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Influence of Arbuscular mycorrhiza fungi and *Bradyrhizobium japonicum* on the fatty acids profile of soybean (*Glycine max* L.) under drought stress conditions

Naser Samsami[1], Ali Nakhzari Moghaddam[1], Ali Rahemi Karizaki[1], and Esmaeil Gholinezhad[2*]

[1]Department of Agronomy, Gonbad Kavous University, Gonbad Kavous, Iran
[2]Department of Agricultural Sciences, Payame Noor University, Tehran, Iran
*Corresponding author; e_gholinejad@pnu.ac.ir

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Abstract

Objective: Water deficiency changes the composition of soybean fatty acids. Arbuscular mycorrhiza fungi and *Bradyrhizobium* play a vital role in the improvement of soybean protein and oil. Therefore, this study aimed to evaluate the effects of different drought levels and inoculation/non-inoculation with mycorrhiza fungi and *Bradyrhizobium* on the soybean oil, protein percentage, and fatty acid composition.

Methods: This experiment was conducted a split-plot factorial layout based on a randomized complete block design with three replications in 2017. The main factor was irrigation after 70, 110, and 150 mm of evaporation from a class A evaporation pan. Subplots included mycorrhiza fungal inoculations with *Funneliformis mosseae, Rhizophagus intraradices,* and without fungus, and bacterium inoculation at two levels *(Bradyrhizobium japonicum* and without bacteria).

Results: Drought stress significantly reduced the oil percentage. The highest oil content (21.94%) was observed in *F. mosseae* mycorrhiza inoculation. The highest amount of unsaturated fatty acid (74.75%) was observed after inoculation with *R. intraradices* and bacteria under full irrigation conditions. Inoculation with mycorrhiza fungi and bacteria increased the unsaturated fatty acids in some cases.

Conclusion: In conclusion, to improve the oil and protein percent, enhance unsaturated fatty acids, and reduce saturated fatty acids in the soybean, the application of mycorrhiza fungi and inoculation with *B. japonicum* may be beneficial.

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Introduction

Soybean (*Glycine max* L.) is the most important oilseed crop in the world. Soybean is a nitrogen-fixing legume that is usually grown in rotation with cereals. Its importance in seed production is increasing due to its high yield potential and lower harvest costs compared to other seeds (Mesquita *et al*. 2007). However, drought stress, as one of the critical abiotic factors, affects soybean production, leading to significant reductions in yield and quality (Lobell *et al.*, 2011; Le *et al*. 2012). Oil content and fatty acid composition are modified by physiological, ecological, and planting conditions (Srivastava and Yadav 2024). Under drought conditions, soybean plants often experience alterations in physiological processes, which can negatively affect their fatty acid composition, particularly the balance between saturated and unsaturated fatty acids. According to Bellaloui *et al*. (2013), drought stress changes soybean fatty acid composition, particularly during the seed-filling period (R5-R6). These fatty acids are essential not only for the nutritional quality of soybean seeds but also for their market value (Wang *et al*. 2022). Studies have shown that drought conditions tend to increase the levels of saturated fatty acids while decreasing the levels of unsaturated fatty acids, which can negatively affect the nutritional quality of the seeds (Boyer 1982; Wang *et al*. 2022). This shift in fatty acid composition is often associated with changes in plant metabolic pathways, particularly those involved in lipid biosynthesis (Ezzati Lotfabadi *et al*. 2022). In addition, drought stress significantly impacts fatty acid synthesis, leading to reduced oil content in plants (Rezvani Moghaddam *et al*. 2014). Under severe water deficiency, both the total fatty acid content and composition decrease (Laribi *et al*. 2009). For instance, drought conditions have been shown to lower seed oil percentages and levels of oleic and linoleic acids, while increasing protein, palmitic, and stearic acid content in safflower seeds (Mohsennia and Jalilian 2012; Sehgal *et al*. 2018). According to Liu *et al*. (2008), changes in seed fatty acids occurred due to drought stress and intolerant cultivars had higher palmitic acid levels and lower levels of linolenic acid. Divsalar *et al*. (2017) indicated that drought severity increases linoleic acid levels while oleic and palmitic acid levels decline. However, the application of some substances, such as humic acid, alleviate the adverse effects of drought stress (Abhari and Gholinezhad 2019). Heshmati *et al*. (2007) reported that under water deficiency conditions during the reproductive stage, applying bio-fertilizers such as phosphorus fertilizer, can enhance unsaturated fatty acids and oil yield. The use of 50 kg ha^{-1} phosphorus fertilizer along with Phosphate Barvar 2, a biological fertilizer, resulted in a significant reduction in palmitic and stearic acid content.

Recent studies have highlighted the roles of mycorrhiza fungi and beneficial bacteria enhancing plant resilience to drought stress. Vatan Doost *et al*. (2018) reported the lowest levels of linolenic acid

(7.12%) and oleic acid (61.08%) in non-inoculated seedlings exposed to drought stress during seed formation. Conversely, the highest levels of palmitic acid (4.56%) and erucic acid (2.89%) under drought conditions without bacterial treatment. However, by applying the beneficial bacteria plant growth and stress tolerance enhance through mechanisms such as phytohormone production and soil health improvement, whereas mycorrhiza fungi improve nutrient and water uptake through their extensive hyphal networks (Smith and Read 2010; Vessey 2003). However, the specific effects of these microbial inoculants on the fatty acid profiles of soybean under drought stress remain poorly explored. The inoculation of soybean with mycorrhiza fungi and beneficial bacteria mitigate some adverse effects of drought stress. For instance, mycorrhiza fungi enhance the synthesis of unsaturated fatty acids by improving the plant's nutrient uptake and overall health (Smith and Read 2010). Similarly, beneficial bacteria can influence the fatty acid composition leading to a higher proportion of unsaturated fatty acids in seeds (Vessey 2003). Recent findings suggest that the synergistic effects of mycorrhiza fungi and beneficial bacteria may lead to an even greater improvement in fatty acid profiles under drought conditions. Moreover, the combined use of Azotobacter and Azospirillum with nitrogen fertilizers can enhance oil content in canola by improving soil characteristics and nutrient absorption (Hasanzadeh Ghorttapeh and Javadi 2016).

Despite the promising results of previous studies, several challenges and research gaps remain. There is a need for comprehensive studies that simultaneously evaluate the effects of mycorrhiza fungi and beneficial bacteria on plant physiology and fatty acid composition under drought stress. In addition, the specific mechanisms by which these microbial partners influence fatty acid biosynthesis in soybean plants are not yet fully understood. Research is also needed to determine the optimal combinations and application methods of these inoculants to maximize benefits. This research aimed to evaluate the effect of inoculation/non-inoculation with mycorrhiza fungi and *Bradyrhizobium* bacterium on soybean oil content, protein percentage, and fatty acid composition under different levels of water deficit.

Materials and Methods

Geographical conditions of the experimental site

The experiment was carried out at the Research Station of the Agricultural High School of Urmia, Iran in 2017. The station is located 12 km away from Urmia city, Iran (longitude: 37° 32′ E; latitude: 45° 2′ N; altitude: 1332 m above sea level). According to meteorological statistics, the area is part of arid and semi-arid climatic conditions with 150 to 180 dry days, cold and humid winters, and hot and dry summers. Some important meteorological features are listed in Table 1.

Some soil properties at the test site

To determine some characteristics of the soil at the experimental site and estimate the soybean fertilizer requirement, five soil samples were taken from the 0-30 cm depth. The soil analyses were carried out according to the literature (Walkley and Black 1934; Olsen 1954; Bouyoucos 1962; Allison and Moodie 1965; Thomas and Hargrove 1984; Bremner 1996; Haluschak 2006), and the results are presented in Table 2.

Experimental design and treatments

The experiment was performed as a split-plot factorial layout based on a randomized complete block design with three replications. The soybean cultivar used was Kowsar. The main factor was irrigation at three levels: normal (irrigation after 70 mm evaporation from class A evaporation pan), moderate drought stress (irrigation after 110 mm evaporation), and severe drought stress (irrigation after 150 mm evaporation). Subplots included the factorial combinations of mycorrhiza fungus at three levels (without mycorrhiza, *Funneliformis mosseae*, and *Rhizophagus intraradices*) and bacterium *Bradyrhizobium japonicum* at two levels (with and without inoculation with *Bradyrhizobium*).

Table 1. The mean monthly temperature, precipitation, evaporation, and humidity during the growing season of soybean in the studied area.

Meteorological parameters	Month					
	March	**April**	**May**	**June**	**July**	**August**
Maximum temperature (°C)	16	23	29	33.3	34.7	32.5
Minimum temperature (°C)	2.9	8	11	16	17	13
Mean temperature (°C)	9.45	15.5	20	24.65	25.85	22.75
Total precipitation (mm)	70.5	16	0.8	0	0.7	0
Total evaporation (mm)	62	160	246	304	281	213
Average humidity (%)	54	54	35	38	36	34

Table 2. Some soil physical and chemical characteristics in the experimental site.

Soil depth (cm)	Soil texture	EC (ds m^{-1})	pH -	C.C.E -	Saturation moisture (%)	Clay (%)	Silt (%)	Sand (%)	Organic carbon (%)	Nitrogen (%)	Phosphorus (mg kg^{-1})	Potassium (mg kg^{-1})
0-30	Loamy	0.85	7.64	23.8	32	40	35	25	1.15	0.12	5.16	211

Planting operations

The field preparation operations included plowing by moldboard tillage and leveling by a tiller. At this stage, based on fertilizer recommendations, 120 kg ha^{-1} potassium sulfate and 100 kg ha^{-1} sulfur were mixed with the soil. Plots and furrows were prepared according to the planting map. The seeds were planted in the second half of May 2017. The row spacing and plant-to-plant spacing were 50 cm and 10 cm, respectively. Each plot consisted of four rows of 4 m long. The distance between sub-plots was 2 m and for the main plots was 4 m. Seeds were sown and covered with 3 cm soil. Mycorrhiza fungi were prepared from Turan Biotech Co. in Shahroud, Iran. Their spores were examined by microscope. The mycorrhiza fungi spores were spread on a sterile culture bed with no living organisms. Inoculum was produced by the trap cultivation on plants such as clover or corn. The mycorrhiza inoculum included sterile sand, mycorrhiza hyphae, spores, and colonized root fragments. Mycorrhiza fungi (10 g) were put in the planting holes in the corresponding plots and then covered with 2 cm soil. The special biological fertilizer for soybean (*Bradyrhizobium* bacterium) was purchased by Mehr Asia Co. There were 10^8 bacteria in each gram of fertilizer.

Seeds were inoculated according to the recommendations on the package of biological fertilizers. Irrigation levels were applied from 2-4 leaf stage (seedling establishment). Thinning was carried out by hand at the 3-4 leaf stage to achieve an optimum density. Weed control was performed manually and twice during the growing season.

Measured traits

Seed yield: After removing the border rows and 50 cm from the ends of each row, the seed yield was measured from a 2 m^2 area in each plot. Harvesting was performed in the second half of September. The seeds of each plot were dried and weighed at 70 °C until a constant weight was achieved.

Oil and protein percentage: The percentage of seed oil was determined by using a Soxhlet oil extractor (Soxtherm 2000 automatic, Germany). Protein content was determined using the Kjeldahl apparatus (VAP 50, Germany) according to the following equations (Fazlara 2009):

$$N\% = \frac{The\ amount\ of\ acid\ used\ in\ titration \times 0.0014}{Sample\ weight} \times 100$$

$$\text{Protein (\%)} = N\% \times 5.63 \text{ (Sosulski and Holt 1980)}$$

Oil and protein yield: By multiplying the oil percentage by the seed yield, the oil yield was calculated. Also, the protein yield was calculated by multiplying the protein percentage by the seed yield.

Fatty acids' profile: The oil samples were initially homogenized with Vertex and 100 mg of each sample was weighed carefully. The fat was then converted to methyl ester by adding 3 ml of methanol potassium hydroxide (2 mM) and then 5 ml of methanol sulfuric acid (12% v/v). The methyl ester was extracted with 1 ml of normal heptane and injected into a gas chromatography (GC) apparatus to analyze the 1 µl of the normal heptane phase of the fatty acid profile. Sigma Company standard fatty acid mixture was used to identify individual fatty acids by comparing the inhibition times. An Agilent 6890N GC apparatus (Agilent Technologies, Wilmington, DE, USA) equipped with an FID detector and split/splitless injector was used for sample analysis. Separations were performed using an HP-88 capillary column (88% - Cyanopropy) aryl-polysiloxane, 100 m length, 0.25 mm i.d., 0.2 µm film thickness (Agilent). ChemStation software was used to process the data. The oven temperature was programmed as follows: 5 min at 140 °C, subsequently increased 4 ˚C per minute to reach 240 ˚C and held for 15 min at 240 ˚C. Nitrogen was used as the carrier and make-up gas, and flow rates were 1 ml per min and 45 ml min, respectively. The temperatures of the injection port and detector were set at 260 ˚C and 280 ˚C, respectively. The injector was set in a split mode (split ratio of 1:30) (AOAC 2000; Barthet *et al*. 2002).

Analysis of variance was carried out after testing for the homoscedasticity of variances with the Bartlett test. Means were compared by the LSD method at the 5% probability level. Statistical analyses were done by SAS software (version 9.1).

Results

The results the analysis of variance revealed that oil and protein percentage, seed yield, oil yield, protein yield, and myristic, palmitic, arachidic, behenic, oleic, and linolenic acids were significantly affected by the irrigation. The effect of mycorrhiza fungi on oil and protein percentage, seed yield, oil yield, protein yield, and palmitic, stearic, arachidic, behenic, palmitoleic, oleic, linolenic, and total unsaturated fatty acids were significant. The effect of the bacterium significantly influenced oil and protein percentage, seed yield, oil yield, protein yield, and myristic, stearic, arachidic, behenic, palmitoleic, oleic, linoleic, linolenic, and total unsaturated fatty acids. The interaction of irrigation × mycorrhiza fungi was significant for protein percentage, protein yield, and myristic, palmitic, stearic, arachidic, behenic, oleic, total saturated, and total unsaturated fatty acids (Table 3). The interaction of irrigation × bacterium significantly influenced seed yield, oil yield, protein yield, and palmitic, stearic, arachidic, behenic, oleic, linoleic, linolenic, and total saturated and total unsaturated fatty acids. The interaction of bacterium × mycorrhiza fungi was significant on seed yield, oil yield, arachidic, behenic, oleic, and total unsaturated fatty acids. The irrigation × mycorrhiza fungi × bacterium interaction

significantly affected palmitic, arachidic, behenic, oleic, linoleic, linolenic, total saturated, and total unsaturated fatty acids (Table 3).

Table 3. Analysis of variance of irrigation and inoculation with mycorrhiza and bacteria for oil content, protein content, seed yield, oil yield, protein yield, and fatty acid composition in soybean.

Source of variation	Mean squares										
	df	% oil	% protein	Seed yield	Oil yield	Protein yield	Myristic acid methyl ester (C14:0)	Palmitic acid methyl ester (C16:0)	Stearic acid methyl ester (C18:0)	Arachidic acid methyl ester (C20:0)	Behenic acid methyl ester (C22:0)
Block	2	19.05	33.06	2960	384.1	1745	0.0008	4.03	3.14	0.14**	0.005
Irrigation (I)	2	5.05**	527.1**	37254**	1990**	17711**	0.0004*	10.88**	0.088	0.023**	0.034**
Error a	4	1.11	13.50	911.4	69.87	575.4	0.0003	1.65	0.48	0.001	0.003
Mycorrhiza (M)	2	12.66**	44.50**	117.24**	1309**	3774**	0.0001	1.46*	2.39**	0.058**	0.048**
Bacteria (B)	1	48.16**	78.62**	66704 **	6455**	14317**	0.001**	0.0001^{ns}	3.47**	0.11**	0.103**
I × M	4	0.88	3.25*	199.7	25.38	212.2**	0.0003*	2.45**	1.06**	0.008**	0.015**
I × B	2	0.38	0.75	628.5*	62.89*	236.9**	0.00001	1.70*	0.53*	0.008**	0.009**
B × M	2	0.22	1.29	1001**	82.2*	93.63	0.00002	0.17	0.39	0.014**	0.016**
I × M × B	4	0.61	0.71	301	33.77	55.48	0.0001	1.14*	0.30	0.002**	0.007**
Error b	30	0.60	1.13	177.3	15.39	32.04	0.0001	0.42	0.12	0.0004	0.001
C.V. (%)	-	3.67	3.23	3.55	4.91	4.51	13.53	6.30	11.09	5.80	13.59

*,**: Significant at 5% and 1% probability levels, respectively.

Table 3. Continued

Source of variation	df	Mean squares					
		Saturated fatty acids	Palmitoleic acid methyl ester (C16:1)	Oleic acid methyl ester (C18:1n9c)	Linoleic acid methyl ester (C18:2n6c)	Linolenic acid methyl ester (C18:3n3)	Unsaturated fatty acids
Block	2	15.12	0.001	2.08	297.2	5.17	349.6
Irrigation (I)	2	7.57	0.0007	70.01**	214.4	22.17*	327.0
Error a	4	2.50	0.0002	1.42	45.16	2.06	68.26
Mycorrhiza (M)	2	9.04**	0.0006**	7.52*	48.78^{ns}	3.93**	125.9**
Bacterium (B)	1	1.56	0.0015**	32.34**	264.3**	7.01**	175.8**
I × M	4	5.67**	0.00004	48.35**	31.52	0.78	146.6**
I × B	2	3.39*	0.00001	41.58**	69.48*	5.07**	254.9**
B × M	2	1.23	0.00001	47.63**	46.34	0.24	166.7**
I × M × B	4	2.20*	0.0001	31.33**	47.11*	1.56**	126.9**
Error b	30	0.78	0.0001	2.03	16.36	0.35	22.64
C.V. (%)	-	6.26	11.87	9.21	15.35	6.67	9.37

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

Oil percentage and yield

Seed oil percentage decreased by drought stress. The highest (21.72%) and lowest amount (20.66%) of oil percent were obtained from normal irrigation and moderate drought stress, respectively. Inoculation with mycorrhiza fungi and bacterium enhanced the oil content. The maximum amount of oil (21.94%) was observed in plants inoculated with *F. mosseae*. The highest (22.11%) and lowest oil percentage (20.22%) were seen in bacterium inoculation and non-bacterium inoculation, respectively. Inoculation with *F. mosseae* compared to non-inoculation of mycorrhiza increased seed oil content by 8.24%. Inoculation with bacterium increased seed oil content by 9.35% compared to non-inoculation conditions (Figure 1A, 1B, 1C). Severe and moderate water deficit reduced oil yield by 24% and 9%, respectively, compared to optimal irrigation (Table 4).

The highest oil yield (100.95 g m^{-2}) was obtained from the interaction of optimal irrigation with inoculation with the bacterium. The lowest oil yield (59.77 g m^{-2}) was obtained from the combination of severe water deficit and no inoculation with bacterium (Table 4). In normal irrigation, moderate drought stress and severe drought stress conditions, inoculation with bacterium increased oil yield by 23%, 27%, and 23%, respectively (Table 4). Also, the highest oil yield (101.54 g m^{-2}) was obtained from *F. mosseae* and bacterium inoculation. The lowest oil yield (61.50 g m^{-2}) was seen in the treatment with no mycorrhizal and bacterial inoculation (Table 5). Inoculation with *F. mosseae* and *R. intraradices* enhanced oil yield by 20% and 12%, respectively, compared to lack of mycorrhiza inoculation (Table 5).

Protein percentage and yield

Inoculation with bacterium rose protein content by approximately 7.6% compared to non-inoculation conditions (Figure 1D). The combination of irrigation and mycorrhiza fungi indicated that mild drought stress increased the protein content, but severe drought stress decreased it. The maximum protein percentage (40.43%) was derived from the combination of moderate stress and inoculation with *F. mosseae*. The lowest protein content (26.71%) was obtained following the treatment with severe drought stress without mycorrhiza inoculation. Moderate drought stress increased protein content by 38.15% compared to optimum irrigation. Severe drought stress reduced protein content by 12.13% compared to optimum irrigation (Figure 2A). Also, severe drought stress reduced protein yield by 31% compared to optimal irrigation (Table 4). The highest protein yield (173.14 g m^{-2}) was obtained from the moderate water deficit under inoculation with F. mosseae (Table 4). The lowest protein yield (80.85 g m^{-2}) was also obtained from the treatment of severe drought stress without mycorrhiza

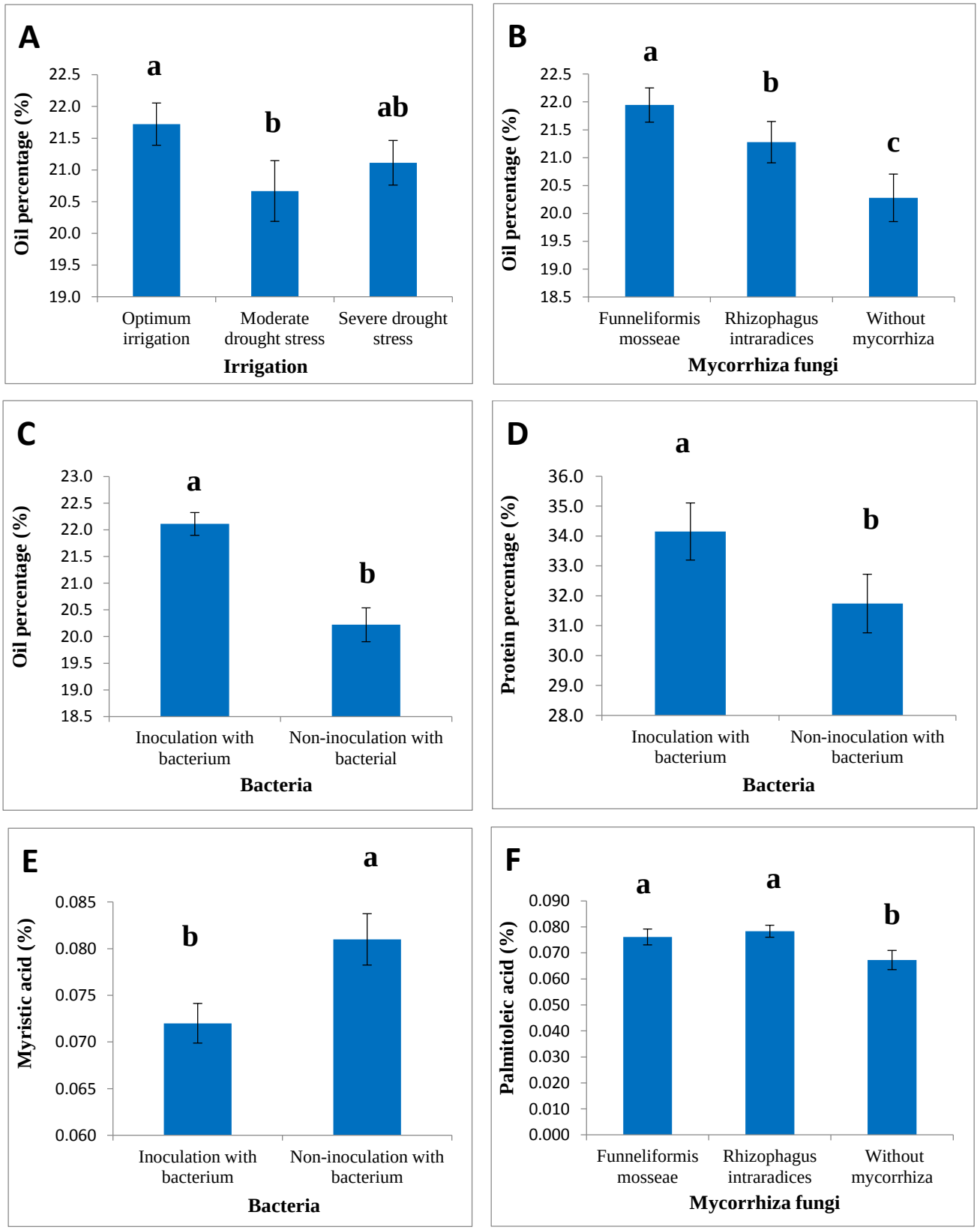

Figure 1. The effects of irrigation on oil percentage (A), mycorrhiza fungi on oil percentage (B) and palmitoleic acid (C), bacterium on oil percentage (D), protein percentage (E), and myristic acid (F); Means followed by the same letter are not significantly different at 5% probability level.

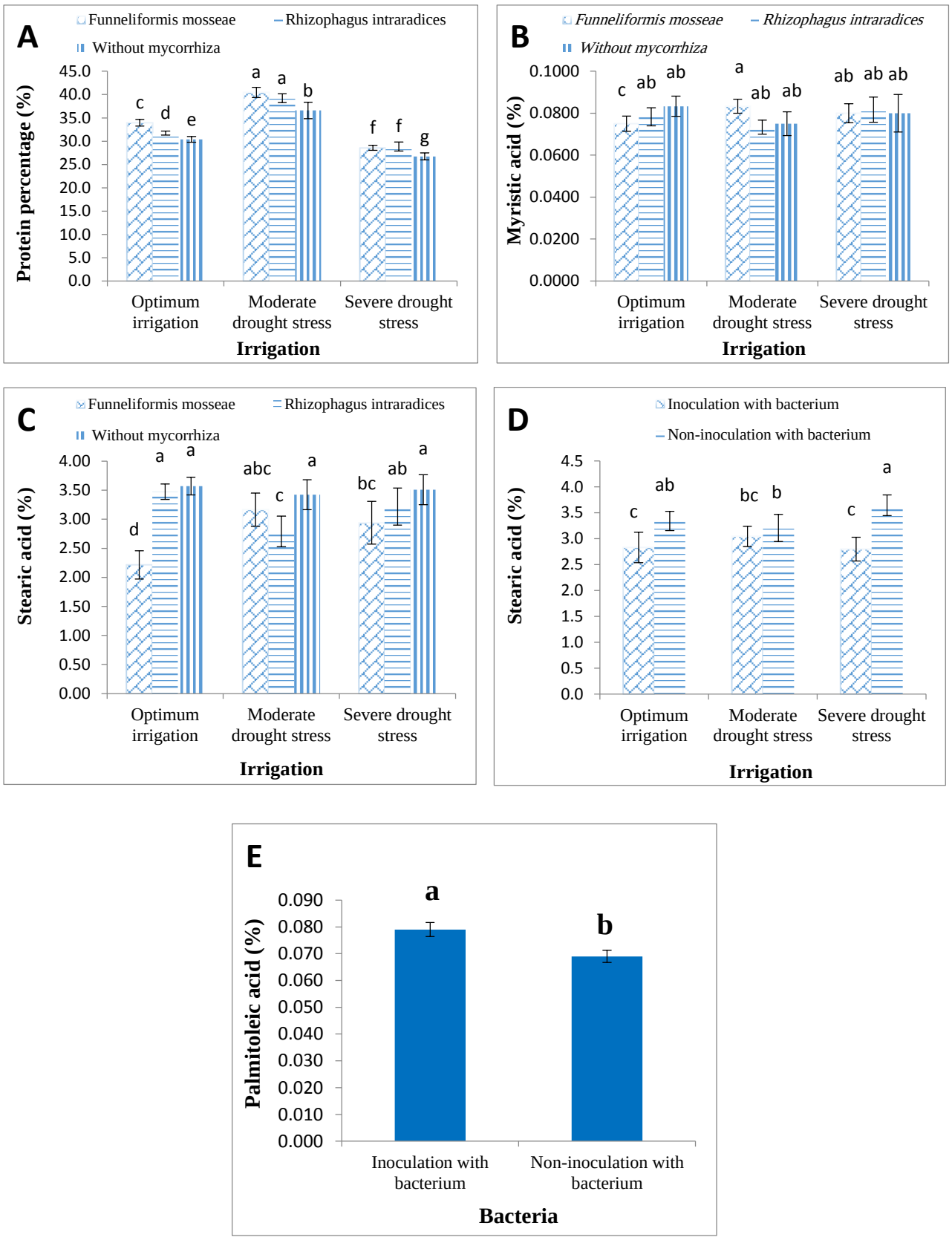

Figure 2. The effect of bacteria on palmitoleic acid (A), interaction effects of irrigation with mycorrhiza on protein percentage (B), myristic acid (C), stearic acid (D), and interaction effects of irrigation with bacterium on stearic acid (E); Means followed by the same letter are not significantly different at 5% probability level.

Table 4. Effects of combination of irrigation (I) with bacterium (B on seed yield, oil yield, and protein yield.

Treatment I×B	Seed yield (g m^{-2})	Oil yield (g m^{-2})	Protein yield (g m^{-2})
$I_1 \times B_1$	446.78 a	100.95 a	148.00 b
$I_1 \times B_2$	372.44 c	77.91 c	115.75 d
$I_2 \times B_1$	431.11 b	94.01 b	173.14 a
$I_2 \times B_2$	351.55 d	69.13 d	132.76 c
$I_3 \times B_1$	351.77 d	77.45 c	103.77 e
$I_3 \times B_2$	294.79 e	59.77 e	78.70 f
$LSD_{5\%}$	12.80	3.77	5.44

I_1= Optimum irrigation, I_2= Moderate drought stress, I_3= Severe drought stress; B_1= Inoculation with bacterium, B_2= Non-inoculation with bacterium; Means with the same letter(s) in each column are not significantly different based on the LSD test (5% probability level).

Table 5. Effects of combination of mycorrhiza fungi with bacterium on seed yield and oil yield.

Treatment M×B	Seed yield (g m^{-2})	Oil yield (g m^{-2})
$M_1 \times B_1$	443.23 a	101.54 a
$M_1 \times B_2$	355.80 d	74.74 d
$M_2 \times B_1$	407.77 b	90.16 b
$M_2 \times B_2$	344.58 d	70.58 e
$M_3 \times B_1$	378.66 c	80.71 c
$M_3 \times B_2$	318.40 e	61.50 f
$LSD_{5\%}$	12.80	3.77

M_1= *Funneliformis mosseae*, M_2= *Rhizophagus intraradices*, M_3= without mycorrhiza; B_1= Inoculation with bacterium, B_2= Non-inoculation with bacterium; Means with the same letter(s) in each column are not significantly different based on the LSD test (5% probability level).

(Table 4). In all three different irrigation conditions, bacterium inoculation increased protein yield compared to non-inoculation (Table 4). Under full irrigation, and moderate and severe drought stresses, bacterium inoculation increased protein yield by 22%, 25%, and 24%, respectively (Table 4).

Seed yield

Severe and moderate water stress decreased seed yield by 22% and 5%, respectively, compared to optimal irrigation. The highest seed yield (446.78 g m^{-2}) was obtained at optimal irrigation in the presence of bacterium inoculation. The lowest seed yield (294.79 g m^{-2}) was observed from the treatment of severe drought stress without bacterial inoculation (Table 4). In favorable irrigation conditions, and mild and severe drought stresses, bacterium inoculation enhanced seed yield by 17%,

19%, and 17%, respectively (Table 4). Also, according to Table 5, the highest seed yield (443.23 g m^{-2}) was obtained from the inoculation with *F. mosseae* and bacterium. The lowest seed yield (318.40 g m^{-2}) was associated with the absence of mycorrhiza and bacterium inoculation (Table 5). Inoculation with *F. mosseae* and *R. intraradices* enhanced seed yield by 13% and 8%, respectively, compared to the non-inoculation of mycorrhiza fungi (Table 5).

Myristic acid

Bacterium inoculation reduced myristic acid by 12.5% compared to its corresponding control (non-inoculation) (Figure 1E). Also, the maximum amount of myristic acid (0.083%) was related to the interaction between moderate stress and inoculation with *F. mosseae*. The lowest myristic acid (0.060%) was obtained at optimum irrigation together with *F. mosseae* inoculation (Figure 2C). With a rising water deficit, myristic acid increased significantly in the presence of *F. mosseae*. (Figure 2B).

Palmitic acid

The highest amount of palmitic acid (11.74%) was obtained from the interaction between moderate drought stress and inoculation with *F. mosseae* and bacterium. The lowest palmitic acid (8.75%) was related to the treatment of optimum irrigation together with the inoculation by *F. mosseae* and bacterium (Table 6). Under optimum irrigation conditions, inoculation of *F. mosseae* alone (without bacterium inoculation) led to a relative increase in palmitic acid content compared with the combination of inoculation by *F. mosseae* and bacterium. Under severe drought stress, there were no significant differences among all treatments in terms of palmitic acid (Table 6).

Stearic acid

The minimum and maximum stearic acid levels of 2.21% and 3.57% were obtained from optimum irrigation × *F. mosseae* and optimum irrigation × non-inoculation with mycorrhiza fungi, respectively (Figure 2C). The maximum amount of stearic acid (3.64%) was related to the non-inoculation of the bacterium under severe drought stress, whereas the lowest (2.79 %) was observed in the bacterium inoculation under severe drought stress (Figure 2D).

Arachidic acid

Our findings of this research showed that the maximum amount of arachidic acid (0.57%) was obtained from the treatment of severe drought stress × non-inoculation with mycorrhiza fungi × bacterium inoculation. The lowest arachidic acid content (0.25%) was associated with three treatments, such as

Table 6. Effects of combination of irrigation, inoculation with mycorrhiza fungi, and bacterium on fatty acid composition.

	Palmitic acid (%)			Arachidic acid (%)			Behenic acid (%)			Saturated fatty acid (%)		
M×B	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**
$M_1 \times B_1$	8.75f	11.74a	9.36ef	0.41c	0.31fgh	0.37d	0.32bc	0.24efg	0.31cd	11.25i	15.48ab	12.38hi
$M_1 \times B_2$	9.95de	10.64bcd	9.92de	0.34def	0.26ij	0.28hij	0.26def	0.16hi	0.24efg	13.35fgh	14.39a-f	14.16b-f
$M_2 \times B_1$	10.82a-d	11.40ab	9.22ef	0.29ghi	0.28hij	0.32efg	0.20ghi	0.23efg	0.25efg	14.85a-e	14.69a-f	12.58ghi
$M_2 \times B_2$	10.79a-d	10.21cde	9.97de	0.25j	0.25j	0.25j	0.15hi	0.21fgh	0.21fgh	14.76a-f	13.62e-f	14.26b-f
$M_3 \times B_1$	11.58ab	11.13abc	9.26ef	0.47b	0.35de	0.57a	0.37b	0.25efg	0.55a	15.82a	15.14a-d	13.90c-g
$M_3 \times B_2$	10.90a-d	11.39ab	9.51ef	0.31fgh	0.29ghi	0.33ef	0.23efg	0.21fgh	0.27cde	15.36abc	15.49ab	13.77d-h
$LSD_{5\%}$	1.087			0.032			0.057			1.478		

M_1= *Funneliformis mosseae,* M_2= *Rhizophagus intraradices,* M_3= without mycorrhiza; B_1= Inoculation with bacterium Non-inoculation with bacterium*;* I1 = Optimum irrigation, I2= Moderate drought stress, I3= Severe drought stress; Means with the same letter(s) in each column are not significantly different based on the LSD test (5% probability level).

Table 6. Continued

	Oleic acid (%)			Linoleic acid (%)			Linolenic acid (%)			Unsaturated fatty acid (%)		
M×B	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**	**I1**	**I2**	**I3**
$M_1 \times B_1$	9.21d	14.79bcd	13.84cd	27.35bcd	31.56 b	24.21cd	9.98a	10.12a	8.32b	46.63cd	56.55b	46.45cd
$M_1 \times B_2$	15.25bcd	12.34cd	24.94a	26.86bcd	24.31cd	23.93cd	10.17a	7.17de	8.19bc	52.37bc	43.90d	57.13b
$M_2 \times B_1$	21.71ab	15.47bcd	13.93cd	42.78a	30.26bc	23.06d	10.17a	10.47a	8.17bc	74.75a	56.29b	45.25cd
$M_2 \times B_2$	17.56abc	11.26cd	17.48abc	24.88bcd	22.60d	24.24cd	10.20a	8.45b	8.08bcd	52.72bc	42.39d	49.88bcd
$M_3 \times B_1$	14.27bcd	12.04cd	17.22bc	30.51bc	25.47bcd	21.76d	10.38a	8.86b	6.55e	55.25b	46.45cd	45.61cd
$M_3 \times B_2$	16.26bcd	14.62bcd	16.68bcd	27.17bcd	22.08d	21.05d	8.94b	8.13bcd	7.22cde	52.45bc	44.90cd	45.02cd
$LSD_{5\%}$	2.373			6.737			0.985			7.925		

M_1= *Funneliformis mosseae,* M_2= *Rhizophagus intraradices,* M_3= without mycorrhiza; B_1= Inoculation with bacterium Non-inoculation with bacterium*;* I1 = Optimum irrigation, I2= Moderate drought stress, I3= Severe drought stress; Means with the same letter(s) in \each column are not significantly different based on the LSD test (5% probability level).

the combination of inoculation with *R. intraradices* fungi and non-inoculation with the bacterium under all irrigation conditions. In addition, under optimum irrigation conditions and moderate and severe drought stresses, bacterium inoculation reduced significantly arachidic acid content compared to non-inoculation with the bacteria (Table 6).

Behenic acid

The maximum amount of behenic acid (0.55%) was obtained from the interaction of severe drought stress × bacterium inoculation × non-inoculation with mycorrhiza fungi. The lowest behenic acid (0.15%) was derived from the treatment of optimum irrigation × inoculation with *R. intraradices* × non-inoculation with the bacterium. Under three irrigation levels, the rate of inoculation with *R. intraradices* × non-inoculation with the bacterium was lower than that of some other treatments. Under

optimum irrigation, moderate drought, and severe drought conditions; inoculation with the bacterium increased behenic acid levels in most cases compared with non-inoculation conditions (Table 6).

Saturated fatty acids

The maximum amount of saturated fatty acid was obtained from the interaction of full irrigation × non-inoculation with mycorrhiza fungi × bacterium inoculation (15.82%), but it was not significantly different from eight other treatments. The lowest saturated fatty acid was related to the treatment of optimum irrigation × inoculation with *F. mosseae* × bacterium inoculation (11.25%) followed by two other treatments. In most cases, non-inoculation with mycorrhiza fungi had higher values than other treatments under three levels of irrigation (Table 6).

Palmitoleic acid

Inoculation with mycorrhiza fungi significantly increased the palmitoleic acid. The maximum amount of palmitoleic acid (0.078% and 0.076%) was obtained from *R. intraradices* and *F. mosseae*, respectively. In these treatments, palmitoleic acid content was increased approximately 16.42 and 13.43%, respectively (Figure 1F). Palmitoleic acid content was higher (16.18%) in bacteria inoculation than in non-inoculation treatment (Figure 2E).

Oleic acid

The maximum amount of oleic acid (24.94%) was related to the interaction of severe drought stress × *F. mosseae* × non-inoculation with the bacterium. The lowest amount (9.21%) was obtained from the treatment of optimum irrigation × *F. mosseae* × bacterium inoculation. Moderate drought stress reduced oleic acid content compared to optimum irrigation, except for the *F. mosseae* × bacterium inoculation (Table 6).

Linoleic acid

Linoleic acid was higher (42.78%) in the treatment of optimum irrigation × inoculation with *R. intraradices* × bacterium inoculation than other treatments. The lowest linoleic acid (21.05%) was derived from the treatment of severe drought stress × non-inoculation with mycorrhiza fungi × non-inoculation with the bacterium, but it was not significant from 13 other treatments. In most cases, moderate and severe drought stresses reduced the linoleic acid content compared to optimum irrigation. Bacterium inoculation enhanced the linoleic acid compared to non-inoculation in most treatments.

Also, inoculation with *F. mosseae* and *R. intraradices* raised linoleic acid content in most instances (Table 6).

Linolenic acid

The results indicated that the maximum amount of linolenic acid (10.38%) was derived from the treatment of optimum irrigation × non-inoculation with mycorrhiza fungi × bacterium inoculation, which was not significantly different from six other treatments. The lowest level of linolenic acid (6.55%) was associated with severe drought stress × non-inoculation with mycorrhiza fungi × bacterium inoculation. With increasing drought stress, linolenic acid levels decreased. In most cases, inoculation with *F. mosseae* and *R. intraradices*, along with bacterium inoculation, enhanced the linolenic acid content compared with non-inoculation by mycorrhiza fungi and bacteria (Table 6).

Unsaturated fatty acids

The maximum amount of unsaturated fatty acid (74.75%) was obtained from the interaction of optimum irrigation × inoculation with *R. intraradices* × bacterium inoculation. The lowest unsaturated fatty acid (42.39%) was related to the treatment of moderate drought stress × inoculation with *R. intraradices* × non-inoculation with the bacterium. Moderate and severe drought stress reduced unsaturated fatty acid levels compared to optimum irrigation, except in the presence of *F. mosseae*. Inoculation with *F. mosseae* and *R. intraradices* increased unsaturated fatty acid content at moderate stress conditions under bacterium inoculation. However, at the severe drought stress, inoculation with *F. mosseae* and *R. intraradices* increased unsaturated fatty acid content under no bacterium inoculation. Bacterium inoculation enhanced unsaturated fatty acid content when accompanied with *R. intraradices* at the moderate drought stress conditions and with *F. mosseae* at the severe stress conditions, compared with the non-inoculation state (Table 6).

Discussion

Recently, inoculation with mycorrhiza fungi and bacteria has been indicated to have considerable advantages for soybean growth and yield in field trials under drought stress conditions (Igiehon *et al*. 2021). However, several studies have focused mostly on the evaluation of the quantity and quality of soybean yield. In the present study, not only yield but also the impact of inoculation with mycorrhiza fungi and bacteria on the oil, protein, and fatty acid profiles of soybean under drought stress conditions was investigated. In line with our research findings, other reports have also shown that treatments with arbuscular mycorrhiza fungi and *B. japonicum* improved the growth and yield of soybean plants under

both drought stress and normal water conditions compared with untreated plants (Sheteiwy *et al*. 2021). It was reported in a study that dual inoculation of soybean plants with beneficial microbes reduces water stress effects, thereby allowing normal plant growth under drought stress conditions (Ashwin *et al*. 2023).

Protein content is a quality trait that increases under water-deficit stress. Moderate water deficit stress enhanced protein content in mycorrhiza fungi, whereas severe drought stress reduced protein content. However, at all irrigation conditions, inoculation with mycorrhiza fungi increased the protein percentage. Drought stress caused by closing the stomata and reducing photosynthesis results in the transfer of less assimilates to the seeds and decreases seed yield. In addition, drought stress may have affected nitrogen fixation and eventually reduced seed yield and protein percentage. These findings are consistent with the results of Navabpour *et al*. (2017). In our study, under water-deficit stress conditions, an increase in protein percentage did not compensate for the decrease in seed yield, resulting in a decrease in protein yield. In the results of other researchers, the highest and lowest yields of seed protein were obtained under normal irrigation conditions and severe drought stress, respectively (Rostami Ajirloo *et al*. 2022).

Based on our research, oil content decreased under drought stress. Inoculation with mycorrhiza fungi and bacteria significantly enhanced oil content. Navabpour *et al*. (2017) also reported that the oil percentage decreased with increasing drought stress and reported a negative relationship between oil percentage and protein. The decrease in oil content and increase in protein content under drought stress (moderate drought stress in our experiment) can be explained by reducing the moisture requirement of the plant and shortening the filling period of the seeds. In addition, oil fills more in seeds under full irrigation conditions than compared to water-deficit conditions. The results of the present study and other research findings were coordinated (Purdehgan *et al*. 2015). In summary, drought stress reduced oil yield. The large decrease in oil yield was due to the effect of water-deficit stress on the capacity of seeds to accumulate oil, reducing the percentage of seed oil, and decreasing seed yield. Some studies have shown that oil yield decreases with increasing of drought stress (Ezzati Lotfabadi *et al*. 2022).

At the severe stress conditions, the palmitic acid content increased significantly or did not change significantly. In this study, severe drought stress enhanced behenic (only in the presence of bacterium), stearic, and myristic acid (only in the presence of *F. mosseae*) compared with optimum irrigation. In a study, drought stress was not found to have a significant effect on the fatty acid composition, except for palmitic acid (Zarei *et al*. 2010). According to researchers, changes in the enzyme activity's involved in the synthesis of fatty acids due to drought stress are the main cause of the changes in the amount of fatty acids (Bellaloui *et al*. 2013). Tohidi-Moghaddam *et al*. (2011) by applying drought

stress from the flowering stage showed a reduction in the percentage of saturated fatty acids in the seed oil (stearic acid and arachidic acid) in six rapeseed cultivars, which could be attributed to shortening the plant growth period under stress conditions

In this study, moderate drought stress increased saturated fatty acids (only in the presence of *F. mosseae* and bacterium, whereas severe drought stress decreased saturated fatty acids in some cases. Water deficit and irregular irrigation increase the percentage of saturated fatty acids but access to water increases the rate of unsaturated fatty acids (Seyed Sharifi 2016).

Based on this research, increasing drought stress reduced the linoleic, linolenic, and unsaturated fatty acid contents in most cases. At moderate drought stress, oleic acid level decreased, but at severe drought stress it increased in some cases. Tohidi Moghadam *et al*. (2011) reported that drought stress reduced rapeseed oil and linoleic acid content. In another study, drought stress increased unsaturated fatty acid content, such as linoleic, linolenic, and oleic acids (Ayoubizadeh *et al*. 2018). In addition, under drought stress conditions, the ratio of saturated to unsaturated fatty acids increased in the seed oil of canola cultivars (Tohidi-Moghaddam *et al*. 2011).

In our research, inoculation with mycorrhiza fungi compared with non-inoculation reduced myristic (only *F. mosseae*), behenic, and saturated fatty acids, while increased palmitoleic, oleic, linoleic, linolenic, and unsaturated fatty acids (in some cases). Inoculation with bacterium also reduced behenic and saturated fatty acids (in some cases), while increased myristic, palmitoleic, oleic, linoleic, linolenic, and unsaturated fatty acids (in some cases). In a study, the stearic acid content decreased under inoculation with *F. mosseae* but increased under non-inoculation conditions (Ghasemi *et al.* 2023). Growth-promoting bacteria reduces the amount of saturated fatty acids (palmitic and stearic acids) and increases unsaturated fatty acids (oleic, linoleic, and linolenic acids) (Seyed Sharifi 2016). Nosheen *et al*. (2013) reported that inoculation with *Azospirillum* bacterium increased oleic and linoleic acid levels but reduced erucic acid in rapeseed. Vatan Doost *et al*. (2018) indicated that the maximum amount of oleic acid and linolenic acid was observed in inoculation with *Azospirillum* and complete irrigation They stated that the application of biofertilizer with triple superphosphate fertilizer during the drought stress at the reproductive stage can be positive on unsaturated fatty acids. Drought stress and nutrient deficiencies in *Pseudomonas aureofaciens* and *Arthrobacter protophormiae* increased the ratio of saturated to unsaturated fatty acids, and raised the ratio of trans fatty acids to cis during 16 days of incubation (Kieft *et al*. 1997).

Conclusion

It can be concluded that seed yield, oil and protein content, and the composition of fatty acids in soybean were affected by water deficit and inoculation with mycorrhiza fungi and bacteria. In three different irrigation conditions, inoculation with *Bradyrhizobium japonicum* bacterium increased seed yield, protein and oil content, and palmitoleic acid and decreased stearic and myristic acids compared with non-inoculation conditions. Mycorrhiza fungi also enhanced seed yield, oil yield, oil content, and protein content. Under moderate drought stress protein content increased significantly, but at the severe drought stress conditions decreased significantly. Under water deficit stress conditions, the oil yield and percentage decreased. In all three irrigation conditions, using mycorrhiza fungi appeared to increase protein percentage and unsaturated fatty acids, and reduce saturated fatty acids in some cases in soybean.

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Ethical considerations

The authors avoided data fabrication and falsification.

Conflict of interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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