# Some physio-biochemical traits of sunflower (*Helianthus annuus* L.) as affected by arbuscular mycorrhizal fungi inoculation under different irrigation treatments

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## Highlights

- *Water deficit negatively affected physio-biochemical traits of sunflower cultivars.*
- *Sunflower cultivars positively responded to arbuscular mycorrhizal fungi inoculation.*
- *The positive effects due to arbuscular mycorrhizal fungi inoculation were more evident under water deficit than well-watered condition.*
- *The improvements caused by arbuscular mycorrhizal fungi were significantly higher under mild than severe water deficit.*

## Abstract

Plant-arbuscular mycorrhizal (AM) fungi association is one of the oldest symbiotic relationships between organisms. This relationship may be more important under stress conditions such as drought and can help the host plant tolerate drought. This study was conducted in 2016 and 2017 at the Agricultural Research Farm of Razi University, Kermanshah, Iran to evaluate the effect of AM fungi (AMF) inoculation (with either *Funneliformis mosseae* or *Rhizophagus intraradices*) on some physio-biochemical traits of three sunflower cultivars under different soil irrigation treatments (severe water deficit stress, mild water deficit stress and well-watered). In both years, water deficit conditions significantly reduced leaf relative water content (RWC), chlorophyll concentrations (a, b and total) and shoot phosphorus concentration (SPC) while simultaneously increasing shoot proline levels and malondialdehyde (MDA) concentrations. AMF inoculation had positive effects on RWC, chlorophyll concentrations and SPC irrespective of sunflower cultivar and irrigation treatment. Shoot proline concentration and MDA reduced more in AM than non-AM plants. In most cases *F. mosseae* performed better than *R. intraradices* in terms of plant performance. Moreover, the improvements caused by AM fungi were more evident under water deficit than well-watered condition. It may be concluded that AM inoculation can alleviate the negative effects of water deficit stress on some important physio-biochemical traits of sunflower grown in the field, and can be considered as a practical and economical approach to improve crop performance in environments exposed to water limitations.

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## Introduction

Sunflower is one of the most important oilseed crops that is widely planted in many areas of the world to supply edible oil and other by-products. It is usually grown during spring and summer when the crop is often subjected to water deficit stress due to a lack of precipitation and high temperatures. Drought is the most serious abiotic stress in many regions of the world and can substantially reduce crop yield and economic return. In recent years, excessive use of ground and surface waters by farmers, as well as a lack of sufficient and regular precipitation due to climate change have posed severe constraints to produce food crops in drylands. Therefore, it is critical to find suitable and practical approaches to increase the efficiency of water use by crops and reduce the adverse effects of drought on their processes and performance. It has been shown that some microorganisms such as arbuscular mycorrhiza (AM) can promote the ability of plants to obtain water from the soil and then enhance tolerance against drought. This is achieved through many morphological, physiological and biochemical modifications of both host plant induced by AM fungi (AMF) and the effect of the AMF on the soil traits. Based on a meta-analysis of 460

studies conducted by Augé *et al*. (2015), AM plants showed 24% higher stomatal conductance compared to non-AM plants. They observed that in comparison with well-watered conditions, AMF inoculation increased stomatal conductance more than two and four times under moderate and severe drought stress, respectively. Liu *et al.* (2016) found that plants inoculated by *Funneliformis mosseae* showed higher root length, diameter, volume and surface area. This can lead to a higher volume of soil explored by roots and consequently greater water uptake by plants (Comas *et al*., 2013).

Generally, AM hyphae can extract soil water that is inaccessible for plant roots. Moreover, AM fungi may influence synthesis and transportation of auxin, thereby positively affecting root hair growth (Liu *et al*., 2018). Increasing cell to cell water transport and root hydraulic conductivity were also reported due to AMF inoculation (Marjanović *et al*., 2005; Bárzana *et al*., 2012). Rahimi *et al*. (2017) found positive effects of AM fungi on photosynthetic pigments and leaf relative water content of borage under drought stress. Zhu *et al*. (2012) observed that AMF inoculation improved relative water content and water use efficiency of corn under water deficit conditions. They concluded that the promotion of photosynthesis and plant water status due to AMF inoculation can mitigate the undesirable effects of drought on corn plants. The promotion of photosynthesis under drought stress in AMF plants is linked with higher chlorophyll concentrations. According to Wu and Xia (2006), plants inoculated with AM showed a higher photosynthetic rate under drought stress than non-inoculated ones. As reported by Zhu *et al*. (2012), chlorophyll concentration of the plants subjected to water deficit stress was enhanced in response to AMF inoculation. Other studies also revealed that chlorophyll concentrations (a, b and total) of the plants affected by drought stress showed higher values when plants were inoculated by AMF (Pal and Pandey, 2016; Rani, 2016). Evelin *et al*. (2009) demonstrated that the adverse effects of drought stress on chlorophyll synthesis were alleviated in mycorrhizal associated plants.

There is a strong link between water status and proline level in plant species. Increasing plant proline concentration as an osmotic adjustment response under water deficit condition has been shown in different studies (Trotel-Aziz *et al*., 2000; Porcel and Ruiz-Lozano, 2004; Szabados and Savoure, 2009; Hazzoumi *et al*., 2015). Accumulation of proline as an important osmolyte has a key role in plant osmotic adjustment under water deficit condition which consequently leads to higher water uptake by plant (Szabados and Savoure, 2009). Higher leaf water potential of soybean plants inoculated by AM under water deficit condition was observed by Porcel and Ruiz-Lozano (2004). However, there are different reports of the AM effects on proline accumulation in host plants: some have suggested an increase (Hu *et al*., 1992; Roosens *et al*., 1998; Ruiz-Sanchez *et al*., 2011) while others found a decreasing effect of AM on plant proline concentration (Wu *et al*., 2013b; Zhang *et al*., 2014; Hazzoumi *et al*., 2015; Wu *et al*., 2017).

Osmotic adjustment due to proline regulation in AMF plants can reduce cell peroxidative damage under drought stress. One of the most important damages caused by drought stress in plants is lipid peroxidation. It is expressed by malondialdehyde (MDA) level as an indicator of peroxidative damage resulting from drought stress (Uzilday *et al.*, 2012). Some studies have shown lower MDA levels in AMF plants under water deficit stress conditions (Porcel and Ruiz-Lozano, 2004; Zhu *et al*. 2011; Li *et al*., 2019) indicating less peroxidative damage and lower membrane degradation in these plants compared to non-AM plants.

Plant nutrient uptake can be influenced by AMF inoculation. Phosphorous is one of the most essential elements needed for plant growth and development. Many studies have shown that AM symbiosis can increase P uptake and accumulation in different host plants (Smith and Read, 1997). According to Wu and Zou (2009) the beneficial effects of AM on plant nutrient uptake were more evident under water deficit than non-stress conditions. Wu *et al*. (2011) also suggested that improved nutrient uptake, especially P due to mycorrhization is a key physiological mechanism that enhances host plant tolerance under water deficit stress conditions. As previously mentioned by Safir *et al.* (1972) enhanced water uptake by the host plant can be a consequence of increased P concentration in the plant tissues due to mycorrhization.

Despite the wide range of reports related to the AM effects on physio-biochemical responses of plants under water deficit condition, there are still uncertainties and sometimes controversial results in this regard. Moreover, we only found a few reports on the AMF inoculation effects on physio-biochemical responses of sunflower to drought stress especially under actual field conditions. Therefore, the present field-based study was conducted to evaluate the physio-biochemical responses of three sunflower cultivars inoculated with two AM species (either *Funneliformis mosseae* or *Rhizophagus intraradices, compared to an uninoculated treatment*) under different irrigation treatments.

## Materials and Methods

A two-year experiment was conducted in 2016 and 2017 at the Agricultural Research Farm of Razi University, Kermanshah, Iran (34°21′30″N, 47°06′15″E; elevation 1319 asl, with an average high temperature of 22.3°C and low temperature of 5.6°C and annual mean precipitation of 478 mm). In each year, the experiment was carried out as split-split plot based on a randomized complete block design with three replications. The main factor was irrigation treatments on the basis of the soil moisture depletion in the root zone [irrigation at 80% available soil moisture depletion (severe stress), irrigation at 60% available soil moisture depletion (mild stress) and irrigation at 40% available soil moisture depletion (no stress)], the sub-factor was sunflower cultivar (Farokh, Hisan and Barzegar) and sub-sub factor was arbuscular mycorrhiza fungi (AMF) (with *Funneliformis mosseae*, *Rhizophagus intraradices* and uninoculated treatment). The soil type was a silty clay with a pH of 7.6 and 1.1% organic carbon. Soil P level was 12.4 mg $kg^{-1}$, which is less than the threshold for optimal plant growth (Li *et al.*, 2011). The land was plowed, disked and harrowed before planting. Weeds were removed by hand as needed and irrigation levels were carried out as explained above. Irrigation treatments were adjusted based on the soil water content at the field capacity. The amount of soil water in the experimental field at the field capacity point was determined by sampling soil from different parts of the field, so that soil samples were poured into pots and saturated with water. The pots were then placed on lattice surfaces for 48 hours to remove gravitational water. The pots were then weighed and placed in an oven at 150°C for 24 hours and their dry weights were measured. The percentage of the soil moisture at the field capacity (FC) point was calculated using the following equation:

$$FC = \frac{FCW - DW}{DW} \times 100$$

where, FCW is the soil sample weight at the field capacity point and DW is the dry weight of the soil sample.

Irrigation at the time of depletion 40% of the available soil moisture was considered as the no stress treatment. This was determined

based on the information obtained from the previous experiments on sunflower. During the growing season, irrigation time in different treatments was determined based on the regular sampling (every two days) and measuring the percentage of soil moisture content.

The inoculum was a commercial product prepared from Organically Plant Protection Center, Asadabad, Hamedan province, Iran. It consisted of a substrate with mycorrhized roots mixed with spores. The number of propagules was 120 spores per 1 gr of the inoculum. Sunflower seeds were planted manually. Before planting, holes of 5 cm deep and 25 cm apart were created by a shovel, then in each hole 20 grams of the inoculum along with sunflower seeds were placed. In the uninoculated treatment no inoculation was carried out. Sunflower was planted on 10 May 2016 and 2017. Each sub-subplot consisted of five sunflower rows of 5 m length, with a row spacing of 75 cm and with 25 cm between plants in the same row. Sunflower physio-biochemical traits were evaluated at the early flowering stage. This stage of measurement was chosen since it is the most sensitive to drought stress during the crop cycle of sunflower (Reddy *et al*., 2003; Göksoy *et al*., 2004; García-López *et al*., 2014; Buriro *et al*., 2015). To assess root colonization, sunflower fine living roots were separated from the soil sampled in each plot, washed with tap water and stained using the method described by Phillips and Hayman (1970). Then the percentage of mycorrhizal colonization was estimated according to McGonigle *et al*. (1990). Leaf relative water content (RWC) was determined in the fully expanded uppermost leaf of sunflower plants using the following equation (Ritchie *et al*., 1990):

$$RWC = \frac{FW-DW}{TW-DW} \times 100$$

where, FW is leaf fresh weight, DW is leaf dry weight (oven-dried at 70°C for 24 hours) and TW is leaf turgid weight (after immersion in distilled water for 24 hours).

Leaf chlorophyll concentrations including chlorophyll a (chl a), chlorophyll b (chl b) and chlorophyll total (chl T) were measured in the green and fully expanded leaves of sunflower plants according to the method proposed by Arnon (1967). A known amount of sunflower leaf tissue (500 mg) was suspended in 20 mL of 80% acetone, mixed well and kept at 4°C overnight in dark. Supernatant was withdrawn after centrifugation (6000 rmp) for 10 min and absorbance was recorded at 663 and 645 nm in spectrophotometer (Model: Varian Cary Bio 300 UV-VIS, Australia) as below:

Chl a = (19.3×A663-0.86×A645) V/100W
Chl b = (19.3×A645-3.6×A663) V/100W
Chl T = Chlorophyll a+Chlorophyll b

Shoot (stem and leaves) phosphorus concentration (SPC) was determined by the Vanadate-Molybdate yellow method (Chapman and Pratt 1961). To evaluate lipid peroxidation induced by drought, the MDA concentration of fresh sunflower leaves was determined using the method described by Heath and Packer (1968) via thiobarbituric acid reaction. The absorbance was read at 532 and 600 nm in above described spectrophotometer.

Leaf proline concentration was measured according to the method proposed by Bates *et al.* (1973). The toluene was used as a blank and the absorbance at 520 nm was read by above described spectrophotometer. At maturity, a 2-m length of the two central rows of each plot was harvested by hand. Sunflower achenes were separated and allowed to dry at 80°C to constant weight and were then threshed, cleaned and weighed. The seed yield in kilograms per hectare was determined.

Data were collected and analyzed using analysis of variance (ANOVA) in SAS software (SAS Institute 2008). According to the Bartllet test, there was a variance heterogeneity between the two years of the experiment, therefore the data of each year were analyzed and the results were reported, separately. Means were compared by least significant difference (LSD) test at the 0.05 level of probability.

## Results

According to ANOVA (Tables 1 and 2), all of the evaluated traits were significantly affected by experimental factors in both years (2016 and 2017). Moreover, there were significant three-way interactions (irrigation treatment×cultivar×mycorrhiza) for all of the traits under study in both years (Tables 1 and 2). Almost, all the evaluated traits showed similar trends in their responses, but different intensities to the experimental treatments. In both years, sun-

**Table 1. Analysis of variance (mean squares) of the traits under study in the first year of the experiment (2016).**

| Source of variance | Mean square | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Relative water content | Chlorophyll a | Chlorophyll b | Chlorophyll total | Shoot P concentration | Proline | Malondialdehyde | Seed yield |
| Replication | 1820.20* | 0.0015 ns | 0.0004 ns | 0.0033 ns | 0.00002 ns | 0.02 ns | 6622.09 ns | 13076.10 ns |
| Irrigation treatment (a) | 20603863.60** | 98.50** | 24.62** | 221.62** | 0.22** | 1181.89** | 4858148.79** | 38568429.30** |
| Error a | 224.80 | 0.0014 | 0.0003 | 0.0031 | 0.000009 | 0.008 | 6113.16 | 6059.50 |
| Cultivar (b) | 40376731.90** | 55.99** | 14.00** | 125.98** | 0.00003 ns | 2609.84** | 30951.94** | 8975298.20** |
| a×b | 20976302.30** | 23.48** | 5.87** | 52.84** | 0.0002** | 253.54** | 3935.46 | 3902706.90** |
| Error b | 2881.10 | 0.0032 | 0.0008 | 0.007 | 0.00001 | 0.007 | 6814.45 | 22265.90 |
| Mycorrhiza (c) | 18220905.40** | 22.33** | 5.58** | 50.24** | 0.16** | 166.69** | 676234.16** | 434702.90** |
| a×c | 41362773.50** | 0.89** | 0.22** | 2.01** | 0.001** | 41.00** | 378176.01** | 47132702.60** |
| b×c | 15337377.30** | 0.11** | 0.03** | 0.25** | 0.0002** | 7.53** | 11782.72 ns | 14163227.80** |
| a×b×c | 18797341.30** | 0.20** | 0.05** | 0.45** | 0.0002** | 5.55** | 14646.79* | 10963234.70** |
| Error c | 3398.80 | 0.003 | 0.0006 | 0.006 | 0.00002 | 0.008 | 6649.72 | 37697.00 |
| CV (%) | 11.85 | 6.14 | 6.24 | 5.87 | 6.03 | 6.80 | 14.38 | 17.03 |

ns, non-significant; *significant at the 0.05 level of probability; **significant at the 0.01 level of probability; CV, coefficient of variation; MS, mean squares.

flower plants were successfully colonized by AMF. Root colonization percentage varied from about 34 to 71% and 33 to 73% in the first and the second year of the experiment, respectively. In general, sunflower roots showed higher colonization percentage under water deficit treatments (especially mild water deficit stress) than no stress condition.

## The first year

Leaf relative water content was reduced by 53.2% compared to well-watered conditions when plants were subjected to the severe water deficit stress, and the reductions were higher in non-mycorrhizal than mycorrhizal plants. In all three irrigation treatments, mycorrhization led to the notable improvements in RWCs, and in most cases *F. mosseae* performed better than *R. intraradices*. Relative water content was improved by 93.8 and 49.7% under mild and severe water deficit stress, respectively in the plants inoculated with *F. mosseae* than in non-AM plants. In general, the highest RWC was recorded when plants were inoculated with *F. mosseae* under mild water deficit stress (Table 3).

Chlorophyll concentrations (a, b and total) also showed notable reductions under both water deficit stress treatments, although the reductions were more evident under severe than mild stress. Generally, severe water deficit stress reduced chl a, chl b and chl T by 20, 36.8 and 25.4% respectively as compared with no stress treatment (Table 3). Among the sunflower cultivars, Hisan had higher chlorophyll values under all three irrigation treatments. Inoculation with AMF drastically improved chlorophyll concentrations under all irrigation treatments and the improvements were higher under water deficit than well-watered conditions (Table 3). *Funneliformis mosseae* showed more positive effects on leaf chlorophyll concentrations of sunflower plants, as the inoculation with this AMF species improved chl a, chl b and chl T concentrations by 15.8, 26.9 and 18% under mild and 10.3, 28.5 and 15.3% under severe stress conditions, respectively (Table 3). The highest values of the chlorophyll pigments were observed in cultivar Hisan in the presence of *F. mosseae* (Table 3).

Shoot phosphorus concentration (SPC) was also influenced by irrigation treatment and mycorrhizal inoculation ($P \leq 0.01$). Water deficit stress led to substantial reductions in SPC (an average reduction of 14 and 47.9% under mild and severe water deficit stress, respectively compared to the well-watered treatment) (Table 3). Mycorrhizal inoculation especially with *F. mosseae* significantly increased SPC under both water deficit stress treatments (an increase of 128.6 and 33.3% under mild and severe stress, respectively) (Table 3). In general, under all irrigation treatments, AMF showed positive effects on SPC, but the effects were more evident under no and mild water deficit stress conditions (Table 3). In other words, severe water deficit stress notably weakened the beneficial effect of AMF on SPC.

Severe water deficit stress considerably increased shoot proline concentration (on average, 98.7% compared to no stress condition). The highest proline value was recorded for Hisan under severe water deficit stress and in the absence of AMF (Table 3). However, under both water deficit conditions, mycorrhization especially with *F. mosseae* led to an essential reduction in shoot proline level (Table 3), and the reductions were 30.8 and 19.7% under mild and severe stress treatments, respectively in the plants inoculated with *F. mosseae* than non-AM plants (Table 3).

Water deficit conditions also increased MDA, a proxy for lipid peroxidation. Highest MDA values were observed under severe drought stress and the lowest ones were recorded in well-watered treatment (Table 3). Arbuscular mycorrhizal inoculation especially with *F. mosseae* notably decreased MDA concentration under all irrigation treatments (Table 3). This AMF species reduced MDA concentration by 29.7 and 50.5% under mild and severe water deficit stress, respectively, compared to the no inoculation treatment, indicating the ability of AMF to alleviate drought induced peroxidative damage in plants.

Seed yield was negatively influenced by water deficit stress, as the lowest seed yields were recorded under water deficit stress (Table 3). However, for all sunflower cultivars and under all three irrigation treatments AMF inoculation improved seed yields. Among the cultivars, Farokh showed better responses to AMF inoculation in terms of seed yield, as the highest seed yield in this year (6399 kg/ha) was observed when this cultivar was inoculated by *F. mosseae* under mild water deficit stress (Table 3).

## The second year

All of the evaluated plant parameters were also significantly influenced by the factors under study in the second year. Severe water deficit stress led to a considerable reduction in RWC (by 32.3%) compared to no stress treatment (Table 4). However, AMF

**Table 2. Analysis of variance (mean squares) of the traits under study in the second year of the experiment (2017).**

| Source of variance | Mean square | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Relative water content | Chlorophyll a | Chlorophyll b | Chlorophyll total | Shoot P concentration | Proline | Malondialdehyde | Seed yield |
| Replication | 1825.05* | 0.002 ns | 0.0005 ns | 0.0042 ns | 0.00004 ns | 0.17** | 6679.15 ns | 128.48 ns |
| Irrigation treatment (a) | 21657428.14** | 100.04** | **26.51** | 225.13** | 0.18** | 1204.54** | 4859453.43** | 4684948.28** |
| Error a | 238.72 | 0.0018 | 0.0004 | 0.0038 | 0.000007 | 0.009 | 6154.51 | 6076.00 |
| Cultivar (b) | 40375313.00** | 63.34** | **15.54** | 131.56** | 0.00006 ns | 2634.21** | 31103.84** | 8970387.84** |
| a×b | 21104175.93** | 25.14** | **6.15* | 54.32** | 0.0005** | 275.43** | 3985.21 ns | 39045184.26** |
| Error b | 3018.45 | 0.004 | 0.0008 | 0.006 | 0.00001 | 0.01 | 6920.66 | 22759.02 |
| Mycorrhiza (c) | 18251832.70** | 24.09** | **7.02** | 52.38** | 0.12** | 178.83** | 676316.31** | 435692.52** |
| a×c | 41371025.16** | 0.97** | **0.27** | 2.07** | 0.003** | 43.24** | 378255.33** | 11548600.21** |
| b×c | 15368510.10** | 0.14** | **0.05** | 0.49** | 0.0004** | 6.67** | 11825.42 ns | 13764311.56** |
| a×b×c | 18799413.77** | 0.26** | **0.08** | 0.53** | 0.003** | 6.77** | 14703.05* | 47096263.31** |
| Error c | 3407.26 | 0.004 | 0.0008 | 0.007 | 0.00004 | 0.012 | 6651.78 | 37929.18 |
| CV (%) | 17.85 | 6.35 | 5.78 | 6.18 | 7.24 | 8.15 | 10.22 | 20.40 |

ns, non-significant; *significant at the 0.05 level of probability; **significant at the 0.01 level of probability; CV, coefficient of variation; MS, mean squares.

inoculation especially under mild stress substantially improved leaf RWC. Relative water content was enhanced by 66.7 and 51.1% under mild and 38.4 and 44.4% under severe stress in the plants inoculated by *F. mosseae* and *R. intraradices*, respectively, as compared with non-inoculated ones (Table 4). Under all irrigation treatments and for all cultivars, non-mycorrhizal plants showed the lowest RWC values (Table 4).

Leaf chlorophyll concentrations were also adversely affected by severe water deficit stress. Chl a, chl b and chl T were reduced by 20.2, 27.9 and 22.6 %, respectively under this stress level when compared to no stress condition (Table 4). The highest chlorophyll concentrations were recorded for cultivar Farokh under mild stress and in the presence of *F. mosseae*. Overall for each cultivar, non-AMF plants showed the lowest chlorophyll concentrations under all irrigation treatments (Table 4). Under mild stress, chl a, chl b and chl T were improved by 13.3, 20.9 and 15.7% respectively in the plants inoculated with *F. mosseae.* However, the improvements induced by this AM species were 13, 33.7 and 18.7%, respectively under severe stress conditions. Under both water deficit treatments, *R. intraradices* showed less beneficial effects on leaf chlorophyll concentrations when compared with *F. mosseae* (Table 4).

Shoot phosphorus concentration was also notably decreased by water deficit stress and the reduction was more intense under severe than mild water deficit (52.2 and 16.4% under severe and mild water deficit stress, respectively compared to well-watered treatment) (Table 4). However, mycorrhization substantially improved SPC under both water deficit treatments. The improvement values were 177.8 and 140.7% under mild and 80 and 95% under severe stress condition, when plants were inoculated by *F. mosseae* and *R. intraradices*, respectively (Table 4). Overall, under all irrigation treatments SPC had significant higher values in mycorrhizal than non-mycorrhizal treatments. However, severe water deficit stress led to dramatic reductions in SPC (even in the presence of AM fungi) when compared with other irrigation treatments (Table 4).

Severe water deficit stress also showed an increasing effect on proline accumulation in sunflower shoot (129.7% compared to no stress treatment). The lowest shoot proline concentrations were measured under well-watered treatment. For all cultivars, AMF inoculation with *F. mosseae* or *R. intraradices* significantly decreased proline concentration irrespective of irrigation treatments (Table 4). The reductions in proline concentration of the plants inoculated with *F. mosseae* and *R. intraradices* were 49.8 and 16.6%, under mild and 8.3 and 12.2% under severe water deficit stress, respectively, compared with non-AM plants (Table 4).

Malondialdehyde responded positively to decreasing available water in the soil. The highest MDA levels were observed under severe stress conditions (an average increase of 293% compared to well-watered treatment). However, under this condition sunflower cultivars didn't show consistent responses to mycorrhizal inoculation in terms of MDA (Table 4). Mild water deficit stress had no significant effects on MDA as compared with no water deficit stress. Moreover, under mild and no water deficit stress MDA concentrations of the cultivars were not significantly affected by mycorrhization with the exception of Hisan which showed a notable

**Table 3. The traits of sunflower cultivars as influenced by irrigation treatment and arbuscular mycorrhiza fungi inoculation in the first year of the experiment (2016).**

| Irrigation treatment | Cultivar | AMF inoculation | RWC (%) | Chl a (mg/g FW) | Chl b (mg/g FW) | Chl T (mg/g FW) | SPC (mg/g DW) | Proline (mg/g FW) | MDA (nmol/g FW) | Seed yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| Severe water deficit stress | Farokh | *F. mossea* | 26.44$^{i}$ | 14.07$^{p}$ | 6.00$^{n}$ | 20.07$^{r}$ | 11.77$^{l}$ | 43.57$^{e}$ | 876.67$^{bc}$ | 3025.65l$^{m}$ |
| | | *R. intraradices* | 40.33$^{g}$ | 13.94$^{pq}$ | 5.77$^{o}$ | 19.71$^{s}$ | 12.39$^{kl}$ | 46.17$^{d}$ | 990.90$^{b}$ | 3302.15l$^{m}$ |
| | | Without AMF | 21.54$^{j}$ | 13.32$^{s}$ | 5.00$^{q}$ | 18.32$^{u}$ | 9.60$^{n}$ | 48.17$^{c}$ | 1476.67$^{a}$ | 1417.35$^{o}$ |
| | Hisan | *F. mossea* | 39.32$^{g}$ | 15.20$^{m}$ | 6.90$^{l}$ | 22.10$^{n}$ | 11.77$^{l}$ | 38.16$^{f}$ | 570.00$^{d}$ | 2768.35$^{n}$ |
| | | *R. intraradices* | 26.54$^{i}$ | 14.43$^{o}$ | 6.11$^{n}$ | 20.54$^{q}$ | 13.32$^{jk}$ | 49.23$^{b}$ | 971.67$^{b}$ | 2746.40$^{n}$ |
| | | Without AMF | 19.23$^{j}$ | 13.69$^{r}$ | 5.24$^{p}$ | 18.93$^{t}$ | 8.67$^{no}$ | 52.82$^{a}$ | 1513.33$^{a}$ | 2696.15$^{n}$ |
| | Baezegar | *F. mossea* | 25.39$^{i}$ | 14.92$^{n}$ | 6.45$^{m}$ | 21.37$^{p}$ | 13.94$^{j}$ | 20.55$^{n}$ | 775.00$^{c}$ | 3372.00$^{l}$ |
| | | *R. intraradices* | 24.13i | 14.03$^{p}$ | 6.01$^{n}$ | 21.04$^{r}$ | 12.39$^{kl}$ | 25.68$^{i}$ | 818.67$^{c}$ | 3379.01$^{l}$ |
| | | Without AMF | 20.12$^{j}$ | 13.05$^{t}$ | 4.82$^{q}$ | 17.87$^{v}$ | 9.29$^{n}$ | 26.41$^{h}$ | 1501.67$^{a}$ | 3221.85$^{m}$ |
| Mild water deficit stress | Farokh | *F. mossea* | 79.48$^{a}$ | 16.58$^{k}$ | 7.90$^{j}$ | 24.48$^{k}$ | 28.81$^{a}$ | 16.54$^{q}$ | 338.33$^{efgh}$ | 6399.05$^{a}$ |
| | | *R. intraradices* | 73.00$^{b}$ | 15.98$^{l}$ | 7.39$^{k}$ | 23.37$^{l}$ | 22.61$^{ef}$ | 23.53$^{kl}$ | 395.00$^{efg}$ | 4525.05$^{g}$ |
| | | Without AMF | 31.29$^{h}$ | 14.06$^{p}$ | 6.03$^{n}$ | 09/20$^{r}$ | 10.22$^{m}$ | 24.23$^{jk}$ | 469.00$^{ef}$ | 3892.80$^{j}$ |
| | Hisan | *F. mossea* | 67.49$^{c}$ | 22.18$^{a}$ | 14.68$^{a}$ | 36.86$^{a}$ | 23.54$^{de}$ | 16.11$^{q}$ | 235.00$^{h}$ | 5176.15$^{de}$ |
| | | *R. intraradices* | 61.30$^{d}$ | 21.13$^{b}$ | 13.56$^{b}$ | 34.69$^{b}$ | 21.37$^{f}$ | 26.76$^{h}$ | 315.00$^{fgh}$ | 5708.15$^{c}$ |
| | | Without AMF | 33.49$^{h}$ | 19.31$^{c}$ | 11.87$^{c}$ | 31.18$^{c}$ | 11.15$^{lm}$ | 28.10$^{g}$ | 400.00$^{ef}$ | 4423.05$^{g}$ |
| | Baezegar | *F. mossea* | 60.55$^{d}$ | 15.84$^{l}$ | 7.00$^{l}$ | 21.84$^{m}$ | 21.68$^{fg}$ | 20.91$^{n}$ | 318.67$^{fgh}$ | 4130.80$^{h}$ |
| | | *R. intraradices* | 68.34$^{c}$ | 15.02$^{n}$ | 6.51$^{m}$ | 21.53$^{o}$ | 19.82$^{g}$ | 23.91$^{kl}$ | 344.33$^{efgh}$ | 5309.35$^{d}$ |
| | | Without AMF | 42.31$^{g}$ | 13.80$^{q}$ | 5.40$^{p}$ | 19.20$^{t}$ | 10.84$^{m}$ | 25.07$^{ij}$ | 399.33$^{fgh}$ | 4095.35$^{hi}$ |
| No water deficit stress (well-watered) | Farokh | *F. mossea* | 74.45$^{b}$ | 18.10$^{f}$ | 10.05$^{f}$ | 28.15$^{f}$ | 20.13$^{g}$ | 18.00$^{p}$ | 268.00$^{fgh}$ | 5947.75$^{b}$ |
| | | *R. intraradices* | 67.00$^{c}$ | 17.32$^{i}$ | 8.76$^{n}$ | 26.08$^{i}$ | 24.47$^{d}$ | 17.60$^{pq}$ | 300.00$^{fgh}$ | 5118.55$^{e}$ |
| | | Without AMF | 48.00$^{f}$ | 16.66$^{k}$ | 7.88$^{j}$ | 24.54$^{k}$ | 16.42$^{hi}$ | 21.91$^{m}$ | 322.33$^{fgh}$ | 4774.15$^{f}$ |
| | Hisan | *F. mossea* | 60.06$^{d}$ | 19.06$^{d}$ | 11.00$^{d}$ | 30.06$^{d}$ | 27.26$^{bc}$ | 21.57$^{m}$ | 288.00$^{fgh}$ | 5349.20$^{d}$ |
| | | *R. intraradices* | 55.72$^{e}$ | 18.53$^{e}$ | 10.56$^{e}$ | 29.09$^{e}$ | 27.88$^{ab}$ | 18.39$^{p}$ | 262.00$^{gh}$ | 5957.60$^{b}$ |
| | | Without AMF | 48.19$^{f}$ | 17.68$^{h}$ | 9.06$^{g}$ | 26.74$^{h}$ | 17.04$^{h}$ | 23.67$^{kl}$ | 281.00f$^{gh}$ | 5164.65$^{de}$ |
| | Baezegar | *F. mossea* | 62.18$^{d}$ | 17.89$^{g}$ | 9.94$^{f}$ | 27.83$^{g}$ | 26.33$^{c}$ | 17.38$^{pq}$ | 265.67$^{gh}$ | 5208.55$^{de}$ |
| | | *R. intraradices* | 54.81$^{e}$ | 17.10$^{j}$ | 8.15$^{i}$ | 25.25$^{j}$ | 24.47$^{d}$ | 18.25$^{op}$ | 291.33$^{fgh}$ | 4986.55$^{e}$ |
| | | Without AMF | 49.28$^{f}$ | 16.03$^{l}$ | 7.30$^{k}$ | 23.33$^{l}$ | 14.25$^{ij}$ | 19.71$^{no}$ | 327.33$^{fgh}$ | 4144.00$^{h}$ |
| LSD (0.05) | | | 4.66 | 0.18 | 0.24 | 0.33 | 1.55 | 1.05 | 135.00 | 138.00 |

AMF, arbuscular mycorrhiza fungi; RWC, relative water content; chl a, Chlorophyll a; chl b, Chlorophyll b; chl T, Chlorophyll total; SPC, shoot P concentration; MDA, malondialdehyde; FW, leaf fresh weight; DW, leaf dry weight; LSD, least significant difference. Means with a letter in common can't be considered different at a P>0.05 according to a least significant difference.

higher MDA in the absence of AM fungi under mild stress condition (Table 4). In both years, sunflower plant traits were adversely affected by water deficit treatments (especially severe stress). While, inoculation with AMF could alleviate the water deficit stress adverse effects and *F. mosseae* performed relatively better than *R. intraradices*. In general, AMF showed higher positive effects under mild than severe stress condition. It seems, AMF efficiency and beneficial effects on plants are highly related to soil water status and may significantly be decreased under severe water deficiency.

In the second year, the lowest seed yields were also obtained from the plots under severe stress (Table 4). In the absence of AMF, higher yields were recorded under well-watered conditions. However, mycorrhization under mild water deficit stress could produce comparable or even higher seed yields when compared with the no stress condition. In general, AMF inoculation notably improved seed yields of all sunflower cultivars irrespective of irrigation treatments, although the beneficial effects were more evident under water deficit stress than well-watered condition (Table 4).

## Discussion

Overall, irrigation treatments had substantial effects on the traits under study. Moreover, the evaluated traits showed notable responses to AMF inoculation. In both years, RWCs were reduced under water deficit conditions, the reductions were mitigated by the mycorrhization. Wu *et al*. (2017) and Rahimi *et al*. (2017) found more leaf relative water contents in the plants inoculated with mycorrhiza. In another study, Aliasgharzad *et al*. (2006) reported higher RWCs in AMF inoculated plants than non-inoculated ones regardless of the soil water status. Mirshad and Puthur (2016) also observed increased leaf water content in mycorrhizal plants.

Improved RWC in mycorrhizal plants in the present experiment can be attributed to an increased water and nutrient acquisition mediated by AMF hyphae (Manoharan *et al*., 2010; Asrar *et al*., 2012; Frosi *et al*., 2016). Augé *et al.* (2001) suggested that in dried soils, mycorrhizal plants can remain more hydrated than non-mycorrhizal plants. Morphological root changes mediated by AMF such as higher root length, surface area, average diameter and volume (Liu *et al*., 2018) can cause higher soil volume explored by plant root and consequently more water and nutrient uptake (Comas *et al*., 2013). These morphological changes can improve root hydraulic conductivity and cell to cell water transportation (Marjanović *et al*., 2005; Bárzana *et al*., 2012). Moreover, AMF extraradical hyphae can increase plant ability to absorb the water that is usually not accessible for non-AM plants (Liu *et al*., 2018).

Chlorophyll concentrations were negatively affected by water deficit conditions, whereas AMF inoculation alleviated the negative effect of drought on clorophyll. Improving effect of AM fungi on leaf chlorophyll concentrations under water deficit condition was shown in corn by Zhu *et al*., (2012). According to Hazzoumi *et al.* (2015) chlorophyll concentrations of basil were significantly reduced under water deficit condition, while mycorrhization notably improved the levels of leaf chlorophyll. Similar results were obtained by Pal and Pandey (2016) and Rani (2016) who

**Table 4. The traits of sunflower cultivars as influenced by irrigation treatment and arbuscular mycorrhiza fungi inoculation in the second year of the experiment (2017).**

| Irrigation treatment | Cultivar | AMF inoculation | RWC (%) | Chl a (mg/g FW) | Chl b (mg/g FW) | Chl T (mg/g FW) | SPC (mg/g DW) | Proline (mg/g FW) | MDA (nmol/g FW) | Seed yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| Severe water deficit stress | Farokh | *F. mossea* | 28.20$^{gh}$ | 16.65$^{h}$ | 7.59$^{j}$ | 24.24$^{i}$ | 9.91$^{i}$ | 44.66$^{c}$ | 1428.18$^{a}$ | 3000.00$^{h}$ |
| | | *R. intraradices* | 32.33$^{fg}$ | 16.35$^{h}$ | 7.21$^{k}$ | 23.56$^{ij}$ | 11.15$^{hi}$ | 45.10$^{c}$ | 900.88$^{b}$ | 3300.61$^{g}$ |
| | | Without AMF | 24.00$^{h}$ | 14.86$^{i}$ | 5.87$^{n}$ | 20.73$^{kl}$ | 6.50$^{k}$ | 48.55$^{b}$ | 745.73$^{c}$ | 1830.11$^{k}$ |
| | Hisan | *F. mossea* | 40.10$^{ef}$ | 17.22$^{h}$ | 8.00$^{i}$ | 25.22$^{h}$ | 10.22$^{i}$ | 49.28$^{b}$ | 561.13$^{d}$ | 2489.14$^{j}$ |
| | | *R. intraradices* | 37.09$^{ef}$ | 15.53$^{hi}$ | 6.57$^{m}$ | 22.10$^{k}$ | 12.39$^{h}$ | 49.13$^{b}$ | 1402.21$^{a}$ | 2820.72$^{i}$ |
| | | Without AMF | 23.15$^{h}$ | 14.66$^{i}$ | 5.53$^{o}$ | 20.17$^{l}$ | 5.58$^{k}$ | 52.46$^{a}$ | 940.47$^{b}$ | 1780.00$^{k}$ |
| | Baezegar | *F. mossea* | 31.00$^{g}$ | 16.03$^{hi}$ | 7.03$^{kl}$ | 23.06$^{j}$ | 13.01$^{h}$ | 40.18$^{d}$ | 810.80$^{bc}$ | 2750.42$^{i}$ |
| | | *R. intraradices* | 34.21$^{fg}$ | 15.87$^{hi}$ | 6.81$^{l}$ | 22.68$^{jk}$ | 12.39$^{h}$ | 34.25$^{e}$ | 886.17$^{b}$ | 3020.90$^{h}$ |
| | | Without AMF | 24.60$^{h}$ | 14.64$^{i}$ | 5.53$^{o}$ | 20.17$^{l}$ | 6.81$^{k}$ | 45.32$^{c}$ | 1443.35$^{a}$ | 2351.12$^{j}$ |
| Mild water deficit stress | Farokh | *F. mossea* | 69.55$^{a}$ | 24.93$^{a}$ | 11.81$^{a}$ | 36.74$^{a}$ | 26.95$^{a}$ | 16.48$^{i}$ | 194.86$^{h}$ | 5237.28$^{a}$ |
| | | *R. intraradices* | 57.02$^{c}$ | 17.80$^{g}$ | 7.39$^{jk}$ | 25.19$^{h}$ | 21.68$^{d}$ | 20.20$^{h}$ | 374.28$^{efg}$ | 4941.11$^{b}$ |
| | | Without AMF | 33.11$^{fg}$ | 22.28$^{c}$ | 10.37$^{c}$ | 32.65$^{c}$ | 7.74$^{ijk}$ | 48.38$^{b}$ | 415.18$^{ef}$ | 3286.14$^{g}$ |
| | Hisan | *F. mossea* | 64.41$^{b}$ | 18.33$^{g}$ | 8.63$^{g}$ | 26.96$^{g}$ | 22.30$^{cd}$ | 24.25$^{g}$ | 276.05$^{fgh}$ | 4904.00$^{b}$ |
| | | *R. intraradices* | 63.30$^{b}$ | 23.88$^{b}$ | 11.28$^{b}$ | 35.16$^{b}$ | 20.44$^{d}$ | 38.41$^{e}$ | 250.12$^{fgh}$ | 4654.34$^{c}$ |
| | | Without AMF | 40.14$^{ef}$ | 15.93$^{hi}$ | 6.93$^{l}$ | 22.86$^{j}$ | 8.673$^{j}$ | 48.20$^{b}$ | 450.45$^{ed}$ | 3170.40$^{g}$ |
| | Baezegar | *F. mossea* | 45.13$^{de}$ | 17.43$^{gh}$ | 8.16$^{i}$ | 25.59$^{h}$ | 20.75$^{d}$ | 30.00$^{f}$ | 320.29$^{efgh}$ | 4583.36$^{c}$ |
| | | *R. intraradices* | 42.00$^{e}$ | 16.92$^{h}$ | 7.81$^{ij}$ | 24.73$^{hi}$ | 18.58$^{e}$ | 29.50$^{f}$ | 218.00$^{gh}$ | 4210.35$^{d}$ |
| | | Without AMF | 34.17$^{fg}$ | 15.33$^{i}$ | 6.33$^{m}$ | 21.66$^{k}$ | 8.98$^{j}$ | 44.46$^{c}$ | 206.21$^{gh}$ | 3010.45$^{h}$ |
| No water deficit stress (well-watered) | Farokh | *F. mossea* | 49.00$^{d}$ | 18.85$^{g}$ | 8.88$^{fg}$ | 27.73$^{g}$ | 24.78$^{b}$ | 20.18$^{h}$ | 252.32$^{fgh}$ | 4918.75$^{b}$ |
| | | *R. intraradices* | 55.00$^{c}$ | 19.07$^{fg}$ | 9.00$^{f}$ | 28.07$^{f}$ | 26.33$^{a}$ | 21.13$^{h}$ | 240.23$^{fgh}$ | 4195.55$^{d}$ |
| | | Without AMF | 41.86$^{e}$ | 17.96$^{g}$ | 8.40$^{h}$ | 26.36$^{gh}$ | 15.49$^{g}$ | 24.63$^{g}$ | 245.33$^{fgh}$ | 3500.68$^{f}$ |
| | Hisan | *F. mossea* | 45.26$^{de}$ | 21.81$^{c}$ | 10.25$^{c}$ | 32.06$^{c}$ | 25.71$^{ab}$ | 15.05$^{i}$ | 200.67$^{gh}$ | 4600.00$^{c}$ |
| | | *R. intraradices* | 42.65$^{e}$ | 21.28$^{c}$ | 9.96$^{d}$ | 31.24$^{d}$ | 23.23$^{e}$ | 16.68$^{i}$ | 322.33$^{efgh}$ | 4580.63$^{c}$ |
| | | Without AMF | 36.68$^{f}$ | 19.44$^{f}$ | 9.11$^{f}$ | 28.55$^{ef}$ | 14.56$^{f}$ | 23.86$^{gh}$ | 265.33$^{fgh}$ | 3760.20$^{e}$ |
| | Baezegar | *F. mossea* | 48.18$^{d}$ | 20.19$^{e}$ | 9.45$^{e}$ | 29.64$^{e}$ | 23.54$^{bc}$ | 16.00$^{i}$ | 264.67$^{fgh}$ | 4120.35$^{d}$ |
| | | *R. intraradices* | 48.00$^{d}$ | 20.85$^{d}$ | 9.67$^{e}$ | 30.25$^{ed}$ | 19.20$^{de}$ | 19.49$^{h}$ | 278.65$^{fgh}$ | 4110.33$^{d}$ |
| | | Without AMF | 39.33$^{ef}$ | 18.42$^{g}$ | 8.72$^{g}$ | 27.14$^{g}$ | 15.18$^{g}$ | 21.03$^{h}$ | 250.27$^{fgh}$ | 3547.19$^{f}$ |
| LSD (0.05) | | | 5.14 | 1.00 | 0.33 | 1.18 | 1.55 | 3.18 | 140.47 | 160.05 |

AMF, arbuscular mycorrhiza fungi; RWC, relative water content; chl a, chlorophyll a; chl b, chlorophyll b; chl T, chlorophyll total; SPC, shoot P concentration; MDA, malondialdehyde; FW, leaf fresh weight; DW, leaf dry weight; LSD, least significant difference. Means with a letter in common can't be considered different at a p>0.05 according to a least significant difference.

reported improved leaf chlorophyll concentrations of mycorrhizal wheat plants under water deficit stress. According to Evelin *et al*. (2009) in AM plants, chlorophyll synthesis is less negatively affected by water deficit stress. In another study, Asrar *et al*. (2012) found higher levels of chlorophyll pigments due to AMF inoculation in water deficit-stressed snapdragon plants. Hu *et al*. (2020) also showed that AM-corn plants had higher chlorophyll concentrations than non-AM ones under both non-stress and water deficit stress conditions. They concluded that higher levels of polyamines in the leaves of AMF inoculated plants may be a key factor for more chlorophyll concentrations in these plants. Beigbeder *et al.* (1995) found a stimulating role for intercellular polyamines in light-independent chlorophyll synthesis from protochlorophyllide. Another reason for more chlorophyll concentrations of AM plants may be higher N levels in their leaves. N is an essential element for chlorophyll synthesis and chlorophyll molecules can notably trap nitrogen (De Andrade *et al*. 2015). AMF association can increase N uptake by partner plant and AMF increasing effect is more obvious under water deficit stress than well-watered condition (Wu and Zou, 2009).

Improved N concentrations in mycorrhizal plants has also been documented by Wang *et al.* (2018) and Smith *et al.* (2011). Others also reported that AM fungi can effectively absorb and transfer N to host plants (Battini *et al*., 2017; Turrini *et al.*, 2018). It can be achieved by an extensive extraradical hyphae produced by AMF in the rhizosphere by which host plant can efficiently uptake N from the soil (Battini *et al*., 2017). Similar results were also obtained by Turrini *et al.* (2018) who reported higher N levels of crop plants in the presence of native mycorrhizal fungi. In general, AM fungi can efficiently obtain nitrogen from soil organic materials and transfer it to the host plant (Hodge and Fitter, 2010). They are also able to absorb and assimilate nitrogen from the soil inorganic sources (Lin *et al*., 2007).

In our study, all sunflower cultivars showed substantial higher shoot phosphorus concentrations due to AMF inoculation irrespective of irrigation treatments. However, AMF inoculation had more positive effects on SPCs under mild drought stress, *i.e.* where higher AMF colonization was recorded. The critical role of AM fungi in increasing the ability of host plant to uptake P has well been documented by several researchers. Hu *et al*. (2020) observed a higher P concentration in AMF-corn plants regardless of irrigation treatments which is in agreement with our finding. Similar results were reported by Bayani *et al.* (2015) in barley, Grumberg *et al.* (2015) in soybean, Zhao *et al.* (2015) and Garces-Ruiz *et al*. (2017) in corn, Rani (2016) in wheat and Liu *et al.* (2018) in potato. Sato *et al.* (2015) also found a higher shoot P concentration in AM-plants than in non-AM ones.

Increasing P uptake by AM plants can be explained by producing acid phosphatase which can hydrolyze soil organic phosphate and convert it to a usable form for host plant (Tawaraya *et al*., 2005). Moreover, polyphosphates may be stored in the vacuoles of AMF hyphae, then hydrolyzed in the form of inorganic P which ultimately can be transported into the host plant (Smith and Gianninazi-Pearson, 1988).

According to Sato *et al.* (2015) releasing acid phosphatase from AMF extraradical hyphae is a major reason for the enhanced P acquisition by mycorrhizal plants. They also suggested that the extension of extraradical hyphae beyond the P-depletion zone of the rhizosphere is another factor explaining the increase P uptake by host plant. Similar results also reported by other researchers (Lambers, 2006; Lambers *et al.,* 2011). An extended AMF hyphae (higher than 10 cm from host root surface) and their lower diameter (20 to 50 μm) can allow them to penetrate and exploit the soil pores which are usually not accessible and exploitable by roots. In other words, AM plants have a root system with higher surface area which can effectively uptake P and other nutrients from the soil (Lambers *et al.*, 2008). Smith and Read (1997) also suggested that increased water intake in AM-plants due to an extensive extraradical hyphae may explain their higher ability to uptake P.

Zhu *et al.* (2010) also observed a higher affinity of AMF hyphae for phosphate compared to plant roots. Increasing proton efflux and decreasing pH to about 6.3 in the rhizosphere (Rigou and Mignard, 1994) can effectively solubilize fixed phosphates in calcareous soils (Bago and Azcon-Aguilar, 1997). This is especially very important in Iran, where the soils of most regions are calcareous with a pH more than 7 in which P is easily fixed by calcium and becomes unavailable for plants.

Releasing extracellular acid phosphatase, excretion of proton hydroxyls and organic anions and redox potential modification resulted from AM fungi symbiotic association are other proposed mechanisms that may facilitate P mobilization and uptake by host plants (Hinsinger, 2001; Rakshit and Bhadoria, 2007). Occupation of sorption sites by organic anions such as malate, citrate and oxalate released by AM fungi and consequently prevention of P fixation and immobilization by other elements such as Ca which can lead to formation of insoluble P compounds, has also been reported by Richardson *et al.* (2011).

In both years, shoot proline concentration showed positive response to increasing water deficit stress level. However, AMF inoculation led to a significant decrease in proline concentration of all cultivars regardless of the soil water status. This is compatible with the results obtained by WU *et al.* (2013b, 2017) who showed lower proline levels in the plants inoculated with AMF. Similar result was reported by Porcel and Ruiz-Lozano (2004) working with soybean. They found 39% lower proline concentration in mycorrhizal plants than non-mycorrhizal ones. Reduced proline concentration in AMF plants can be related to a decrease of glutamate synthetic pathway mediated by AMF which enhances the catabolism of proline (Wu *et al.* 2017).

Accumulation of organic osmolytes such as proline in order to osmotic adjustment is an efficient mechanism for plant survival under drought condition. However, AMF plants usually show lower levels of proline under water deficit stress condition reflecting a higher ability to avoid drought stress. (Augé, 2001). In other words, less proline concentrations in AMF plants indicates a better water status of these plants and consequently a lower damage resulted from water deficit condition (Augé and Moore, 2005).

However, our finding is incompatible with the results reported by Ruiz-Sanchez *et al.* (2011) and Yooyongwech *et al.* (2013) who reported higher proline levels in AMF plants under water deficit condition. They argued that it is an adaptation mechanism in order to a better osmotic adjustment which can consequently lead to a higher plant ability for tolerance of drought stress.

MDA is an indicator of lipid peroxidation and notably increased when the soil water deficit was intensified. At the one time, mycorrhizal plants had significant lower MDA concentrations compared to non-mycorrhizal ones. According to Porcel and Ruiz-Lozano (2004) non-AM plants had a substantial higher lipid peroxidation under drought stress, whereas at the same time AMF plants showed 55% lower lipid peroxidation than non-AMF ones. Similar results were reported by Zhu *et al.* (2011) who found an alleviating effect of AMF on MDA concentration of corn leaf.

MDA production is a criterion of lipid peroxidation which is occurred under environmental stress such as drought and it reflects membrane degradation and dysfunction (Lacan and Baccou, 1998; Ali *et al.*, 2005). It is used as an indicator to determine the peroxidative damage intensity due to water deficit condition (Uzilday *et*

*al.*, 2012). Lipid peroxidation is usually lead to electrolyte leakage which shows the loss of cell membrane integrity (Kormanik *et al.,* 1980). Li *et al.* (2019) suggested that AMF plants had lower MDA concentration (by 32%) under water deficit stress condition. In their study, MDA reduction was occurred simultaneously with increases in the activity of antioxidant enzymes such as catalase and superoxide dismutase.

In both years, severe water deficit stress significantly reduced sunflower yields and reductions were notably higher in no mycorrhizal treatments (Tables 3 and 4). Overall, under all irrigation treatments AMF inoculation improved seed yields indicating other beneficial effects of AMF aside from their alleviating role on water deficit stress in sunflower plants. As our findings showed, AMF colonization has improving effects on plant chlorophyll and phosphate concentrations (Tables 3 and 4) which can ultimately be reflected in sunflower yield. Moreover, although in the absence of AMF, higher yields were recorded under well-watered condition, but mycorrhization under mild water deficit stress led to the comparable or even higher yields indicating improved crop water status caused by AMF as revealed by RWC data (Tables 3 and 4).

In general, beneficial effects due to AMF inoculation were notably higher under mild than severe stress condition indicating a significant effect of soil water level on AMF efficiency. This was in agreement with the colonization data where sunflower plants showed higher AMF colonization under mild than severe stress condition. There are many reports regarding to adverse effects of soil water deficit on AMF development and activity. According to Daniels and Trappe (1980) the germination of AMF spores was significantly repressed when water potential of soil dropped below the field capacity. In another study, Douds and Schenck (1991) found that spore germination of *F. mosseae* and *R. intraradices* (two AM species used in our study) was prevented at low soil matric potentials. Based on an extensive literature review, Augé (2001) suggested that root colonization by AMF can notably be reduced under long term soil water deficit condition. According to Neumann *et al.* (2009) extraradical hyphal length was highly suppressed under soil water deficit condition. Wu *et al.* (2013a) also reported that root AMF development can significantly be inhibited by drought stress. Turrini *et al.* (2016) found that AMF colonization level is influenced more by environmental factors than genetics.

## Conclusions

In general, our findings revealed that all of the sunflower plant traits under study including relative water content, chlorophyll concentrations, shoot phosphate concentration, shoot proline levels and MDA accumulation were significantly affected by soil water status, as water deficit condition had negative effects on relative water content, chlorophyll concentrations and shoot phosphate concentration, while shoot proline levels and MDA accumulation positively respond to this condition. For all cultivars, inoculation with AMF (*F. mosseae* or *R. intraradices*) improved plant traits regardless of irrigation treatments. Although, in most cases *F. mosseae* performed relatively better than *R. intraradices.* The improvements caused by AM fungi were more evident under water deficit than well-watered condition. Moreover, the positive effects resulted from AMF inoculation were significantly higher under mild than severe water deficit stress. This was in agreement with the colonization data where sunflower plants showed higher AMF colonization under mild stress condition. This indicates that efficiency and beneficial effects of AMF are highly dependent on soil water status and can notably be reduced when soil water deficit is intensified. However, further studies are needed to recognize the mechanisms and other physio-biochemical processes involved in plant response to AMF inoculation under water deficit stress condition.

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