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HAJAR AMINI

AHMAD ARZANI

MOSTAFA KARAMI

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Effect of water deficiency on seed quality and physiological traits of different safflower genotypes

Hajar AMINI, Ahmad ARZANI*, Mostafa KARAMI
Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

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Abstract: Safflower is one of the most adapted oilseed crops to the drought-prone arid lands. This experiment was carried out to evaluate the effects of water deficiency on some physiological traits such as proline content, soluble carbohydrate content, relative water content (RWC), and seed quality using 64 safflower genotypes grown under normal and water-deficient field conditions. Plants were grown under normal irrigation until the branching growth stage, when water deficiency was applied to the plants. Results of combined analysis of variance showed the significant effects of genotype, water deficiency, and their interactions on the tested traits. RWC, seed oil content, and oil yield significantly decreased whereas proline, soluble carbohydrate, and protein content increased in response to water deficiency. Cluster analysis divided genotypes into 3 groups of sensitive, tolerant, and semitolerant genotypes under water-deficient conditions. The second group (tolerant group) possessed the lowest seed yield loss due to water stress and produced the highest seed protein content and seed oil yield under water-deficient conditions. The superior seed yield and quality was closely related to the physiological properties of the plants, resulting in higher leaf proline, carbohydrate, and RWC under water-deficient conditions. The results suggest that leaf proline content, carbohydrate content, and RWC would be useful traits to select for water stress-tolerant plants in safflower.

Key words: Safflower, drought stress, proline, seed oil, soluble carbohydrate

1. Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop that can tolerate environmental stresses including salinity and drought (Dwiedi et al., 2005).

Drought is considered as one of the main environmental factors that cause osmotic stress and inversely influence plant performance as well as global crop production (Hojati et al., 2011; Saruhan Güler et al., 2012). It induces many physiological, biochemical, and molecular responses in plants resulting in the modification of some metabolic pathways (Delauney and Verma, 1993). In terms of intensity and duration of water shortage, plants are able to develop tolerance mechanisms to adapt to stress conditions (Aspinall and Paleg, 1981).

Numerous physiological and metabolic responses occurring during drought help plants to overcome injuries caused by osmotic stresses (Bray et al., 2000). Among them is the accumulation of low-molecular-weight soluble compounds including proline. Accumulation of proline is one of the drought avoidance mechanisms to counteract the decrease in pressure potential in a wide range in various plants when subjected to water deficiency (Aspinall and Paleg, 1981).

Accumulation of proline may play a role in osmotic adjustment, which is often considered to be involved in protection of enzymes and cellular structures. Proline acts as a free radical scavenger and stabilizes biological membranes, which results in adjustment of cell metabolism and growth in response to stress conditions and in dehydration tolerance of plants (Verbruggen and Hermans, 2008). Proline accumulation is a stress-inducing tolerance in many plant species (Bandurska, 2001).

Changes in soluble sugar contents under drought stress were also reported for a number of species. For example, Koutroubas et al. (2004) observed the positive role of total soluble sugar contents in safflower accessions differing in drought stress. Moreover, the combined effects of soluble carbohydrate and proline content were observed in several drought stress plant species and have been attributed to a reduction in the rates of protein synthesis and an increase in proteolytic activity, both of which tend to cause an increase in the total soluble nitrogen. The accumulation of compatible solutes is a prerequisite for the adaptation of plants to osmotic stress imposed by drought stress (Serraj and Sinclair, 2002). Water content and water potential of plant tissue are considered as the

* Correspondence: a_arzani@cc.iut.ac.ir

physiologically appropriate integrators of drought effects; thus, relative water content (RWC) has been nominated as a suitable screening tool for drought-tolerant crops (Jones, 2007; Terzi et al., 2013). It has been reported that water loss can lower leaf water potentials, leading to reduced turgor, stomatal conductance, and photosynthesis, and finally to reduced seed yield. Movahhedy-Dehnavy et al. (2009) observed that drought-tolerant genotypes of safflower possessed higher RWC than others under water-deficient conditions.

It is worth mentioning that the ultimate purpose of safflower cultivation is oil and protein production. Considering the factors responsible for increasing crop economic yield, environmental factors such as drought stress are the most important (Blum, 1997). Water-deficient conditions increase protein and decrease oil contents. Reduction in oil content of soybean seed has also been reported to take place under drought stress (Maclagan, 1993).

Due to the fact that tolerance of water deficiency is considered as a primary breeding goal for crop plants to be grown in arid areas, it seems that a deeper understanding of the physiological and biochemical mechanisms employed by water stress-tolerant genotypes is necessary for a plant breeder to select plants with high and stable yield performance. Therefore, the objective of this study was to increase our understanding of the effect of water-deficit stress on some physiological traits and seed protein and oil contents of 64 safflower genotypes.

2. Materials and methods

2.1. Plant material and growth conditions

A total of 64 safflower genotypes, including 46 native and 18 exotic genotypes, were grown in 2 growing seasons (2011 and 2012) at the research farm of Isfahan University of Technology located at Lavark, Najaf-Abad, Iran (40 km southwest of Isfahan, 32°32′N, 51°23′E, 1630 m a.s.l.). A square lattice design (8 × 8) with 2 replications was used for each water-deficit stressed and nonstressed (normal) field experiment. The mean annual precipitations and mean annual temperature were 149 mm and 15.4 °C, respectively. The soil type at the experimental site is a silty clay loam, Typic Haplargids of the arid tropic with pH 7.3–7.6. The nitrogen, phosphorus, and potassium fertilizers were applied at 120:60:60 kg/ha in terms of urea, super phosphate, and potash, respectively, in 2 split applications prior to planting and at the early branching stage.

Plants were grown under full irrigation until the branching growth stage, when water stress was applied. This is a common problem affecting safflower and other spring season crops in the region. Irrigation treatments were applied based on the maximum allowable depletion (MAD) percentage of the soil available water (SAW). Plants were irrigated at 50% and 80% MAD of SAW in nonstressed and stressed plots, respectively (Stegman, 1983). In the full irrigation treatment (normal conditions), irrigation was done when 50% of the SAW was depleted from the root zone. The deficit irrigation treatment (stress condition) was irrigated when 80% of the SAW was depleted (Allen et al., 1998). Due to temporal variation of evapotranspiration, the number of days between 2 irrigations during the growing season was variable. Soil samples were taken from soil depths of 0–30 and 30–60 cm for both normal and water-deficient conditions and were analyzed for soil moisture contents. The depth of irrigation was calculated using the following equations:

$$SAW = (\theta_{fc} - \theta_{pwp}) \times D \times 100, \quad (1)$$

$$I_d = SAW \times p, \quad (2)$$

$$I_g = I_d \times 100 / Ea. \quad (3)$$

SAW is soil available water (cm); θ_{fc} and θ_{pwp} are the volumetric soil water content (%) at field capacity (0.03 MPa) and permanent wilting point (1.5 MPa), respectively; D is the soil layer depth (cm); I_d is the irrigation depth (cm); p is the fraction of SAW (50% and 80%) that can be depleted from the root zone; I_g is the gross depth of irrigation (cm); and Ea is the irrigation efficiency averagely assumed as 65%.

Precipitation and cumulative amount of water applied for each water-deficient stressed and nonstressed (normal) field experiment in 2 growing seasons are presented in Figures 1 and 2.

Leaf proline content, soluble carbohydrate content, RWC, and seed quality of the studied safflower genotypes at the 50% flowering stage were assessed using the following procedures.

2.2. Proline content

Free proline content was determined according to Bates et al. (1973). Approximately 0.2 g of leaf was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 10,000 rpm. Supernatant was used for the measurement of proline content. The reaction mixture consisted of 2 mL of acid ninhydrin and 2 mL of glacial acetic acid, which was boiled at 96 °C for 1 h. After the termination of the reaction in an ice bath, the reaction mixture was extracted using 4 mL of toluene and the absorbance of the pink-red upper phase was recorded at 520 nm against a toluene blank using a spectrophotometer (U-1800, Hitachi, Japan). A standard curve for proline in the range 0.01–1.5 mM was constructed to determine the proline concentration in each sample. Free proline content was expressed in mg/g of fresh leaf weight.

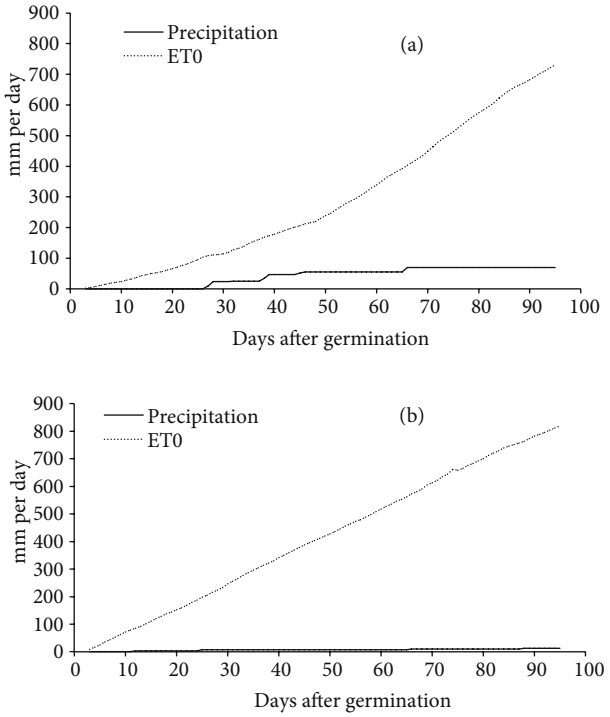

Figure 1. Precipitation for 2011 (a) and for 2012 (b). ET0 represents evapotranspiration.

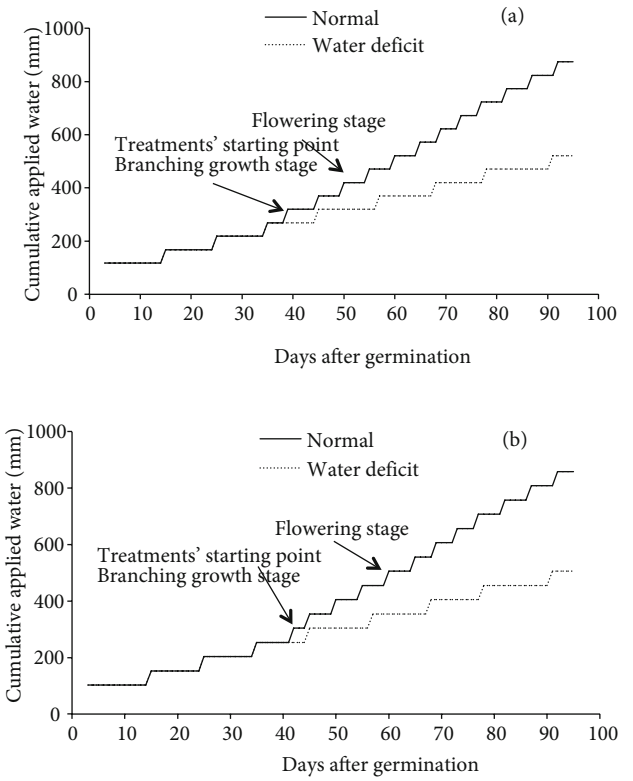

Figure 2. Cumulative amount of water applied for irrigation regimes (normal and water-deficit stress) for 2011 (a) and for 2012 (b).

2.3. Soluble carbohydrate content

Carbohydrates were extracted from dry leaf of safflower in warm water. Quantifications of total soluble carbohydrates were carried out at 490 nm according to Dubois et al. (1956), using glucose (Sigma Chemicals) as a standard. Soluble carbohydrate content was expressed in mg/g of dry leaf weight.

2.4. Relative water content

RWC was determined according to Barrs and Weatherley (1962) using the following equation:

(fresh weight – dry weight) /
(turgid weight – dry weight) × 100.

Turgid weight was determined after imbibition of the tissue in distilled water for 4 h. Dry weight was determined after incubation of the leaf segments in an oven at 80 °C for 48 h.

2.5. Seed oil content and seed oil yield

Ten grams of ground seeds was used to extract the oil, using petroleum ether for 6 h in a Soxhlet system according to the AOCS method (AOCS, 1993), and then the oil content as a percentage was calculated for each sample. Oil yield (kg/ha) was calculated as the product of seed oil content and seed yield.

2.6. Seed protein content

The seed protein content was calculated by multiplying total nitrogen content by a factor of 5.30. Total nitrogen content of the seeds was determined by the micro-Kjeldahl method (Jackson et al., 1973) and then the protein content as a percentage was calculated for each sample (Bremner and Mulvaney, 1982).

2.7. Data analysis

The data were tested for homogeneity and normality of residuals using the Kolmogorov–Smirnov and Bartlett tests, respectively. Afterwards, a combined analysis of variance (ANOVA) was used to compare the effects of water deficiency and nonstressed treatments and genotypes by environmental (normal and water deficiency) condition interactions for 2 years using PROC GLM of SAS 9. Mean comparisons were conducted using Fisher's least significant difference ($LSD_{0.05}$) test. Cluster analysis was conducted using Ward's method based on linkage distances with SPSS 19.0.

3. Results

Since there were no significant differences between the 2 growing seasons (years) for the tested traits except RWC and seed protein content ($P < 0.05$), 2-year data were pooled and used for the mean comparisons. Results of combined ANOVA showed a significant effect of water-deficit stress on all characteristics that were assessed in this study (Table 1). Analysis of combined ANOVA also indicated significant differences among genotypes for all the traits under both normal and water-deficient conditions (Table 1). Moreover, significant genotype × year interactions were observed for oil yield and seed yield (Table 1). In addition, the mean comparisons of genotypes under water-deficiency stress and nonstressed conditions of the traits revealed a cross-over genotype × environment interaction (G × E), meaning that genotypes recognized as suitable for a normal environment would not be so for the water-deficient conditions.

Safflower genotypes varied significantly for proline and soluble carbohydrate contents under both normal and water-deficient stress conditions (Table 1). Means of proline content were significantly increased by water stress and ranged from 0.38 to 3.1 mg/g leaf fresh weight and 1.3 to 3.1 mg/g leaf fresh weight under normal and water-deficient conditions, respectively (Table 2).

There was a negative correlation between seed yield and proline content under water-deficient conditions ($r = -0.49^{**}$). The C411, Kino-78, and PI-250190 genotypes not only possessed the highest proline content (Table 2) but also had the least seed yield loss (Table 3).

Water deficiency stress increased the mean soluble carbohydrate content in the vast majority of safflower genotypes (Table 2). On the other hand, water deficiency stress decreased the mean RWC in the vast majority of genotypes (Table 2). Moreover, the genotypes varied significantly for RWC under both environmental conditions (Table 1). In the present study, the C411 genotype was a member of the group having the highest leaf RWC and proline contents in water-deficient conditions (Table 2).

Field water deficiency stress caused decreases in the mean oil content and mean oil yield in the vast majority of safflower genotypes used in this study (Table 3). Genotypes varied significantly for oil contents and oil yield under both environmental conditions (Table 1).

Oil contents of safflower genotypes ranged from 24.4% to 32.95% under normal and 21.6% to 31% under water-deficient field conditions (Table 3). The mean oil yield reduction due to water-deficit stress was 206.1 kg/ha (36%) and the overall mean oil content reduction was 8.8% (Table 3). In the present study, the highest seed oil yield belonged to the C411 line, which was significantly superior compared to the other genotypes under both normal and water-deficit stress conditions. However, its oil content in both water treatments was inferior. The Hamedan 17 genotype produced the greatest amount of oil content (32.9%) under both normal and water-deficient field conditions (Table 3). The IL genotype possessed the lowest oil content under both nonstressed and water-deficient conditions (Table 3). It is interesting to note that the IL genotype had the lowest oil content under contrasting normal and sa-

Table 1. Combined analysis of variance for physiological traits and seed quality in safflower genotypes grown under 2 environmental conditions (normal and water-deficit stress) in 2 growing seasons of 2011 and 2012.

Source of variation	df	Mean square						
		Proline	Soluble carbohydrate	RWC	Protein content	Oil content	Oil yield	Seed yield
Environment (E)	1	39.81**	18753**	18706*	680.1*	800**	19,074,689**	45,908,372**
Year (Y)	1	0.22^{ns}	699.3^{ns}	53505*	3746*	0.02^{ns}	2,783,086	28,203^{ns}
E × Y	1	0.0001^{ns}	0.03^{ns}	14535**	148.7**	0.00001^{ns}	4,141,510**	0.00001^{ns}
Block / (E × Y)	4	0.75**	368.7^{ns}	3743**	133.5**	3.01**	102,254**	942,701**
Genotype (G)	63	2.02**	1073**	3591**	25.5**	19.71**	49,585**	933,564 **
G × E	63	1.02**	880.9**	151.4**	11.24**	3.35**	24,379**	310,340**
G × Y	63	0.001^{ns}	7.71^{ns}	52.3^{ns}	2.05^{ns}	0.005^{ns}	21,990**	124,552*
G × E × Y	63	0.0001^{ns}	0.01^{ns}	60.1*	0.99^{ns}	0.00001^{ns}	6054^{ns}	0.00001^{ns}
Residual	252	0.21	208.4	45.72	2.42	0.69	14,113	89,601

RWC: relative water content.
ns, *, and ** represent nonsignificant and significant at $P < 0.05$ and $P < 0.01$, respectively.

Table 2. Mean of physiological traits in 64 safflower genotypes grown under normal and water-deficient field conditions.

No.	Genotypes	Origin	Proline (mg/g fw)		Soluble carbohydrate (mg/g dw)		Relative water content (%)	
			Normal	Stress	Normal	Stress	Normal	Stress
1	C111	Iran	1.58	2.88	75.30	110.14	84.20	62.90
2	C116	Iran	2.06	1.39	79.25	60.17	73.10	58.56
3	C411	Iran	1.12	3.03	43.78	80.04	84.20	82.50
4	C444	Iran	2.29	2.90	76.13	56.49	63.49	53.99
5	C4110	Iran	1.40	2.95	65.11	80.69	68.44	54.13
6	S6-58/41-168	Iran	3.01	2.54	83.97	85.99	74.99	75.98
7	S6-697-307	Iran	1.53	2.33	90.45	95.10	65.93	62.51
8	S6-697-324	Iran	3.06	2.80	95.79	101.11	69.99	53.34
9	IL	Iran	1.37	2.83	55.85	98.18	75.65	58.43
10	N/27	Iran	2.16	2.92	64.44	82.10	73.81	59.10
11	73-14-34	Iran	1.18	2.86	45.41	67.89	62.95	84.44
12	PI-405985	Iran	2.15	2.88	62.10	97.54	77.59	56.87
13	LRV-51-51	Iran	1.10	2.13	117.35	89.61	62.32	49.46
14	LRV-55-295	Iran	1.26	2.94	77.87	69.15	77.96	72.24
15	Hamedan17	Iran	0.83	1.61	69.05	63.83	73.78	69.43
16	Hamedan21	Iran	3.10	2.82	64.15	78.21	73.46	61.79
17	Hamedan38	Iran	1.80	2.78	65.22	88.79	81.46	57.71
18	Hamedan40	Iran	2.81	2.81	91.12	118.69	64.80	65.91
19	Kordestan1	Iran	2.33	2.80	94.67	60.70	68.46	58.75
20	Kordestan2	Iran	1.27	3.01	74.04	83.07	68.67	68.11
21	Kordestan3	Iran	1.29	2.80	55.21	108.61	75.23	49.75
22	Kordestan4	Iran	3.06	2.60	70.16	66.56	73.71	56.72
23	Kordestan5	Iran	0.68	1.41	80.70	60.77	68.11	62.45
24	Kordestan6	Iran	2.72	2.83	78.93	91.05	82.61	60.59
25	Kordestan7	Iran	1.52	2.42	39.43	60.72	67.19	52.37
26	Kordestan8	Iran	2.70	2.67	78.99	105.12	57.07	55.60
27	Kordestan9	Iran	2.58	2.85	89.57	59.83	61.74	61.55
28	Darab1	Iran	2.87	2.81	69.76	66.86	67.50	55.02
29	Darab2	Iran	2.52	2.61	55.82	80.70	72.50	61.61
30	Darab4	Iran	3.07	2.80	75.97	79.24	58.95	50.68
31	Darab9	Iran	1.75	2.98	42.62	61.12	69.04	47.68
32	Khorasan62	Iran	1.51	1.80	51.68	88.46	69.54	59.87
33	Khorasan330	Iran	1.13	1.65	55.16	106.32	65.88	62.97

Table 2. (Continued).

No.	Genotypes	Origin	Proline (mg/g fw)		Soluble carbohydrate (mg/g dw)		Relative water content (%)	
			Normal	Stress	Normal	Stress	Normal	Stress
34	Khorasan376	Iran	1.46	2.10	67.05	68.60	75.85	75.36
35	Khorasan508	Iran	2.97	3.01	48.35	85.61	67.43	56.59
36	Kermanshah	Iran	2.24	2.73	69.67	91.63	73.40	60.34
37	Kermanshah44	Iran	1.50	2.47	38.61	91.19	61.57	63.05
38	Kermanshah46	Iran	1.46	2.92	64.26	73.89	76.36	58.75
39	Kermanshah47	Iran	2.91	2.92	75.16	80.56	74.70	62.95
40	Kemanshah60	Iran	3.11	2.90	47.02	61.61	57.10	68.76
41	Esfahan4	Iran	2.06	2.70	104.32	76.05	61.85	54.10
42	Esfahan Kuse	Iran	2.22	2.97	74.03	79.22	86.50	51.86
43	Marand	Iran	2.73	3.14	47.00	61.92	67.21	58.03
44	Zarghan	Iran	2.99	2.87	59.74	97.78	67.17	61.13
45	Sina	Iran	2.93	2.36	79.29	106.81	77.10	58.94
46	Arak	Iran	2.97	2.22	92.08	69.74	65.14	69.16
47	Dincer	Turkey	1.93	2.86	59.42	73.69	73.22	51.13
48	Yinice	Turkey	2.66	2.90	84.79	69.99	72.28	59.25
49	C1055	Turkey	0.38	2.87	57.63	85.15	70.58	55.68
50	PI-198844	France	2.68	2.57	49.53	57.66	72.37	61.56
51	PI-253384	Palestine	2.93	2.83	56.07	73.87	68.47	50.46
52	PI-250190	Pakistan	2.60	2.96	62.96	76.16	59.35	56.99
53	PI-250537	Egypt	1.47	2.75	78.48	65.70	67.43	50.79
54	PI-506426	China	2.93	2.44	60.75	70.69	73.52	58.99
55	Cyprus Bregon	Cyprus	2.96	2.85	56.68	81.56	67.28	68.91
56	Syrian	Syria	2.98	2.90	62.01	102.20	70.23	72.51
57	PI-258417	Portugal	2.70	2.76	63.48	70.94	67.71	56.24
58	Hartman	USA	1.69	2.68	57.96	60.98	53.31	50.23
59	Gila	USA	0.93	2.45	79.66	92.19	55.79	48.53
60	CW-4440	USA	3.08	2.22	86.41	78.06	64.75	82.90
61	S-541	USA	2.19	2.31	62.84	77.56	66.12	52.21
62	PI-537636-S	USA	2.93	2.96	54.59	76.54	76.39	51.27
63	PI-537636	USA	0.89	2.91	81.26	108.70	68.51	48.14
64	Kino-76	Mexico	0.50	2.99	74.07	70.81	68.01	78.82
	LSD 0.05		0.99	0.74	30.49	24.13	8.90	12.45

Table 3. Mean of seed oil, protein, and oil yield in 64 safflower genotypes grown under normal and water deficiency stress field conditions.

Genotype	Protein content (%)		Oil content (%)		Oil yield (kg/ha)	
	Normal	Stress	Normal	Stress	Normal	Stress
C111	15.70	15.50	28.90	25.34	574.1	260.2
C116	14.30	15.31	27.38	23.85	513.8	323.1
C411	15.75	20.76	26.78	22.65	610.1	574.1
C444	20.90	21.52	28.86	26.75	525.3	210.4
C4110	19.45	17.31	28.01	26.50	593	304.1
S6-58/41-168	14.25	17.52	26.78	23.45	583.4	296.8
S6-697-307	14.45	16.51	29.61	27.75	525.3	313.2
S6-697-324	16.75	16.59	26.71	25.10	472.1	412.8
IL	18.35	21.32	27.70	22.45	833.8	516.9
N/27	16.25	19.57	27.72	25.65	467.9	225.1
73-14-34	17.60	15.30	27.80	27.00	499.8	362.1
PI-405985	16.20	18.81	25.38	23.26	363.7	190.1
LRV-51-51	15.05	15.70	27.60	26.05	497.2	392.7
LRV-55-295	16.65	18.18	28.42	25.85	606.2	424.5
Hamedan17	17.20	15.82	32.95	31.05	668.3	447.1
Hamedan21	14.40	16.10	27.55	26.81	674.1	483
Hamedan38	16.35	20.50	29.97	22.80	758.8	480.4
Hamedan40	15.40	15.80	28.33	25.05	510.0	266.1
Kordestan1	19.65	20.25	29.90	28.50	644.7	569.2
Kordestan2	16.25	18.45	28.29	26.45	513.4	395.2
Kordestan3	17.15	20.10	29.04	26.40	453.6	475.8
Kordestan4	18.95	17.70	25.19	22.80	404.6	296.3
Kordestan5	20.10	21.82	27.99	24.95	692.6	491.1
Kordestan6	18.25	19.55	28.40	25.35	627.5	355.9
Kordestan7	13.56	15.45	26.97	23.85	516.8	299.1
Kordestan8	15.15	16.40	30.24	26.95	495.5	478.4
Kordestan9	15.95	17.22	27.65	24.25	580.2	337.5
Darab1	16.30	17.24	27.05	25.65	454.2	265.8
Darab2	14.60	16.87	26.73	25.10	565.5	365.4
Darab4	15.95	17.86	27.98	23.35	587.5	272.3
Darab9	14.35	19.95	29.40	26.10	552.3	281.8
Khorasan62	16.65	18.16	30.37	27.45	934.7	476.5
Khorasan330	15.55	17.36	28.52	27.20	667.3	337.3

Table 3. (Continued).

Genotype	Protein content (%)		Oil content (%)		Oil yield (kg/ha)	
	Normal	Stress	Normal	Stress	Normal	Stress
Khorasan376	14.9	16.95	29.02	25.85	676.1	306.3
Khorasan508	13.40	15.40	29.50	27.05	457.4	407.1
Kermanshah	15.95	16.89	28.20	28.45	705.8	459.7
Kermanshah44	14.55	17.17	28.60	25.30	522.6	267.2
Kermanshah46	13.10	16.81	27.87	25.30	482.8	237.3
Kermanshah47	19.00	18.10	24.63	21.65	885.7	469.4
Kemanshah60	15.55	16.37	26.51	24.45	376.9	275.7
Esfahan4	17.95	16.85	27.83	28.00	514.5	473.1
Esfahan Kuse	17.8	20.22	25.28	23.85	423.5	314.6
Marand	14.65	14.17	27.56	25.20	439.1	268.4
Zarghan	15.85	15.43	26.51	25.15	571.6	387.8
Sina	15.05	17.33	28.66	27.25	536.3	471.6
Arak	18.90	18.25	25.24	25.30	386.9	368.7
Dincer	21.25	15.42	27.57	23.35	589.4	282.8
Yinice	15.90	15.65	29.63	27.80	741.8	411.2
C1055	16.00	17.04	30.70	26.99	703.8	397.6
PI-198844	14.10	18.34	27.50	25.05	544.9	376.8
PI-253384	19.05	17.31	24.42	22.75	455.7	425.6
PI-250190	16.30	18.83	28.34	27.05	494.6	422.1
PI-250537	14.55	20.79	27.67	24.95	599.5	401.1
PI-506426	13.90	18.21	32.15	26.65	866.7	373.9
Cyprus Bregon	15.70	15.25	28.17	25.80	557.3	315.7
Syrian	13.00	12.86	26.87	23.00	636.7	476.5
PI-258417	15.30	19.89	28.44	25.30	513.5	383.9
Hartman	16.6	12.36	28.93	26.05	490.8	202.1
Gila	14.2	20.18	27.97	26.55	509.2	252.9
CW-4440	13.90	13.77	31.41	27.99	541.7	316.1
S-541	14.70	17.02	29.70	26.75	728.8	288.3
PI-537636-S	17.40	14.89	27.28	25.80	417.3	239.1
PI-537636	19.70	16.45	28.60	27.25	514.7	277.3
Kino-76	12.45	17.54	27.78	26.45	443.5	381.6
LSD 0.05	2.24	2.46	1.64	1.69	181.6	157.1

linity-stress field conditions, as well (Yeilaghi et al., 2012). In addition, the Iranian safflower genotypes Kermanshah 47, Hamedan 38, and Kordestan 1 had the highest oil yield under both normal and water-deficient conditions (Table 3). Thus, these genotypes could perform well under both environmental conditions.

Seed protein content was significantly affected by water-deficit stress (Table 1). The 64 safflower genotypes used in the present study displayed increased seed protein content with water deficiency (Table 3). Significant variations were observed among genotypes with respect to protein content under both environmental conditions. Protein content of safflower genotypes ranged from 12.45% to 21.25% under normal and 12.36% to 21.82% under water-deficient field conditions (Table 3). The highest protein content was produced by Kordestan 5, while the lowest was produced by Hartman under water-deficit stress (Table 3). There was a negative correlation between seed oil and protein content under water-deficient conditions ($r = -0.46^{**}$). The results of cluster analysis based on the tested traits divided the genotypes into 3 groups of sensitive, tolerant, and semitolerant (Figure 3). The highest seed oil yield and protein content belonged to the genotypes in the second group. Thus, Hamedan 38, C411, Kordestan 1, and others located in the second cluster could be recommend as having high seed yield and quality under water-deficient conditions.

4. Discussion

Numerous physiological and metabolic responses occurring during drought stress may play a role in improvement of injuries caused by osmotic stresses. In this study, leaf proline content was increased due to water-deficit stress. This result is in agreement with that of Ninganoor et al. (1995), who reported an increase of proline content of safflower cultivars under water-deficit stress. Considering accumulation of proline as a widespread stress response is one of the most frequently reported modifications in response to drought stress in plants and it is often considered to be involved in stress tolerance mechanisms (Verbruggen and Hermans, 2008). Increasing soluble carbohydrate and proline contents in several drought-stressed plant species have been related to a reduction in the rates of protein synthesis and an increase

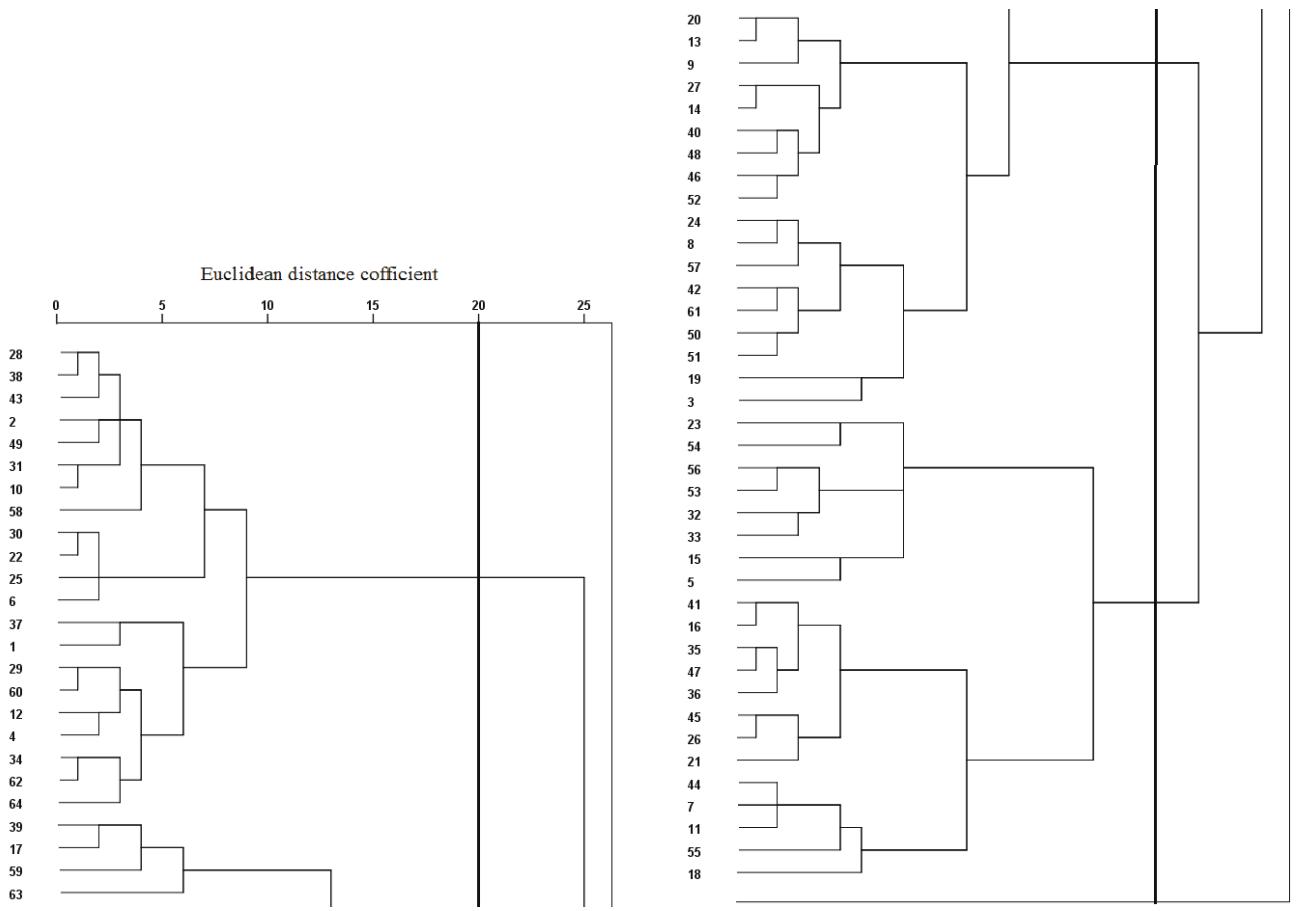

Figure 3 Cluster analysis of safflower genotypes on the basis of physiological and seed quality traits under water-deficit stress. The numbers 1 to 64 correspond to the genotypic numbers listed in Table 2.

in proteolytic activity, both of which tend to cause an increase in the total soluble nitrogen (Serraj and Sinclair, 2002). Thus, the negative correlation observed between proline content and seed yield under water-deficit stress is a reasonable finding. This result is consistent with that of Bandurska (2001), who demonstrated the benefits of proline accumulation in tolerating water deficiency stress in safflower genotypes.

A positive relationship was observed between leaf proline content and RWC under water-deficit stress. Moreover, the greatest level of RWC in the second group was accompanied with the greatest seed yield of this genotype, providing further evidence supporting its strong association with seed yield under both environmental conditions (Figure 3). Accumulation of higher proline content in the leaves may due to lower RWC (Clarke and McCaig, 1982).

The soluble carbohydrate content was positively influenced by water-deficit stress (Table 2). This finding is consistent with that of previous researchers who reported an increase in carbohydrate content due to water stress (Hongbo et al., 2006; Yau, 2006; Zhang et al., 2008).

The high RWC of tolerant genotypes was probably the result of their better ability for water uptake at low soil water potential (Kumar and Sharma, 2010). These results are in agreement with the findings of Hojati et al. (2011), who reported high RWC under drought in safflower tolerant genotypes, suggesting RWC as a good indicator of plant performance under drought stress conditions (Kumar and Sharma, 2010; Schonfeld et al., 1988). The decline in RWC could be attributed to an imbalance between water loss from the leaves due to evapotranspiration in the plant canopy and replenishment by irrigation (Jones, 2007).

The reduction in seed oil content observed under water-deficit stress is not only consistent with the previous works conducted under drought stress but also consistent with a previous work using the same 64 genotypes, in which the oil content of safflower seeds was found to be decreased due to salinity stress (Yeilaghi et al., 2012). These results are in agreement with those of Ashrafi and Razmjoo (2010) in safflower and Dornbos and Mullen (1992) in soybean, who also observed oil content reductions due to drought stress. Oil concentration is not only affected by drought stress but was also shown to highly depend on the genotype (Blum, 1997). The results of significant genotypic variation for oil content under both environmental conditions are in agreement with the findings of Premchandra et al. (1990), who reported that the seed oil content varied considerably among safflower cultivars.

Reductions in oil yield as the economic yield may be related to reductions in seed production, oil content of seeds, or both (Yeilaghi et al., 2012). According to our results, it seems that oil content is of minor contribution to the overall oil yield and accordingly less affected by drought stress, which is consistent with the findings of Abbadi et al. (2008), who observed similar effects for nitrogen supply. The seed oil content ranges observed for safflower genotypes agree with those reported for safflower cultivars or accessions by other researchers (Knowles and Ashri, 1995; Weiss, 2000). The oil content was found to not be a stable yield component, which is in support of previous observations by Francois (1996).

The positive effect of water-deficit stress on seed protein content observed in this study is in agreement with the findings of Foroud et al. (1993), who observed the significant increase of seed protein content in soybean. Water-deficient conditions increased protein and decreased oil contents in rape due to changes in the embryo endosperm and testa (Henry and MacDonald, 1978). It is noteworthy that oil and protein production is a fundamental aim in the oilseed cultivation of plants like safflower. The negative correlation between seed oil and protein content is known from various oilseeds, and this association may be due to tight linkage between oil and protein alleles in the repulsion phase or to pleiotropic effects (Yu et al., 2012). Environmental factors during the seed-filling period and even during the flowering stage can widely affect seed yield and seed quality of oilseed crops (Monotti, 2003).

Because water deficiency is a worldwide problem seriously affecting global crop production and it will become even more important due to global climate change, developing tolerant genotypes with high yield and quality is a very essential approach to support crop production. In this study, the second cluster obtained from the cluster analysis possessed the highest seed yield, proline content, carbohydrate content, RWC, seed protein, seed oil yield, and seed yield. Therefore, this group of genotypes can be further exploited to improve genotypes tolerant to water deficiency in a safflower breeding program. In conclusion, the above physiological traits were in accordance with relevant least seed yield reduction and could be useful for screening of genotypes for water-deficit tolerance in safflower.

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