

CHANGES IN GROWTH PARAMETERS, OIL YIELD, FATTY ACID COMPOSITION AND MINERAL CONTENT OF TWO SAFFLOWER (*CARTHAMUS TINCTORIUS* L.) GENOTYPES IN RESPONSE TO WATER STRESS

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Abstract. The present study was designed to investigate the effects of water stress on growth and development parameters, oil yield coupled with their fatty acid composition as well as mineral content of two safflower genotypes including Remzibey-05 and Gokturk. Accordingly, significant differences regarding plant length, branch number per plant, capsule number per plant, capsule seed number, 1000-seed weight and seed yield per pot were assessed. However, first branch length did not exhibit significant differences between genotypes in response to the treatments. Furthermore, Oil yield was not significantly affected by severe water stress for Remzibey-05 even a 1.60% increase was observed under severe water stress but the yield was significantly increased by 2.37% in Gokturk. In terms of oil composition unsaturation degree, UFA/SFA significantly increased with stress for both genotypes. Concerning mineral content; Remzibey -05 genotype had the highest value of K and Na whereas Gokturk genotype had the highest values of Mg, Fe, Cu and Zn. Finally, the experimental groups were discriminated and identified using PCA and heatmap visualization.

Keywords: *fatty acid unsaturation degree, drought, Remzibey-05, Gokturk, water stress*

Introduction

Water stress resistance of crops is one of the great challenges for the world's agricultural systems. Water stress-induced loss in crop yield probably exceeds losses caused by other severe environmental constraints. Thereby, numerous researches in plants responses to water scarcity has been important interest for scientists for many years in order to develop, improve or explore drought-tolerant plant species or cultivars, genotypes within the same species for a sustainable agricultural production (Hamrouni et al., 2001; Turk et al., 2004; Al-Barrak, 2006; Bettaieb et al., 2009; Ozkan and Kulak, 2013; Zandalinas et al., 2018; Fahad et al., 2017; Shi et al., 2017; Ahmed et al., 2016; Ozkan, 2018; Rubin et al., 2017; Vurukonda et al., 2016; Pouri et al., 2019; Cetinkaya et al., 2016).

Water stress alters the biochemical synthesis pathways and subsequently production of primary and secondary metabolites is influenced. It is worthy to remark that industrially, medicinally or economically important metabolites may be accumulated beyond the decline in the growth and productivity of crops (Ozkan and Kulak, 2013; Hamrouni et al., 2001; Laribi et al., 2009). Of those, the variations in lipid content and its composition have been reported under varying irrigation degrees (Laribi et al., 2009; Bettaieb et al., 2010; Laribi et al., 2011; Ozkan and Kulak, 2013).

Safflower (*Carthamus tinctorius*) belonging to Asteraceae family is of the oldest cultivated oilseed crop but not common and not very popular when compared to other oil crops such as soybean, sunflower and peanut (Canavar et al., 2014; Bortolheiro and Silva, 2017). Of the oilseed crops, safflower has been well-documented to possess

ability in moderate tolerance of environmental constraints but changes in oil yield and subsequently fatty acid composition have been reported, indicating that water constraints water deficient condition might cause increase protein and decrease oil contents of safflower (Nabipour et al., 2007; Amini et al., 2014; El Sabagh et al., 2019). Seed oil of safflower is well characterized with a large amount of saturated (palmitic and stearic) and unsaturated (oleic, linoleic and linolenic) fatty acids and composition. However, the percentage and content of the compounds and oil yield are particularly affected by water stress (Bortolheiro and Silva, 2017; Nabipour et al., 2007; Ashrafi and Razmjoo, 2010; Eslam, 2011; Amini et al., 2014; Ozkan and Kulak, 2013; Nazari et al., 2017). Since each species of the plants have been comprised of genotypes, cultivars, varieties, populations and chemotypes, many studies can be performed on the different genotypes of the same species. As well-known and documented that, plants have been adapted to environmental conditions through not only based their genetic inheritance and but also the primary and secondary metabolites inside. Through profiling the metabolite content, plants have been sorted as tolerant or sensitive. Along with the present study, two different safflower genotypes were used in order to determine their oil yield combined with fatty acid compositional profiling in response to water stress conditions. In addition to the oil and its composition, mineral content or accumulation of seed samples belonging to both genotypes were determined. Furthermore, growth and developmental parameters of genotypes were compared after subjection water stress and normal life span under irrigated conditions (control).

Materials and methods

Plant material and water deficit treatment

Experiments were performed under greenhouse conditions with pots including 2 kg soil mixture of 3:1:1 (soil-peat-perlite) at Gaziantep, Turkey. The experiments were carried out with three replicates corresponding 10 plants for each replicate. Each pot included one plant seedling. The effects of severe water stress (SWD) on safflower genotypes (Remzibey-05 and Gokturk) were investigated. The safflower genotypes are of the significant oil crops. The materials used herein are of the developed in Turkey. Herewith, the studies regarding adaptation of the genotypes in arid and semi-arid regions corresponding to the limited water sources are required. In this context, the genotypes were screened for their low water content tolerance corresponding to the oil yield coupled with their fatty acid composition in addition to basic agricultural parameters.

For the experimental processes, similar methods with Bettaieb et al. (2009) were followed. In brief, during the first 35 days of the study, plants were irrigated with tap water, and subsequently exposed to different water regimes: 100% (control group: C; 600 ml) 25% (severe water deficit group: SWD; 150 ml) of field capacity (FC). The properties of experimental soils were as follows: Potassium (297.75 ppm), organic matter (1.16%), salt (0.059) at a set pH (8.0). Experiments were conducted in a greenhouse with a 14-h photoperiod and performed between October 13, 2018 and July 26, 2019. Mean temperature was kept at 25 ± 2 °C during daytime and 14 ± 2 °C at nighttime, respectively, with a relative humidity of 70%. After harvest, seeds were airdried and stored at 4 °C until use for further analysis.

Growth and yield parameter measurements

For each treatment, measurement of plant length, seed number per capsule, capsule number, 1000-seed weight, total weight per pot, and single plant yield were evaluated by harvest of six randomly selected plants from each experimental replicate (Ozkan and Kulak, 2013; Ozkan, 2018).

Oil extraction and fatty acid composition analysis

Oil extraction and then fatty acids of oil were determined according to our previous methods applied (Ozkan and Kulak, 2013; Ozkan, 2018). Briefly, oils were extracted from safflower seeds (each 2 g sample) with n-hexane for 4 h using a Soxhlet extraction apparatus. Subsequently, the solvent was evaporated under reduced pressure and temperature using a Rotary evaporator (Heidolph). 0.1 g of safflower oil was added to 2 ml n-heptanes into a screw-capped tube for esterification. The fatty acid analyses were conducted according to the official method COI/T.20/Doc.no.24 2001. 0.1 g of safflower oil was taken into screw-capped tube. 2 ml n-heptane was added and shaken. After 0.2 ml methanolic potassium hydroxide was added for esterification, tubes were vigorously shaken for 30 s after the vials were closed. The supernatant of the solution was taken followed after 1 h of incubation at room temperature. Then, the supernatant was put in 2 ml vials for injection.

Fatty acid composition profile determination

GC-FID analyses of fatty acids methyl esters were carried out on a Shimadzu gas chromatography (GC2010 series) equipped with a Supelco SP 2380 fused silica capillary column (100 m, 0.25 mm i.d., 0.2 µm film thickness). Helium was used as carrier gas, at a flow rate of 3 mL/min. The injection and detector temperature were 140 °C and 240 °C, respectively. The oven temperature was held isothermal at 140 °C for 5 min, then raised to 240 °C at 4 °C/min and held isothermal at 240 °C for 15 min. Injection volume of Diluted samples [1/100 (v/v) in n-heptanes] of 1.0 µL were injected automatically in the split mode (1/100). The identification of the constituents was based on comparison of the GC-retention times with those of available analytical standards (Larodan Fine chemicals, mixture of 37 components of fatty acids methyl esters). Peak area was used to obtain the percentage of individual fatty acid.

Sample preparation and measurement for mineral content

The harvested seed samples were cleaned, washed by de-ionized water, and dried. Pre-dried samples were de-moisturized at 70 °C for 48 h in an oven and ground for chemical analysis. 0.2 g of ground powdered seed samples were immediately placed into burning cup with 5 ml HNO₃ 65% and 2 ml H₂O₂ 30%. After the incineration process, the solution was cooled at room temperature for 45 min. The extracts were passed through a Whatman 42 filter paper. These filtrates were collected by de-ionized water in a 20 ml-polyethylene bottles and kept at 4 °C in laboratory for ICP-OES analysis. Each sample was analyzed in triplicate.

Statistical analysis

SPSS statistical program was used to determine statistical significance levels by employing the independent one-way ANOVA followed by Duncan multiple range test

and the differences between individual means were considered to be statistically important at $p < 0.05$. Moreover, specifically for each genotype, control and stress group of plants were compared using independent t-test in order to determine to clarify their behaviour against stress. Also, the discrimination for experimental groups was done with the principal component analysis using SPSS software. Finally, heatmap was 209 constructed using ClustVis (<https://biit.cs.ut.ee/clustvis/>).

Results and discussion

Effect of water stress on plant growth and development parameters

Based on independent t-test, there were significant differences between plant length ($t = 2.274$; $p < 0.05$), capsule seed number ($t = 3.688$; $p < 0.01$), 1000-seed weight ($t = 2.333$; $p < 0.05$), seed yield per pot ($t = 5.231$; $p < 0.01$) for Remzibey-05 genotype in response to water stress. However, first branch length, branch number per plant and capsule number per plant did not exhibit significant differences between treatments ($p > 0.05$) (Table 1).

Table 1. t-test analysis of growth and yield parameters of Remzibey-05 genotype exposed to water stress

Growth and yield parameters	Control	SWD	t-statistic	p value
Plant length (cm)	37.50±6.02	29.33±6.41	2.274	0.046*
First branch length (cm)	18.50±6.29	17.33±5.57	0.340	0.741
Branch number per plant	4.83±1.33	3.83±1.17	1.384	0.197
Capsule number per plant	5.83±2.23	3.83±1.17	1.947	0.080~
Capsule seed number	30.17±9.70	15.34±1.70	3.688	0.004**
1000-seed weight (g)	51.66±14.09	35.20±10.01	2.333	0.042*
Seed yield per pot (g)	5.99±1.44	2.40±0.87	5.231	0.000**

** $p < 0.01$; * $p < 0.05$; ~ $p < 0.10$; SWD: severe water deficit

Severe water stress significantly affected plant length ($t = 3.353$; $p < 0.01$), branch number per plant ($t = 3.835$; $p < 0.01$), capsule number per plant ($t = 4.715$; $p < 0.01$), capsule seed number (3.664; $p < 0.01$), seed yield per plant ($t = 8.871$; $p < 0.01$) in Gokturk genotype. However, first branch length ($t = 0.902$; $p > 0.05$) and 1000-seed weight ($t = 1.987$; $p > 0.05$) did not differ in Gokturk genotype in response to severe water stress (Table 2).

Table 2. t-test analysis of growth and yield parameters of Gokturk genotype exposed to water stress

Growth and yield parameters	Control	SWD	t-statistic	p value
Plant length (cm)	34.00±5.514	22.83±6.014	3.353	0.007**
First branch length (cm)	18.17±6.113	14.83±6.68	0.902	0.388
Branch number per plant	4.83±.41	3.17±.98	3.835	0.003**
Capsule number per plant	6.00±1.10	3.17±.98	4.715	0.001**
Capsule seed number	23.67±7.71	11.74±2.03	3.664	0.004**
1000-seed weight (g)	49.85±7.21	40.91±8.34	1.987	0.075~
Seed yield per pot (g)	5.29±.82	1.54±.64	8.871	0.000**

** $p < 0.01$; ~ $p < 0.10$; SWD: severe water deficit

Furthermore, independent t-test was performed in order to determine the differences between control and stress groups of genotypes. No differences were observed for the parameters of both genotypes in control groups (*Table 3*). Also, there were no significant differences-except capsule seed number between genotypes under severe stress conditions (*Table 4*).

Table 3. *t*-test analysis of growth and yield parameters of both genotypes under irrigated conditions (control)

Growth and yield parameters	Remzibey-05 control	Gokturk control	t-statistic	p value
Plant length (cm)	37.50±6.03	34.00±5.52	1.050	0.319
First branch length (cm)	18.50±6.29	18.17±6.11	0.093	0.928
Branch number per plant	4.83±1.33	4.83±0.41	0.000	1.000
Capsule number per plant	5.83±2.23	6.00±1.10	-0.164	0.873
Capsule seed number	30.17±9.71	23.67±7.71	1.285	0.228
1000-seed weight (g)	51.66±14.1	49.85±7.21	0.281	0.785
Seed yield per pot (g)	5.99±1.44	5.3±.82	1.045	0.320

Table 4. *t*-test analysis of growth and yield parameters of both genotypes under severe water stress

Growth and yield parameters	Remzibey-05 SWD	Gokturk SWD	t-statistic	p value
Plant length (cm)	29.33±6.41	22.83±6.02	1.812	0.100
First branch length (cm)	17.33±5.57	14.83±6.68	.704	0.497
Branch number per plant	3.83±1.17	3.17±0.98	1.069	0.310
Capsule number per plant	3.83±1.17	3.17±0.98	1.069	0.310
Capsule seed number	15.34±1.70	11.75±2.03	3.327	0.008**
1000-seed weight (g)	35.20±10.00	40.90±8.34	-1.073	0.308
Seed yield per pot (g)	2.40±0.875	1.54±0.64	1.959	0.079~

**p < 0.01 ~p < 0.10; SWD: severe water deficit

One-way variance analysis reveals significant differences in terms of plant length ($F = 6.708$; $p < 0.01$), branch number per plant ($F = 3.750$; $p < 0.05$), capsule number per plant ($F = 5.719$; $p < 0.01$), capsule seed number ($F = 10.289$; $p < 0.01$), 1000-seed weight ($F = 3.413$; $p < 0.05$) and seed yield per pot ($F = 28.921$; $p < 0.01$). However, first branch length did not exhibit significant differences between genotypes in response to the treatments (*Table 5*).

In many and various studies regarding water stress and agronomical yield attributes interaction, stress conditions have been reported to exhibit inhibitory impacts on stem growth and plant height due to shrinkage in output changes in cellular water status of the plant. The effects of the water stress on the plant parts are variable for the examined parameters. The effects can be observed at certain developmental stages of the plant, a trait of the plant or genotypes of the same plant species (Prasad and Staggenborg, 2008).

Table 5. One-way variance analysis of growth and yield parameters of genotypes in response to treatments

Growth and yield parameters	Remzibey-05 control	Remzibey-05 SWD	Gokturk control	Gokturk SWD	ANOVA F-statistic
Plant length (cm)	37.50±6.02a	29.33±6.40bc	34.00±5.51ab	22.83±6.01c	6.708**
First branch length (cm)	18.50±6.29	17.33±5.57	18.17±6.11	14.83±6.68	.432
Branch number per plant	4.83±1.33 a	3.83±1.17 ab	4.83±.401 a	3.17±.98 b	3.750*
Capsule number per plant	5.83±2.229 a	3.83±1.169 b	6.00±1.095 a	3.17±.983 b	5.719**
Capsule seed number	30.17±9.70 a	15.34±1.70 b	23.67±7.71 a	11.74±2.03 b	10.289**
1000-seed weight (g)	51.66±14.09 a	35.20±10.01 b	49.85±7.21 a	40.90±8.34 ab	3.413*
Seed yield per pot (g)	5.99±1.44 a	2.40±0.88 b	5.30±0.82 a	1.54±0.64 b	28.921**

Means±SD in the same row by the same letter are not significantly different to the test of Duncan ($\alpha = 0.05$). ** $p < 0.01$ * $p < 0.05$; SWD: severe water deficit

Correlation analysis of growth and yield parameters of safflower

Table 6 presents the correlation analysis of plant length, first branch length, number of branches, capsule number, seed number, 1000-seed weight and seed yield in *Carthamus tinctorius* genotypes. Significant and positive relation was observed by 64.2% between plant height and first branch length ($r = 0.642$; $p < 0.01$). Also, 69.9% ratio between plant length and branch number was found to be significant with positive correlation ($r = 0.699$; $p < 0.01$). Plant length was also positively correlated with capsule number per plant by 80.4% ($r = 0.804$; $p < 0.01$). Plant length was furthermore positively correlated with seed yield per pot ($r = 0.782$; $p < 0.01$). moreover, significant and positive correlation coefficients between first branch length and capsule number per plant ($r = 0.417$; $p < 0.05$), first branch length and seed yield per pot ($r = 0.465$; $p < 0.05$), branch number per plant and capsule number per plant ($r = 0.900$; $p < 0.01$), branch number per plant and seed yield per pot ($r = 0.742$; $p < 0.01$), capsule seed number and seed yield per pot ($r = 0.790$; $p < 0.01$), capsule seed number and seed yield per pot ($r = 0.727$; $p < 0.01$) as well as 1000-seed weight and seed yield per pot ($r = 0.508$; $p < 0.05$) were observed. The remained coefficients were all positive but not significant. No negative correlations were found between investigated parameters (Table 6).

Table 6. Correlation analysis of growth and yield parameters of safflower

	Plant length	First branch length	Branch number per plant	Capsule number per plant	Capsule seed number	1000-seed weight	Seed yield per pot
Plant length	1						
First branch length	.642**	1					
Branch number per plant	.699**	0.39	1				
Capsule number per plant	.804**	.417*	.900**	1			
Capsule seed number	0.395	0.228	0.322	0.294	1		
1000-seed weight	0.336	0.219	0.288	0.279	0.26	1	
Seed yield per pot	.782**	.465*	.742**	.790**	.727**	.508*	1

** $p < 0.01$, * $p < 0.05$

Effects of water stress on oil yield and fatty acid composition

Oil yield was not significantly affected with severe water stress in Remzibey-05 even a 1.60% increase was observed under severe water stress but the yield was significantly augmented by 2.37% in Gokturk. The non-significant changes in oil percentage could be attributed to stress timing at vegetation of the plant. In the current study, severe water stress was applied at the flowering. Nazari et al. (2017) also reported that drought stress at the flowering stage of some safflower did not influence seed oil percentages. In the studies performed on different genotypes of safflower, six different safflower lines were exposed to water stress and subsequently followed by rehydration. The oil yield increased from 24.18 ± 5.01 to 26.61 ± 6.21 (Bortolheiro and Silva, 2017). However, oil yield significantly decreased under water stress (Nabipour et al., 2007; Ashrafi and Razmjoo, 2010; Ensiye and Khorshid, 2010; Eslam, 2011; Amini et al., 2014). Ozkan and Kulak (2013) reported a non-significant slight increase in oil content of seeds of sesame seedlings exposed to severe water stress. Furthermore, drought stress did not cause significant changes in percentage of seed oil content of safflower species (Nazari et al., 2017).

After oil esterification, the fatty acid compounds in oil were identified using gas chromatography. Of the fatty acid compounds identified for both genotypes, oleic acid, linoleic acid, palmitic acid, stearic acid and behenic acid were of compounds with percentage over 1% (Table 7).

Their total percentage for treatments were 98.62% for Remzibey-05 control, 99.02% for Remzibey-05 SWD, 98.80% Gokturk control and 99.12% Gokturk SWD. The yield and percentage can change but the composition of the oil was not affected by severe water stress.

Of the compounds, palmitic acid percentage significantly decreased with severe water stress by 28.30% for Remzibey-05 and 15.61% in Gokturk genotype. No differences regarding percentage of the palmitic acid in control and SWD groups of both genotypes, respectively were found. The decline in palmitic acid under water stress was also reported (Ashrafi and Razmjoo, 2010; Petcu et al., 2001). However, Nazari et al. (2017) reported slight increases in palmitic percentages of for safflower species.

Stearic acid also exhibited similar manner with palmitic acid in both genotypes in response to severe water stress. Stearic acid decreased by 45.36% in Remzibey-05 and 26.66% in Gokturk genotypes. The decreases in percentage of stearic acid were documented (Ashrafi and Razmjoo, 2010). However, Laribi et al. (2009) noted an increase in palmitic acid (110%) and stearic acid (269%) and a decline in petroselinic acid (18.47%) in *Carum carvi* under severe water stress.

Oleic acid percentage significantly increased by 7.85% with severe water stress in Remzibey-05 but the percentage did not significantly vary with severe water stress in Gokturk genotype. Ashrafi and Razmjoo (2010) reported a decline in oleic acid percentage in different safflower genotypes but an increase was documented by Gao et al. (2009). In the study by Ozkan and Kulak (2013), two sesame genotypes were exposed to moderate and severe water stress, documenting that no significant changes in percentage of oleic acid in Cumhuriyet genotype were found while there was a slight but significant change Özberk genotype under severe water stress.

Linoleic acid percentage was not affected by severe water stress in Remzibey-05 genotype but increased by 1.80% in Gokturk under severe stress conditions. Linoleic acid percentage did not significantly vary in sesame (Cumhuriyet genotype) but increased with stress in sesame (Özberk genotype) (Ozkan and Kulak, 2013). Ashrafi

and Razmjoo (2010) also reported a decline in percentage of linoleic acid in safflower genotypes.

Oleic acid and linoleic acid ratio increased in Remzibey-05 under severe water stress (ratio: 0.511 and 0.545 for control and SWD, respectively) but it decreased in Gokturk (0.554 and 0.545 for control and SWD, respectively). Laribi et al. (2009) reported decreases in oleic acid and linoleic acid ratio under severe water stress but an increased ratio of oleic acid/linoleic acid was reported (Talha and Osman, 1975).

Percentage of behenic acid increased by 10.64% and 17.19% in both genotypes in response to water stress Remzibey-05 and Gokturk, respectively. Behenic acid did not change in sesame seeds in response to water stress (Ozkan and Kulak, 2013; Ozkan, 2018).

Table 7. Effects of water stress on oil yield and fatty acid composition of both safflower genotypes

Fatty acid compounds	Remzibey-05 control	Remzibey-05 SWD	Change (%)	Gokturk control	Gokturk SWD	Change (%)
Myristic acid	.0762a	.0201b	-73.62%	.0460	.0314	-31.74%
Palmitic acid	6.3492a	4.5521b	-28.30%	4.4915a	3.7903b	-15.61%
Palmitoleic acid	.0665b	.0918a	38.05%	.0759	.0932	22.79%
Heptadecanoic acid	.0316	.0256	-18.99%	.0244	.0163	-33.20%
Cis-10Heptadecanoic	.0674	.0757	12.31%	.0750b	.0825a	10.00%
Stearic acid	2.1725a	1.1871b	-45.36%	1.2532a	.9191b	-26.66%
Oleic acid	29.8967b	32.2424a	7.85%	32.7382	32.8169	0.24%
Linoleic acid	58.4864	59.1422	1.12%	59.0845b	60.1460a	1.80%
Arachidic acid	.2454a	.1163b	-52.61%	.1557	.1136	-27.04%
Cis-11Eicosenoic acid	.0688	.0762	10.76%	.0727	.0762	4.81%
Linolenic acid	.1985b	.2755a	38.79%	.2591	.2748	6.06%
Heneicosanoic acid	.1916a	.0724b	-62.21%	.0484	.0331	-31.61%
Behenic acid	1.7107b	1.8928a	10.64%	1.2362	1.4487	17.19%
Lignoceric acid	.0970	.0985	1.55%	.1034	.1055	2.03%
Oilyield (%)	27.89	29.49	1.60	25.48b	27.85a	2.37%

SWD: Severe water deficit; Means for same genotypes with different superscripts (a-b) are significantly different according to Student t test ($\alpha = 0.05$)

Fatty acid unsaturation degree

Based on our experimental data (Table 8), lipids extracted from safflower seeds are dominated by C16 and C18 fatty acids which are common in higher plants (Rebey et al., 2017). Analysis of fatty acid composition indicated that safflower seeds in control plants of Remzibey-05 genotype were characterized by a high proportion of polyunsaturated fatty acids (PUFA) (58.68%) versus 30.10% of monounsaturated fatty acids (MUFA) and 10.87% of saturated fatty acids. SWD significantly decreased saturated fatty acids (SFA) whereas significantly increased MUFA, UFA and UFA/SFA. However, SWD did not elicit any significant changes in PUFA in Remzibey-05 genotype.

In control groups, safflower seeds in control plants of Gokturk genotype were characterized by a high proportion of polyunsaturated fatty acids (PUFA) (59.34%)

versus 32.96% of monounsaturated fatty acids (MUFA) and 7.36% of saturated fatty acids. SWD significantly decreased saturated fatty acids (SFA) whereas significantly increased PUFA, UFA and UFA/SFA. However, SWD did not elicit any significant changes in MUFA in Gokturk genotype.

Fatty acid unsaturation degree has been considered important in maintenance of membrane fluidity and providing optimal environment for proper membrane functions (Xu and Beardall, 1997; Rebey et al., 2017). In safflower seeds, under control and SWD, the unsaturated fatty acid (UFA) to saturated fatty acid (SFA) ratio was 8.17 and 11.54, respectively for genotype Remzibey-05. UFA/SFA was 12.55 (control) and 14.48 (SWD) for Gokturk genotype. Both genotypes exhibited similar manner regarding fatty acid unsaturation degree.

Table 8. Fatty acid unsaturation degree of genotypes in response to water stress

Fatty acid unsaturation degree	Genotype: Remzibey-05			Genotype: Gokturk		
	Control	SWD	Change (%)	Control	SWD	Change (%)
SFA	10.87a	7.96b	-26.77%	7.36a	6.46b	-12.23%
MUFA	30.10b	32.49a	7.94%	32.96	33.07	0.33%
PUFA	58.68	59.42	1.26%	59.34b	60.42a	1.82%
UFA	88.78b	91.90a	3.51%	92.31b	93.49a	1.28%
UFA/SFA	8.17b	11.54a	41.25%	12.55b	14.48a	15.38%

SWD: Severe water deficit; Means for same genotypes with different superscripts (a-b) are significantly different according to Student t test ($\alpha = 0.05$)

However, UFA/SFA was significantly decreased with rising water deficit levels, indicating that severe water stress causes a decline in the passive membrane permeability (Rebey et al., 2017) and coupling with an increase of cellular membrane rigidity (Monteiro de Paula et al., 1993). Herewith, plant might protect itself against possible accumulation oxidative stressors through modifying or re-structuring the membranes with less unsaturated fatty acids.

Correlations between major fatty acids and oil yield

Along with the present study, two correlation analyses for fatty acids were done (Tables 9-10). In the first correlation analysis (Table 9), all data obtained from both genotypes were pooled- not considering the experimental groups- and then correlated. According to the correlation coefficients, palmitic acid was positively correlated with stearic acid ($r = .955$; $p < 0.01$), oleic acid ($r = .882$; $p < 0.01$) but negatively correlated with behenic acid ($r = -.680$; $p < 0.05$). Stearic acid was positively correlated with oleic acid ($r = .938$; $p < 0.01$) but negatively correlated with linoleic acid ($r = -.917$; $p < 0.01$) and behenic acid ($r = -.650$; $p < 0.05$). Oleic acid was negatively correlated with linoleic acid ($r = -.0996$; $p < 0.01$).

According to the second correlation analysis done in order to determine whether correlation coefficients vary depending control and stress groups (Table 10), in control group, palmitic acid was positively and significantly correlated with linoleic acid ($r = .850$; $p < 0.05$) but in stress group, palmitic acid was again positively correlated but not significant. Also, palmitic acid was negatively and significantly correlated with

behenic acid ($r = -0.831$; $p < 0.05$) but in stress groups, the coefficient was .060 and not significant. Oleic acid was positively and significantly correlated with behenic acid ($r = 0.980$; $p < 0.01$) in control group. However, the coefficient strongly shifted towards negative correlation from .980 to -.494. As expected, opposite changes were observed between linoleic acid and behenic acid from -.993 to .537 (Table 10).

Table 9. Correlations between major fatty acids and oil yield in safflower seeds ($n = 12$)

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Behenic acid	Oil yield
Palmitic acid	1					
Stearic acid	.955**	1				
Oleic acid	.882**	.938**	1			
Linoleic acid	-.917**	-.961**	-.996**	1		
Behenic acid	-.680*	-.650*	-.376	.442	1	
Oil yield	-.332	-.296	-.181	.217	.302	1

* $p < 0.05$ ** $p < 0.01$

Table 10. Correlations between major fatty acids in control and SWD respectively ($n = 6$)

	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Behenic acid	Oil yield
Palmitic acid	1					
Stearic acid control SWD	.185 -.730	1				
Oleic acid control SWD	-.882* -.820*	-.426 .317	1			
Linoleic Acid control SWD	.850* .639	.418 -.189	-.990** -.924**	1		
Behenic acid control SWD	-.831* .060	-.335 .603	.980** -.494	-.993** .537	1	
Oil yield control SWD	-.175 -.399	-.437 .241	.276 .481	-.267 -.246	.201 .056	1

* $p < 0.05$; ** $p < 0.01$; SWD: severe water deficit

Effects of water stress on seed mineral content of safflower

The changes mineral contents in both genotypes with respect to the water stress treatments were statistically significant (Table 11). For Remzibey-05 genotype, K and Mg content significantly increased and as expectedly Na content significantly decreased. However, micro elements did not significantly differ as a response to severe water stress. For Gokturk genotype, K content significantly increased with severe stress. In similar to the Remzibey-05 genotype, there were decreases in Na content and increase in Mg content in Gokturk genotype. However, those changes were not statistically significant. Mg content diminished with drought (Grabaová and Martinková, 2001). Of the micro elements, Cu content significantly decreased with stress. The changes in content of micro elements were significant.

Any external stimuli-mediated internal changes in plant metabolism cause perturbations and pose impairments in mineral uptake and their translocation in the plants. Hu and Schmidhalter (2005) highlighted that the water stress induced- nutrient reductions might be attributed to the low transpiration ratio, decreased active transport and disrupted membrane permeability. In the former reports, K content decreased under drought conditions (Mahouachi, 2007). However, Ozkan and Kulak (2013) examined the K content in seeds of two genotypes of sesame, genotypes exhibited opposite behaviour of K accumulation in response to severe water stress. Arjenaki et al. (2012) reported the changes mineral elements of two wheat varieties contrasting drought stress, documenting that tolerant varieties had the highest value of K while sensitive variety had the highest value of Na. Along with the study, Remzibey-05 had the highest values of both K and Na in comparison with Gokturk. Furthermore, when evaluated the growth and yield, all parameters except 1000-seed weight favoured for Remzibey-05 genotype, which seems to be more tolerant against severe water stress.

Overall, it must be highlighted that the mineral uptake and sequential accumulation or partitioning of the minerals thought out the complex plant system are dependent many factors including species, genotypes, cultivars, age of the plant and then stress type and duration of the stress, timing of the stress (pre-flowering, flowering and post-flowering vegetative stage). Up to best knowledge and survey of the literature, the studies regarding with interaction of mineral uptake and following processes in response to water stress are scarce even though there are many studies focusing only the last accumulation and content of the status. Forthcoming studies are required to focus on the whole plant system response in terms of minerals and their fate after subjection to the water stress.

Table 11. Water stress treatments on the mineral content of safflower seed (mg kg^{-1})

	Remzibey-05		Gokturk		F values
	Control	SWD	Control	SWD	
K	6872.00±128.01b	7510.67±173.50a	6405.00±213.58c	7004.67±121.78b	36.162**
Na	831.00±10.13 a	660.19±60.67b	347.80±7.700c	318.83±23.99c	167.464**
Mg	624.90±10.55c	685.37±15.84b	890.63±34.40a	928.40±14.70a	152.702**
Fe	40.62±3.49b	42.30±6.14b	50.61±4.02a	51.28±3.36a	4.754*
Cu	4.63±0.26 c	4.52±0.28c	14.66±0.81a	12.33±1.67b	91.204**
Zn	32.24±2.99b	36.96±5.16b	47.28±2.45a	50.15±4.05a	14.796**

SWD: severe water deficit. Means \pm SD in the same row by the same letter are not significantly different to the test of Duncan ($\alpha = 0.05$). ** $p < 0.01$; * $p < 0.05$

Principal components analysis (PCA) of genotypes

PCA is of the powerful statistical tools in discrimination of the samples based on identified components. Along with the study, two genotypes under two experimental groups with their fatty acid profiles were submitted to Principal Component Analysis. Accordingly, components extracted (Table 12), total variance explained (Table 13) and 2-D visualization of the experimental groups (Fig. 1) were obtained. Herewith, the first component (factor) explained 76.476% of total variances. The second and third components explained 18.965% and 45.596% of total variances respectively. Experimental groups were well-discriminated, clarified and identified using PCA,

documenting that any external deviation from regular irrigation of the plants would cause changes in fatty acid metabolites with their percentage. Herein, genotype Remzibey-05 grown under control groups was quite differentiated from other groups (Fig. 1).

Table 12. Components extracted based on fatty acid compounds corresponding to the their loadings

Components	PC 1	PC 2	PC 3
Myristic acid	-0.26061	-0.2416	0.2832
Palmitic acid	-0.294	0.049671	0.045667
Palmitoleic acid	0.26381	0.25974	0.11928
Heptadecanoic acid	-0.27315	0.10474	-0.40634
Cis-10Heptadecanoic	0.28756	-0.024918	0.2695
Stearic acid	-0.29412	0.0032959	0.10548
Oleic acid	0.28051	-0.10805	-0.3063
Linoleic acid	0.26744	-0.042402	0.50497
Arachidic acid	-0.28525	-0.1282	0.17041
Cis11Eicosenoic acid	0.27601	0.2089	-0.053246
Linolenic acid	0.28407	0.087706	-0.27679
Heneicosanoic acid	-0.28516	0.10642	0.22612
Behenic acid	-0.089875	0.56431	0.046164
Lignoceric acid	0.23315	-0.34263	0.24914
Oilyield (%)	0.0057603	0.57592	0.28634

Table 13. Total variance explained

PC	Eigenvalue	% variance
1	114.714	76.476
2	28.447	18.965
3	0.683934	45.596

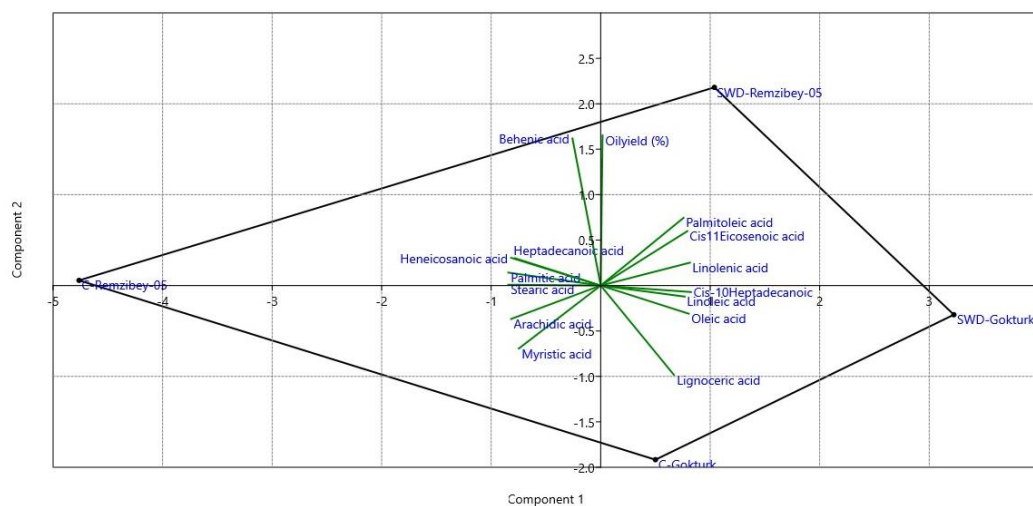


Figure 1. Principal component analysis of genotypes corresponding to the control and severe water stress

Heatmap construction for genotypes for their fatty acid profile corresponding to control and stress conditions

According to the heatmap (Fig. 2), arachidic acid, myristic acid, stearic acid, palmitic acid and heneicosanoic acid were more pronounced under control conditions but SWD suppressed their content. SWD increased behenic acid, linoleic acid, cis-11-eicosanoic acid and palmitoleic acid in Remzibey-05 genotype.

SWD topped the percentage of linoleic acid, cis-10-heptadecanoic acid, lignoceric acid, linoleic acid, oleic acid, cis-11-eicasenoic acid and palmitoleic acid in comparison with the control group of Gokturk.

As seen in Figure 2, control and SWD experimental groups exhibited clear different behaviour corresponding to the fatty acid profiles, which were also coupled and supported with principal component analysis (Fig. 1).

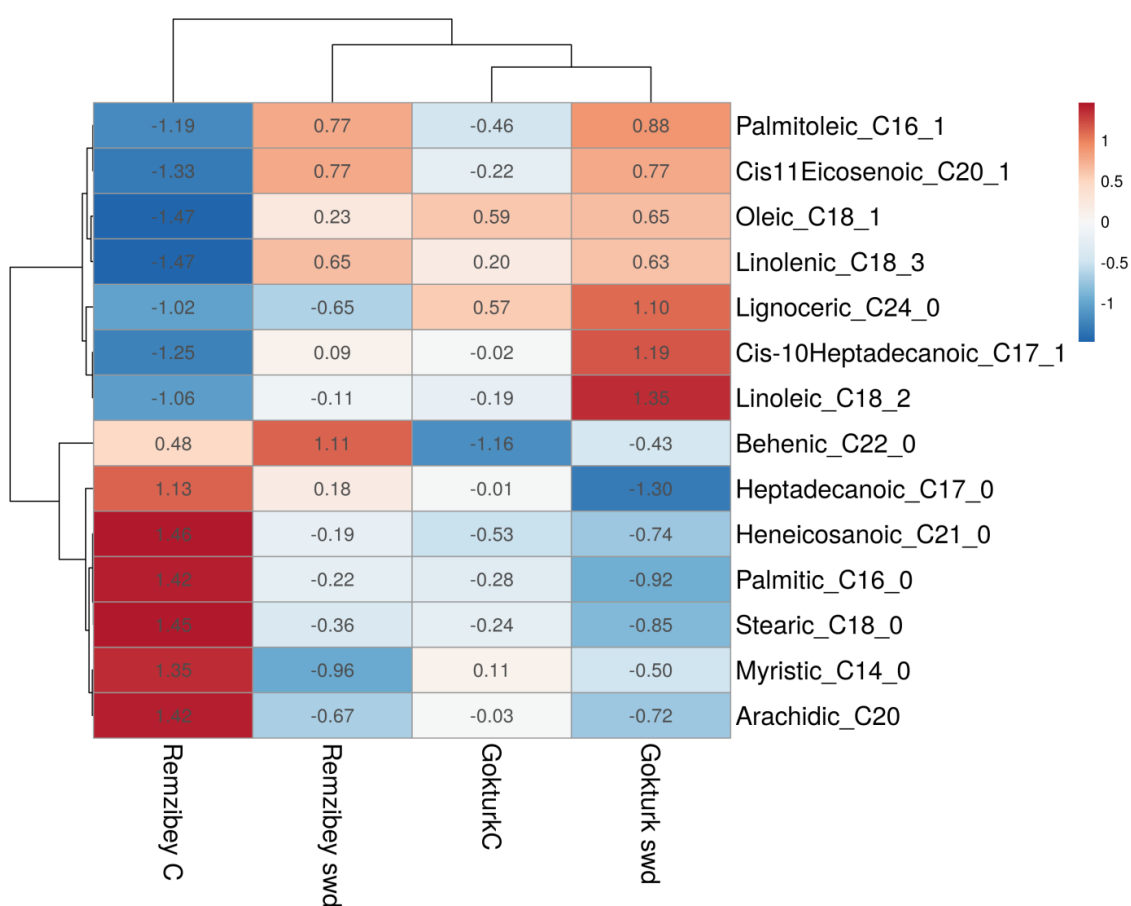


Figure 2. Heatmap constructed for both genotypes concerned with fatty acid profile corresponding to control and stress groups

Conclusion

The present study was designed to investigate the effects water stress on oil yield combined with their fatty acid profile and mineral contents of seeds of two safflower genotypes. The most important conclusions of this study are:

- Regarding growth and yield parameters under stress, Remzibey-05 genotype had higher values except 1000-seed weight but the values were not significant.

- Capsule number differences were reported to be significant under stress.
- Oil yield increased under stress conditions for both genotypes.
- UFA/SFA was significantly increased with stress for both genotypes.
- For Remzibey-05 genotype, K and Mg content significantly increased and as expected Na content significantly decreased. However, micro elements did not significantly differ as a response to severe water stress. For Gokturk genotype, K content significantly increased with severe stress.

Finally, genotypes exhibited similar behaviour regarding unsaturation degree of the oil, also increments in their oil yield.

For the forthcoming studies, field experiments regarding those genotypes should be performed since the present study was carried under controlled green house conditions. The study can be deemed as preliminary study to ascertain some basic properties of the two safflower genotypes under limited water sources. Moreover, more genotypes of the safflower can be screened for their performance and then a selection concerning high tolerant against low water content of soils for genotypes can be made.

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