Influences of Water Deficit Stress and Symbiosis with Growth-Promoting Bacteria on Seed Biochemistry of *Camelina sativa*

ABSTRACT

Camelina sativa, being a flowering plant in the family Brassicaceae, is traditionally cultivated as an oilseed crop due to its exceptionally high level (up to 45%) of omega-3 fatty acids, which is uncommon in vegetable sources. In the recent years camelina has been growing more than before because of its potentiality in the production of biodiesel and bioproduct. An aforementioned experiment was designed in three irrigation levels (100%, 75% and 50% of field capacity) and *Micrococcus yunnanensis* was used as plant growth-promoting bacteria during the reproductive phase. The bacteria were incubated in a nutrient broth medium at the temperature of 28°C for 24h on shaker ceaselessly. When sowing, seeds were treated with 1.0 ml bacteria suspension in the inoculated groups. Water stress was applied to each pot in the levels of 75 and 50 % FC from the budding stage to full maturity in generative phase. Camelina silique yield was harvested at the maturity stage for analysis of seed quality and biochemical responses. Total soluble carbohydrate was extracted thrice from 100 mg of mature seed using extraction soluble including glacial acetic acid, methanol, and water. Oil and protein content were measured using Near-Infrared Reflectance spectrometer. Seed fatty acid contents were determined using gas chromatography. The results showed a significant relation between the highest proportion of fatty acid and the polyunsaturated fatty acid (55.12 to 65.66%) in particular linolenic acid. The increase of polyunsaturated fatty acid and saturated fatty acid was coincided with the decrease of monounsaturated fatty acid under water deficit stress. The application of plant growth-promoting bacteria is proven to increase protein with 50% of field capacity. In general, water deficit stress and plant growth-promoting bacteria have significant effects on the remobilization of nutrients from the soil to developing seed and following metabolism synthesis.

Keywords: Drought, **Irrigation**, *Camelina*, Plant growth-promoting bacteria (PGPB), *Micrococcus*, Seed, Biochemistry

1. INTRODUCTION

Water deficit stress in plants, being the insufficiency of water to maintain plant growth, photosynthesis, and transpiration, is a major abiotic threat impacting directly gene expression, cell metabolism, physiology, morphology, and biochemistry features, resulting in the reduction of plant growth, and consequently leading to poor crop productivity (Reddy et al., 2004; Guo et al., 2018; You et al., 2019). Based on the studies flowering and seed filling stages have been recognized to be most susceptible stages to water deficit stress (Sehgal et al., 2018). It is well documented that plant growth–promoting bacteria (PGPBs) is an all-purpose option in the growth, development, and increase of the crop yield; besides, PGPBs handles biotic and abiotic stress-induced climacteric conditions. Some reports have shown the significant effects of PGPB on physical (weight and number of seeds), and chemical (oil, protein, and mineral elements) characteristics of oilseeds (Olivera et al., 2018; Etesami and Maheshwari, 2018).

Camelina sativa L. Crantz (or false flax), being an oilseed crop belonging to the Brassicaceae family, has a notable potency of cultivation in different climates in all over the world. Great agronomic performance and seed quality attributes of camelina which has been highly concerned in the last decades are their low- demand nutrients, short- term duration of growth, nutrient retention (Anderson et al., 2019) and their high resistance to biotic and abiotic stresse (Soorni et al., 2017; Yuan et al., 2017).

As for high genetic conservation (Lohaus et al., 2020) of camelina with *Arabidopsis*, researchers recognized camelina as a suitable genetic model in the study of genes function and transgenic plants (Heydarian et al., 2016). Camelina seed is an underutilized rich source of oil, protein and mineral nutrients. Based on the studies, camelina oil with a relatively high level of unsaturated fatty acid content (≤85%) and a desirable level of erucic acid (≥3%) is underlined as a superior vegetable oil with multiple potentials in the industries such as; feedstock, biodiesel, cosmetic, medicine, and biopolymers (Waraich et al., 2013; Obour et al., 2017; Jankowsky et al., 2019; Wiwart et al., 2019). There is not enough information on the effect of water deficit during the reproductive phase, nor on the effect of PGPB symbiosis on growth, seed quality and seed composition; therefore, the objective of the present experiment is to evaluate the effect of water deficit stress on Camelina "Soheil cultivar" in 75 and 50% field capacity levels from bud to seed maturation stage and also the effect of *Micrococcus yunnanensis* on growth, protein and oil content of seed, and also the fatty acid profile and some minerals of them.

2. MATERIALS and METHODS

2.1. Growth conditions and treatments

This research was organized at the Research Greenhouse of Biology Department, Shiraz University, Shiraz, Iran from December to February 2018. Camelina "Soheil cultivar" was selected as plant material and obtained from Bisetoon Shafa Co., Kermanshah, Iran. Research was designed based on applying drought and plant growth-promoting bacteria on seed biochemistry of *C. sativa*. So seed total soluble carbohydrate, oil and protein content and fatty acid contents were determined.

Before planting, the soil samples were sieved with a 2 mm sieve and were autoclaved for 3h. The results of soil analysis have been presented in Table 1. According to the results, essential elements were added to all pots each containing 5kg soil. Firstly, all of the pots were divided into two groups on the basis of PGPB presence. Humidity monitoring was done daily by field capacity. To prepare PGPB suspension, the bacteria *Micrococcus yunnanensis* was supplied from the Department of soil science, Shiraz University. The bacteria were incubated in a nutrient broth medium at the temperature of 28°C for 24h on shaker ceaselessly. When sowing, seeds were treated with 1.0 ml bacteria suspension (9×10^7 CFU ml^{-1}) in the inoculated groups. Water stress was applied to each pot in the levels of 75 and 50 % FC from the budding stage to full maturity in generative phase. Camelina silique yield was harvested at the maturity stage for analysis of seed quality and biochemical responses in the treatment of water stress and PGPB.

2.2 Total soluble carbohydrate

Total soluble carbohydrate (TSC) was extracted thrice from 100 mg of mature seed using extraction soluble including glacial acetic acid, methanol, and water. It was centrifuged at 3000 rpm for 10 min. The mixture of 0.5 mL of the extraction was heated with 0.5 mL of 5% (v: v) phenol solution and 2.5 mL of pure sulfuric acid at the temperature of 90°C for 30 min (Dubois et al., 1965). The absorbance was read at 490 nm, eventually TSC content was recorded as mg^{-1}dry weight.

2.3 Oil and protein content

Oil and protein content were measured using Near-Infrared Reflectance spectrometer (NIR)**.** Collecting data simultaneously at all wavelengths (450- 1650 nm), it used an advanced optics based on diode array (7200) technology.

2.4 Fatty acid profile

Seed fatty acid contents were determined using gas chromatography (GC) following trans- methylation of fatty acids and production of fatty acid methyl ester (FAME) by the modified method of Xue et al., 2013. In short, dried seeds (300 mg) were heated in 500 µl of methanol containing H_2SO_4 (49:1 v,v) at the temperature of 80°C for 2h. When

the mixture was cooled at room temperature, FAMEs was separated with 150 µl of hexane and 100 µl of %0.9 NaCl. After being vortexed and centrifuged at 3000rpm for 5min, supernatants (hexane phase) were injected to GC analyzer using a DB-225 column (30m × 250µm × 0.25 µm) on Agilent 7890B.

2. 5 Statistical analysis

This work was conducted in a completely randomized design with a factorial arrangement and three replicates. These obtained data were then subjected to analysis of variance (ANOVA) followed by Least-squares means differences students tests (LS Means Student's t) to determine the significant difference at $P \leq 0.05$ using the SAS software version 9.4 and LSD (p= 0.05) test. The regression analysis was used to show the correlation of oil and protein, total soluble carbohydrate, DPPH, saturated fatty acid, monounsaturated fatty acid, and polyunsaturated fatty acid, and also of 1000-seed weight and oil, protein and total soluble carbohydrate. It was assessed by Pearson's correlation coefficient (r^2). The curves were prepared in the Minitab18 software.

3. RESULT

3.1. Growth and yield quality

To evaluate the impact of water stress in the reproductive phase, we defined three levels of drought (75, 50, 25% FC) for oilseed camelina in the first year. According to the ultimate results it was shown that a high level of stress (25% FC) had disrupted many flower buds and therefore there weren't enough seed collected for all of our analyses. So, we followed our experiment without this level of drought.

3.2 Total soluble carbohydrate content

Water deficit stress exhibited a significant increase ($P < 0.001$) in the TSC content; while PGPB and PGPB* drought interaction had no significant effect on that. The TSC content had a negative correlation with oil because the enhancement of the TSC content was coincided with the decrease in oil content (Fig 1). The results showed that drought stress increased TSC content by 52.3% and 46.6% in B0D2 and B1D2 respectively (Fig. 1).

3.3 Oil and protein content

Analysis of variance (anova) revealed that water deficit stress ($P < 0.001$) affected the oil and protein content significantly (Table 2), while PGPB effect was significant in neither of the traits. Based on the data obtained from NIR, a negative correlation was observed between oil and protein content (r^2= 0.83) during all of the treatments (Fig 2). The highest oil content was recorded with 31.94% and 31.28% for B0D0 and B1D0 treatment respectively, while the highest protein content was related to B1D2 (27.16%) (Fig 2). According to the results obtained from Fig 2,

there was a meaningful ($P < 0.001$) negative correlation of the oil content and protein (r^2=- -0.873), carbohydrate (r^2= - 0.692), DPPH (r^2= - 0.746) and PUFA (r^2= -0.512) ($P < 0.01$).

3.4 Fatty acid profile

The effect of water stress and PGPB treatment on the obtained fatty acid profiles in gas chromatography is depicted in Table 3. The water-deficit stress, PGPB and their interactions significantly was increased on SFA content ($P < 0.001$) the total saturated fatty acids (SFA) content including palmitic acid (C16:0), stearic acid (C18:0) and arachidic acid (C20: 0) (Table 2 and 3). The least palmitic, stearic and arachidic acid (7.85, 2.85, and 1.49 respectively) were related to B1D0. A decrease in water supply led to a reduction in the detected MUFAs content namely oleic acid (C18:1), ecosanoic acid (C20:1) and erucic acid. PGPB and its interaction with drought decreased the content of oleic acid and eicosanoid acid, except erucic acid which was not significantly increased in B1D0. Analysis of variance revealed that the drought stress had a significant effect on MUFA ($P<0.001$) and PUFA ($P<0.01$), while there was a significant effect of PGPB on MUFA ($P<0.001$) and its interaction with drought on MUFA and PUFA ($P<0.001$) (Table 2). The decrease of erucic acid content was coincided with the increase of drought stress levels for both inoculated and no-inoculated plants. The highest proportion of fatty acids was related to polyunsaturated acid (PUFA) including linolenic acid (C18:3) and linoleic acid (C18:2). Linoleic acid content was increased in plants grown in 75% FC irrigation, while the PGPB treatment significantly decreased linoleic acid content (Table 3). The linolenic acid content ranged from 33.40 to 43.44%. Both the PGPB and water deficit treatment significantly increased the linolenic acid content. Linolenic acid (18:3) content exhibited a negative correlation with linoleic acid (18:2) in the inoculated plants because the highest linolenic acid (43.44%) was coincided with the least linoleic acid (18.53%) content.

4. DISCUSSION

Although there is some record that camelina can be well adapted to semi-arid environmental condition, the present results showed that severe water deficit (25% FC) particularly during the reproductive phase significantly disrupted flower buds and declined seed yield of the plant. Based on the recent studies, PGPBs are pivotal candidates in sustainable agricultural management under water deficit conditions. During water deficit stress, the TSC content was increased as compared to the control.

It is in agreement with our results that water deficit stress–induced alternation of carbon and nitrogen content-coincided with the decrease of oil and increase of protein and TSC; additionally, rising TSC content, as the main

resource for oil synthesis in the seed, is considerable for acclimation to drought stress (Ni et al., 2019). Results presented in *Brassica napus* (Aslam et al., 2009), *C. sativa* (Obour et al., 2017; Hossein et al., 2019), *Brassica junica* (Elferjani and Soolanayakanahally 2018) *B. napus*, and *Brassica junicea* are similar to the present report given for the negative correlation of oil with both protein and TSC content under stress and non-stress conditions. Nakagawa and workers (2018) have reported that water deficit during the reproductive stage resulted in the reduction of oil and protein content and the increase of carbohydrate in soybean seed. It might be related to the increase of gene expression involving in lipid degradation, along with the decrease of gene expression active in lipid biosynthesis

A part of the mechanisms underlying plant resilience to water deficits is the alternation of membrane structure and fluidity with remodeling oil content and fatty acids profile (Mohamed and Latif 2017). The decrease of photosynthesis and carbon remobilization leads to the reduction of seed oil content in the drought stress (El Sabagh et al., 2019). The present study have confirmed the previous findings indicating the effect of water deficit stress on the significant reduction of camelina oil by about 14.74% (Rebey et al., 2012; Elferjani and Soolanayakanahally 2018; Hatzig et al., 2018). Additionally, the decrease of camelina oil has been coincided with the increase of SFA (C16:0, C 18:0 and C20:0) and PUFA (C18:2 and C18:3) on one hand, and the decrease of MUFA (C18:1, C20:1) on the other. Our finding is coincided with the previous results that have shown water deficit stress increasing linoleic and linolenic acid at the reproductive phase (Aslan et al., 2009; Gharechaei et al., 2019). The increase in unsaturated fatty acid (linoleic acid and linolenic acid) could be due to the activation of the lipase enzyme followed by developing membrane fluidity for the adaptation to water deficit stress (Upchurch 2008). Desaturation enzymes activated in PUFA synthesis have stability under the biotic and abiotic stress (Nayeri and Yarizade 2014). The stress-induced increase trend in C16:0 and C18:0 was quite similar in both of the inoculated and non-inoculated plants. These results were in agreement with the obtained report by Laribi et al., 2009. Based on the previous findings in soybean (Mohamed and Latif 2017) and sunflower (Petcu et al., 2001) increase of palmitic acid coincided with the decrease in stearic acid under water deficit stress. In the present study, oleic acid, eicosanoid acid, and erucic acid were detected as MUFA. In our experiment, MUFAs content was positively correlated with camelina oil under drought stress. It is suggested that a remarkable decrease in oleic acid content could have been due to water deficit stress–induced reduction of Δ^9 desaturase activity and related – gene expression in the synthesis pathway of oleic acid from stearic acid. Additionally, a decrease in oleic acid, along with an increase in linoleic acid

and linolenic acid could be due to the higher enhancement of Δ^6 and Δ^{12} desaturase than to Δ^9 desaturase activity for strong tolerance to drought stress. Similar results were reported for canola under drought stress (Aslam et al., 2009). Furthermore, there is a significant decrease in eicosanoid acid content in the drought- stressed plant compared to the control. It may be due to association of the desaturation activity with elongase enzyme which converts oleic acid to linoleic acid or eicosanoid acid respectively. Erucic acid (EA) (C22:1), present at high concentrations mainly in the seeds of species of the Brassicaceae family (Velioglu et al., 2017), is created by FAE (e2) elongating eicosanoid acid. Our finding showed that the most abundant erucic acid in B1D0 (by about 3.03%) is still lower than the limited content of food consumption. It can be due to lower levels of FAE (e2) in camelina than to other brassica species (Enjalbert et al., 2013). The current study showed that seed inoculation by *M. yunnanensis* resulted in different alterations of seed composition. The decrease of oil content was coincided with the increase of PUFA and the decrease of SFA and MUFA in the inoculated in comparison to no-inoculated plant.

Unsaturated acids fatty acids (USFA), oleic acid and linolenic acid contents increased with PGPB, while linoleic acid decreased. the results are in a line with those already reported by Silva et al., 2013 in soybean seed and Sharifi et al., 2017 in safflower seed about the effect of PGPB on the reduction of SFA (palmitic acid and stearic acid) and enhancement of USFA (oleic acid and linolenic acid) in soybean seed. Based on the obtained reports, PGPBs, increasing involvement of the nutrients in the synthesis of composition seed, increased seed filling duration (Sharifi et al., 2017).

5. CONCLUSION

In this study, seed yield and seed composition of camelina has been focused when it is grown in water deficit condition and symbiosis with *M. yunnanensis*. From our findings, it might be concluded that camelina yield is significantly affected by water-deficit stress at the reproductive stage. Our findings have confirmed that the proportion of oil and protein seeds are adversely altered as a consequence of disparate partition of nutrients in particular carbon and nitrogen in various environmental conditions. In the fatty acid profile, the most proportion has been related to PUFA in particular linolenic acid (18:3) by 50%. Saturated fatty acid and polyunsaturated fatty acid content are relatively parallel when camelina is grown in water deficit stress condition. Similar to oil, the monounsaturated fatty acid content is reduced in stressed plants. Based on the science, the PGPB exhibited various changes in the growth and the physical and biochemical characteristic of camelina in both of the well-watered and water-limited plants.

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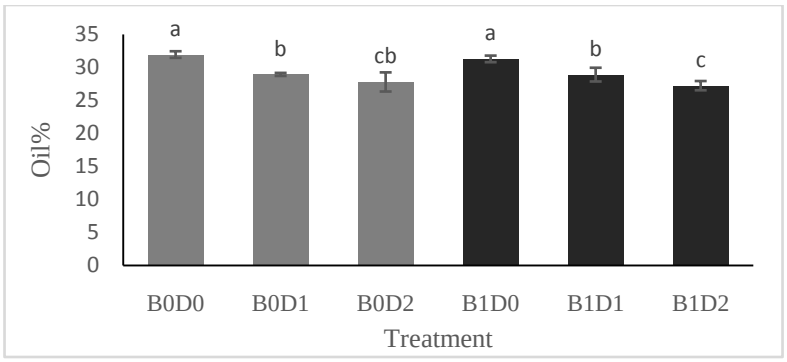

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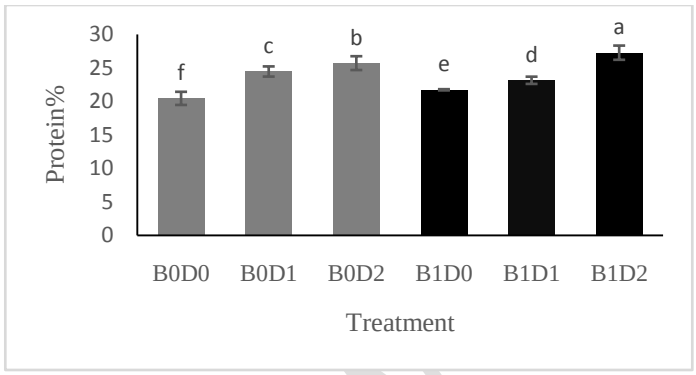

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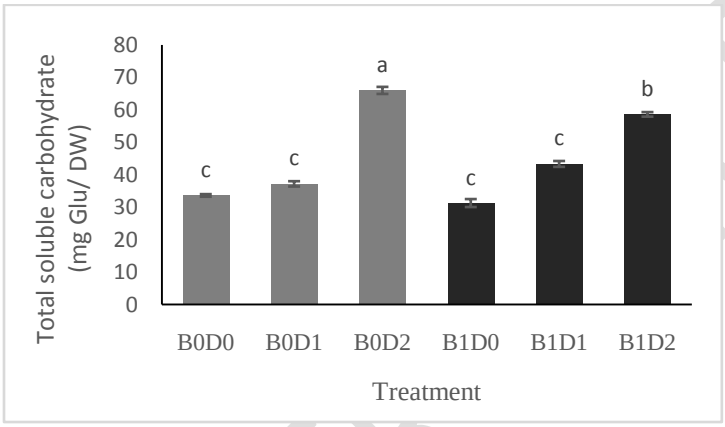

(C)

Figure 1. Oil % (A), Protein% (B) and Total soluble carbohydrate (C) in seed of Camelina sativa grown under drought stress and in the presence of PGPB. (D0: 100%FC, D1: 75%FC, D2: 50% FC) (B0: no-inoculated; B1: inoculated). Vertical bars indicate the standard error of the mean. The value with same letters have no significant difference ($p < 0.05$) at LSD test

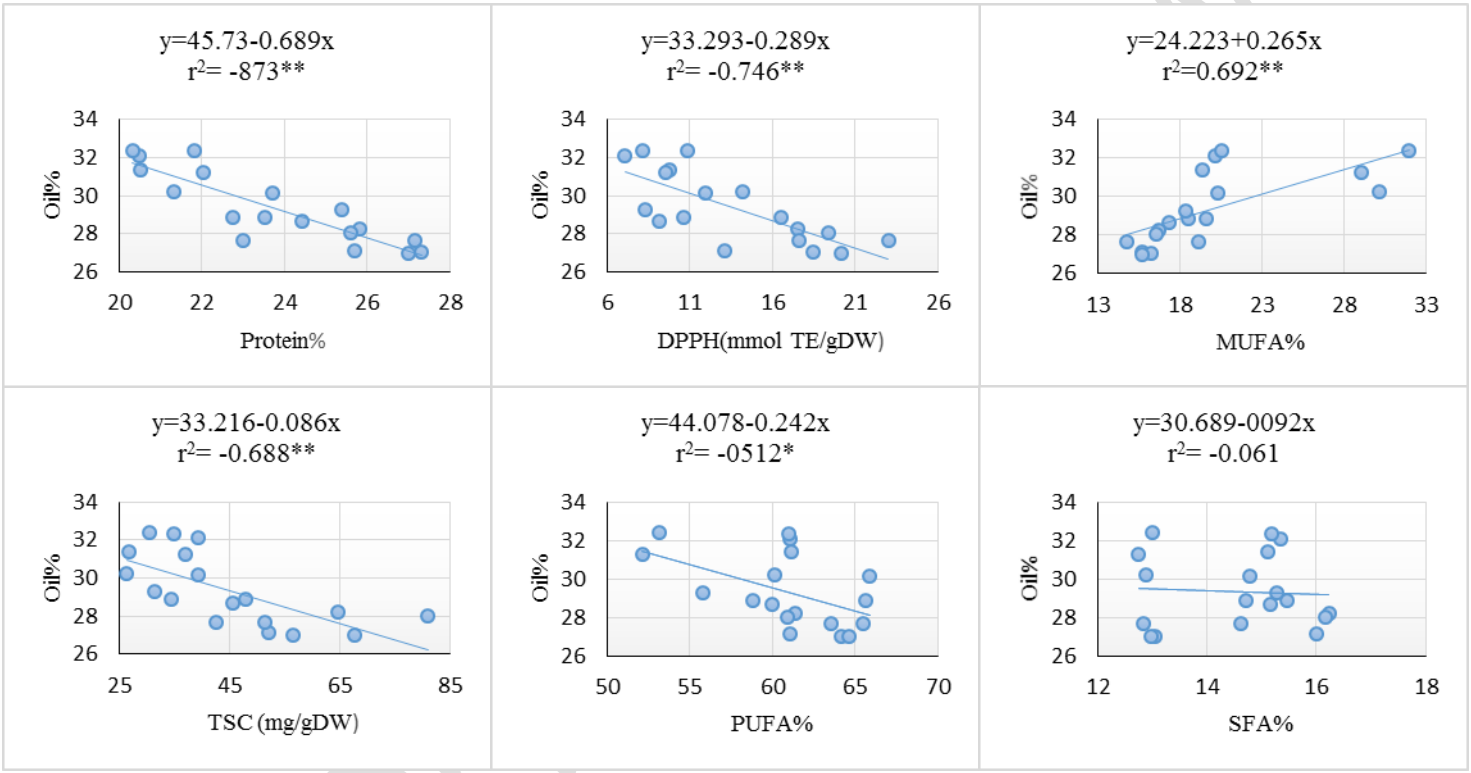

Figure 2. Correlation between Oil % with protein (%), total soluble carbohydrate content (TSC) (mg-1dry weight) and saturated fatty acid (SFA), mono un saturated fatty acid (MUFA), poly unsaturated fatty acid (PUFA), and DPPH (%) in water deficit stress and inoculation with PGPB

Table 1. Physical and chemical properties of the soil

Soil texture (%)			Elements (mg kg^{-1})						pH	EC (dS m^{-1})	OM %	TN %	FC %
Sand	Silt	clay	K	P	Fe	Mn	Cu	Zn					
25.60	63.40	11.00	345.05	10.49	1.40	3.20	0.72	3.00	7.98	0.64	0.71	0.04	16.78

Table 2. Analysis of variances (ANOVA) of oil content, protein content, total soluble carbohydrate content (TSC), total phenol content saturated fatty acid (SFA), mono-unsaturated fatty acid (MUFA), poly- unsaturated fatty acid (PUFA), and DPPH *Camelina sativa* grown under drought stress and in the presence of PGPB.

Source	DPPH	TPC	PUFA	MUFA	SFA	TSC	Oil	Protein
Drought	21.557***	415.921***	8.939**	194.956***	97.624***	20.512***	43.884***	201.024***
PGPB	15.337**	50.245***	2.615	90.558***	1325.373***	0.093	1.339	4.832
Drought *PGPB	0.574	26.610***	17.452***	74.57***	185.734***	1.001	0.294	16.908***

*p<0.05; **p<0.01; ***p<0.001

Table 3. Effect of water deficit stress and PGPB symbiosis on fatty acid profile in Camelina sativa. C16:0 palmitic acid, C18:0 stearic acid, C18:1 oleic acid, C18:2 linoleic acid, C18:3 linolenic acid, C20:1 eicosanoid acid,C22:1 erucic acid, SFA saturated fatty acid, MUFA monounsaturated fatty acid, PUFA poly unsaturated fatty acid,(D0: 100%FC, D1: 75%FC, D2: 50% FC, B0: Non inoculated, B1: inoculated)

	B0D0	B0D1	B0D2	B1D0	B1D1	B1D2
C16:0	9.5 ±1.2a	9.6 ±0.09a	9.9 ±1.01a	7.8 ±0.75a	9.1±1.03a	8.1 ±1.1a
C18:0	3.5±0.7a	3.5±0.9a	3.6±1.2a	2.8±0.9a	3.4±1.01a	3.1±1.5a
C18:1	3.17±1.0a	2.76±1.3b	1.69±1.1d	1.83±0.9c	3.13±0.7a	1.81±0.6c
C18:2	22.6±0.3a	23.2±0.5a	22.8±0.5a	18.53±0.3b	22.3±0.4a	20.12±0.3b
C18:3	37.5±0.7c	40.8±0.4ab	37.9±0.5bc	33.4±0.3d	41.3±0.5a	43.4±0.2a
C20:0	1.72± 0.57a	1.67±0.93a	1.95± 0.8a	1.49±1.03a	1.76±0.6a	1.51±0.3a
C20:1	14.1±0.9ab	13.1±0.9ab	12.5±1.05bc	11.3±1.02c	14.3±0.5a	10.99±0.2c
C22:1	2.6±0.4ab	2.1±0.7bc	2.1±0.4bc	3.03±0.5a	2.1±0.3bc	1.64±0.7c

SFA	15.2±0.5b	15.3±1b	16.1±0.1a	12.8±0.1d	14.7±0.3c	12.9±0.5d
MUFA	20.05±0.6b	18.08±1.7c	16.36±1.6d	30.38±0.6a	19.69±1.5b	15.59±1.3d
PUFA	61.05±0.08bc	58.19±2.1dc	61.1±6.17bc	55.12±4.3d	65.66±0.2a	64.13±0.5ab

The values presented are mean ±SE from three replicates of each treatment. Different letters indicate significant differences at $P \leq 0.05$