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Impact of mycorrhizal fungi and water stress on oil and protein harvest index in sesame

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Abstract

Drought stress is one of the most important environmental stresses affecting plant growth, yield and crop production around the world. It is believed that arbuscular mycorrhizal fungi are used for protecting plants against drought damage. Vesicular-arbuscular mycorrhizal fungi have been used in recent years to cope with water stress in many plants. In this study, the relationship between water deficit stress and mycorrhizal fungi were analyzed with mycorrhizal dependence index and chlorophyll stability in sesame (*Sesamum indicum* L.). The experiment was conducted in a split-plot design based on randomized complete blocks with three replications in the research field of Agricultural Research Center, West-Azerbaijan during years 2015 and 2016. The main factors consisted of normal irrigation, moderate and severe water stress and subplots included two different species of mycorrhizal fungi namely, *Funneliformis mosseae* and *Rhizophagus intraradices*. A non-inoculated plant served as the control. Mean comparison based on 2-years data showed that with increasing severity of water stress, biological water use efficiency (WUBE), oil harvest index (OHI) and protein harvest index (PHI) decreased. Using two kinds of mycorrhizal fungi *F. mosseae*, *R. intraradices* compared to non-inoculated, caused an increase in WUBE and PHI about 28 and 20% and 6 and 2%, respectively. Also in three different irrigation conditions, the effect of *F. mosseae* and *R. intraradices* was similar on chlorophyll b stability index (CSI_b). The maximum and minimum WUBE (0.96 and 0.43 kg/m^3), OHI (17.61 and 10.03%) and PHI (9.36 and 5.80%) were obtained under optimal irrigation and severe drought stress conditions, respectively. The maximum (34.69%) and minimum (20.26%) of mycorrhizal dependence index based on biological yield (MDIBY) were observed under severe drought stress and optimal irrigation conditions, respectively. Therefore, inoculation with mycorrhizal fungi (measured by MDIGY and MDIBY) under drought stress caused an increase in the chlorophyll (measured by TCSI). Increasing the chlorophyll led to an enhancement in the photosynthesis and promoted WUEE and WUBE. Improvement of the WUEE and WUBE caused an increase in oil and protein (measured by OHI and PHI). In severe and moderate water stresses mycorrhizal dependence index based on grain yield (MDIGY) and MDIBY increased compared to optimal irrigation. It can be concluded that for achieving high WUEE, WUBE, OHI and PHI, TCSI and as a result tolerance to the water stress can be increased.

Key words: Biological water use efficiency, Mycorrhiza, Oil harvest index, Sesame, Total chlorophyll, Water Stress.

INTRODUCTION

Sesame (*Sesamum indicum* L.) is a very old crop and often named “the queen of oil seeds” because it has a high oil quantity and quality (Dossa *et al.*, 2017a). In fact, it is progressively adapted because its cultivation is comparatively easy. Sesame can grow on several types of soil, the need for irrigation is low and it can withstand extreme temperatures and heat. Sesame is suitable for crop rotation (Dossa *et al.*, 2017b). It is widely grown in different parts of the world. For many centuries, sesame seeds have been used as a source of oil, protein, vitamins, and minerals for human and animal nutrition (Movahhedi Dehnavi, 2017). The seed is not only rich in oil (42–45%) but also has high protein (20%) and carbohydrates (14–20%) content. The micronutrient content of sesame seeds generally follows the order Fe>Cu>Zn>Mn (Suresh *et al.*, 2013). Seed yield is a quantitative polygenic and complex trait and is a result of different factors (Emamgholizadeh *et al.*, 2015). Pre-transplant inoculation with mycorrhizal fungi increased root colonization in tomato at flowering and harvest compared to the non-inoculated plants (31.8 vs 23.6%) (Mugendi Njeru *et al.*, 2017).

Water stress is one of the most important abiotic stresses which affect several aspects of plant growth and developments (Golestani and Pakniyat, 2015). Previous reports have shown that water stresses has adverse effects on plant growth and productivity (Bahrami *et al.*, 2012; Amani *et al.*, 2012). In assessing the water use efficiency of sesame cultivars under different irrigation conditions, the highest water use efficiency was obtained under severe water deficit conditions (Eskandari *et al.*, 2010). It has also been shown that researchers in the evaluation of the effect of irrigation regimes and mycorrhiza on the WUE of sesame declared that the highest WUEE (0.44 kg m^{-3}) was related to 80% irrigation regime and inoculated with mycorrhizal fungus (Ahmadnezhad *et al.*, 2013). It is reported that the highest water use efficiency of 1.307 kg/m^3 was observed under 90% irrigation application. Whereas, 1.299 kg/m^3, 1.194 kg/m^3 and 1.071 kg/m^3 were observed under 100% application, 80% application and 70% application, respectively (Gezae, 2018). In a study, researchers indicated that the maximum (1.47 kg/m^3) and minimum (1.36 kg/m^3) WUEE were obtained in 75 and 50% irrigation requirement, respectively. Also the highest (4.26 kg/m^3) and lowest (3.29 kg/m^3) WUBE were observed under 50% irrigation requirement and full irrigation, respectively (Asvadi *et al.*, 2018). Researchers in assessing the influence of mycorrhizal symbiosis on growth and proline content in Leek (*Allium porrum* L.) and two genotypes of Persian Leek (*Allium ampeloprasum* ssp. persicum L.) under water stress stated that among leek genotypes, Shadegan genotype had more mycorrhizal dependence in all levels of water stress than the two other populations with a weaker root system (Ghasem Jokar *et al.*, 2015). An indicator for assessing tolerant plants to water stress is to measure the chlorophyll stability index (CSI). CSI exhibits how chlorophyll functions under water stress conditions. A superior CSI aids plants to tolerate water stress via better access of chlorophyll content by keeping more biomass production, and preferable fertility (Baroowa and Gogoi, 2012). Ghahramani *et al.* (2015) reported that the effect of water stress was significant on chlorophyll stability index (CSI) and it decreased the amount of CSI. The highest CSI (94%) was related to KFS2 genotype under 65% field capacity. Water stress reduced chlorophyll a, chlorophyll b, total chlorophyll and CSI (Rana and Kumari, 2016). The highest (0.60 kg/m^3) and lowest (0.32 kg/m^3) water use efficiency were observed in plants irrigated after 200 and 50 mm of evaporation, respectively (Habibzadeh *et al.*, 2012).

The important method that protects plants against water stress is symbiosis with mycorrhizal fungi (Auge *et al.*, 2015). One of the possible mechanisms for increasing tolerance to water stress in mycorrhizal plants is increasing hydraulic conduction of roots (Tian *et al.*, 2013), increasing water absorption in moisture deficit due to the expansion of fungi, creating osmotic balance and keeping turgor pressure (Singh *et al.*, 2011), increasing photosynthetic activity, carbohydrates accumulation and proline content and increasing micro and macro-nutrients absorption (Deepika and Kothamasi, 2015). Thus, it is necessary that investigators work on water utilization to take complete growth in crops and maximum water use efficiency (WUE) (Rodrigues Pereira *et al.*, 2017). Water stress in the reproductive stage reduced the amount of chlorophyll a (Mahrokh *et al.*, 2016). Several studies have shown that mycorrhiza can modify adverse effects of water stress in plants (Barea, 1992). Haghighatnia *et al.* (2012) stated that mycorrhizal colonization, especially by *F. mosseae*, improves resistance to water stress and compensates for some of the reduction in yield. The dependence of host plants to mycorrhizal fungi depends on environmental factors such as light intensity, temperature, soil conditions, and root morphology characteristics and plant physiology (Smith and Read, 2008). The maximum index of chlorophyll stability in the stress condition was obtained in wheat lines: "Homa» ,«Ohadi« and »Unknown 11« (Sharifi *et al.*, 2012). Researchers reported that mycorrhizal plants compared to the non-mycorrhizal plants cause improvements in harvest index of proteins, and ecosystem water use efficiency (Habibzadeh *et al.*, 2012). Dorostkar and Pirzad (2018) reported that the highest protein was obtained from inoculated plants with *R. intraradices*. So this experiment was conducted to investigate the relationship between water stress and mycorrhiza in sesame (*Sesamum indicum* L.) " Darab 2" cultivar with mycorrhizal dependence index and chlorophyll stability.

MATERIALS AND METHODS

Geographic location and date of the experiment

The study was carried out during two successive-years 2015 and 2016 in the research field of Agricultural Research Center, West-Azerbaijan with 37°32′ N and 45°5′ E and 1352 meters above the sea level. The experiment was conducted, in an arid and semi-arid region 25 km from Urmia. According to the long-term meteorological data, the average annual rainfall is 390 mm, the average temperature is 11.3 °C and the relative humidity is 75%. Some of the meteorological parameters from planting to harvesting for two years (2015 and 2016) are presented in supplementary Table 1.

Experimental design and treatments

This experiment was performed as a split-plot based on a randomized complete block design (RCBD) with three replications. The main factor consisted of three levels of irrigations, normal irrigation (irrigation after 70 mm evaporation of crop (ETc)), moderate water stress (irrigation after 90 mm ETc) and severe water stress (irrigation after 110 mm ETc). Subplots included two different species of mycorrhizal fungi, namely, *Funneliformis mosseae* (Nicol. and Gerd) BEG 12 and *Glomus intraradices* (Schenck and Smith; the new name is *Rhizophagus irregularis*). Non-inoculated plants served as the control. The mycorrhizal inocula were a mixture of sterile sand, mycorrhiza hyphae, spores (20 spores per gram) and colonised root fragments. Approximately 10 g of the appropriate inocula were placed inside the hole below each seed and then covered with soil. For the control, sesame plants were sown with 10 g of killed inocula. The seeds were cultivated on May 20, 2015 and May 13, 2016 with plant spacing of 50 cm×15 cm containing 133 333 plants ha^{-1}. Each plot contained four rows of 4 meter long. Cultivation and irrigation were performed by furrowing and leakage method, respectively. At the time of planting, three seeds were placed in each clump and then thinned in 2–4 leaf stages. All treatments were irrigated to 2–4 leaves. After this stage, different water stress levels were applied. The distance between subplots and main plots was approximately 1 and 2 m, respectively. Therefore, the area of each subplot and the main plot was 10 and 96 m^2, respectively. Considering the total area of the experiment, the intervals between the experimental units and irrigation canals were approximately 3000 m^2. The nutritional elements required in the two years of cultivation, were added to the soil during tillage, which are listed in the supplementary Table 2. To maximize the infection of sesame roots with mycorrhiza for increased colonization percentage, phosphorus fertilizer was not added to the soil. The first and second years of planting were performed on May 10, 2015 and May 3, 2016 by hand in a wet planting manner. The first irrigation was conducted approximately 10 days after planting. Weeding was performed manually in two stages, 20 and 40 days after planting. No specific disease or pests were found in the field. Some reported characteristics of the studied mycorrhizal species are presented in supplementary Table 3.

Chemical and physical properties of soil in the experimental site

To ensure that the experimental area contains a low mycorrhizal population, the spore density was measured using a standard method (Habibzadeh *et al.*, 2012). The physical and chemical properties of the soil in the experimental site are presented in supplementary Table 4. Soil analysis was performed in accordance with the methodology proposed by EMBRAPA (1997) and Rayment and Higginson (1992) (supplementary Table 4). According to the table, the soil type of the experimental site was loam-clay loam, pH=8, and Ec was approximately 1.5 ds/m, which was suitable for sesame cultivation.

Method of application of different irrigation treatments

To determine the field capacity, the permanent wilting point (PWP) and bulk density were measured using the method reported by Mousavi and Akhavan (2008). Bulk density, field capacity and PWP were calculated as 1.37 g/cm3, 25% and 12%, respectively. The readily available water (RAW) was calculated by Equation 1.

$$\text{(1)} \qquad \mathrm{RAW} = \frac{\mathrm{FC-PWP}}{100} \times \rho \times \mathrm{D} \times \mathrm{MAD}$$

Where RAW is the readily available water (mm), FC is the field capacity (%), PWP is the PWP%, ρ is the bulk density, D is the root zone depth (mm) and MAD is the coefficient of management allowable depletion. In loam-clay loamy soil, the soil capacity was 25 and the PWP was 12. The root development depth in sesame was 600 mm. The coefficient of water easy to use is F or MAD or θ. *RAW*=(25-12)/100×1.37×600×0.65.

MAD=coefficient of management allowable depletion is the same as water that can be used between field capacity and PWP. The coefficients were 0.65, 0.8 and 0.95 for optimal irrigation, moderate and severe water stress, respectively. RAW was 70, 85 and 100 mm under the optimal irrigation, moderate and severe water stresss, respectively, which can be considered equivalent of ET_C or evapotranspiration. ETO and ETC were calculated by Equation 2.

(2) $$ETo = ETp \times Kp \quad , \; ETc = ETo \times Kc$$

Where, ETo is the potential evapotranspiration, ETp is the pan evapotranspiration, ETc is the crop evapotranspiration, Kp is the pan coefficient and Kc is the crop coefficient of sesame.

Irrigation was measured using type III flumes (Washington State College) with a throat width of 304.8 mm and head of 30 mm (Chamberlain, 1952). Parameters measured for defining the moderating effect of mycorrhizal fungi under water stress in sesame are presented in Table 1.

Statistical analysis

Combined analysis of factorial split-plot experiments based on randomized complete block design was performed using SAS 9.2 software. The homogeneity of error variances was tested using Bartlett test. To reduce type 1 error, Bonferroni correction was carried out for variance analysis and correlation coefficients. A comparison of the means was carried out by SNK test at 5% level by MSTATC software.

RESULTS

Variance analysis

Combined analysis of data revealed that the impact of irrigation and mycorrhiza on WUEE, WUBE, MDIGY, MDIBY, OHI, PHI, TCSI, CSIa and CSI_b were significant at 1% probability level ($P<0.01$). The interaction effect of irrigation and mycorrhiza on WUEE, MDIGY, TCSI, CSIa and CSIb were significant (Table 2). The maximum and minimum values for WUEE, WUBE, MDIGY, MDIBY, PHI, TCSI, CSIa and CSI_b were observed in 2016 and 2015, respectively (Table 3). The rates of TCSI and CSI_b were higher in the first year than in the second year (Table 3).

Mean comparions

Mean comparisons based on two years data showed that with increasing the severity of water stress, WUBE, OHI and PHI decreased significantly but MDIBY increased. So that severe and moderate water stress compared to optimal irrigation reduced WUBE, OHI and PHI about 56, 44 and 38 percent, respectively (Table 4). Severe water stress compared to optimal irrigation increased MDIBY by about 42 percent (Table 4).

Table 1. Parameters measured for defining the moderating effect of mycorrhizal fungi under water stress in sesame.

Parameter	Abbreviation	Formula	Refernce
Economic water use efficiency	WUEE	the ratio of grain yield to irrigated water based on kg/m^3	Katerji *et al.*, (2014)
Biological water use efficiency	WUBE	as the ratio of biological yield to irrigated water based on kg/m^3	Katerji *et al.*, (2014)
Mycorrhizal dependence index based on grain yield	MDIGY	$= \frac{GYIMF - GYNIMF}{GYNIMF} \times 100$ Where GYIMF is seed yield of inoculated mycorrhizal fungi, GYNIMF is grain yield of non-inoculated mycorrhizal fungi.	Menge *et al.*, (1978)
Mycorrhizal dependence index based on biological yield	MDIBY	$= \frac{BYIMF - BYNIMF}{BYNIMF} \times 100$ Where BYIMF is biological yield of inoculated mycorrhizal fungi, BYNIMF is biological yield of non-inoculated mycorrhizal fungi.	Menge *et al.*, (1978)
Oil harvest index	OHI	the oil yield divided by biological yield×100	
Protein harvest index	PHI	the protein yield divided by biological yield×100	
Total chlorophyll stability index	TCSI	TCSI=(Total Chl under stress/Total Chl under control)×100	Sairam *et al.*, (2008)
Chlorophyll a stability index	CSIa	CSIa=(Chla under stress/ Chla under control)×100	Sairam *et al.*, (2008)
Chlorophyll b stability index	CSIb	CSIb=(Chla under stress/ Chlb under control)×100	Sairam *et al.*, (2008)

Table 2. Variance analysis of the studied traits during two successive years.

Source of variation	df	Mean of square								
		Economic water use efficiency	Biological water use efficiency	Mycorrhizal dependence index based on grain yield	Mycorrhizal dependence index based on biological yield	Oil harvest index	Protein harvest index	Total chlorophyll stability index	Chlorophyll a stability index	Chlorophyll b stability index
Year (Y)	1	0.018**	0.2251**	2706.82**	914.39**	1.92^{ns}	0.98**	178.14**	204.28**	5624.89**
Block/year	4	0.00042	0.602	109.17	911.20	200.60	55.44	75.46	159.43	108.10
Irrigation (I)	2	0.262**	1.300**	2484.43**	1170.48**	268.49**	60.23**	11335.88**	15750.79**	5348.00**
Year×irrigation	2	0.0038**	0.0096^{ns}	72.51^{ns}	61.48^{ns}	28.05**	8.14**	47.44**	51.35**	1413.19**
Error a	8	0.00014	0.100	127.43	92.41	40.29	11.41	24.73	62.48	31.81
Mycorrhizal (M)	2	0.033**	0.237**	12536.02**	9965.59**	0.61^{ns}	0.98**	327.50**	219.77**	953.25**
I×M	4	0.0017**	0.0036^{ns}	730.55**	318.97^{ns}	0.82^{ns}	0.29^{ns}	107.82**	89.40**	240.63**
Y×M	2	0.0044**	0.021**	938.07**	354.23^{ns}	1.53^{ns}	0.44^{ns}	336.78**	286.92**	542.37**
Y×I×M	4	0.00073*	0.0040^{ns}	185.10^{ns}	51.28**	0.99^{ns}	0.30^{ns}	98.65**	118.43**	136.62**
Error b	24	0.00022	0.0037	76.85	144.80	1.22	0.157	5.08	9.71	18.65
Coefficient of variation (%)	-	6.34	8.37	31.08	47.38	7.77	5.07	3.00	4.31	5.37

**,* and ns: Significant at 1%, 5% and non-significant probability levels, respectively.

Table 3. Mean comparison of different traits between 2 years of cultivation.

Year	WUEE (kg/m^3)	WUBE (kg/m^3)	MDIGY (%)	MDIBY (%)	PHI (%)	TCSI (%)	CSI_a (%)	CSI_b (%)
2015	0.22^b	0.67^b	21.12^b	21.28^b	7.70^b	76.84^a	70.35^b	90.62^a
2016	0.25^a	0.80^a	35.28^a	29.51^a	7.96^a	73.20^b	74.24^a	70.20^b

Means followed by the same letter in each column are not significantly different.

Table 4. Comparison of means for simple effects of irrigation and mycorrhiza on the studied traits during two successive years.

Treatment	Biological water use efficiency (kg/m^3)	Mycorrhizal dependence index based on biological yield (%)	Oil harvest index (%)	Protein harvest index (%)
Irrigation				
Optimal irrigation	0.96^a	20.26^b	17.61^a	9.36^a
Moderate water stress	0.79^a	21.23^b	15.09^a	8.32^{ab}
Severe drought stress	0.43^b	34.69^a	10.03^b	5.80^b
Mycorrhizal				
Funneliformis mosseae	0.83^a	46.45^a	14.39^a	8.08^a
Rhizophagus intraradices	0.75^b	29.73^b	14.30^a	7.77^b
Non- inoculated (control)	0.60^c	00.00^c	14.04^a	7.62^b

Mean in each column followed by the same letter(s) are not significantly different at 1% probability level according to SNK Test.

Table 5. Comparison of means of irrigation and mycorrhizal interaction on the studied traits during two successive years.

Treatment (I×M)		Economic water use efficiency (kg/m^3)	Mycorrhizal dependence index based on grain yield (%)	Total chlorophyll stability index (%)	Chlorophyll a stability index (%)	Chlorophyll b stability index (%)
Irrigation	Mycorrhizal					
Optimal irrigation×	Non-inoculated (control)	0.281^c	00.00^e	100.0^a	100.0^a	100.0^a
	Funneliformis mosseae	0.405^a	45.09^{bc}	100.0^a	100.0^a	100.0^a
	Rhizophagus intraradices	0.346^b	23.32^d	100.0^a	100.0^a	100.0^a
Moderate water stress×	Non-inoculated (control)	0.210^e	00.00^e	70.29^d	72.85^c	62.18^d
	Funneliformis mosseae	0.285^c	35.53^c	75.93^c	73.62^c	82.09^b
	Rhizophagus intraradices	0.261^d	24.82^d	79.52^b	80.76^b	76.71^c
Severe water stress×	Non-inoculated (control)	0.071^h	00.00^e	40.25^f	33.34^e	54.65^e
	Funneliformis mosseae	0.131^f	76.27^a	53.98^e	43.69^d	76.83^c
	Rhizophagus intraradices	0.111^g	48.75^b	55.19^e	46.38^d	71.21^c

Mean in each column followed by the same letter(s) are not significantly different at 1% probability level according to SNK Test.

Using two kinds of mycorrhizal fungi *F. mosseae* and *R. intraradices* caused increases in WUBE (38 and 20%) and PHI (6 and 2%) compared to non-inoculated (control) conditions, (Table 4). Under optimal irrigation conditions, inoculation with mycorrhizal fungi improved the WUEE (31 and 19%) compared to the non-inoculated plants. Under moderate water stress conditions, inoculation with mycorrhizal fungi caused increases in WUEE by 27 and 20% in comparison to the non-inoculated plants. However, in severe water stress conditions, inoculation with mycorrhizal fungi caused increases in WUEE by 46 and 36% compared to the non-inoculated plants (Table 5).

Under optimal irrigation, moderate and severe water stress conditions, inoculation with mycorrhizal fungi

Table 6. Mean and variations percentage of the studied traits in sesame under different levels of water stress.

Traits	Optimal irrigation		Moderate water stress		Severe water stress		Percentage of variations in traits for moderate water stress	Percentage of variations in traits for severe water stress
	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation		
WUEE	0.34	0.06	0.25	0.04	0.10	0.03	26.47	70.58
WUBE	0.96	0.14	0.79	0.10	0.43	0.10	17.70	55.20
MDIGY	22.80	22.55	20.12	18.23	41.67	38.62	11.75	-82.76
MDIBY	20.27	19.25	21.23	19.51	34.69	31.87	-4.73	-71.13
OHI	17.61	0.27	15.09	0.20	10.03	0.51	14.31	43.04
PHI	9.36	0.38	8.32	0.07	5.80	0.34	11.11	38.03
TCSI	100	0	75.25	4.65	49.81	8.30	24.75	50.19
CSI_a	100	0	75.74	4.36	41.14	6.88	24.26	58.86
CSI_b	100	0	73.66	10.30	67.56	11.53	26.34	32.44

Table 7. Correlation coefficients of the studied traits in sesame.

Traits	1	2	3	4	5	6	7	8	9
1. WUEE	1								
2. WUBE	0.99**	1							
3. MDIGY	-0.04^{ns}	0.008^{ns}	1						
4. MDIBY	0.009^{ns}	0.06^{ns}	0.99**	1					
5. OHI	0.94**	0.93**	-0.28^{ns}	-0.24^{ns}	1				
6. PHI	0.96**	0.95**	-0.23^{ns}	-0.18^{ns}	0.99**	1			
7. TCSI	0.94**	0.93**	0.15^{ns}	0.11^{ns}	0.97**	0.95**	1		
8. CSI_a	0.63^{ns}	0.66*	0.19^{ns}	0.25^{ns}	0.48^{ns}	0.52^{ns}	0.42^{ns}	1	
9. CSI_b	0.83**	0.82**	0.16^{ns}	0.18^{ns}	0.78**	0.76**	0.89**	0.28^{ns}	1

**,* and ns: Significant at 1%, 5% and non-significant probability levels, respectively.

improved the MDIGY by 49, 31 and 36%, respectively (Table 5). It seems that MDIGY and MDIBY in *F. mosseae* were higher than *R. intraradices*. The highest protein harvest index was obtained in the mycorrhizal plants compared to the nonmycorrhizal plants (Table 4). Severe water stress decreased TCSI, CSI_a and CSI_b about 34, 46 and 9%, compared to the moderate water stress, respectively (Table 5). Under severe water stress conditions, inoculation with mycorrhizal fungi increased TCSI, CSI_a and CSI_b about 26%, 24 and 29%, and 28%, 29 and 24%, respectively compared to the non-inoculated plants (Table 5).

Correlation analysis

These results indicate that in order to achieve high WUEE and WUBE, it is possible to select cultivars with a higher PHI and TCSI. Investigating the percentage of the variation in the studied traits (Table 6) indicated that the highest and lowest variation percentages of traits in moderate water stress conditions were related to WUEE (26.47%) and MDIBY (-4.73%). Also, the maximum and minimum percentages of variation of traits in severe water stress conditions were observed in MDIGY (-82.76%) and CSIb (32.44%) (Table 6). The highest correlation coefficient was observed between WUEE and WUBE. There was a positive correlation among WUEE with WUBE, PHI and TCSI (Table 7).

DISCUSSION

In this research, results showed that with increasing the severity of water stress, WUEE and WUBE decreased significantly. The results of other researchers were consistent with the findings of this research (Gholinezhad *et al.*, 2009). Also, other researchers in evaluation of the effect of irrigation regimes and mycorrhiza on the WUE of sesame declared that the highest WUEE (0.44 kg m^{-3}) was related to 80% irrigation regime and the inoculated plants with mycorrhizal fungi (Ahmadnezhad *et al.*, 2013). It seems that under optimal irrigation conditions, the absorption of nutrients increased due to the improved water availability to the plants, which led to an increase in the WUEE. Our findings were consistent with the results of other researchers (Ahmadnezhad *et al.*, 2013;

Rodrigues-Pereira *et al.*, 2017). Under all different levels of water stress conditions, two mycorrhizal fungi *F. mosseae* and *R. intraradices* caused increases in WUEE and WUBE, compared to non-inoculated (control) plants. These results show that the presence of mycorrhizal fungi in the soil and its symbiosis with the sesame roots not only increases the water absorption by the root of the plants, but also decreases water loss by reducing the plant evapotranspiration rate (Smith and Read, 2008). Increasing WUE due to symbiosis of mycorrhizal fungi has been reported in other plants such as sesame (Kocheki *et al.*, 2015) and almond (*Prunus dulcis*) (Aghababaee and Raiesi, 2012). It represents that agronomic operations, such as the selection of suitable species and cultivars (Alizadeh and Alizadeh, 2007), can greatly improve the WUE by preventing water loss (Ritchie and Basso, 2008). Regarding the WUEE equation, any factor that can improve grain yield will result in increased WUEE. Several factors affect the physiological processes of the plant and therefore, influence the grain yield, as well as the amount of water losses from the plants, and thus affect the WUEE (Ritchie and Basso, 2008). Conversely, Rafiee and Kalhor (2016) reported that WUEE showed an increase with increasing water deficit stress. Higher susceptibility of total dry matter than GY to water stress caused WUBE to be reduced by increasing water deficit stress (Zamani *et al.*, 2014). Under different irrigation conditions, inoculation with *F. mosseae* for improving the MDIBY was more effective than the *R. intraradices*. In severe and moderate water stress mycorrhizal dependence index based on grain (MDIGY) and biological (MDIBY) yield increased compared to optimal irrigation. The length of the hairy roots can be an indicator of the degree of mycorrhizal dependence. That is, short hairy roots show a higher degree of mycorrhizal dependence compared to long hairy roots. Due to the fact that water stress reduces the length of hairy roots, it can be justified to increase the mycorrhizal dependence with increasing water stress (Baylis, 1975). In investigating the mycorrhizal symbiosis in leek (*Allium porrum* L.) and two Iranian garlic chives (*Allium ampeloprasum ssp. Persicum* L.), the researchers stated that mycorrhizal dependence at all levels of water stress was more than normal (Ghasem Jovkar *et al.*, 2015). In our work, using two kinds of mycorrhizal fungi *F. mosseae* and *R. intraradices* led to increases in OHI and PHI in comparison to non-inoculated (control) conditions. Severe and moderate water stress decreased OHI and PHI compared to optimal irrigation conditions. It seems that the distribution of photosynthetic material to generate seed protein was constant in all different levels of irrigation (Habibzadeh *et al.*, 2012). In our research, severe water stress decreased TCSI, CSI_a and CSI_b compared to moderate water stress. Under moderate and severe water stress conditions, inoculation with mycorrhizal fungi of *F. mosseae* and *R. intraradices* increased TCSI, CSI_a and CSI_b compared to the non-inoculated plants. Our findings were consistent with those of other researchers mentioning that tolerant and moderately tolerant cultivars and hybrids showed a lesser reduction (6 and 12%) in CSI at 50 percent available soil moisture than control (Surendar *et al.*, 2013). Our findings were also consistent with the results of other researchers reporting that water stress decreased TCSI. The highest TCSI was obtained by "KFS2" genotype under normal irrigation and the lowest TCSI was related to "KFS17" genotype under severe water stress (Ghahramani *et al.*, 2015). During water stress, chlorophylls are decomposed in chloroplast and thylakoid structures disappeare. On the other hand, water stress disrupts enzyme systems of depleting active oxygen and increases the peroxidation of fats, resulting in damage to cell membranes and degradation of pigments (Ruiz-Sanchez *et al.*, 2011). Mycorrhizal plants tolerate oxidative stress induced by water stress that led to enhance the production of chlorophyll a and b (Pedranzani *et al.*, 2016). Also, it has been reported that mycorrhizal fungi by facilitating the absorption of elements such as nitrogen and magnesium (the main component of the molecular structure of chlorophyll), help to increase chlorophyll content (Moghaddasan *et al.*, 2015).

CONCLUSION

Based on our study, with increasing severe water stress, WUEE, WUBE, OHI, PHI, TCSI, CSI_a and CSI_b decreased but at the same time MDIGY and MDIBY increased. Inoculation with mycorrhizal fungi (*F. mosseae* and *R. intraradices*) compared to non-inoculated mycorrhizal plants led to improvement in WUEE, WUBE, OHI, PHI, TCSI, CSI_a and CSI_b. MDIGY and MDIBY in *F. mosseae* species were higher than *R. intraradices* species. Inoculated plants with *F. mosseae* species has been operating better than the *R. intraradices* species in enhancing WUEE, WUBE, OHI, PHI, TCSI, CSI_a and CSI_b. There was a positive correlation among WUEE with WUBE, PHI and TCSI. It seems that inoculation with mycorrhizal fungi especially *F. mosseae* assisted sesame in water stress tolerance by increasing water absorption and nutrients. Besides, inoculation with mycorrhizal fungi (measured by MDIGY and MDIBY) under drought stress caused improvement in the chlorophyll content (measured by TCSI). Increasing the chlorophyll content led to enhanced photosynthesis and promoted WUEE and WUBE. Improvement in the WUEE and WUBE increased oil and protein (measured by OHI and PHI) contents. Generally, increasing microbial population of the soil such as inoculation with mycorrhizal fungi that improve the root system can increase WUEE and WUBE in sesame plants.

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SUPPLEMENTARY DATA

Supplementary Table 1. Environmental conditions at the experimental site during 2-years of the study.

Parameter	Month											
	Apr.		May		June		July		Aug.		Sept.	
	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016	2015	2016
Highest temperature (°C)	17.05	15.18	21.4	22.94	29.48	26.58	33.53	31.28	34.35	32.56	28.50	30.13
Lowest temperature (°C)	3.01	3.42	8.6	9.14	12.26	10.99	17	16.13	15.54	15.95	12.66	11.92
Mean temperature (°C)	10.03	9.30	15	16.04	20.87	18.78	26.25	23.70	24.94	24.25	20.58	21.02
Sum of Rainfall (mm)	13.94	63.55	40.34	52.35	6.23	31.12	0	5.54	0	0	10.51	0.01
Sum of evapotranspiration (mm)	68.2	64.1	206.3	172.2	278	193.2	279.4	254.1	288.1	241.2	164.6	184.9
Mean relative humidity (%)	52.67	61.42	50.84	53.59	42.20	49.44	37.48	47.13	36.62	45.59	53.46	45.48

Supplementary Table 2. Fertilizer elements used in two years of testing.

Fertilizer	Year	Source fertilizer	Fertilizer rate (kg/ha)	Application time	Fertilizer use method
Nitrogen	2015	Urea	200	One third before planting - one third at 6-8 leaf - one third before flowering	Direct spraying in the soil
	2016	Urea	250	One third before planting - one third at 6-8 leaf - one third before flowering	Direct spraying in the soil
Sulfur	2015	Yellow Tio Bacillus	200	Before planting	Direct spraying in the soil
	2016	Yellow Tio Bacillus	300	Before planting	Direct spraying in the soil
Animal manure is completely rotten	2015	Cow manure	5000	Before planting	Direct spraying in the soil
	2016	Cow manure	7000	Before planting	Direct spraying in the soil
Zinc sulfate	2015	Zinc sulfate	20	Before planting	Direct spraying in the soil
	2016	Zinc sulfate	30	Before planting	Direct spraying in the soil

Supplementary Table 3. Some reported characteristics of the studied mycorrhizal species.

Name of mycorrhizal	Kingdom	Phylum	Class	Order	Family	Color of spore	Figure of spore	Dimeter of spore (μm)	Figure of hyphae
Glomus mosseae	Fungi	Glomeromycota	Glomeromycets	Glomerals	Glomeraceae	Brown-yellow	Irregular spherical	100-260	Irregular funnel
Glomus intraradices	Fungi	Glomeromycota	Glomeromycets	Glomerals	Glomeraceae	Pink-brown yellow	Irregular oval	40-140	Irregular cylindrical

Reference: (Smith and Read, 2008).

Supplementary Table 4. Chemical and physical properties of soil before planting in two years of testing from 0-30 cm depth.

Properties of soil	Units	2015	2016
Ec	Ds/m	1.18	1.99
pH	-	7.79	8.13
Saturation percentage	%	49	49
lime	%	16.8	16
clay	%	33	23
silt	%	50	50
sand	%	17	27
Organic carbon	%	1.16	0.78
Nitrogen	%	0.12	0.08
phosphorus	mg/kg	8.15	2.82
potassium	mg/kg	774	407
Soil field capacity	%	25.5	25.5
Soil texture	-	loam - clay loam	loam - clay loam