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Mycorrhizal inoculation under water stress conditions and its influence on the benefit of host microbe symbiosis of *Terminalia arjuna* species

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Abstract

Background Entisol is a very poor, compact, and low-water-holding capacity soil. They are obstacles to the plant's root system's penetration and the availability of water, particularly in dry months. However, Arbuscular mycorrhizae fungi (AMF) is used for seedling growth and reduces water stress in the plant.

Results In this experiment, the growth parameters and the physiological activities of the plant were changed for the well watering (WW), fractionated watering (FW), and stopped/no watering conditions of the *T. arjuna*seedling. This experiment demonstrated higher mycorrhizal dependency (24.90%) under the FW condition than that of the WW condition (18.58%). Also the root colonization was higher (67%) under FW plants compared to WW plants (53%) associated with AMF+ in *T. arjuna* seedling. Photosynthesis was found 24.27% more with FW than the WW condition. Experiment' shows posivitivecorrelation between the photosynthesis and interval of no watering for AMF− plants (r2 = 0.873 for AMF− (control) and comparatively very weak for plants with AMF+ (r2 = 0.259 for AMF+ plants).

Conclusions The findings confirms the use of AMF in entisol soil to improve plant growth and biomass by reducing edaphic stress.

Keywords Forest species, Entisol soil, Mycorrhizae, Water stress, Physiology

Background

Forest conservation refers to preserving a forest's natural resources that benefit both humans and the environment. Forests are essential to human life because they provide a variety of resources. They store carbon and act as carbon sinks; they also produce oxygen, essential for life on Earth, so they are appropriately referred to as "earth lungs" (Olivero et al. 2016). They help regulate the hydrological cycle and the global climate by purifying water, providing habitat for wildlife, reducing global warming, absorbing toxic gases and noise, reducing pollution, conserving soil, and mitigating ecosystem (Watson et al. 2018). At present, forest cover is changing rapidly due to a variety of factors, including the expansion of agriculture, timber plantations, other land uses such as pulp and paper plantations, urbanization, road and industry construction, fire, and drought, all of which together pose the greatest and most serious threat to the forest, resulting in a significant reduction in forest cover (Kumar et al. 2022). *Terminalia arjuna (Roxb.)* is a member of the Combretaceae family, commonly known as Arjuna. This large, evergreen tree can be found in the Sub-Himalayan tract, Chota Nagpur, Orissa, West Bengal, Punjab, Deccan, and Konkan, along rivers

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throughout the Indian Peninsula (Soni and Singh 2019). It can be grown in many soil types, but it prefers fertile alluvial loam and deep, sandy, well-drained soil (Ramesh and Palaniappan 2023). Entisol is a broad category of soils that all have one thing in common the development of narrow horizons. Entisol soils found in areas where a dry or cold climate (Sumono Loka and Nasution 2018).

Water shortage is one of the main problems of entisol soil. It inhibits the percolation of rainwater due to its high compactness, bulk density, coarse texture, and preponderance of large volumes of gravel. This results in poor survival of most forest species and has stunted growth. It is thought that most plants benefit from mycorrhizal symbiosis under water stress conditions by improving water status and uptake by increasing the absorptive capacity (Omirou et al. 2013; Wu et al. 2013; Boyer et al. 2015; Darro et al. 2022). The host's physiology changed after association with AMF (Begum et al. 2019), as the fungus demand somewhat 10–20% of the photosynthesis compound from the host plant to maintain the symbiosis relationship (Al-Hmoud and Al-Momany 2017). As a result, competition for photosynthetic energy between fungus and host is the primary factor responsible for positive or negative impacts on plant growth (Bhardwaj et al. 2023). Mycorrhizae improve plant growth photosynthesis rate in mycorrhized plants in nutrient balance (Jixian et al. 2017). Mycorrhizal fungus associated plants also control transpiration rates, enabling drought resistance in plants compared to nonmycorrhizal fusngus associated plants (Caitlyn et al. 2023; Chandra and Kumar 2023).

At the same time, AMF infection in roots evolves numerous mechanisms in the host plant, viz. photosynthesis and water absorption due to osmotic potential alleviation during stress conditions (Rashidi et al. 2021). Root hydraulic conductivity, leaf gas exchange and expansion, Phyto-hormone regulation, and leaf conductance were affected by interaction with arbuscularmycorrhiza (Nasaruddin 2018). Fungal mycelium is involved in water transport, especially at low soil potential, which forms colonization in arid and tropical landscapes and coal mine soil (Vieira et al. 2020; Deepika and Kothamasi 2021). Plants colonized by AMF can improve their mineral nutrition, water supply, and tolerance to various environmental stresses (Joseph et al. 2022). Furthermore, AM symbiosis has been shown to improve plant performance under drought stress and alter plant-water relations in well-watered and drought-stressed conditions (Kevin et al. 2022). AMF induced tolerance to water deficits has been linked to several mechanisms in AM plants, including improved stomatal conductance (Kemmelmeier et al. 2022).

Methods

The experiment was setting to find out the effect of AMF on plant growth and physiology. The study conducted several experiments in the nursery of the Forestry Department of GGV Bilaspur, Chhattisgarh, India. The entisol soil was brought from the bhataland of Chakarbhatha for polypot experiments to Guru GhasidasVishwavidyalaya, Bilaspur, Chhattisgarh, India. The implementation of the study began in October 2016 and ended in January 2017. The seeds of *T. arjuna* collected from the Bilaspur provenance were used. The experiment consisted were RBD (Random Block Design) of 7 treatments with 10 replication. Seeds of these species were directly sown in polythene bags with 2.5 kg of sterilized entisol soil without any amendments at 15 psi/h for 45 min twice to ensure the absence of pre-existing mycorrhizal spores. On October 4th, seeds were sown, and after 1 month of sowing, singling was done and maintained only one seedling in each polythene bag. At the same time, 20 g of mixed inoculum of AMF was placed in the root zone of the seedling by making holes around the plant, followed by refilling with sterilizedentisol soil. In this experiment, they were used in two treatments, i.e., AMF inoculation and an un-inoculated control. The mixed inoculation consisted of *F. mosseae*, *R. intraradices,* and *A. Scrobiculata* were added equally to 20 g of inoculum, consisting of infected roots, spores, and soil substrate and containing approximately 1000 spores in each seedling of both the tree species. Control seedlings also received the same amount of sterilized inoculum to equalize the similar quantity of substrate as control plants.

In addition, water stress was studied by using two levels of irrigation: well-watered [WW] and fractionated water [FW] (regulated deficit irrigation—water stressed). Thus, four treatments covered the two experimental factors, including un-inoculated control.Total of 160 seedlings were tested for AMF+ and AMF− treatments, with both WW and FW conditions included.

When the plants were sufficiently established, and AMF was inoculated at the age of 40 days, fractionated water (FW) was applied to half of the plants while the other half were watered daily. Before the drought treatment began, all the pots got the same treatment and were watered once a day. Two of the irrigation lines were randomly assigned to the WW treatment (2 l/d water/plant, receiving 100% of the water estimated to have been lost through evapotranspiration) and the FW treatment (2 l/plant at two day intervals, receiving 60% of the water of the WW treated plants). Watering was scheduled as per treatment needs between 9 and 11 a.m.

A third experiment was also conducted as a consequence of the WW experiment to increase the drought plants' stress when experiments A and B were not showing significant signs of drought stress. Similar procedures were followed in this experiment as in experiment A [WW], inoculated AMF, regular watering of 2 l/day/plant, maintained for 120 days, but in experiment C, there was no watering (no watering) until the plants died. After stopping watering, daily observations on photosynthesis, transpiration, and other physiological parameters, as well as soil moisture, were recorded for five regular days on which plant leaves reached 100% senescence.

After 120 days of plant age and 90 days of AMF inoculation, five plants were randomly selected from each treatment, one from each replicate, and the substrate was carefully washed from the root mass. Before harvesting plants, physiological observations were taken, and the same plants were used for other measurements related to growth and biomass. Fifth leaf from the top to the bottom of each plant was selected for the measurements of photosynthesis variables. On a sunny day, gas exchange parameters, including net photosynthetic rate, stomatal conductance, intercellular CO_2 concentration, transpiration rate, and leaf temperature, were determined from 9 to 11 a.m. Entire data recorded during the experiment were statistically analyzed by the using of SPSS 20.0 software.

The moisture of the soil was analyzed by moisture meter model GB-2283656**.** All physiological attributes were recorded using the apparatus LC Pro$^+$ photosynthetic analyzer by ADC Bio-scientific Limited, Serial No. 32480.

Results

Experiment A: well water condition (WW)

Effect on plant growth and biomass

In this experiment (WW), 2 L of water were manually irrigated to each seedling of the treatments for 120 days, including AMF+ and AMF− seedlings, and plant experimental data were collected after this age. The experimental results are summarized in Tables 1 and 2 and reveal that the inoculation of AMF was found beneficial for all the attributes of plant growth and biomass, significantly ($P<0.05$) except for root length, which showed a non-significant difference between treatments. The seedling height of the AMF+ plant was found to be 37.24 ± 1.24 cm, which was 23% higher than the height rendered by the seedling under AMF−. For this attribute, the F value was calculated at 13.89, indicative of a treatment difference at $P<0.05$. (Table 1). The collar diameter of the AMF− exhibited a 46% lower value (4.10 mm/plant) compared to the AMF+ seedling (6.02 mm/plant). Root length was found to be 3.39 cm higher in inoculated seedlings (32.34 cm/plant) than control seedlings; however, it was statistically non-significant ($P<0.184$) (Table 1). However, fresh root weight rendered significant results due to AMF+ under the WW condition. As far as total fresh weight was concerned, it was 41% higher in plants inoculated with AMF+ than in AMF− seedlings. Dry weight was also taken into account, which also proved to be significantly higher in AMF+ in comparison to un-inoculated plants. The result shows that the total dry weight of the AMF− seedling was 6.62 g/plant,

Table 1 Growth and biomass of *T. arjuna* inoculated with AMF under well water condition [WW]

Treatments	Height (cm)	Diameter (mm)	Root length (cm)	Fresh weight (g/pl)		Total fresh weight (g/pl)
				Shoot	Root	
AMF−	30.23 ± 1.42^{bc}	4.10 ± 0.21^{bc}	28.95 ± 1.48^{a}	8.19 ± 0.75^{ab}	5.26 ± 0.56^{ab}	13.44 ± 0.95^{b}
AMF+	37.24 ± 1.24^{a}	6.02 ± 0.35^{a}	32.34 ± 1.80^{a}	11.69 ± 1.10^{a}	7.33 ± 0.70^{a}	19.02 ± 0.71^{a}
F value	13.891	21.805	2.112	6.945	5.315	21.66
Significant level	0.006	0.002	0.184	0.030	0.050	0.002

Data represent ± indicates standard error while data of same column with same letter indicates non-significant difference ($P<0.05$)

Table 2 Dry biomass of *T. arjuna*inoculated with AMF under well water [WW] conditions

Treatments	Dry weight (g/pl)		Total dry weight (g/pl)	Mycorrhizal dependency (%)
	Shoot	Root		
AMF−	3.78 ± 0.14^{b}	2.84 ± 0.24^{b}	6.62 ± 0.31^{b}	–
AMF+	4.91 ± 0.39^{a}	2.93 ± 0.15^{a}	7.85 ± 0.53^{a}	18.58
F value	13.656	14.896	18.464	18.453
Significant level	0.006	0.005	0.003	0.003

Data represent ± indicates standard error while data of same column with same letter indicates non-significant difference ($P<0.05$)

which increased to 7.85 g/plant with AMF inoculation, with an 18.58% net improvement in the total dry weight of the plant with the presence of AMF in the rhizosphere of the seedling (Table 2). A significant difference between treated and untreated plants was observed for dry shootand root dry weights. Thus, in this experiment, plant growth and biomass showed a positive impact of AMF inoculation, which was significantly different from the value of un-inoculated plants. AMF colonization in the roots of inoculated plants was 53%, and there was no colonization in AMF− plants under well water conditions (Fig. 1F). Mycorrhizal dependency was calculated at 18.58%, which also proved the positive contribution of AMF on the growth and development of *T. arjuna* under seedling age.

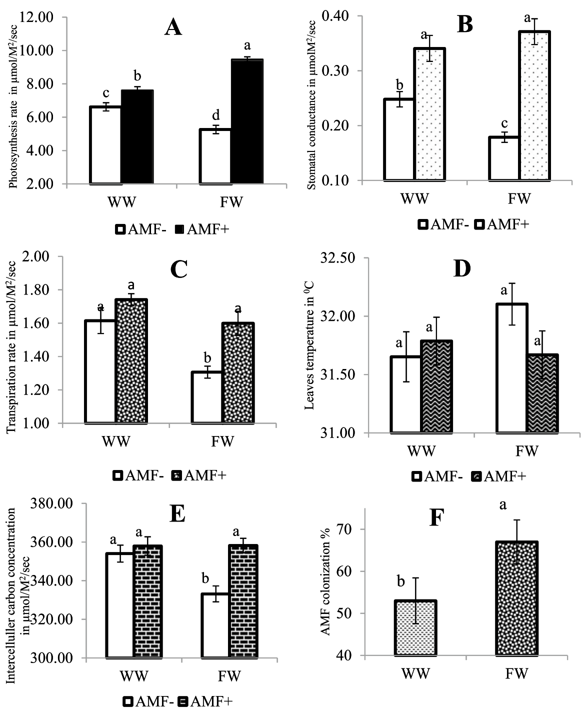

Fig. 1 Effect of AMF application on **A** photosynthesis rate, **B** stomatal conductance, **C** assimilation rate, **D** leaf temperature and **E** inter cellular carbon concentration **F** AMF root colonization well watering (WW) and fractionated watering (FW) condition. Figure showed mean ± Standard error, diagram bar in figures indicated with same letter does not show significant difference at $P \leq 0.5$ levels

Effect on plant physiology

The impact of AMF inoculation under WW conditions was also evaluated on physiological parameters of the plant, and the results are given in Fig. 1. The photosynthesis rate of the AMF+ inoculated *T. arjuna* seedling recorded 7.62 µmol/meter^2/s), which was 15% higher than the photosynthesis rate of the AMF− seedling (6.62 µmol/meter^2/s) (Significance at $P<0.05$) (Fig. 1A). Similarly, the AMF+ plant had a significantly higher rate of stomatal conductance (36% higher) than the AMF− plant. However, the transpiration rate, leaf temperature, and intercellular CO_2 gave non-significant results at $P<0.05$, though 8% and 0.44% higher transpiration and leaf temperature were recorded under AMF+ seedlings than under AMF− (Fig. 1C, D). Intercellular CO_2 concentration (ci) showed a lower and non-significant value in the treatment factor of AMF+ plants at $P<0.05$ (Fig. 1E).

Experiment B: fractionated water condition (FW)

Effect on plant growth and biomass

In this experiment, FW plants received 2 L of water at an interval of 2 days to determine the combined effects of reduced watering and AMF on plant growth, biomass, and physiology. The data results are statistically analyzed and summarized in Tables 3 and 4. In contrast of A experiment, in B experiment, the growth and plant biomass was decreased with the fractionated watering. However, the treatment difference showed significantly positive results of AMF inoculation for plant height, diameter, total fresh, and total dry weight of the plant at $P<0.05$. The plant height increased by 21% with AMF+ compared to AMF− as the plant height in AMF+ plants was 32.56 cm, and it was 26.84 cm/plant with AMF− (significance level $P<0.05$) (Table 3). Collar diameter increased by 34% with fractionated watering, followed by AMF+, while root length showed no significant difference between AMF+ and AMF− treatments. The treatment effects could not be found effective for root length,

Table 3 Growth parameters and fresh weight of *T. arjuna* inoculated with AMF under fractionated water condition [FW]

Treatments	Height (cm)	Diameter (mm)	Root length (cm)	Fresh weight (g/pl)		Total fresh weight (g/pl)
				Shoot	Root	
AMF−	26.84 ± 1.97^c	3.67 ± 0.42^c	30.35 ± 0.71^a	6.82 ± 0.76^{bc}	4.59 ± 0.33^b	11.40 ± 0.94^b
AMF+	32.56 ± 0.81^b	4.95 ± 0.19^b	30.22 ± 0.45^a	10.34 ± 1.40^c	6.79 ± 1.01^a	17.13 ± 1.02^a
F value	7.25	7.776	0.023	4.291	4.290	16.97
Significant level	0.027	0.024	0.884	0.057	0.072	0.003

Data represent ± indicates standard error while data of same column with same letter indicates non-significant difference ($P<0.05$)

Table 4 Dry biomass of *T. arjuna* inoculated with AMF− and AMF+ under fractionated water [FW] conditions

Treatments	Dry weight (g/pl)		Total dry weight (g/pl)	Mycorrhizal dependency (%)
	Shoot	Root		
AMF−	2.85 ± 0.21[b]	1.60 ± 0.18[b]	4.45 ± 0.13[b]	–
AMF+	3.69 ± 0.31[b]	1.87 ± 0.23[b]	5.56 ± 0.47[b]	24.90 ± 10.55[b]
F value	4.958	0.831	5.170	5.170
Significant level	0.057	0.389	0.053	0.053

Data represent ± indicates standard error while data of same column with same letter indicates non-significant difference ($P < 0.05$)

as in inoculated plants, the root length was 30.22 cm, which was 0.13 mm/plant less than plants of AMF− (Table 3). Total fresh weight in the AMF+ plant was rendered at 17.13 g/plant compared to the AMF− plant (11.40 g/plant), which showed a significant difference at $P < 0.05$. Similar to the previous experiment, there was an almost 50% increment in fresh weight in AMF+ seedlings compared to AMF− seedlings (Table 3). Shoot and root dry weight, and total dry weight of AMF + seedlings and control seedlings under FW condition showed a non-significant difference at $P < 0.05$, but AMF+ plants had higher values of all these parameters than un-inoculated plants (Table 4). AMF colonization in roots of inoculated plants was 67%, and there was no colonization in AMF− plants under fractionated water conditions (Fig. 1F). Under fractionated water conditions, the plant's mycorrhizal dependency for biomass production was 24.90%. It proves the contribution of AMF to the biomass production of *T. arjuna.*

Effect on plant physiology

The physiological attributes of *T. arjuna* seedlings under AMF and FW conditions were significantly improved compared to the un-inoculated control plant without any inoculation at $P < 0.05$. In contrast to experiment A, the impact of AMF on photosynthesis and other attributes was more apparent, and thus the contribution of AMF under FW was more prominent than the WW condition. Control seedlings showed decreased photosynthesis rate, stomatal conductance, transpiration, and intercellular CO_2 content under FW conditions compared to WW conditions. The results of FW in *T. arjuna* indicate that the net improvement of 80% photosynthesis with AMF+ compared to AMF− was significantly higher ($P < 0.001$) (Fig. 1A). The photosynthesis rate recorded in AMF+ plants was 9.47 μmol/m^2/s while it was 5.26 μmol/m^2/s in AMF− plants (Fig. 1A). Similarly, stomatal conductance was increased by 105.5%, and intercellular CO_2 was also increased by 18.78% due to AMF inoculation, which was lacking in plants without AMF (Fig. 1B).

It was also found that transpiration rate is adjusted by the plant at moisture stress conditions such as FW, as a lower rate of transpiration was reported in this experiment compared to experiment A. The transpiration rate of the AMF+ seedling was higher than that of the AMF− seedling, which was significant at $P < 0.001$ (Fig. 1C). It probably indicates that for high photosynthesis of plants, higher transpiration is required, and AMF meets the enhanced requirement for water for transpiration. Leaf surface temperature was also lower in AMF+ plants than in AMF− plants, but it was not significant at $P < 0.05$ (Fig. 1D). The AMF+ plant's intercellular CO_2 concentration was also non-significantly higher than that of the AMF− plants (Fig. 1E).

It is clear from the above two experiments that AMF dependency increases (24.90%) in *T. arjuna* under FW conditions than under WW conditions (18.58%), which indicates the importance of AMF, especially when the plant suffers due to water stress. When plants receive enough water to meet their needs, they grow faster and form a poor to moderate level of symbiosis. When nutrients or water are deficient, the plant grows slowly, and a strong symbiosis is formed to meet the plant's needs. Similarly, physiological parameters were also found to have a better impact on AMF under FW than WW conditions, regardless of treatment factors.

Experiment C: no watering condition

In the WW condition of the present study, plants did not exhibit any stress as the water was provided daily, while in the FW condition, watering was done at a 2 day interval for all the *T. arjuna* plants experimented on to assess the impact of AMF+ and AMF− on plant growth and physiology. As a result, the results of AMF− inoculated plants were significantly higher than those of AMF− control plants, but most of the parameters showed comparatively lower growth than the WW experimented plants. The results of both experiments were enough to prove the role of AMF in plant growth and physiological responses in entisol soil. However, the variation in results demands more evidence. Therefore, experiment C was conducted to prove the effect of AMF inoculation on physiological activities under chronic stress of water by not watering the plants. It was observed that the plant showed wilting

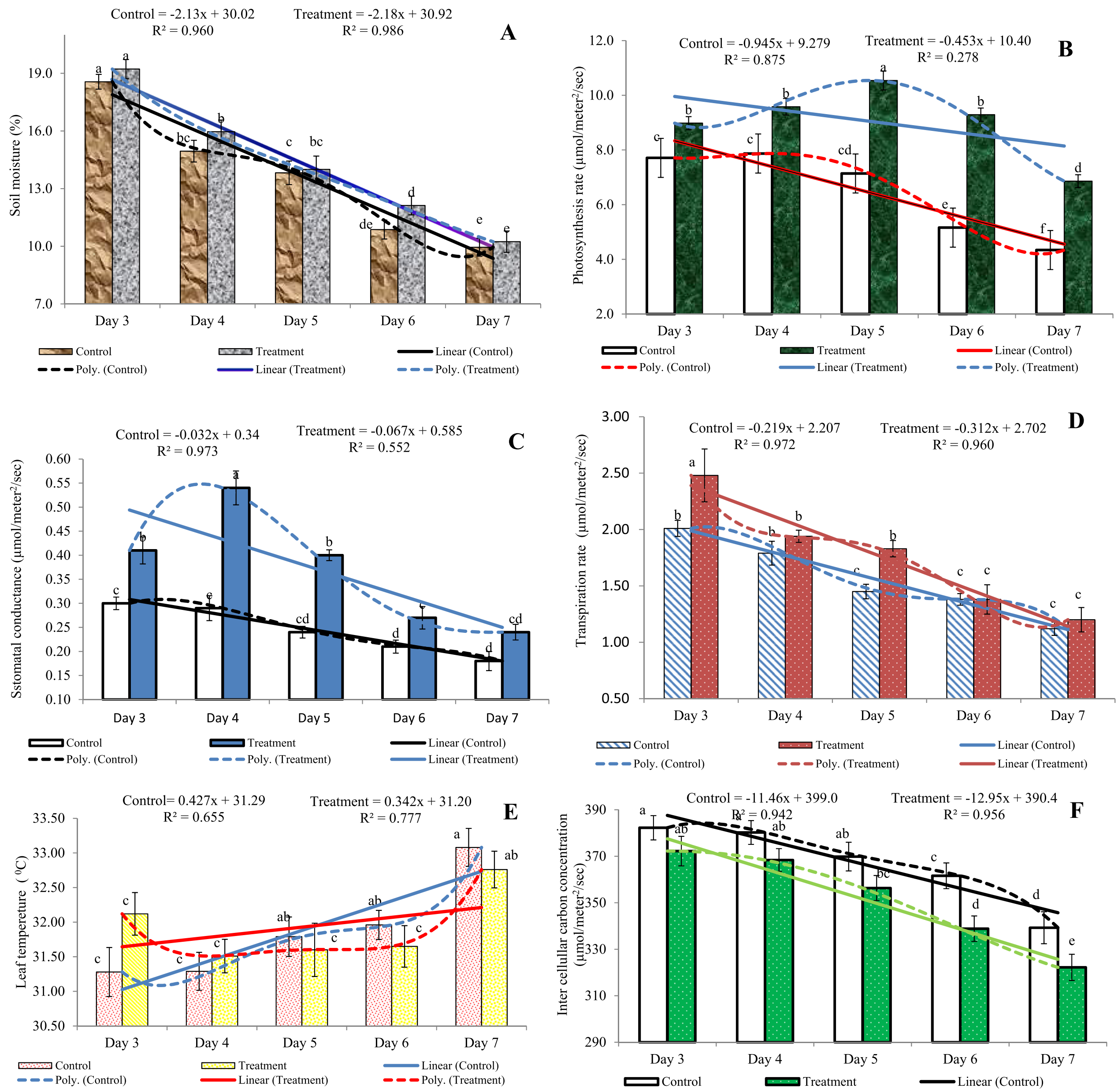

Fig. 2 Effect of AMF application on **A** soil moisture %, **B** photosynthesis rate, **C** stomatal conductance, **D** transpiration rate, **E** leaf temperature and **F** inter cellular carbon concentration with no watering. The observations were taken from 3rd to 7th days of no watering when seedling expressed wilting on leaves. Data showed mean ± SE. Diagram bar with same letter shows non-significant difference between each other as per DMR test at $P < 0.5$ levels. Straight line indicates regression curve and dotted line shows polynomial trend line

symptoms just after 3 days of watering, which continued till the 7th day, and then the plant showed complete senescence (leafless) and died due to the shortage and stress of water. Observations were recorded after 3 days of not watering the plant till the death, i.e., 7 days after watering. The results of experiment C are represented in Fig. 2.=

Moisture content of soil (%)

No watering plants showed temporary wilting on the third day between 12 and 3 p.m. (evening), and afterward, plants exhibited permanent wilting due to moisture loss due to soil moisture stress. Data from the results reveal that the soil moisture after 3 days of no watering was recorded at 19.22% in AMF+ soil, which was

3.5% higher than in AMF− soil (18.56% soil moisture) (Fig. 2A). Finally, soil moisture decreased to 9.95% and 10.24% on the seventh day of no watering in AMF− and AMF+ soils, respectively, compared to their respective initial values on the third day of no watering. At this stage of soil moisture, plants become leafless and show permanent wilting. Overall, moisture loss was 86.53% in plants without AMF− and 87.69% with AMF+ in *T. arjuna* plants. It was found that soil moisture declined consistently and rapidly after no watering in AMF+ and AMF− conditions, with a non-significant difference at $P<0.05$. However, DMR values indicated differences due to treatment factors but rendered significant results compared with soil moisture with an increasing number of days of no watering to plants at significant level < 0.001 (Fig. 2A). Soil moisture fluctuated, resulting in inconsistent trends of decline in AMF+ and AMF− inoculated plants grown in entisol soil under nursery conditions. There was a significant positive relationship between days without watering and soil moisture percent for AMF+ and AMF− treatments (r2 = 0.960 and r2 = 0.986 for AMF− and AMF+ treated plants, respectively, Fig. 2A).

Photosynthesis

It is one of the most important parameters determining the food synthesis in plants and was found to have significantly positive effects of AMF inoculation (Fig. 2B), as there was higher photosynthesis in AMF+ plants than AMF−. On the fourth day of no water to the plants, it measured 8.98 μmol/m^2/s for AMF+ rather than 7.71 μmol/m^2/s in AMF− plants. The photosynthesis rate maintained an increasing trend for up to 4 days in AMF− plants while it consistently increased for up to fifth days in AMF+ plants, which depicts the alleviated rate of photosynthesis due to AMF symbiosis under drought and water stress conditions. Not with standing, it was observed that in AMF+ plants, the decline in photosynthesis rate was prolonged (29.02%) with the increasing days of no watering, while it was exponential (77.64% decline) in non-inoculated plants. 5 days after watering, photosynthesis was recorded at 7.14 μmol/m^2/sec in AMF− plants while it was 10.54 μmol/m^2/s in the same days with AMF+, clearly indicating the increased rate of photosynthesis due to AMF+. The observation made on the seventh day of no watering indicates an abysmal rate of photosynthesis in AMF− plants (4.34 μmol/m^2/s/plant) due to prominent symptoms of water stress on the leaves of AMF− plants, but AMF+ plants exhibited a photosynthesis rate of 6.96 μmol/m^2/s/plant with increased resistance against drought and stress symptoms were also poorly observed on plants. The difference in photosynthesis rate due to increasing days of no watering and the effects of treatment factor both gave significant results at $P<0.05$. Experimental data shows that AMF plants benefited in two ways: one through maintaining the increasing trend of photosynthesis and, secondarily, a prolonged decrease in photosynthesis rate with increasing days of no watering compared to AMF− plants. The positive correlations r2 = 0.875 and r2 = 0.278 for AMF− and AMF+ plants, respectively, clearly show that the decline in photosynthesis rate was greater in AMF− plants than in AMF+ plants.

Stomatal conductance

The results are presented in Fig. 2C, which reveals that AMF significantly increases stomatal conductance compared to plants without having the benefits of AMF in all the observations recorded from the 3rd to seventh days of no watering. Stomatal conductance was measured at 0.41 mol/m^2/s in the AMF+ plant and 0.30 mol/m^2/s in the AMF− plant after the third day of no watering. This attribute declined continuously till the last observation, i.e., seventh day of no watering in both AMF+ and AMF− plants, and reached the minimum level of 0.24 and 0.18 μmol/m^2/s in AMF+ and AMF− plants, respectively. Exceptionally, stomatal conductance in plants with AMF+ showed increasing trends from observations recorded on the third day. The rate of decline in stomatal conductance in plants was 60–70% for *T. arjuna* AMF− and AMF+ plants. In contrast to photosynthesis, this attribute declined faster in mycorrhized plants than in non-mycorrhizal ones. The treatment effects found at 36.60% and 33.30% on the third and seventh days of no watering indicated the consistent positive contribution of AM on stomatal conductance of plants, especially under water stress conditions. According to Fig. 2C, AMF+ plants alleviate stomatal conductance better than AMF− plants, as the rate of this attribute was significantly lower in AMF− plants (r2 = 0.973) than in AMF+ plants (r2 = 0.552).

Transpiration

Inoculation of AMF was found to alleviate the transpiration rate compared to non-inoculated plants of *T. arjuna*, but the results were non-significant in all the observations except the 5th day of observation after no watering to the plants (Fig. 2D). The rate of transpiration at the time of 3-day observation was 2.48.0 and 2.0 μmol/m^2/s in plants with and without AMF, respectively, which rendered almost 23.40% higher results due to AMF+. Similarly, in the last observation, i.e., 7 days of no watering, the transpiration rate was 1.20 and 1.12 μmol/m^2/s for AMF+ and AMF− plants, respectively, with a difference of 12.5%. AMF+ plants consistently showed a higher rate of transpiration than those without AMF, but the decline noticed in this was attributed to increasing days of no watering in both AMF+ and AMF− plants. It was also noticed that with the increase in drought and stress, AMF+ plants also experienced adversities. The effectiveness of AMF on this attribute decreased compared to the observation recorded on the 3rd day of no watering. The DMR test showed a significant decline in the transpiration rate with the increasing number of days of no watering to plants, which was also clearly indicated by the regression equation analysis in Fig. 2D that the transpiration rate declined continuously and significantly as the number of no watering days increased ($r2 = 0.972$ and $r2 = 0.960$ for AMF− and AMF+ plants).

Leaf temperature

The surface temperature of the leaf plays a determining role in plants' physiology, and this attribute usually increases under drought and water stress conditions, which results in poor photosynthesis and transpiration compared to a well-watered plant. The results of the present investigation are summarised and represented in Fig. 2E, which reveals that the leaf temperature fluctuated inconsistently, resulting in a non-significant difference between treatment and nontreatment at $P < 0.05$. Leaf temperatures of AMF− plants ranged from 31.78 to 33.58 °C, while it was between 31.62 and 33.26 °C in AMF+ plants. Data shows that leaf temperature decreased in plants with AMF+ compared to AMF− plants in all the observations recorded during 3–7 days of no watering. AMF+ had a leaf temperature of 0.16 °C lower on the third day and 0.32 °C lower on the 7th day after no watering conditions compared to AMF− plant. The increasing stress of water increased the leaf temperature continuously in AMF− plants, but the rate of increment was somewhat slower in AMF+ than in AMF− plants. Leaf temperature was found to have a significant positive relationship with increasing days of no watering in both AMF− and AMF+ plants, with r2 values of 0.655 and 0.777, respectively.

Internal cellular CO_2 concentration

This attribute showed decreasing trends in both the plants of the experiments, i.e., AMF+ and AMF−, and the treatment difference was found non-significant at $P < 0.05$ (Fig. 2F), but the DMR test indicates a significant difference with the decrease in leaf CO_2 with increasing days of no watering. The internal CO_2 of the leaf was found to be 372.22 and 382.25 μmol/m^2/s with AMF+ and AMF− respectively, which is indicative of the lowering of CO_2 in leaves during stress time to the presence of AMF+. This trend was observed to decline consistently with the increasing days of no watering. The rates of decreases in the internal level of leaf CO_2 in plants with AMF+ were 2.69% and 5.27% on the third and seventh days of observation, respectively, after no watering. Thus, it can be established that AMF+ exhibited lowering effects on leaf CO_2 during water stress, which were more pronounced (15.52%) with an increase in days of no watering compared to the AMF− plant (12.69%). When no watering days were increased, plants' attributes showed a significantly positive relationship ($r2 = 0.942$ and $r2 = 0.956$, respectively, for AMF− and AMF+ plants).

Discussion

Water serves as a raw material for various physiological processes in plants, including maintaining cell turgidity for structure and growth, transporting nutrients throughout the plant, and serving as a raw material for photosynthesis and transpiration (Brendel 2021). Its deficiency impacts plant growth, development, and physiological activities (Luvaha et al. 2008). Species have drought avoidance mechanisms and the ability to acclimate to moisture stress conditions through active osmoregulation by maintaining metabolic activity under suboptimal conditions during establishment when roots have not reached deep soil water (Takahashi et al. 2020). Plants with AM fungi aid in developing growth and biomass, particularly in drought and moisture-stressed conditions (Smith et al. 2010; Zhu et al. 2012). Entisol soil is known for its water stress and lack of nutrients, resulting in a significant reduction in plant survival, growth, development, physiological activities, and plantation failure. The effects of water stress and its influence on the growth and physiological activities of *T. arjuna* were investigated in this study to see if AMF could mitigate the impact of water stress on normal plant growth and physiological function under WW and FW conditions. The results showed that under WW and FW conditions, *T. arjuna* seedlings formed symbiotic relationships with AMF, resulting in significant improvements in plant growth and biomass. It was also discovered that AMF colonization was 26% higher in *T. arjuna* under FW conditions than WW conditions, demonstrating the strong symbiotic relationship between plants and bacteria when underwater stress. The impact of adversities caused by FW condition on plant characteristics, such as height, fresh weight, and dry weight, was visible in the tree species, and the stress was effectively mitigated by higher root colonization in plant roots grown in the presence of AMF+ compared to AMF− plants under FW condition. It was confirmed that plants in *T. arjuna* were more mycorrhizal dependent in FW conditions than in WW conditions. In the species used in the experiment, most of the parameters related to plant growth and biomass showed higher values in the presence of AMF+ under WW conditions. It was due to AMF's important role in plant growth, which allowed for more efficient water and nutrient absorption through the hyphal network (Shi et al. 2016; Guo et al. 2020). Wu and Xia 2006; Fidelibus et al. 2001; Dell'Amico et al. 2002; Wu et al. 2008; Asrar and Elhindi 2011; Shi et al. 2016) all confirmed the findings of this study. Furthermore, as Shi et al. (2016) reported that increased biomass by mycorrhization could greatly increase the absorption surface and thus nutrient uptake capacity under FW conditions.

Under WW and FW conditions, the impact of the AM fungus on physiological activities was investigated. When compared to the WW condition, the FW condition decreased photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration while increasing leaf temperature in *T. arjuna* plants without AMF−. Mycorrhizal plants had significantly higher photosynthesis rate, stomatal conductance, and non-significant but higher transpiration and intercellular CO_2 concentration than AMF plants, while leaf temperature was lower regardless of water treatments. The positive effects of AMF in improving physiological activities in any condition (Fig. 1) demonstrate that AMF has a positive effect in any situation. *T. arjuna*plants treated with FW increased their photosynthetic rate by 24.27% compared to WW plants treated with AMF+, while AMF+ plants treated with FW increased their stomatal conductance by 8.82% compared to WW plants treated with AMF+. This effect of AMF+ plants was due to a higher degree of symbiosis in plant roots under FW conditions compared to WW conditions, allowing plants to absorb water optimally as needed for normal physiological functioning even under water stress conditions like FW. Auge 2021 also suggested that the rate of gas exchange is essential for plant growth because mycorrhizal plants have a higher rate of photosynthesis than non-mycorrhizal plants, implying that AMF colonization increases the number of photosynthesis units and the rates of photosynthetic storage and export. Zhu 2012 made similar observations in *Zea mays* under drought stress conditions, reporting that AM symbiosis could enhance photosynthesis and increase transpiration fluxes, implying that AMF+ plants could keep their stomata open longer than AMF− plants (Khan et al. 2022). Our findings show that the efficiency of AMF increases with increasing levels of water stress, as evidenced by WW and FW conditions, which is also supported by (Porcel and Ruiz-Lozano 2004), who found that AMF symbiosis can improve the water status of the host plant, resulting in higher leaf water potential under drought conditions when compared to non-mycorrhizal plants.

Drought stress [FW] increased leaf temperature in AMF− plants significantly, whereas the same attribute decreased with AMF inoculation in all species except *T. arjuna* under WW conditions, which could be due to high leaf water potential FW conditions due to AM symbiosis, as [36], have suggested. External hyphal extraction of soil water, stomatal regulation through hormonal signals (Aroca et al. 2008), more significant osmotic adjustment (Wu and Xia 2006), and higher hydraulic conductivity (Caitlyn et al. 2023) may all contribute to mycorrhizal plants' better water status. Furthermore, (Campo et al. 2020) demonstrated that plants accumulate a high concentration of low molecular mass organic solutes such as soluble sugars, proline, and other amino acids to regulate cell osmotic potential, aiming to improve water absorption under drought stress by maintaining a favorable gradient for water entry into the roots (Abbaspour et al. 2012). Previous studies (Wu and Xia 2004; Shi et al. 2016; Begum et al. 2019) have found that in the presence of AMF plants, photosynthesis and stomatal conductance are higher than in non-AMF plants under water stress. Stopping/no watering in *T. arjuna* plants grown in entisol soil under nursery conditions was investigated to confirm further the benefits of AMF on the regulation of physiological characteristics under acute water shortage, and the results are presented graphically (Fig. 2). High water stress was created by not watering the plants, and physiological activities were recorded in both AMF+ and AMF− plants from 3 days of no watering until senescence, i.e., the eighth day of no watering. Wilting symptoms appeared in AMF− plants after only 3 days of no watering, but this stage appeared in AMF+ plants after the fifth day of no watering. It is a sign that mycorrhized plants' survived higher due to improved water use efficiency and drought resistance in AMF+ plants. Longer stomatal openings and higher photosynthesis rates of the plant may be due to hyphae that penetrate pores inaccessible to roots beyond the root zone (Barzana et al. 2012) and the effectiveness of AMF for better exploitation of bound water in dry conditions, and AMF can sometimes provide soil water below the plant's permanent wilting point (Smith and Read 2008).

In the current experiment, no watering significantly reduced soil moisture percent in both plants, regardless of the AMF treatment factor. However, when plants were inoculated with AMF+, soil moisture was significantly increased ($P < 0.05$) compared to control plants. Soil moisture decreased gradually as the number of days without watering increased, and between 3 and 8 days without water, more than 85% of soil moisture was lost. It showed a strong positive correlation for both species, which resulted in plant wilting regardless of the treatment effect (Fig. 2A).

Similarly, Wu et al. (2015) have reported the importance of AMF on soil aggregate stability and high moisture content due to binding through an extensive hyphal network and release of glomalin to the soil, both of which play a crucial role in soil moisture conservation, especially under moisture stress conditions exploited more efficiently by AMF+ plants than non-AMF− plants. In contrast to AMF− plants, which showed significantly decreasing trends in photosynthesis and stomatal conductance, no watering in AMF+ plants found to enhance the rate of photosynthetic and stomatal conductance results in consistently increasing trends even on the fifth and fourth days of no watering, respectively, for these two attributes. Nonetheless, the strong positive correlation between photosynthesis and days without watering for AMF− plants (r2 = 0.873 and 0.875 for AMF− (control) and a comparatively weak relationship in AMF+ plants (r2 = 0.259 and 0.278 for AMF+ plants) confirms the critical role of AMF during stress time in maintaining and regulating lifesaving activities, such as photosynthesis rate. Similarly, stomatal conductance found similar trends in AMF+ and AMF− plants, which agrees with other researchers' findings (Zhu et al. 2012; Shi et al.2016; Wang et al. 2016). However, after 5 days of no watering, AMF+ plants could not maintain the same rate of photosynthesis and began to decline gradually, whereas control plants' rate was exponential. Shi et al. (2016) observed similar results under AMF association, owing to higher leaf chlorophyll, carotenoid, and photosynthesis, allowing more excellent C fixation and carbohydrate accumulation. AMF, according to Gavito et al. (2019), promotes plant sunlight capture and thus better photosynthetic production. Our findings are also in line with Bhardwaj et al. (2023). AMF's faster water transportation resulted in higher stomatal conductance (Talaat and Shawky 2014; Kemmelmeier et al. 2022). In contrast to the characteristics as mentioned above, the transpiration rate of AMF+ plants was higher than that of AMF− plants, but it decreased steadily as the number of days without water increased. The entire experiment demonstrates AMF's beneficial role in reducing the adverse effects of water stress, lowering the transpiration rate in AMF+ plants. When AMF+ plants created a conducive environment for essential physiological activities during acute water stress conditions, leaf temperature was lower in AMF+ plants than in non-AM plants in the current study. However, AMF was unable to lower the leaf's surface temperature after that, resulting in a consistent increase in leaf temperature as the number of days without watering increased in both AMF+ and AMF− plants.

This could result from a combination of AMF symbiosis and high water use efficiency of plants by maintaining enhanced water potential in plants and leaves (Sharma et al. 2021). They showed that AMF+ plants could withstand drought stress by accumulating large amounts of organic solutes and sugars to control cell osmotic potential. This result demonstrates that AMF+ plants have better water stress management skills than non-AMF plants, resulting in a higher survival rate, drought resistance, and delayed senescence even after no watering. Under WW and FW conditions, intercellular CO_2 concentrations varied with AMF treatment but decreased with increasing days without watering in *T. arjuna* species regardless of treatment factor. AMF− treated plants, on the other hand, were found to have a lower rate of CO_2 concentration than AMF− plants on the same day of no watering. Regardless of water and salt stress conditions (Zhu et al. 2012; Bhardwaj et al. 2023), and even in rocky areas, these results were consistent with maize and alfalfa (Chen et al. 2014). The influence of symbiosis on the CO_2 dynamics of the host leaf may also be related to AMF promotion of stomatal conductance, as 20% of the assimilations contributed to maintaining symbiosis and bidirectional benefit movement to both partners (He et al. 2017; Chandrasekaran et al. 2019). They also stated that, as in the current study, faster CO_2 movement from the leaf results in lower CO_2 in the leaf in AMF plants than in non-AM plants. Overall, mycorrhizal fungi regulate water relations with their nonmycorrhizal counterparts and influence plant growth (Caitlyn et al. 2023; Saboor et al. 2021). Higher stomatal conductance and transpiration have been reported in mycorrhizal situations (Mena-Violante et al. 2006). More efficient water exploration by mycorrhizal fungi could lead to more extreme wet/dry cycles, which could have significant implications for carbon fixation during water stress, especially important for plant growth in arid environments.

Conclusions

Water is limited in entisol soil, and its scarcity influences plant development, resulting in a high degree of AMF interaction with the plant to develop a mechanism for efficient utilization of water. The plant growth attributes and physiological activities have been evaluated under WW (well watering), FW (fractionated watering), and SW (stopped/no watering) conditions, and the result shows higher mycorrhizal dependency (24.90%) under FW condition than that of WW condition (18.58%) in *T. arjuna*respectively. AMF root colonization was also higher (67%) under FW plants than WW plants (53%) treated with AMF+ in *T. Arjuna*. These indicate the importance of AMF, especially when plants suffer water stress. *T. arjuna's* photosynthetic rate increased by 24.27% under FW conditions compared to WW conditions.AMF inoculation was found to alter the physiological activities of the plant. It showed a significantly higher photosynthesis rate, stomatal conductance, and non-significant but higher transpiration and intercellular CO_2 concentration while leaf temperature was lowered regardless of WW and FW conditions, which indicates the positive effect of AM in ameliorating physiological activities. The linear correlation between photosynthesis and days without watering showed a very strong relationship in AMF− plants ($r2=0.873$ for AMF− (control) and a comparatively weak relationship in AMF+ plants ($r2=0.259$ for AMF+ plants), confirming the importance of AMF during stress time in maintaining and regulating lifesaving activities. The study confirms the benefits of AMF in entisol soil for ameliorating the adverse edaphic conditions and improving the growth and biomass of plants. As AMF is scanty in entisol, an apparent result is not obtained, but once the status of AMF is manipulated, the positive response is ascertained.

Abbreviations

AMF	Arbuscular mycorrhiza fungi
WW	Well watering
FW	Fractionated watering
SW	Stopped/no watering

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