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ORIGINAL ARTICLE

Safflower's (*Carthamus tinctorius* L.) physio-biochemical mechanisms to improve its drought tolerance

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Abstract

Drought is a main stressor affecting plant production worldwide. Safflower (*Carthamus tinctorius* L.) is known to exploit biochemical strategies to tolerate drought stress. However, the little so far known about these strategies does not guarantee safflower yield stability in future. To fill the gap, changes in the biochemical traits and antioxidant activities of safflower were monitored using 100 genotypes under the two non-stress and drought-stress field conditions in two subsequent years (2017 and 2018). While drought stress was observed to give rise to reversible increases in total phenolics (TPC), total flavonoids (TFD), total flavonols (TFL), total anthocyanin (Ant), proline, malondialdehyde (MDA), and antioxidant activity, it decreased total chlorophyll (ChlT) and total carotenoid (Car) contents in safflower. Under drought stress, the highest values for TPC (21.55 16.07 mg GAEg^{-1} fresh weight [FW]), Car (0.08 mg g^{-1} FW), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity (98%) were measured in G_{16}, averaged over the two study years. Also the highest values for TFD (5.17 mg QEg^{-1} FW), TFL (1.99 mg QEg^{-1} FW), Ant (234.1 µmol g^{-1} FW), ChlT (0.67 mg g^{-1} FW), and proline (851 µmol g $^{-1}$ FW) were recorded for G_{80}, G_{60}, G_{23}, G_{62}, and G_{33}. The least MDA content (2.8 µmol g^{-1} FW) was denoted to G_{91} under drought stress. The results of both principal component and correlation analyses demonstrated the effective role of total flavonoids in safflower drought tolerance. The high genetic variance was seen to result in the high heritability of biochemical traits under drought stress, thereby improving drought tolerance in safflower cultivated in drought prone regions. The significant genetic variations in all the biochemical traits indicated that these traits, especially TPC and TFD, could be used as screening criteria for genotypic selection in arid climates.

Keywords Antioxidant activity · Biplot · Correlation · Total phenolics content · Total flavonoids

Introduction

Drought is considered as a deleterious abiotic stress that hampers crop growth and yield in about one-third of the world's cultivated land (Basu et al. 2016). Global warming, declining regional precipitation, and anthropogenic activities in near future are expected to increase the intensity and frequency of drought events in most geographical regions of the world (Fang and Xiong 2015). Disruption of cellular redox homoeostasis under drought stress will naturally cause changes in proteins, nucleic acids, and cell structure due to the associated increase in reactive oxygen species (ROS) at the cellular level (Anjum et al. 2017). Moreover, the intensity of oxidative damage to cell membrane leads to lipid peroxidation (LP) (Chakhchar, et al. 2015) which is reflected in plant malonyldialdehyde (MDA) levels.

To cope with the harmful effects of drought stress, plants employ different adaptation mechanisms as biochemical,

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morphological, physiological, and molecular levels (Sharma et al. 2012; Anjum et al. 2017). This is while osmotic adjustment, pigment synthesis, ion accumulation, and enhanced antioxidant activity as well as accumulation of osmolytes (as proline), polyols, betaines, and secondary metabolites (SMs) are considered as the adaptive mechanisms contributing to drought tolerance in plants (Anjum et al. 2017; Fang and Xiong 2015). While phenolic compounds, as the main group of SMs, enable plants to combat drought stress by scavenging ROS through catalyzing the oxygenation reactions (Mittler 2002; Fang and Xiong 2015), osmotic adjustment regulates the synthesis and accumulation of compatible solutes such as proline under drought stress (Hayat et al. 2012; Singh et al. 2015).

Plant responses to drought stress depend on a number of genetic (namely, species, genotype, and developmental stage) and environmental factors (e.g., stress severity and duration) (Tester and Langridge 2010). To improve drought tolerance in plants, many physio-biochemical traits need to be thoroughly evaluated because of the low heritability of such traits, high genotype × environment interactions, and the complex nature of drought tolerance (Blum 2011; Basu et al. 2016).

Considering the scarcity of water as a serious constraint, exploiting new genetic resources might facilitate the selection of drought-tolerant genotypes. Unfortunately, however, the initial biochemical effect of water deficit, as an appropriate selecting marker for drought tolerance in plant species, is not well understood.

Safflower (*Carthamus tinctorius* L.) is an oily crop with a long history of food, industrial, cosmetic, ornamental, and medicinal applications (Golkar and Karimi 2019; Hussain et al. 2016). It is cultivated as a main crop in many (semi-) arid regions of the world with extremely low irrigation or agricultural potentials (Golkar and Karimi 2019). The total safflower seed yields in the world and in Iran are reportedly 9033 (hg ha^{-1}) and 12,896 (hg ha^{-1}), respectively (FAOSTAT 2016). Safflower is grown in arid and semi-arid areas around the world where droughts can occur at any growth stages of the plant (Hussain et al. 2016; Santos et al. 2017). A crucial concern for safflower breeders, however, is its further improvement for subsistence under drought conditions with long days.

Previous reports demonstrated changes in the different safflower traits including its agronomics traits such as seed yield and yield components (Istanbulluoglu et al. 2009; Santos et al. 2017) physiological traits such as electrolyte leakage, water potential, relative water content and water use efficiency (Bortolheiro and Silva 2017; Yeloojeh et al. 2020), enzymatic antioxidants such as superoxide dismutase catalase and peroxidase (Hojati et al. 2011; Yeloojeh et al. 2020), morphologic properties such as plant height and plant weight (Ahmadzadeh et al. 2012), phenological traits such as days to maturity (Ahmadzadeh et al. 2012) and oil yield and quality (Ashrafi and Razmjoo 2010; Santos et al. 2017) under drought stress. To the best of the present authors' knowledge, no investigation has yet been published on the effects of drought stress on changes in some SMs of safflower including total phenolics, total flavonoids, total flavonols and total anthocyanins. Nevertheless, there is no report on genotypic differences for these traits under drought stress. It seems that an in-depth study is required to identify the mechanisms employed by the plant at the biochemical level under drought tension. The identification of biochemical responses involved in adopting of safflower genotypes to drought-stress condition can play an important role in safflower breeding programs. The knowledge, thus, gained may then be exploited to determine superior genotypes of safflower for cultivation in (semi-)arid regions. Meanwhile, new findings regarding the genotypic variation in safflower germplasm with respect to its drought tolerance might both accelerate safflower improvement and increase the efficiency of genotypic selection in safflower breeding programs based on biochemical traits.

The objective of the present study is twofold: (1) to investigate the responses of biochemical traits in a broad range of safflower germplasm to drought stress to unveil the basic mechanisms involved in their drought tolerance, and (2) to identify the responses of different safflower genotypes to drought stress that might help select superior genotypes with the highest potential for adaptation to (semi-) arid climates.

Materials and methods

Site description and plant material

Seeds from one hundred safflower genotypes collected from different geographical regions (Table 1) were cultivated under non-stress and drought-stress field conditions in the two consecutive years of 2017 and 2018. The experimental field was located at the Lavark Research Farm of Isfahan University of Technology, 40 km southwest of Isfahan, Iran (32° 32′N, 51° 23′ E, 1630 m above sea level), where the soil is silty clay loam characterized by a bulk density of 1.3 g cm^{-3} (in the top 50 cm) and a pH range of 7.4–7.9. The meteorological data at the site including mean monthly precipitation, maximum and minimum monthly temperatures, and relative humidity are reported in Table S1. The seeds were sown by hand in MidApril, 2017 and 2018, in rows 3 m long and spaced 25 cm from each other to yield a plant density of 40 plants m^{-2} in the plots. While no fungicides were applied, fertilization included surface application of 25 kg ha^{-1} of phosphorous and 130 kg ha^{-1} of nitrogen in both non-stress and drought-stress treatments with an additional 55 kg ha^{-1} of nitrogen applied during the rosette stage.

Table 1 Geographical origins of the 100 safflower genotypes used in this study

Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin	Genotype code	Genotype name	Geographical origin
G1	A2	Iran (Azerbaye-jan)	G34	Car159	Germany	G67	Car64	Slovakia
G2	Ac- Stirling	Canada	G35	Car160	Russia	G68	Car67	Germany
G3	AC-sunset	Canada	G36	Car161	Russia	G69	Car68	Germany
G4	Arak 2811	Iran (Markazi)	G37	Car169	Hungary	G70	Car70	Lybyan
G5	C111	Iran(Isfahan)	G38	Car175	India (Kusum)	G71	Car72	North Korea
G6	Car118	India	G39	Car181	India	G72	Car74	North Korea
G7	Car 116	India	G40	Car188	Poland	G73	Car75	North Korea
G8	Car 9	Czechoslovakia	G41	Car19	Poland	G74	Car76	North Korea
G9	Car100	Italy	G42	Car190	Iran (Isfahan)	G75	Car77	North Korea
G10	Car106	Spain	G43	Car198	Azerbaijan	G76	Car78	Hungary
G11	Car114	India	G44	Car199	Korean republic	G77	Car79	Japan
G12	Car117	Sudan (tozi)	G45	Car200	unknown	G78	Car80	North Korea
G13	K21	Iran (Kordestan)	G46	Car201	Sudan	G79	Car83	Tajikistan
G14	Car124	Pakistan	G47	Car210	Spain	G80	Car86	Tunisia
G15	Car125	Russia	G48	Car211	Germany	G81	Car87	Romania
G16	Car126	Belgium	G49	Car214	Poland	G82	Car89	Tunisia
G17	Car127	Germany	G50	Car215	Germany	G83	Car94	Spain
G18	Car129	Germany	G51	Car216	Germany	G84	GE62918	Germany
G19	Car130	Morocco	G52	Car217	Germany	G85	Gila	USA
G20	Car131	Paraguay	G53	Car218	Germany	G86	Hartman	USA
G21	Car132	Germany	G54	Car219	Germany	G87	IL111	Iran(Auroumieh)
G22	Car135	Portugal	G55	Car221	Germany	G88	Isf-14	Iran (Isfahan)
G23	Car137	Pakistan	G56	Car224	Germany	G89	Isf28	Iran(Isfahan)
G24	Car138	Poland	G57	Car226	Germany	G90	K21	Iran (Kordestan)
G25	Car146	Egypt	G58	Car227	Germany	G91	KMS 36	Iran (karaj)
G26	Car147	Pakistan	G59	Car228	Germany	G92	Mex.17–45	Mexico
G27	Car148	Pakistan	G60	Car230	Germany	G93	Mex.7–147	Mexico
G28	Car151	India	G61	Car24	Morocco	G94	Mex.7–38	Mexico
G29	Car152	Iraq	G62	Car37	Sudan	G95	Mex-13–216	Mexico
G30	Car155	Russia	G63	Car42	Sudan	G96	Mex2-138	Mexico
G31	Car156	Pakistan	G64	Car49	Spain	G97	Mex22-191	Mexico
G32	Car157	Morocco	G65	Car55	Poland	G98	Mex6-97	Mexico
G33	Car158	Paraguay	G66	Car56	Nebraska 8 (USA)	G99	PI 301,055	Turkey
						G100	Saffire	Canada

Experimental design and irrigation regimes

The experiment was carried out as a split plot design based on a randomized complete block design with two replications in each year. From planting to budding stage, all experimental plots were properly irrigated every week. At these stages, the irrigation was done when soil moisture (%) reached at 50% field capacity (FC) (Allen 1998). Irrigation water was supplied from a pumping station via polyethylene pipes to a volumetric counter. From the budding stage up to full physiological maturity, the non-stress treatment received irrigation until 50% of the total available water was depleted from the root zone (50% FC), while irrigation in the drought-stress treatment continued until 75% of the total available water had been depleted (75% FC) (Allen, 1998). Soil water moisture in each treatment was calculated using soil samples from depths of 0–60 cm. Irrigation depth (I) was accordingly determined using the formula: $I = \left[\left(\theta_{FC} - \theta_i\right)/100\right] D \times B)$, where I represents irrigation depth (cm), $\theta_{FC}(-0.03\,MPa)$ is soil gravimetric moisture percentage at field capacity (22%), $\theta i(-1.5\,MPa)$ is soil gravimetric moisture percentage at irrigation time (10%), D is root-zone depth (50 cm), and B is soil bulk density at the root zone (1.3 g cm^{-3}) (Clarke et al. 2008). The volume of

irrigation depth (I_d) was monitored using $I_d = I \times p$, where p is the fraction of I that can be depleted from the root zone. The volume of water used in each irrigation treatment was measured using a volumetric counter. The total values of irrigation water applied were 6910.2 and 4128 m^3 ha^{-1} in 2017, as well as 6230.3 and 3140 m^3 ha^{-1} in 2018, in non-drought and drought-stress conditions, respectively. The biochemical traits at physiological maturity were determined at 50% flowering stage according to the procedures described below.

Leaf extraction procedure

About 0.3 g of dried leaf from each replication was powdered in liquid nitrogen before it was homogenized in 3 mL of methanol (99%, Merck, Germany). The samples, thus, obtained were centrifuged at 4500 rpm for 25 min. The supernatant was subsequently separated and stored at −20° C until analysis to determine TPC, TFD, TFL, and photosynthetic pigment contents.

Total phenolic content (TPC) assay

The total phenolic content of safflower was determined using the method described in Sarker and Oba (2018). Briefly, the Folin–Ciocalteu reagent (Merck, Germany) was initially diluted at a 1:4 ratio of reagent: distilled water. Then, 50 µl of the leaf methanolic solution and 1 mL of the diluted Folin-Ciocalteu reagent were poured into a test tube and mixed thoroughly. After three min, 1 mL of $NaCO_3$ (10%) was added to the mixture and allowed to stand for 1 h in the dark. Absorbance was then read at 760 nm using a spectrophotometer (U-1800, HITACHI, Tokyo, Japan). The standard curve ($r^2 = 0.98$; 5–50 mg mL^{-1}) was constructed using gallic acid (Merck, Germany). Finally, TPC concentration was reported as mg gallic acid equivalent in the fresh leaf extract (mg of GAE/ mg of extract).

Total flavonoid (TFD) and total flavonol (TFL) contents

Total flavonoid content (TFC) was determined according to the calorimetric method (Sarker and Oba 2018) with minor modifications. For this assay, 500 µl of leaf extract, 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate (1 M), and 2.8 mL of distilled water were transferred into a test tube. Absorbance of the reaction mixture was read at 415 nm after 30 min at room temperature. Quercerin (Sigma-Aldrich) was used as the standard compound. Total flavonoid content was reported as quercetin equivalent (QE) in mg per fresh leaf weight (mg QEg^{-1} FW).

Total flavonol (TFL) content was estimated as described in Golkar et al. (2019) and absorbance was measured at 445 nm using the standard curve of quercetin for calibration. Finally, total flavonol content was reported as quercetin equivalent (QE) in mg per fresh leaf weight (mg QEg^{-1} FW).

Measurement of photosynthetic pigments and anthocyanin

Photosynthetic pigments (total chlorophyll and carotenoids) were estimated according to the method described in Lichtenthaler and Wellburn (1983) and anthocyanin content was measured according to Wagner (1979). Briefly, 0.1 g of leaf sample was homogenized in 10 mL of acidified methanol [methanol: HCL 99:1 v/v]. The solution was kept at 25° C for 24 h in the dark before centrifugation for 5 min at 4000 rpm. The absorbance of the mixture was then read at 550 nm using a spectrophotometer. An extraction coefficient of 33,000 mol^{-1} cm^{-1} was used.

Malondialdehyde assay

The intensity of cell membrane damage was determined based on estimated malondialdehyde (MDA) values (Shanazari et al. 2018). Briefly, 200 mg of fresh leaf sample was initially homogenized in a 0.1% trichloroacetic acid (TCA) solution. The homogenate thus obtained was then centrifuged at 4500 rpm and the supernatant was separated. A new mixture was subsequently produced by mixing 500 µl of the supernatant in 2 mL of thiobarbituric acid (TBA) (0.5%) and TCA (20%). Absorbances were finally read at 532 and 600 nm using a spectrophotometer and MDA content was reported as µmol g^{-1} FW.

DPPH assay

The radical scavenging activity of leaf samples was evaluated using the DPPH (1,1-diphenyl- 2-picrylhydrazyl) method (Aparadh et al. 2012). For this purpose, 20 µL of each leaf methanolic extract was added to 2 mL of 50 µM DPPH solution in methanol and mixed to prepare plant extracts ranging from 0 to 1,500 µg mL^{-1}. The mixtures were then stored for 20 min in the dark. Reduction of DPPH absorption (as the standard) was measured at 515 nm and the inhibition percentages (IP%) of the extracts were calculated according to the following formula: IP(%) = (OD control − OD sample/OD control) × 100.

Proline assay

To calculate leaf proline content (Bates 1973), fresh leaf samples (500 mg) were ground in 10 mL of 3% (w/v) aqueous sulfosalicylic acid. The solution was then centrifuged at 4000 rpm for 10 min at 4° C before they were mixed with 2 mL of ninhydrin and 2 mL of glacial acetic acid. The mixtures were subsequently incubated for one hour at 100° C in a water bath and cooled on ice before 4 mL of toluene was added to each mixture. Finally, absorbance was read at 520 nm using a spectrophotometer against toluene used as blank. Proline content was expressed as μ mol g^{-1} FW.

Statistical analysis

Analysis of variance and estimation of the genetic parameters were performed using SAS statistical software (SAS ver 9.1) (SAS 2004). Different genetic variance components were calculated according to Fehr (1978). Expected response to selection (R^2) was calculated using the formula: $R = ih^2\sigma_p$ (Falconer and Mackay 1996). The intensity of selection (i) was considered as 1.69 in breeding programs. The CORR-PROC of SAS was used to estimate correlations between different traits. Finally, principal component analysis (PCA) and cluster analysis were performed using R- software (ver 3.4.3) (Team 2017).

Results

Analysis of variance revealed the significant ($P < 0.01$) effects of both year and treatment (non-stress and drought stress) on all the studied traits (Table 2). Moreover, all the studied traits exhibited significant genotypic variabilities (Table 2). The interaction effects of year × treatment were found significant ($P < 0.01$) for TPC, total chlorophyll (ChlT), total carotenoids (Car), and MDA. The interaction effects of genotype × year were significant for ChlT, Car, and MDA (Table 2), implying differences in genotypic behavior between the two study years. The interaction effects of genotype × environment were also significant for all the studied traits.

Effects of drought stress on the studied traits

Comparisons of means of all the studied traits under the two environmental conditions (non-stress and drought stress) are reported in Table 3. Clearly, the mean comparisons for the interaction effects of year × environment demonstrated that the values obtained in 2018 for all the studied traits, except for Ant, DPPH, and proline, were higher than those obtained in the first year.

Drought stress was found to have a reducing effect on photosynthetic pigments (ChlT and Car) in both study years, while the other traits (i.e., TPC, TFD, TFL, Ant, Pro, MDA, and DPPH) increased under the effect of drought stress relative to those of the control. It is also seen in the same Table that total phenolic content showed a significant increase from the control value of 7.71 mg $GAEg^{-1}$ FW to that of 10.96 mg $GAEg^{-1}$ FW under drought conditions in 2017. The same increasing trend was observed for 2018 (namely, 11.63 mg $GAEg^{-1}$ FW under the non-stress treatment *vs.* 16.26 mg $GAEg^{-1}$ FW under drought conditions). Based on the comparisons of means, TFD content under drought-stress conditions showed increases of about 16% and 21% in 2017 and 2018, respectively, relative to those of the control. Similarly, increases from 0.36 mg QEg^{-1} FW to 0.57 mg QEg^{-1} FW in 2017 and from 0.51 mg QEg^{-1} FW to 0.73 mg QEg^{-1} FW in 2018 were recorded for TFL under the control and drought-stress conditions, respectively (Table 3). Ant content showed significant increases of about 23% and 24% in 2017 and 2018, respectively, relative to the values measured in the control treatment. Both ChlT and Car contents under the drought-stress treatment showed significant decreases in both study years relative to those measured under the non-stress conditions. This is while proline content experienced significant increases of about 1.56- and 1.57-fold in 2017 and 2018, respectively, relative to the corresponding control values. Moreover, MDA content, as the final product of plant cell membrane peroxidation, showed increases of about 2.26-fold and 2.19-fold in 2017 and 2018, respectively, relative to the corresponding control values. Finally, significant increases in DPPH activity under drought conditions were also observed in both study years relative to those obtained under the non-stress treatment; this is evidenced by the increase from 65.81% under the control to 81.18% under drought stress in 2017 and that from 66.25% under normal conditions to 81.30% under drought stress in 2018 (Table 3).

Genotypic mean comparisons

Mean comparisons among the genotypes evaluated under the non-stress treatment are reported in Table S2. Under normal conditions, the highest values (reported as averages of the two study years) of TPC (16.07 mg $GAEg^{-1}$ FW), TFD (3.5 mg QEg^{-1} FW), TFL (0.8 mg QEg^{-1} FW), Ant (259.52 μmol g^{-1} FW), ChlT (0.715 mg g^{-1} FW), Car (0.116 mg g^{-1} FW), Pro (515.75 μmol g^{-1} FW), and DPPH activity (99.01%) were recorded for G_{80}, G_9, G_{72}, G_{87}, G_{20}, G_{30}, G_{20}, and G_{35} genotypes, respectively (Table S2). This is while the highest (6.73 μmol g^{-1} FW) and lowest (0.51 μ mol g^{-1} FW) values of MDA belonged to G_{23} and G_{50}, respectively (Table S2).

Table 2 Analysis of variance for the traits of safflower genotypes studied under drought and non-drought conditions over the two study years of 2017 and 2018

	df	TPC	TFD	TFL	Ant	ChlT	Car	MDA	Pro	DPPH
Year	1	4250.42**	8.77**	4.83**	14.15	0.073**	0.01**	46.28**	693.11	12.45
Replication (years)	2	13.07*	0.98**	0.025	6455.978	0.047	0.0003	1.61	14,031.46*	1.92
Environment¥	1	3106.94**	28.63**	8.85**	220,062.38**	9.069**	0.161**	3263.001**	4,669,759.04**	46,077.06**
Year × environment	1	95.91**	0.8	0.00009	51.32	0.41**	0.0003**	2.57**	41.24	3.40
Environment × Rep (years)	2	0.81635	0.1	0.0007	455.78	0.0006	0.0002**	0.087	0.98	156.60**
Genotype	99	32.07**	2.18**	0.12**	4431.07**	0.037**	0.0009**	23.92**	76,763.81**	1524.34**
Year × genotype	99	0.19	0.12	0.012	25.14	0.013**	0.0003**	0.48*	49.569	15.0465
Environment × genotype	99	11.89**	6.63**	0.08**	2476.16**	0.032**	0.0006*	12.91**	33,483.83**	818.81**
Year × Environment × genotype	99	0.65	0.05	0.006	40.18	0.016**	0.00028**	0.49**	27.691	17.06
Residual	396	1.13	0.27	0.022	242.22	0.002	0.00003	0.26	3853.9	31.21
C. V (%)		9.15	23.53	27.55	10.02	17.47	10.16	9.67	18.06	7.58

¥ Environment (non-stress and drought-stress conditions), *TPC* Total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* anthocyanin, *ChlT* total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *Pro* proline, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *C.V* coefficient of variation

* and ** significant at $p < 0.05$ and $p < 0.01$, respectively

Table 3 The mean values obtained for the traits studied under non-drought and drought conditions in two consecutive years

Year	Environment	Secondary metabolites				
		TPC (mg GAEg^{-1} FW)	TFL (mg QEg^{-1} FW)	TFD (mg QEg^{-1} FW)	Ant (μ mol g^{-1} FW)	Pro (μmol g^{-1} FW)
2017	Non-stress	7.71^{b} ± 0.17	0.36^{b} ± 0.007	1.94^{b} ± 0.05	138.82^{b} ± 2.5	268.81^{b} ± 7.2
	Drought-stress	10.96^{a} ± 0.14	0.57^{a} ± 0.01	2.26^{a} ± 0.09	171.49^{a} ± 1.8	421.29^{a} ± 10.2
2018	Non-stress	11.63^{b} ± 0.17	0.51^{b} ± 0.01	2.09^{b} ± 0.06	138.58^{b} ± 2.6	266.63^{b} ± 7.2
	Drought-stress	16.26^{a} ± 0.16	0.73^{a} ± 0.01	2.53^{a} ± 0.09	172.26^{a} ± 1.8	418.98^{a} ± 10.2

Year	Environment	Photosynthetic pigments	
		ChlT (mg g^{-1} FW)	Car (mg g^{-1} FW)
2017	Non-stress	0.37^{a} ± 0.004	0.07^{a} ± 0.0008
	Drought-stress	0.20^{b} ± 0.006	0.042^{b} ± 0.0008
2018	Non-stress	0.43^{a} ± 0.012	0.078^{a} ± 0.01
	Drought-stress	0.17^{b} ± 0.01	0.05^{b} ± 0.0007

Year	Environment	MDA (μ mol g^{-1} FW)	DPPH (%)
2017	Non-stress	3.10^{b} ± 0.11	65.81^{b} ± 1.4
	Drought-stress	7.02^{a} ± 0.17	81.18^{a} ± 0.9
2018	Non-stress	3.47^{b} ± 0.12	66.25^{b} ± 1.5
	Drought-stress	7.61^{a} ± 0.19	81.30^{a} ± 0.9

¥Abbreviations: *TPC* Total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* Anthocyanin, *ChlT* Total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *Pro* proline, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

Under drought conditions, the highest values (reported as averages of the two study years) of TPC (21.55 mg GAEg^{-1} FW), TFD (5.16 mg QEg^{-1} FW), TFL (1.99 mg QEg^{-1} FW), Ant (234.1 μmol g^{-1} FW), ChlT (0.67 mg g^{-1} FW), Car (0.08 mg g^{-1} FW), Pro (851 μmol g $^{-1}$ FW), and DPPH (98%) were measured in G_{16}, G_{80}, G_{60}, G_{23}, G_{62}, G_{16}, G_{33}, and G_{16}, respectively (Table S3); while G_{93}, G_{88}, G_{13}, G_{74}, G_{88}, G_{31}, G_{66}, and G_{52} recorded the lowest two-year average values of TPC (8.38 mg GAEg^{-1} FW), TFD (0. 1 mg QEg^{-1} FW), TFL (0.27 mg QEg^{-1} FW), Ant (111.3 μ mol g^{-1} FW), ChlT (0.02 mg g^{-1} FW), Car (0.016 mg g^{-1} FW), proline (169.57 μmol g $^{-1}$ FW), and DPPH (40.69%), respectively (Table S3). Finally, MDA had its highest (14.67 μmol g^{-1} FW) and lowest (2.8 μ mol g^{-1} FW) values in G_{71} and G_{91}, respectively.

Genetic parameters

Estimated values of the different genetic parameters for all the studied traits under the non-stress and drought-stress treatments are reported in Table 4. Comparisons of the genetic and genetic × environment effects on TPC, TFD, DPPH, and Pro revealed the magnitude of genotypic variance, illustrating the high heritability of these traits under both non-drought and drought-stress conditions. Clearly, the highest (0.97) and lowest (0.29) values of broad-sense heritability under the non-drought condition are observed for DPPH and ChlT, respectively; while the highest (0.98) and lowest (0.79) values under drought conditions belong to MDA and TFL, respectively. The greatest benefits of response to selection (R) belong to Pro under non-stress (158.18%) and drought-stress (221.01%) conditions, respectively (Table 4). It is, thus, concluded that the greatest genetic advance in response to selection might be expected for proline activity under drought stress.

Table 4 Two-year average estimates for the genetic parameters of the various characters of safflower under non-drought field conditions

	Traits								
	TPC	TFL	TFD	Anthocyanin	ChlT	Carotenoids	Proline	MDA	DPPH
Non- stress									
Mean	9.67	0.44	2.02	138.70	0.40	0.075	267.34	3.28	66.06
δ^2_g	5.19	0.008	0.48	1137.11	0.002	0.00017	9278.15	2.45	417.00
$\delta^2_{g\times y}$	0	0.002	0	0	0.012	0.0002	0	0.21	0
δ^2_y	1.13	0.007	0.33	293.96	0.003	0.00003	2378.23	0.15	36.80
δ^2_P	5.48	0.01	0.56	1210.60	0.010	0.0002	9872.71	2.60	426.20
GCV	23.56	20.51	34.48	24.31	13.58	13.70	36.03	47.67	30.90
PCV	24.20	23.87	37.37	25.084	25.02	21.34	37.16	49.06	31.24
h^2	0.94	0.73	0.85	0.93	0.29	0.41	0.93	0.94	0.97
R	3.76	0.13	1.08	55.36	0.05	0.011	158.18	2.57	34.21
Drought stress									
Mean		0.65	2.39	171.87	0.18	0.04	420.13	7.32	81.24
δ^2_g	5.58	0.03	1.67	573.35	0.007	0.0001	18,264.44	6.50	160.76
$\delta^2_{g\times y}$	0	0	0	0	0	0	0	0.01	0
δ^2_y	1.13	0.03	0.20	190.49	0.002	0.000004	5329.60	0.36	25.63
δ^2_P	5.86	0.047	1.72	620.97	0.007	0.0001	19,596.84	6.60	167.16
GCV	17.34	29.93	53.93	13.93	44.07	24.22	32.16	34.82	15.60
PCV	17.78	33.47	54.741	14.49	45.67	25.27	33.31	35.08	15.91
h^2	0.95	0.79	0.97	0.92	0.93	0.91	0.93	0.98	0.96
R	3.90	0.29	2.15	38.97	0.13	0.018	221.01	4.28	21.06

δ^2_g:Genetic variance,δ^2_y: year variance,$\delta^2_{g\times y}$: Genetic × year variance, δ^2_P: Phenotypic variance, *GCV* Genotypic coefficient of variability, *PCV* Phenotypic coefficient of variability, h^2 Broad-sense heritability, *R* expected response to selection (%), *TPC* total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *ChlT* total chlorophyll, *MDA* malondialdehyde, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

Table 5 Principal component analysis of the 100 safflower genotypes under the non-stress and drought-stress treatments; the values are reported as average values obtained over the two study years of 2017 and 2018

Trait	Non- drought				Drought stress			
	PC_1	PC_2	PC_3	PC_4	PC_1	PC_2	PC_3	PC_4
TPC¥	0.51	−0.26	0.19	−0.09	0.45	−0.39	0.06	−0.03
TFL	0.15	−0.51	0.47	−0.19	−0.04	−0.46	0.50	−0.10
TFD	−0.43	−0.36	0.06	−0.24	0.49	−0.02	−0.28	0.14
Ant	0.0003	−0.415	−0.16	0.66	−0.23	−0.37	0.03	0.46
ChlT	0.04	−0.28	−0.58	−0.13	− 0.38	−0.27	−0.43	−0.23
Car	0.13	−0.26	−0.55	−0.40	−0.16	−0.39	−0.58	0.06
MDA	−0.41	−0.14	0.20	−0.37	−0.34	−0.24	0.28	−0.39
DPPH	0.52	−0.08	0.09	−0.09	0.40	−0.43	0.01	−0.07
Proline	0.22	0.42	−0.06	−0.33	0.17	0.06	−0.18	−0.72
Eigen value	2.60	1.75	1.34	0.96	2.39	1.73	1.43	1.08
Variance (%)	28.98	19.50	14.92	10.76	26.66	19.24	15.95	12.0
Cumulative variance (%)	28.99	48.49	63.42	74.18	38.98	58.10	75.35	85.40

¥: *TPC* total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* anthocyanin, *ChlT* total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *Pro* proline, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

Principal component analysis

The results of principal component analysis (PCA) showed that the first four principal components explained about 74% and 85.40% of the total variability under non-stress and drought-stress conditions, respectively (Table 5). Under non-stress conditions, DPPH and TPC had the most positive loading values on PC1, while MDA and TFD had the

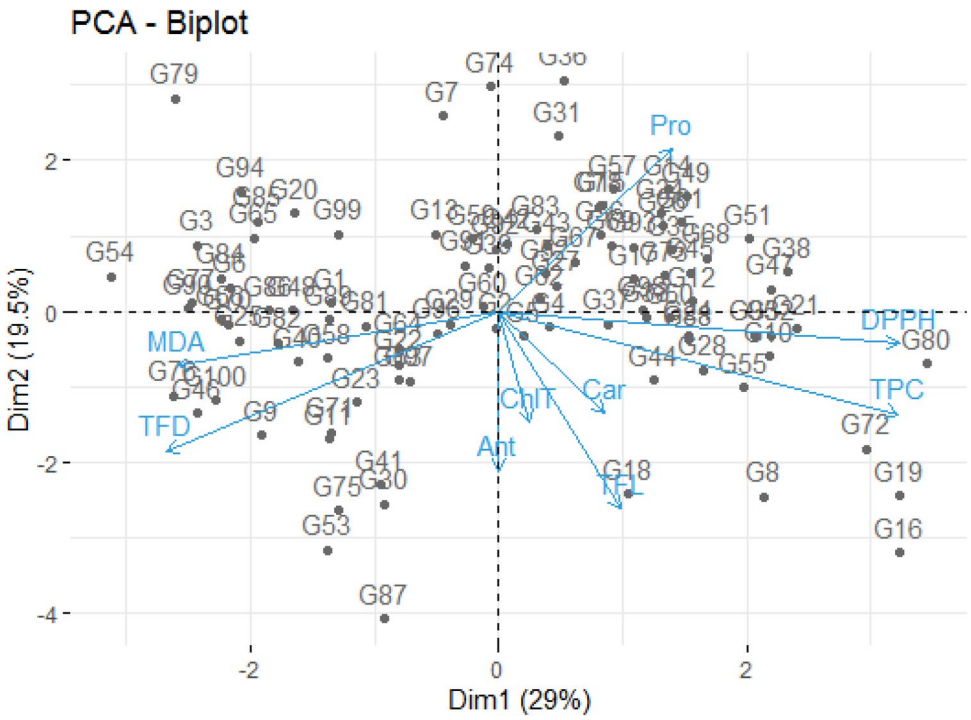

Fig. 1 Genotype–trait biplot for the 100 safflower genotypes under the non-stress treatment; the values are reported as average values of measurements over the two study years. The code for the genotypes are presented. Traits abbreviation: *TPC* total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* anthocyanin, *ChlT* total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *Pro* proline, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

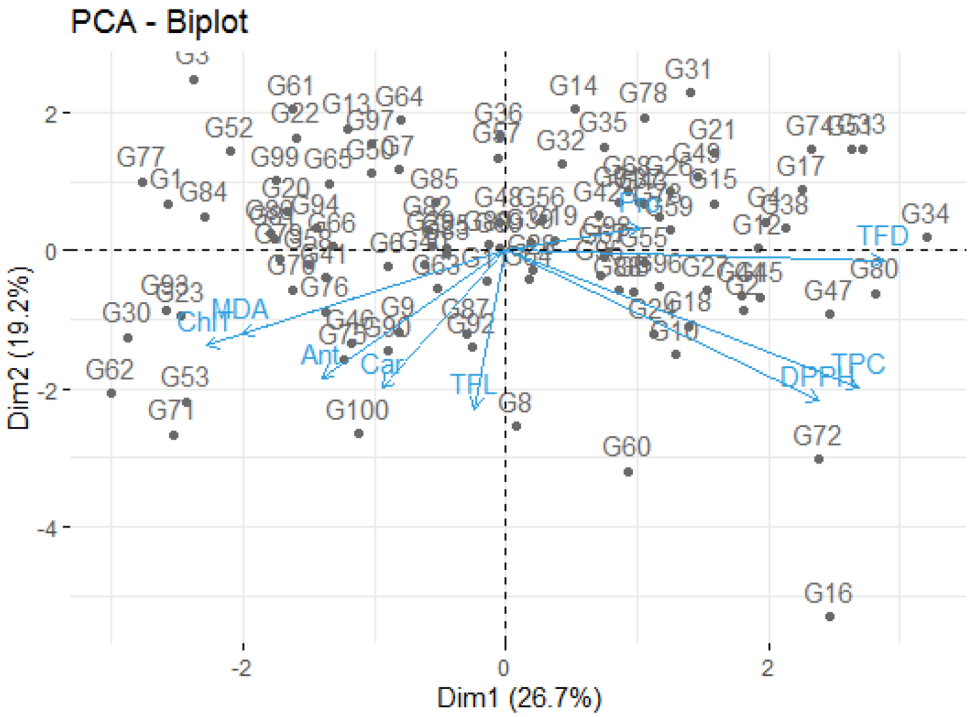

Fig. 2 Genotype–trait biplot for the 100 safflower genotypes under the drought-stress treatment; the values are reported as averages of measurements over the two study years. The code for the genotypes are presented. Traits abbreviation: *TPC* total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* anthocyanin, *ChlT* total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *Pro* proline, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

most negative loading values on PC2. Under drought stress, TFD had the most positive loading values on PC1 while TFL was the trait with the most negative loading values on PC2 (Table 5). A biplot, as an effective tool for the interpretation of data, was constructed based on the first and second principal components for safflower genotypes under the two non-drought and drought-stress conditions (Figs. 1 and 2). Based on the biochemical traits represented in the biplot, the first (PC1) and second (PC2) components explained about 29% and 19.5% of the total variance of the variables, respectively (Fig. 1); thus, both PCs cumulatively explained 48.49% of the total variance of all the variables analyzed. As shown in Fig. 1, the genotypes G^{16}, G^{19}, G^{72}, and G^{80} had the highest positive values for DPPH and TPC under non-stress

conditions. On the other hand, the genotypes G_{46}, G_{54}, G_{76}, G_{79}, and G_{100} had the highest positive values for MDA and TFD (Fig. 1). The Pro content showed a positive correlation with PC2 while TFL and Ant showed the highest negative correlation with PC2 (Fig. 1). Finally, the genotypes G_{74}, G_{36}, G_{31}, and G_7 had the highest values for proline but G_{87}, G_{53}, G_{16}, G_{75}, and G_{53} had the highest values for Ant and TFL (Fig. 1).

Under drought stress, PC1 and PC2 explained about 26.7% and 19.2%, respectively, of the total variance (Fig. 2); therefore, both PCs explained 38.98% of the total variance in all the variables investigated (Table 5). Under drought stress, TFD, TPC, and DPPH had the most positive effects on PC1 (Fig. 2). Based on this biplot, the genotypes G_{80}, G_{34}, G_{47}, G_{16}, and G_{72} had the highest values for TPC, DPPH, and TFD; while MDA and ChlT had the most negative loading effects on PC1 and the genotypes G_1, G_{30}, G_{77}, G_{84}, G_{90}, and G_{92} had the highest values for MDA (Fig. 2). As regards PC2, TFL and DPPH had the highest negative loading effects. The genotypes G_{16}, G_{72}, G_{60}, G_{100}, and G_8 had the highest values for TFL and DPPH under drought stress, while G_{16}, G_{93}, and G_{76} showed the highest values for Car and Ant (Fig. 2).

Correlation analysis

Under the non-stress and drought-stress treatments, 13 and 14 significant correlations, respectively, were detected

Table 6 Simple correlations among the traits investigated in 100 safflower genotypes (two-year mean values) treated under non-stress (the upper triangle in bold type) and drought-stress (the lower triangle) conditions

	TPC¥	TFL	TFD	ChlT	Car	MDA	DPPH	Proline	Ant
TPC	1.00	0.56**	−0.38**	0.05	0.19*	−0.40**	0.70**	0.12	0.08
TFL	0.27**	1.00	0.22**	0.02	−0.04	0.08	0.26	−0.17	0.15
TFD	0.46**	−0.25**	1.00	0.05	0.06	0.51**	−0.47**	−0.36**	0.11
ChlT	−0.24**	−0.03	−0.27**	1.00	0.34**	−0.07	−0.02	−0.05	0.18
Car	0.05	−0.03	0.01	0.57**	1.00	−0.10	0.21	−0.02	0.09
MDA	−0.15	0.28**	−0.40*	0.33**	−0.02	1.00	−0.38**	−0.19	−0.05
DPPH	0.63**	0.22	0.41**	−0.12	0.08	−0.08	1.00	0.19*	0.00
Proline	0.10	−0.06	0.17	−0.01	−0.01	−0.06	0.09	1.00	−0.25**
Ant	−0.08	0.24**	−0.13	0.17	0.27**	0.17	−0.04	−0.23*	1.00

¥: *TPC* total phenolic content, *TFL* total flavonols, *TFD* total flavonoids, *Ant* anthocyanin, *ChlT* total chlorophyll, *Car* carotenoids, *MDA* malondialdehyde, *DPPH* 2,2-diphenyl-1-picrylhydrazyl

* and ** Significant at $p < 0.05$ and $p < 0.01$; respectively

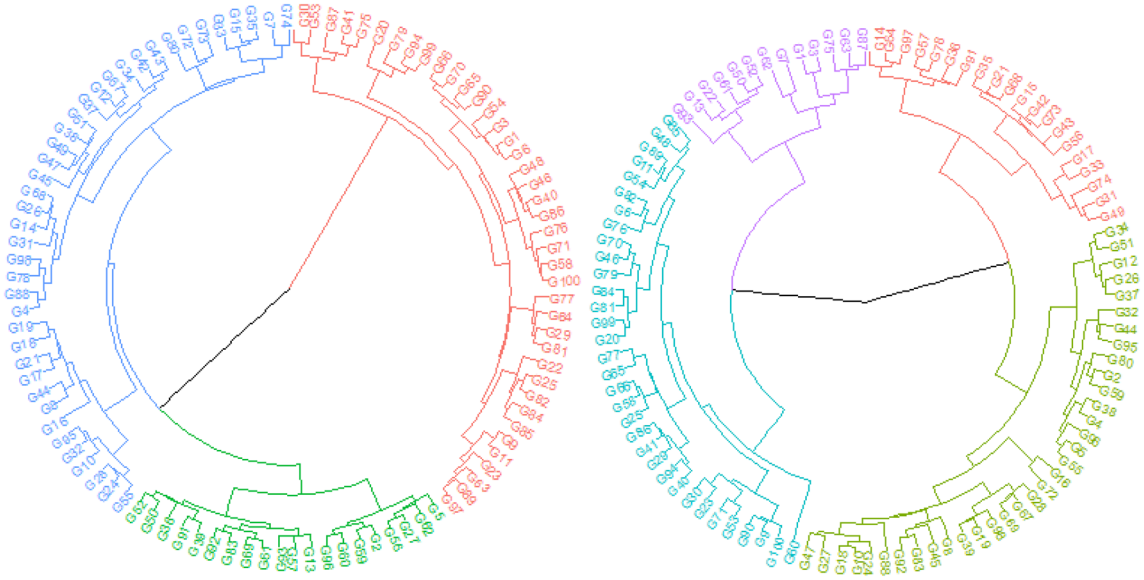

Fig. 3 Dendrogram generated using the Ward's method based on all the studied traits for the safflower genotypes treated under both non-stress (left) and drought-stress (right) conditions; the values are reported as averages of measurements over the two study years

(Table 6). A significant and positive correlation was observed between TPC and TFL under both treatments (Table 6). TPC and TFD established a negative and significant correlation (0.38^{**}) under the non-stress treatment but a positive and significant one (0.46^{**}) under the water-deficit treatment. This is while TPC and TFL exhibited positive and significant correlations under both treatments. Also, TPC and TFD exhibited a significant and positive correlation with DPPH activity under drought stress. Finally, a positive and significant correlation was established between MDA and TFD under normal conditions but a negative and significant under drought stress (Table 6).

Cluster analysis

Genotype clustering is an efficient tool for minimizing the plant gene pool during the selection process. A dendrogram was drawn based on the cluster analysis and the Ward's method applied to all the traits studied under both treatments (Fig. 3). The cluster analysis classified the 100 safflower genotypes investigated into three distinct groups: one containing 40%, another containing 20%, and the last one including 40% of the genotypes subjected to the non-stress treatment (Fig. 3: left). The smallest group (marked in green color) includes G_{13}, G_{61}, G_{33}, G_{58}, G_{47}, G_{11}, G_{24}, and G_3 characterized by the highest TPC, TFD, Ant, Car, and DPPH values. The genotypes in this same group are regarded as superior ones because they are rich in secondary metabolites under normal conditions. Clustering of the genotypes subjected to the drought-stress treatment classified them into four groups: one with 20, another with 34, a third one with 33, and the last one with 13 genotypes (Fig. 3: right). The genotypes within the green-colored group might be considered as the superior drought-tolerant ones due to their TPC, TFL, and Ant accumulation in addition to their highest antioxidant activities. The genotypes in the red-colored and purple-colored groups are identified as those with moderate to high sensitivity to drought stress.

Discussion

Development of drought-tolerant crop cultivars has been limited by the unavailability of effective selection procedures, insufficient genotypic variation, and low heritability of candidate traits (Blum 2011; Anjum et al. 2017). This state of affairs particularly applies to the identification of biochemical traits contributing to drought tolerance in safflower, the literature on which is scant. The traits investigated in the present study (i.e., phenolics, photosynthetic pigments, proline, and antioxidant activity) might be effectively exploited to identify drought-tolerant safflower genotypes, rather than merely to determine their seed yields under field conditions. Indeed, the data obtained reveal the responses of the studied biochemical traits in safflower to water stress under field conditions. Based on the present findings, a high genetic variation in the biochemical traits exists among the genotypes subjected to the non-stress and drought-stress treatments, averaged over two years of study. However, the lack of adequate information on the heritability of biochemical traits limits development of ideotypes genotypes for drought tolerance in plants. According to literature review, heritability values for phenolics (TPC, TFD, TFL and Ant) is not reported yet in safflower. Drought stress increased heritability values for mentioned traits. Generally, higher genotypic variance and heritability estimates for traits under drought-stressed conditions signify that genotypes expressed higher genetic potential under less favorable growing conditions. This higher genetic variation and the better responses of the genotypes to selection under drought stress indicate that selection in this germplasm under drought stress can be successful since the different secondary metabolites (SMs) are found to be sufficiently heritable.

The higher mean values of TPC in 2018 rather than 2017 could be attributed to the lower mean air temperatures and higher mean values of relative humidity and rainfall in growing months (April–July) in 2018 (Table S1), similar with previous reports for barberry (Gholizadeh Moghaddam et al. 2017) figwort (Zargoosh et al. 2019) and oil seed in safflower (Zemour et al. 2019). Regarding this finding, it could be noted that the production of phenolics, although under the control of genetic factors, is significantly affected by the climatic factors (Zargoosh et al. 2019). Also the higher growing temperature in 2018 resulting in higher amount of TFD, TFL and Ant due to the effect of temperature forcing on safflower to produce extra flavonoids, flavonols and anthocyanins a as a defense strategy against the environmental changes. The expression levels of many flavonoid biosynthesis pathway genes were upregulated independently by either low temperature or light treatment (Azuma et al. 2012).

The environment × genotype interaction for phenolics (TPC, TFD, TFL and Ant) was investigated here by the first. The significant interaction effects of genotype × year on ChlT, Car, and MDA point out the differences in the behavior of the genotypes over the two study years. The significant interaction effects of genotype × environment on mentioned traits might have been due to such factors as genetic variation (Shanazari et al. 2018), different patterns of gene expression (Bhargava and Sawnat 2013; Nakabayashi et al., 2014), and environmental factors (e.g., air temperature and humidity) (Waskiewicz et al. 2013).Results of analysis of variance demonstrated at higher magnitude of environment effect on these studied traits rather than genotypic effects (Table 1). Hence, this interaction effect might be usefully exploited as

an important selection index to increase drought tolerance in safflower through the selection of superior genotypes.

Phenolic compounds, as non-enzymatic antioxidants, are able to scavenge directly molecular species of reactive oxygen in plants subjected to environmental stresses (Waskiewicz et al. 2013; Naikoo et al. 2019). So, enhanced phenolic content (including TPC, TFD, total TFL, and Ant) exploits metabolic alterations in cells to protect them against the negative effects of not only ROS but also protein denaturation, DNA damage, and LP under environmental stresses (Mittler 2002; Nascimento and Fett-Neto 2010).

Likewise, and for the first time, the contents of total phenolics, TFD, TFL and Ant were assessed in this study to identify new genotypic sources of phenolics in safflower at reproductive stage. The significant variation observed among the safflower genotypes was probably due to the great genetic variation in the accumulation of phenolic compounds (TPC, TFD, TFL, and Ant) that provided a better protection for the cells against the detrimental factors in drought stress. The significant increase in TPC in the safflower genotypes might be explained with recourse to the fact that the drought tolerance mechanism in safflower at the reproductive stage is controlled by an increase in endogenous phenolic compounds. This is confirmed by other authors reporting on safflower (Yaginuma et al. 2002; Farooq et al. 2020), canola (Shafiq et al. 2014) at vegetative stages, chrysanthemum (Hodaei et al. 2018), edible amaranth (Sarker and Oba 2018), and rye (Czyczyło-Mysz and Myskow 2017). The magnitude and diversity of the phenolic compounds generated are reportedly related to such factors as differences between and within species (Nascimento and Fett-Neto 2010; Quan et al. 2016), development phase of the plant (Weidner et al. 2000), duration of stress (Waskiewicz et al. 2013), and differences in soil–water content (Czyczyło-Mysz and Myskow 2017) as well as environmental factors (Waskiewicz et al. 2013).

Flavonoids belong to the family of polyphenolic compounds with a broad range of functions such as ROS scavenging under environmental stress (Falcone Ferreyra et al. 2012). Similar to this finding, significant increases in TFD under drought stress have been reported in such other medicinal plants as edible amaranth (Sarker and Oba 2018) buckwheat (Siracusa et al. 2017), rice (Quan et al. 2016), and chrysanthemum L. (Hodaei et al. 2018). The positive and significant correlation between TPC and TFL under both drought and non-drought conditions was explained not only by their similar biosynthetic pathways in safflower but also by the fact that they both belong to the family of polyphenols (Falcone Ferreyra et al. 2012), which bestows them a synergic role in drought tolerance.

Flavonols in plants are considered to be low-molecular-weight antioxidants that underlie plant antioxidant activity (Martinez et al. 2016). The findings of the present study indicate significant increases in TFL content under drought conditions in safflower, similar to what has been observed in Arabidopsis (Nakabayashi et al. 2014).

Increases in Ant compounds, as natural sources of non-enzymatic antioxidants, under drought stress have been attributed to their optical protective role in the direct removal of ROS during oxidative stress (Zhang et al. 2010; Ma et al. 2014). Similar to this finding in safflower, an increasing trend has been reported in the Ant content of chrysanthemum *L.* (Hodaei et al. 2018), *Labisia pumila* Benth (Jaafar et al. 2012), wheat (Ma et al. 2014), and Arabidopsis (Nakabayashi et al. 2014). Positive and significant correlations between TFL and Ant (0.24**) could be resulted due to the alignment in the synthesis pathways (phenylpropanoid) of these two substances under drought-stress conditions.

Low levels of phyto-inhibition in the photosynthetic system reportedly resulted in negligible decreases in chlorophyll and carotenoid contents under drought stress (Fang and Xiong 2015). The significant reductions in ChlT and Car contents observed in the present experiment might have been due to such drought-induced events as faster decomposition of chlorophyll in safflower, likely transformation of chlorophyll into pigment–protein complexes, and increased enzyme (chlorophyllase and peroxidase) activities responsible for photosynthesis (Fang and Xiong 2015). In agreement with the present results, a number of authors reported decreases in ChlT and Car contents in safflower (Javed et al. 2013; Farooq et al. 2020; Yeloojeh et al. 2020) and such other crops as bread wheat (Shanazari et al. 2018), triticale (Shanazari et al. 2018), and canola (Akram et al. 2018). According to the findings, a decrease in ChlT concentration could have stimulated the production of TPC in safflower under drought stress, as shown by the negative correlation coefficient (Table 6) between ChlT and TPC ($r^2 = -0.24^{**}$). A possible explanation to this might be that the decrease in chlorophyll content, as a symptom of photosynthesis rate, could have increased the shikimic acid pathway that enhanced the production of TPC in safflower, similar to reports by Ibrahim et al. (2011) in *Labisia pumila* Benth.

Proline has been identified as a unique osmolyte compound generated in response to water deficit through osmotic adjustment in a wide variety of plant species (Hayat et al. 2012). The significant increase in proline content observed in the present study is consistent with those previously reported on safflower (Farooq et al. 2020; Yeloojeh et al. 2020), chrysanthemum (Hodaei et al. 2018), and rye (Czyczyło-Mysz and Myskow 2017). This observed increases in proline might have been due to: (1) its significantly enhanced biosynthesis in the genotypes studied, (2) its inhibited oxidation by other antioxidant systems, (3) the declining requirement for protein synthesis, and (4) compensation for its decrease due to the enhanced protein turnover machinery under water-deficit conditions (Hayat et al. 2012). As reported in Table 6,

variations in proline content failed to affect any of the biochemical parameters under drought stress, which could be the result of the independent biosynthetic pathways of proline (glutamate pathway) (Hayat et al. 2012) and phenolic compounds (shikimate/phenylpropanoid pathway) (Martinez et al. 2016) in safflower.

Similarly, drought-induced increases in MDA content, as an indicator of LP in cell membranes, have been observed in safflower (Javed et al. 2013; Farooq et al. 2020), bread wheat (Shanazari et al. 2018), *Labisia pumila* Benth (Jaafar et al. 2012), chrysanthemum (Hodaei et al. 2018), and argan (Chakhchar, et al. 2015). Interestingly, the drought-tolerant genotypes showed the least MDA content under drought stress, which is in agreement with the findings reported by Shanazari et al. (2018) on bread wheat, Sarker and Oba (2018) on edible amaranth and Akram et al. (2018) on canola. The positive and significant (0.28^{**}) correlation established between TFL and MDA demonstrated the non-supportive or inadequate effects of flavonols as antioxidants to reduce the deleterious effects of drought tolerance in safflower.

Antioxidant activity has a crucial role in maintaining the equilibrium between the production and scavenging of free radicals. The enhancements in DPPH activities observed in the safflower genotypes studied indicated that different antioxidant (both enzymatic and non- enzymatic) activities are stimulated in safflower in response to water-deficient conditions. This finding is consistent with similar findings reported by Abdallah et al. (2013) on safflower when subjected to saline conditions or by Hodaei et al. (2018) on chrysanthemum, and Ma et al. (2014) on wheat subjected to drought stress. The rather high variation in DPPH activity under drought stress in safflower could be explained by the high variation in polyphenolic composition in response to drought stress observed in different genotypes. As a new finding, the positive and significant correlation of TPC and TFD with DPPH under drought stress and that between MDA and TFD under drought stress demonstrated the important ROS scavenging roles played by TPC and TFD under drought stress in safflower, as also observed in wheat (Ma et al. 2014). Moreover, this new finding further suggests that TPC and TFD are closely related to the drought tolerance of safflower as drought stress triggers more reactions of total phenolics and flavonoids to counteract the negative effects of drought tension. It may, therefore, be concluded that the antioxidant effect of safflower leaf can be attributed to the presence of phenolics and flavonoids compounds, among others, as a novel finding. These findings are in agreement with previous reports on safflower by Abdallah et al. (2013) who found that many flavonoid compounds have significant contributions to antioxidant activity in safflower under salinity stress.

Given the fact that drought tolerance in any species might be associated with improved activity of antioxidant compounds, it is concluded that higher heritability and selection responses of phenolic compounds (i.e., TPC, TFD, TFL, and Ant) in safflower under drought stress might accelerate breeding for the direct selection of these traits under water-deficit conditions so that drought tolerance could be judged based on phenolic traits rather than on overall plant tolerance.

The results of the PCA conducted and the distribution of the genotypes on the biplots derived were used to identify the superior genotypes under each (non-stress or drought stress) condition. Accordingly, the genotypes G_{16}, G_{34}, G_{47}, G_{72}, and G_{80} exhibiting the highest values for TPC, DPPH, and TFD were found capable of tolerating drought stress through TPC and TFD accumulation that bestowed them a high antioxidant capacity (Fig. 2). Moreover, the genotypes G_3, G_{14}, G_{31}, G_{61}, G_{64}, and G_{78} were identified as drought-sensitive (Fig. 2). The biplots of the genotypes subjected to drought stress revealed that the genotypes with high TPC, TFD, and DPPH contents (namely, G_{16}, G_{34}, G_{47}, G_{80}, and G_{72}) could be hybridized with those containing high levels of TFL namely, G_{60} and G_{100}) to achieve superior hybrids with elevated TPC, TFD, and TFL levels as well as improved antioxidant activity revealed by the DPPH method.

Furthermore, cluster analysis revealed more patterns of genetic variation among the accessions subjected to drought stress. For instance, the genotypes with good drought tolerance were assigned to the green group, while those assigned to the red and purple groups were found sensitive to drought stress. Based on the cluster analysis performed, it was found that hybridization of genotypes with the greatest genetic distances (including the ones in the green group with G_{80} in the red and/or purple groups) would produce new hybrids for mapping the population of the studied safflower traits under drought conditions.

Conclusion

The biochemical traits of safflower were used to demonstrate the high genetic variation among the genotypes in terms of their drought tolerance. Based on the results obtained, it was concluded that the drought-tolerant genotypes (e.g., G_{16}, G_{72}, and G_{80}) were the ones with superior drought tolerance due to their mechanisms of TPC and TFD accumulation. The genotypes identified as drought tolerant may be recommended for cultivation in (semi-) arid regions. It was also found that phenolic compounds, especially total flavonoids, served as useful tools for defining drought tolerance in safflower genotypes and for selecting superior genotypes. It was suggested that superior safflower genotypes could be

selected on the basis of their high total phenolics, total flavonoids, and drought tolerance simultaneously. Future research is recommended to investigate the trend changes in phenolic acid derivatives for enhancing drought tolerance in safflower. Another topic of interest will be producing bioactive reagents to enhance safflower yield under water scarcity using the knowledge to be gained on how different phenolics react under drought stress.

Author contribution statement P.G designed research, did field experiments, data analysis and wrote the main body of the manuscript. S.A.M. Mirmohammadi Maibody prepared technical materials of experiment and revised the manuscript. The field experiments was carried out by E.H under the supervision of P.G and S.A.M. Mirmohammai Maibody. Traits measurement was carried out by E.H and M.T.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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