the means, with a significance level of $P<0.05$, using the SPSS Software Version 24.0.

Results

Classification of detected arbuscular mycorrhizal fungi (AMF) species

Five *Glomus* (*G.)* species were isolated and identified based on morphological characteristics of spores, such as color, shape, size, wall layer, and subordinate hyphae as presented in Table 1. The identified species were: *G. invarmaium*, *G. xanthium*, *G. intraradices*, *G. mosseae*, and *G. macrocarpum* (Figure 1). The microscopic investigation revealed that the proportion of onion roots infected with the AM fungus ranged between 80 and 95 percent (Figure 2).

Impact of AMF on tomato plants under drought stress

Root and shoot traits

Microscopically, root colonization was identified in all AMF inoculations, while no mycorrhizal colonization was detected in non-inoculated control plants (Figure 3). Without stress treatment, AMF inoculation plants reached 82% at harvest time, which was the highest colonization, followed by 66% for inoculated plants under drought stress. The water shortage led to a considerable reduction in the colonization intensity (Figure 3). The shoot and root fresh and dry weights of inoculated plants with AMF were significantly higher than those of non-inoculated plants under drought stress conditions (Figure 3). The highest values were assigned for inoculated plants with AMF under well-watered conditions, followed by non-inoculated plants with AMF under well-watered conditions and inoculated plants with AMF under drought stress conditions. On the other hand, non-inoculated plants with AMF under drought stress conditions exhibited the lowest value of the shoot and root fresh and dry weights.

Table 1. Morphological characterization of AMF spores isolated from soil samples

No	Spore characteristics				Subtending hypha			AMF species
	Color	**Shape**	**Size (μm)**	**Wall layers/ size**	**Shape**	**Width (μm)**	**Wall**	
1	Light Brown to Brown	Globose to subglobose	100-240	Two L1 (1.0-2.2 μm) L2 (3.7-5.6 μm)	Cylindrical	15-28	Two L1 (1.6-2.0 μm) L2 (2.5-1.0 μm)	*G. invermaium* Hall
2	Light yellow to yellow	Globose to subglobose	45-100	Three L1 (1.2-2.7 μm) L2 (0.2-1.2 μm) L3 (0.7-2.7 μm)	Cylindrical	20-40	Two L1 (1.2-2.7 μm) L2 (0.5-1.5 μm)	*G. xanthium* (Blaszk. V. Blanke, C. Renker & F. Buscot)
3	Pale yellow to greyish yellow	Globose to subglobose	50-150	Three L1 (0.5-3.0 μm) L2 (1.5-5.0 μm) L3 (3.0-7.0 μm)	Cylindrical	10-16	Three L1 (0.5-3.0 μm) L2+L3 (3.0-6.4 μm)	*G. intraradices* (N.C. Schenck & G.S. Sm.)
4	Pale yellow to golden yellow	Globose to subglobose	140-280	Three L1 (0.5-2.0 μm) L2 (0.8-1.8 μm) L3 (2.8-7.2 μm)	Funnel	14-30	Three L1 (1.0-1.5 μm) L2+L3 (2.4-4.8 μm)	*G. mosseae* (Nicol. & Gerd.) Gerd. & Trappe
5	Yellow	Globose to subglobose	110-130	Two L1 (1.2-1.7 μm) L2 (4.7-7.6 μm)	Cylindrical	17-26	Two L1+L2 (7.0-15 μm)	*G. macrocarpum* (Tul. & C. Tul.)

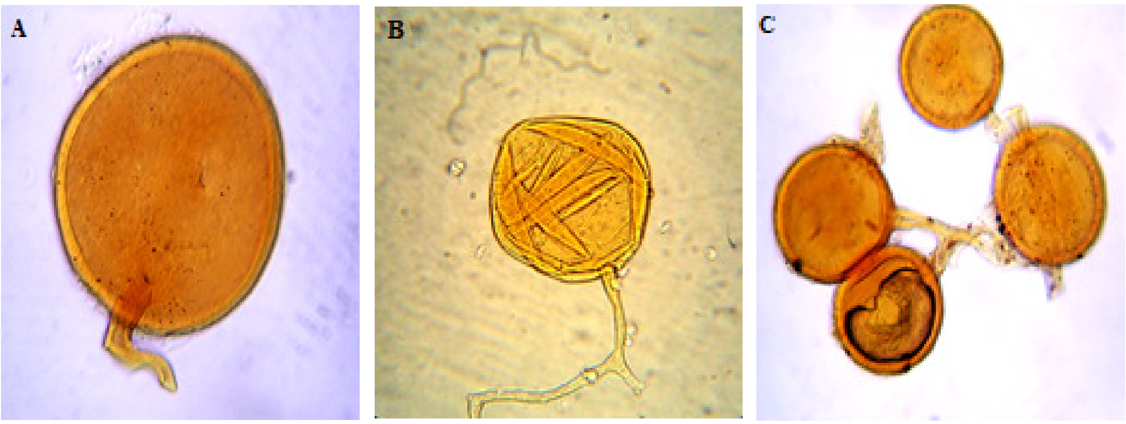

Figure 1. Morphology of spores from detected arbuscular mycorrhizal fungi at 400× magnification: *G. invermaium* (A), *G. xanthium* (B), *G. intraradices* (C), *G. mosseae* (D), *G. badium* (E)

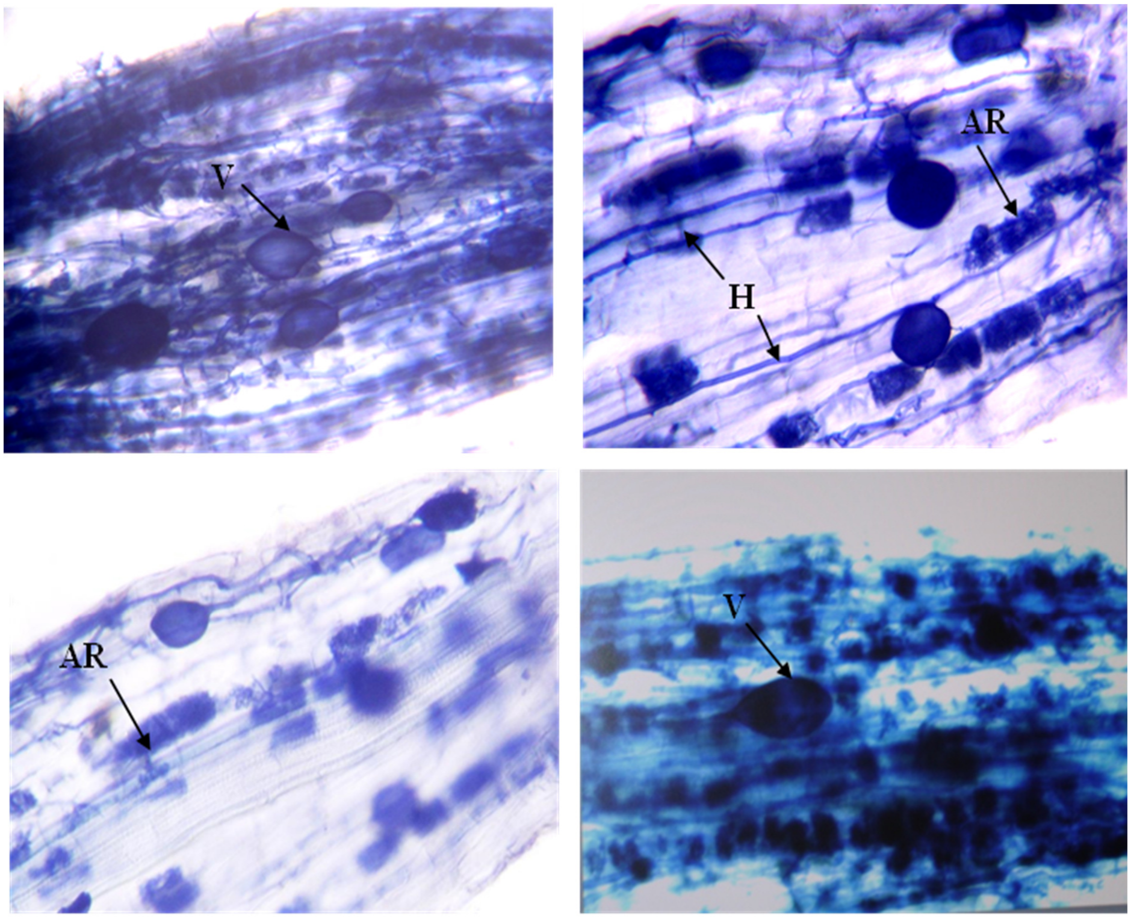

Figure 2. *Alium cepa* roots infected with AMF showing typical structures of AMF within the root at 200× magnification. AR - arbuscular, V - vesicle and H - hypha

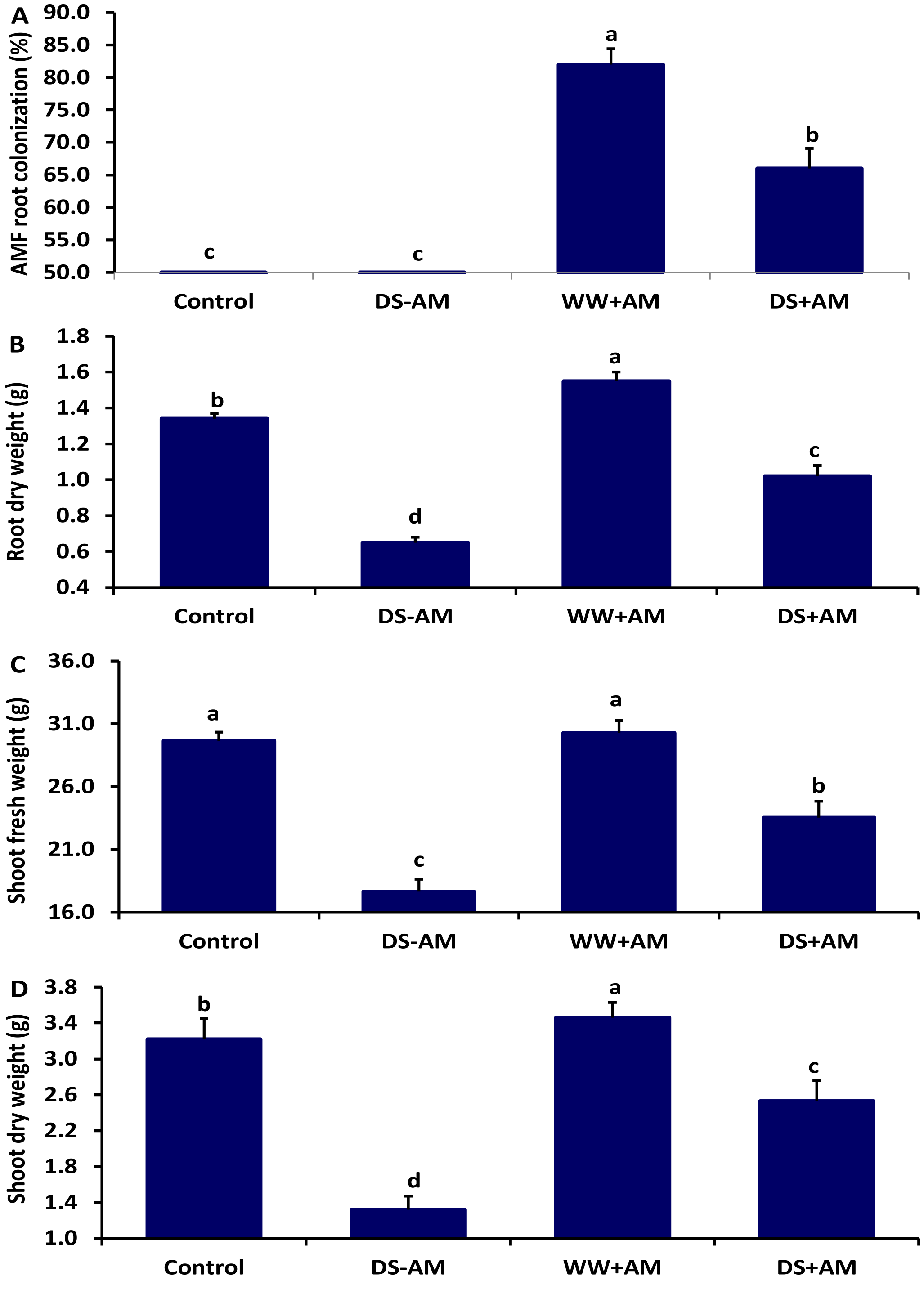

Figure 3. Impact of inoculation with AMF on root traits and shoot traits of tomato plants under well-watered and drought stress conditions. WW-AM: non-inoculated plants with AMF under well-watered conditions; DS-AM: non-inoculated plants with AMF under drought stress; WW+AM: inoculated plants with AMF under well-watered conditions; DS+AM: inoculated plants with AMF under drought stress conditions. The bars above columns denote standard deviation. Different letters differ significantly according to Tukey's HSD test ($p < 0.05$)

Physiological parameters

Inoculated plants exhibited considerably higher leaf chlorophyll concentration than non-inoculated controls under both irrigation treatments. Mycorrhization of tomato plants generated a rise in leaf chlorophyll, which was always significantly greater than in non-mycorrhizal plants under stressful conditions (Figure 4A).

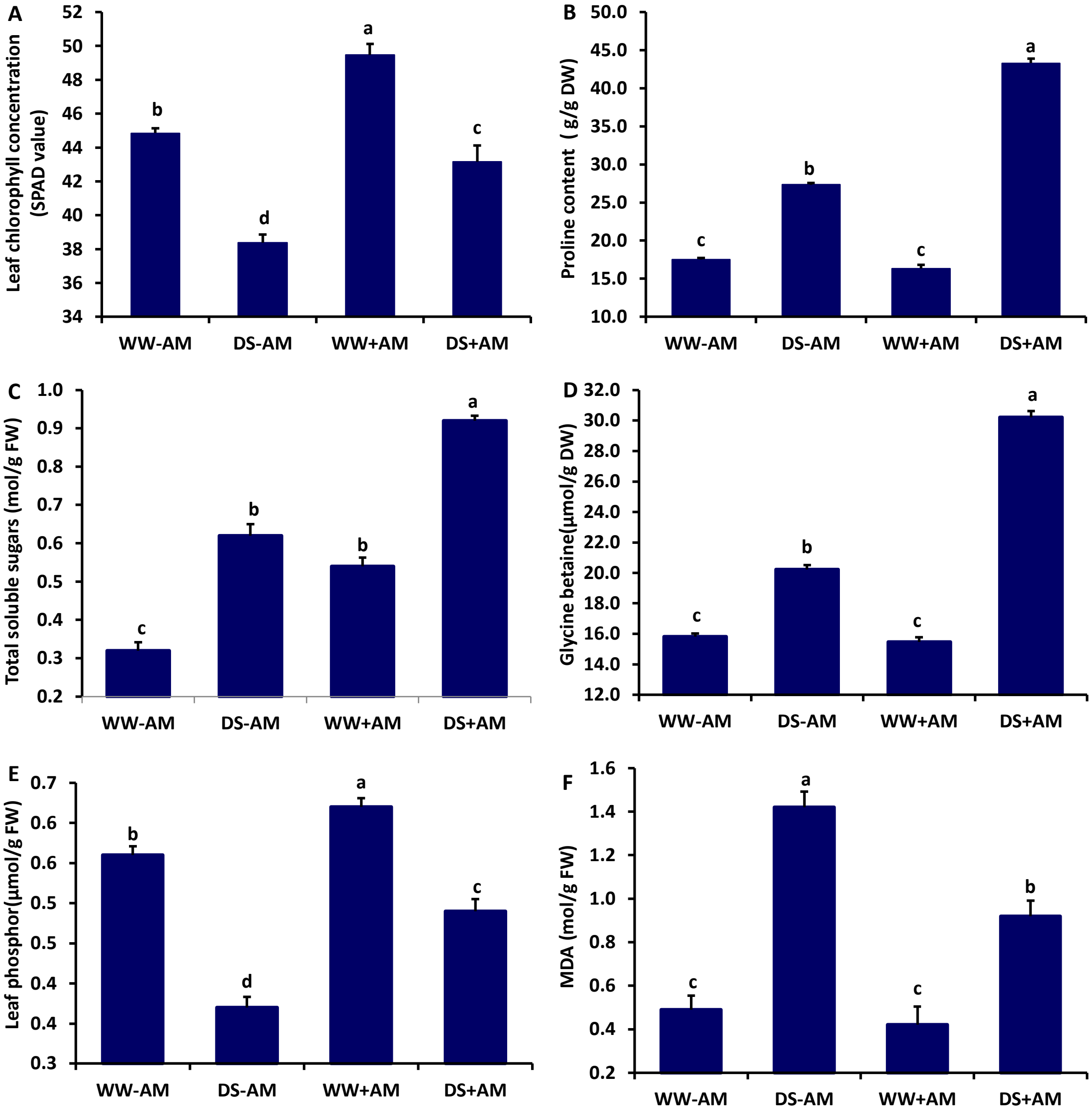

Figure 4. Impact of inoculation with AMF on leaf chlorophyll concentration (A), proline content (B), total soluble sugars (C), glycine betaine (D), leaf phosphorus concentration (E), and malondialdehyde, MDA (F). WW-AM: non-inoculated plants with AMF under well-watered conditions; DS-AM: non-inoculated plants with AMF under drought stress; WW+AM: inoculated plants with AMF under well-watered conditions; DS+AM: inoculated plants with AMF under drought stress conditions. The bars above columns denote standard deviation. Different letters differ significantly according to Tukey's HSD test ($p < 0.05$)

The highest leaf proline concentration was observed under stress conditions, with a declining trend under well-watered conditions. As a result of drought stress, the proline concentration in the leaves of inoculated plants was higher than that of non-inoculated plants under drought stress conditions (Figure 4B). Likewise, the stressed plants contained more total soluble sugars than those that were grown under well-watered conditions. The inoculated plants exhibited higher total soluble sugars in leaves than non-inoculated plants under drought stress (Figure 4C). A considerable increase in the amount of glycine betaine was accumulated under drought stress. The accumulation of glycine betaine was higher in inoculated plants than in non-inoculated controls under drought stress conditions (Figure 4D). The leaf phosphorus concentration of inoculated plants with AMF was considerably higher than that of non-inoculated plants under drought stress conditions (Figure 4E). The highest values were assigned for inoculated plants under well-watered conditions, followed by non-inoculated plants under well-watered conditions and inoculated plants under drought stress conditions. On the other hand, non-inoculated plants with AMF under drought stress conditions exhibited the lowest values of leaf phosphorus concentration. The largest quantity of lipid peroxidation, as shown by the malondialdehyde (MDA) assay, was under drought stress without inoculation. Under the AMF application, the quantity of MDA was reduced compared to the non-inoculation control (Figure 4F).

Thermal images and canopy temperature

There were differences in the canopy temperature between the studied treatments (Figure 5). Readings of canopy temperature were observed to be greater in the non-inoculated plants than in inoculated plants under different water treatments. In particular, non-inoculated plants exhibited higher canopy temperatures under drought stress conditions than inoculated plants. Notably, inoculation with AMF substantially impacted tomato canopy temperature compared to non-inoculated control plants under drought stress conditions.

Discussion

Glomus (G) species, the dominant arbuscular mycorrhizal fungi (AMF) in neutral to alkaline agriculturally significant soils, play a vital role in various ecosystems by enhancing plant growth and development through improved nutrient uptake (Fall et al., 2022). In this study, several G. species associated with tomato plants grown across different locations in the Ismailia Governorate, Egypt, were identified. Their physiological and morphological benefits were evaluated under drought stress conditions.

Likewise, several published reports have elucidated the presence and dominance of G. species in the crop rhizosphere (Bansal *et al.*, 2012; Jie *et al.*, 2013; Islam *et al.* 2022; Cheng *et al.* 2024). The present study assessed the beneficial impacts of five identified G. species on tomato plants, particularly focusing on their responses to drought stress.

Drought stress significantly reduced AMF colonization in tomato roots. This decline is attributed to inhibited spore germination, reduced hyphal growth, and limited hyphal distribution under water-deficient conditions (Bahadur *et al.*, 2019). Since most of the energy for AMF hyphal development is derived from plant photosynthesis, drought-induced reductions in photosynthetic activity can decrease carbohydrate availability to the roots, thereby affecting colonization (Mathur *et al.*, 2019).

In the current study, five AMF species were examined for their ability to enhance drought resistance in tomato plants. Inoculation with AMF significantly improved morphological and physiological parameters compared to non-inoculated controls under drought stress.

Drought conditions normally inhibit root system development, reduce shoot growth, and overall plant performance. However, AMF inoculation mitigated these negative effects, enhancing root biomass, root length, and root branching. In addition, inoculated plants showed better shoot growth performance under drought conditions.

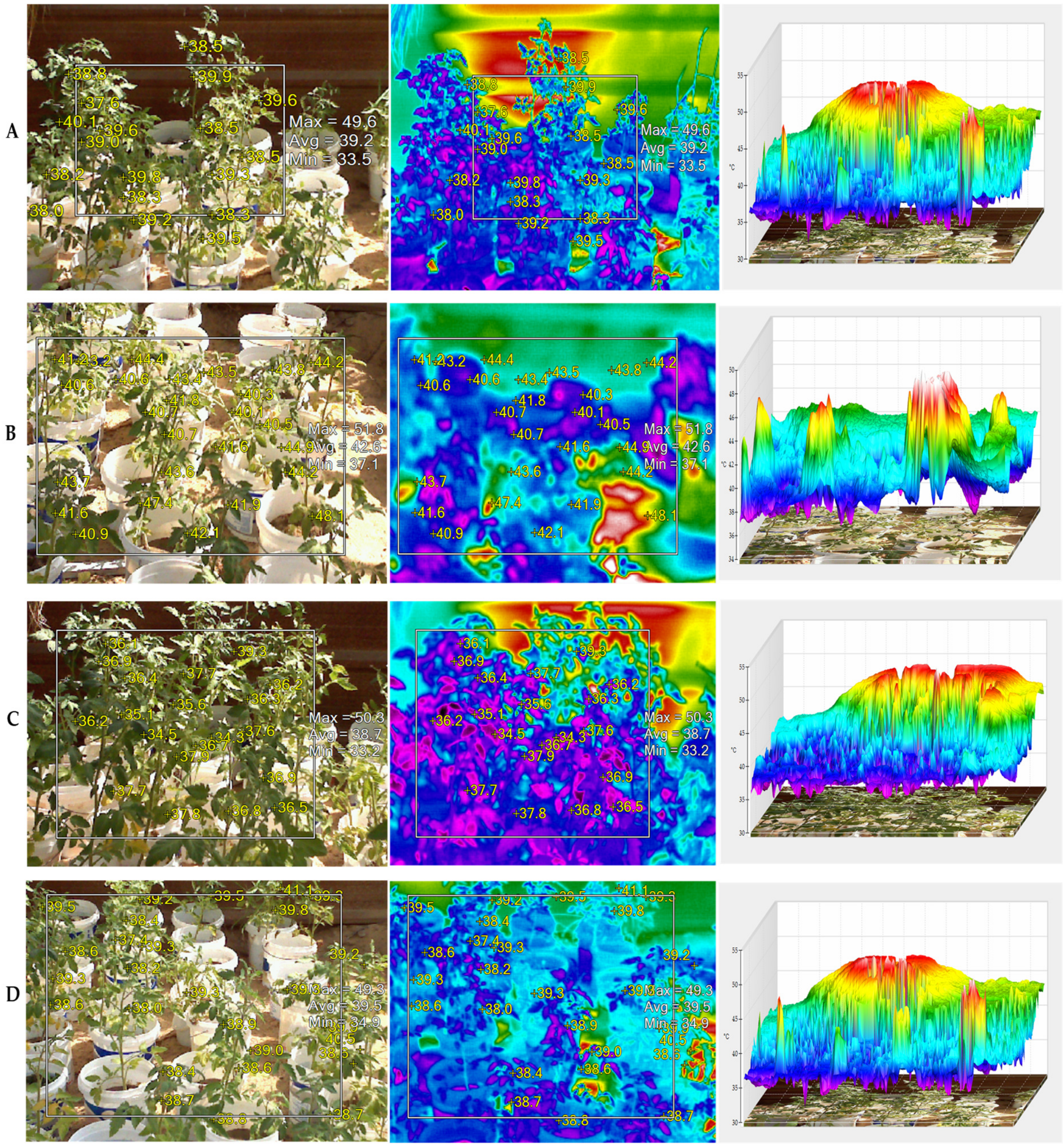

Figure 5. Thermal images and canopy temperature of non-inoculated tomato plants under well-watered conditions (A), non-inoculated plants under drought conditions (B), inoculated plants under well-watered conditions (C), and inoculated plants under drought conditions (D)

Photosynthetic pigments are crucial indicators of plant health under abiotic stress (Desoky *et al.*, 2021; Morsi *et al.*, 2023). In this study, drought stress led to a significant reduction in chlorophyll concentrations in leaf tissue. Conversely, AMF-inoculated plants under drought stress exhibited significantly higher chlorophyll levels, likely contributing to improved photosynthetic efficiency (Vafadar *et al.,* 2014). Improving the expression of photosynthetic pigments is essential for sustaining plant growth and productivity under environmental stress (Ozturk *et al.*, 2021; Mansour *et al.*, 2021).

Under drought conditions, plants undergo osmotic adjustments involving solutes like proline, sugars, proteins, and glycine (Desoky et al., 2020; Ozturk *et al.,* 2021). AMF symbiosis enhances the plant's ability to perform these adjustments, as inoculated plants accumulated higher concentrations of these osmolytes (Zou *et al.,* 2021). Increased leaf proline content under water deficit suggests that proline contributes to osmotic regulation during drought (Desoky *et al.,* 2023). Higher proline levels in AMF-inoculated plants may thus correlate with greater drought tolerance. Abdelmoneim *et al.* (2014) reported a similar trend, with significantly higher proline concentrations in inoculated plants compared to non-inoculated ones.

Similarly, drought conditions led to increased accumulation of total soluble sugars in tomato plants. AMF application further enhanced glycine betaine content under stress. Soluble sugars also serve as signaling molecules that activate regulatory pathways and influence growth and the translocation of photosynthetic products during abiotic stress (Khan *et al*., 2019). Abdelmoneim *et al.* (2014) also observed that AMF significantly enhanced concentrations of sucrose, fructose, and glucose in trifoliate orange, and glucose in *Ephedra foliate* under drought conditions.

Glycine betaine helps protect cell membranes from drought-induced damage and contributes to osmotic balance (Mansour *et al.,* 2023). These findings suggest that AMF mitigate water stress by improving the accumulation of key osmolytes, including proline and glycine betaine.

Malondialdehyde (MDA) is a marker of lipid peroxidation and cellular damage. In the present study, AMF-inoculated tomato plants exhibited significantly lower MDA levels compared to non-inoculated controls. These findings are consistent with earlier reports indicating that AMF colonization helps reduce MDA concentration (Beltrano and Ronco, 2008). Lower MDA levels reflect less damage to cell membranes, suggesting that AMF effectively protect plants against oxidative stress and contribute to enhanced drought tolerance.

While the results clearly demonstrate the potential of AMF inoculation to enhance drought tolerance in tomato plants, some practical limitations hinder widespread application. One major challenge is the cost of AMF inoculants, which may be prohibitive for small-scale farmers, particularly in developing regions. Scalability is another concern, as effective AMF use depends on factors such as inoculant availability, compatibility with local soil conditions, and technical knowledge for proper implementation.

Although AMF show great promise for improving crop resilience under abiotic stress, further research is essential to optimize inoculation protocols, lower costs, and ensure their practical viability in diverse agricultural systems. Addressing these challenges will be key to unlocking the full potential of AMF in sustainable agriculture

Conclusions

Water shortage displayed devastating effects on all evaluated morphological and physiological parameters. However, the inoculation with AMF significantly alleviated the destructive impacts of water stress by enhancing the concentration of chlorophyll, proline, MDA, glycine betaine, total soluble sugars, and leaf phosphorus of tomato. The positive impacts of inoculation with AMF were reflected in the shoot and root fresh and dry weights of inoculated tomato plants were significantly higher than those of non-inoculated controls under drought stress conditions. Additionally, canopy temperature was greater in the non-inoculated plants than in inoculated plants under different drought stress. As a result, the inoculation with AMF significantly positively influenced tomato plants compared to non-inoculated control plants under drought stress conditions.

Authors' Contributions

Conceptualization A.F.S, K.M.A. N.K and O.M.G; methodology, A.F.S, N.K and O.M.G; software, A.F.S, K.M.A and O.M.G.; validation, A.F.S, K.M.A, N.K and O.M.G.; investigation, A.F.S, K.M.A. N.K and O.M.G; resources, A.F.S, K.M.A, N.K and O.M.G.; data curation, A.F.S, K.M.A, N.K and O.M.G.; writing—original draft preparation, A.F.S, K.M.A, and O.M.G. and T.M.A.; writing—review and editing, A.F.S, K.M.A. N.K and O.M.

All authors have read and agreed to the published version of the manuscript.

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Availability of Data and Materials

The data are available from the corresponding author upon reasonable request

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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