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RESEARCH ARTICLE

Impact of Silicon Foliar in Some of Antioxidant Enzymes and Oil Yield in Safflower Cultivars Exposed to Drought Stress

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ABSTRACT

Drought stress is considered as a main restriction to have the best potential crops performance in arid and semi-arid regions in the world. Hence, some mechanistic strategies are required to manage drought stress detrimental influences. Silicon as an essential mineral nutrient, plays an important role in physiology, metabolism, and function of crops exposed to drought stress. This study was carried out to evaluate the influences of various irrigation regimes and silicon foliar on three safflower cultivars to find the optimal irrigation level and silicon fertilizer. Three levels of irrigation (0, 2 and 4 times irrigation during growth) were main plots and sub plots were three silicon foliar levels (0, 1 and 2 mM) and three safflower cultivars (Goldasht, Padideh and Golmehr). This experiment was performed in Firouzabad city, Fars Province, Iran with latitude 28°51' N and longitude 52°36' E, during 2018 and 2019. Malondialdehyde content (MDA), polyphenol oxidase (PPO), superoxide dismutase (SOD) and ascorbate peroxidase (APX), oil yield, oil content and seed yield were measured on these cultivars. Interactive effects of irrigation, silicon and cultivars were significant ($p<0.01$) for MDA, APX, oil yield and oil content. Drought stress in the all safflower cultivars caused a decline in seed yield, oil content and oil yield. On the other hands, silicon was mediated to decrease effect of drought stress and increased amount of seed yield, oil content, oil yield, PPO and SOD. The highest seed and oil yield were observed in Goldasht cultivar under full irrigation and 2mM silicon. Thus, it can be suggested that 1 mM of silicon foliar may ameliorate the performance of safflower exposed to drought stress.

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Introduction

Safflower (*Carthamus tinctorius* L.), a member of the *Asteraceae* family, is one of the important cultivated crops with high quality edible oil which is partially tolerated to arid and semi-arid zones [1]. Total content of safflower seed oil is approximately 27–32%, which includes oleic, linoleic and linolenic acids [2]. Furthermore, safflower is utilized for coloring and flavoring foods, herbal medicine, and fodder [3].

Drought is one of the major abiotic stresses restricting crop yield and leads to little yield in many grain crops exposed to stress during vegetative to grain fill growth. However, crops are sensitive to drought stress during reproductive growth stage and yields will be impacted during this period [4]. Safflower with valuable agronomic properties can be considered as a suitable alternative crop in dryland agro-ecosystems. Several commercially cultivars of safflower which have tolerance to drought is available as substitute agronomic crops [5].

Safflower has the same nutrient requirements as winter cereal such as barley and wheat. However, this plant in comparison with other annual crops has a longer growth period. Deep root system of safflower is an advantage in this plant compared to winter cereal which allows safflower to absorb nutrients and water from deeper layer of the soil.

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While, longer growth period, is a weakness of this plant in arid and semi-arid zone, which can negatively reduce the seed yield. Because of high evapotranspiration rate, lower precipitation and limited access to water in this regions, study on effects of water-deficit stress can help to find or improve appropriate methods for ameliorate crop yield [6]. Developing drought-tolerant varieties, using of suitable fertilizers and ameliorating the soil water holding capacity are effective strategies to enhance productivity [7].

Silicon is the second greatest abundant element after oxygen on the earth's crust (28.2 vs 48.4) on a weight basis [8], and it has been affirmed to have various valuable influences on plant growth [9]. Aggregation of silicon improves crop resistance to various abiotic stress, including metal toxicity, salinity, and ultraviolet irradiation, and biotic stress due to pests and pathogens [10]. Deleterious effects of drought stress can be also decline by application of silicon in rice, sorghum, and wheat [11-13]. These positive effects of silicon on these crops exposed to drought stress are related to an improved water use efficiency [11] and evapotranspiration rate by a restriction in the loss of water, decreased electrolyte leakage and oxidative damage, and enhanced overall photosynthetic performance and biomass yield [14].

A complex of enzymatic and non-enzymatic mechanisms, such as antioxidants production and reactive oxygen species (ROS) scavenging enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX) and polyphenol oxidase (PPO) is a clear response strategy to deal with oxidative stress. Consequently, these physiological activities could increase potential of plant to tolerate abiotic and biotic stresses [15].

The aim of this study was to evaluate the influences of various irrigation regimes and silicon on three safflower cultivars to find the appropriate irrigation level and silicon fertilizer requirements by measurement of Malondialdehyde content (MDA), PPO, APX, SOD, oil yield, oil content and yield.

Materials and Methods

The fields are distributed in Firouzabad city, Fars Province, Iran with latitude 28°51' N and longitude 52°36' E. A split plot factorial experiment based on randomized complete blocks design with three replicates was carried out at 2018 and 2019. Main plots were three levels of irrigation (0, 2 and 4 times during growth) and sub plots were three silicon foliar application levels (0, 1 and 2 mM) and three safflower cultivars (Goldasht, Padideh and Golmehr). Irrigation levels were considered as follow: Control group was irrigated four times at stemming, budding, flowering, seeding stage, moderate drought stress was irrigated two times (stemming and budding stages), and in severe drought stress, crop relied on rainfed condition. Treflan herbicide (2.5 L ha^{-1}) was used to control the weeds. Soil analysis was carried out and mineral nutrition contents were presented in Table 1. Urea (300 Kg ha^{-1}), super phosphate (100 Kg ha^{-1}), and potassium sulfate (50 Kg ha^{-1}) were applied to the soil.

Table 1. Physicochemical properties of the research station soil

Manganese	**Potassium**	**Phosphorus**	**Cu**	**Fe**	**pH**	**Soil Depth**
ppm						**(cm)**
4.96	441	5.22	3.89	5.05	7.24	0-45

Structure	Salinity	Sand	Silt	Clay	Sampling Depth
	(dS/m)	%			(cm)
Clay loam	0.414	30	31	39	0-45

Malondialdehyde Content

The MDA content was assessed by homogenizing of 200 mg leaf samples in 10% trichloroacetic acid. Then, Supernatant was filtrated and heated in 0.25% thiobarbituric acid. Absorbance at 532 nm wavelength was used to record the MDA content [16].

Polyphenol Oxidase

Extractions were performed by homogenizing of 100 mg fresh leaf in a cold mortar in 1 ml of 50 mM Na-phosphate buffer (pH 7) containing 2% polyvinylpyrroli, 2 mM EDTA, 50 mM Tris-HCl, 0.2% triton X100 and 2 mM a-dithiothreitol for 15 minutes. These extractions were immediately applied to evaluation of enzyme activities.

The PPO activity was evaluated by mixture of 2.5 ml of 200 mM sodium phosphate buffer (pH 6.8), 200 µl of 20 mM pyrogallol and 50 µl of enzymes extract at 40°C [17]. The increase in absorbance was detected at 430 nm wavelength using a spectrophotometer U-1800 (Hitachi, Japan). The PPO activity was expressed as 1 µM of pyrogallol oxidized per minutes per mg protein.

Ascorbate Peroxidas

The APX activity was calculated according to the method of Nakano with some modifications [18]. Briefly, 50 µl of enzyme extract was mixed to 3 ml of 50 mM Na-phosphate buffer (pH 7.8) containing 100 µl of 5 mM ascorbate and 4.51 µl of H2O2 (30%). The decrease in absorbance at 290 nm wavelength was monitored every 30 seconds for 2 minutes. The APX activity was defined as nanomole of H2O2 decomposed per mg of protein per minute.

Superoxide Dismutase

The SOD activity was measured according to Giannopolitis and Riess with some modifications [19]. 50 µM nitro-blue tetrazolium chloride, 1.3 µM riboflavin, 75 µM EDTA,13 mM methionine, and 50 mM phosphate buffer were mixed in 50 µL of each sample and irradiated under light at 78 µmol m^{-2}s^{-1} for 15 minutes. Finally, each sample was recorded at 560 nm wavelength.

Oil content and Oil Yield

Oil content was determined using a time-domain nuclear magnetic resonance in an SLK-SG-200 spectrometer (Spin Lock Magnetic Resonance Solutions, Malagueno, Córdoba, Argentina) at room temperature [20]. Results were mentioned on a dry basis (% DB). Oil yield (kg ha^{-1}) was calculated by multiplying oil content with seed yield.

Statistical Analysis

A general linear model (GLM) was used for data analysis using the GLM procedure in SAS v. 9.1 software. Means were compared by Duncan multiple range test at $P < 0.05$. Data from each growing year was analyzed separately. Bartlett test was used to evaluate the homogeneity of variances before running the combined analyses.

Results and Discussion

The combined analysis of variances indicated that year had not significant effect on all evaluated traits in the safflower (Table 2). While, drought stress condition, Silicon treatment, and different cultivars had significant effect on all evaluated traits, ($p<0.01$, Table 2).

Table 2. Combined analysis of variance for plant traits in different irrigation regimes, silicon foliar and safflower cultivars

SOV	df	Mean square						
		Seed yield	MDA	PPO	APX	SOD	Oil yield	Oil content
Year	1	71871^{ns}	1.32^{ns}	0.01^{ns}	0.67^{ns}	0.15^{ns}	60.78^{ns}	51.98^{ns}
Rep(Year)	4	12478	453.89	11.00	77.27	21.87	1775.17	101.94
Drought	2	88931**	13211.56**	17.23**	131.77**	31.30**	7512.96**	116.53**
Year×drought	2	26619^{ns}	5.72^{ns}	0.03^{ns}	0.18^{ns}	0.38^{ns}	149.81^{ns}	49.79*
Error a	8	7562	7.08	0.30	1.49	0.25	34.54	10.20
Silicon	2	37401*	213.95**	1.31**	40.06**	2.31**	2479.20**	87.80**
Year×Silicon	2	27768^{ns}	20.04^{ns}	0.14^{ns}	0.65^{ns}	0.06^{ns}	35.43^{ns}	9.26^{ns}
Drought ×Silicon	4	19947^{ns}	33.01**	0.04^{ns}	5.87**	0.86*	5611.46**	22.81*
Year×drought ×Silicon	4	22509^{ns}	15.34^{ns}	0.05^{ns}	0.06^{ns}	0.21^{ns}	133.97**	22.85*
cultivar	2	61.83**	1118.68**	0.33*	16.37**	2.29**	20775.84**	23.56*
Year×Cultivar	2	27201^{ns}	1.79^{ns}	0.03^{ns}	2.52^{ns}	0.01^{ns}	11.71^{ns}	14.88^{ns}
Drought ×cultivar	4	19947^{ns}	87.69**	0.05^{ns}	7.76**	1.63**	709.71**	43.94**
Year×drought ×cultivar	4	20627^{ns}	2.37^{ns}	0.23*	0.61^{ns}	0.04^{ns}	130.23**	20.95^{ns}
Silicon×cultivar	4	18134^{ns}	40.78**	0.01^{ns}	9.29**	0.29^{ns}	7170.11**	27.40*
Year×Silicon×cultivar	4	26181^{ns}	4.66^{ns}	0.16^{ns}	0.06^{ns}	0.22^{ns}	37.18^{ns}	16.16^{ns}
Drought ×silicin×cultivar	8	16774^{ns}	24.75**	0.03^{ns}	6.08**	0.17^{ns}	4085.95**	51.30**
Year× drought ×silicon×cultivar	8	16887^{ns}	4.16^{ns}	0.04^{ns}	0.33^{ns}	0.21^{ns}	92.69**	19.48*
Error b	96	11334	8.49	0.07	0.87	0.27	18.45	8.65
ns, *and** show no significant and significant at 5 and 1% statistically level								

Seed Yield

Drought stress reduced the seed yield by 19.64%, in comparison to normal irrigated group (Table 3). Also, seed yield was increased by 20.59% when applying 1 mM silicon foliar treatment. However, the highest seed yield was observed in by applying 2 mM silicon in Goldasht cultivar under full irrigation condition (Fig. 1).

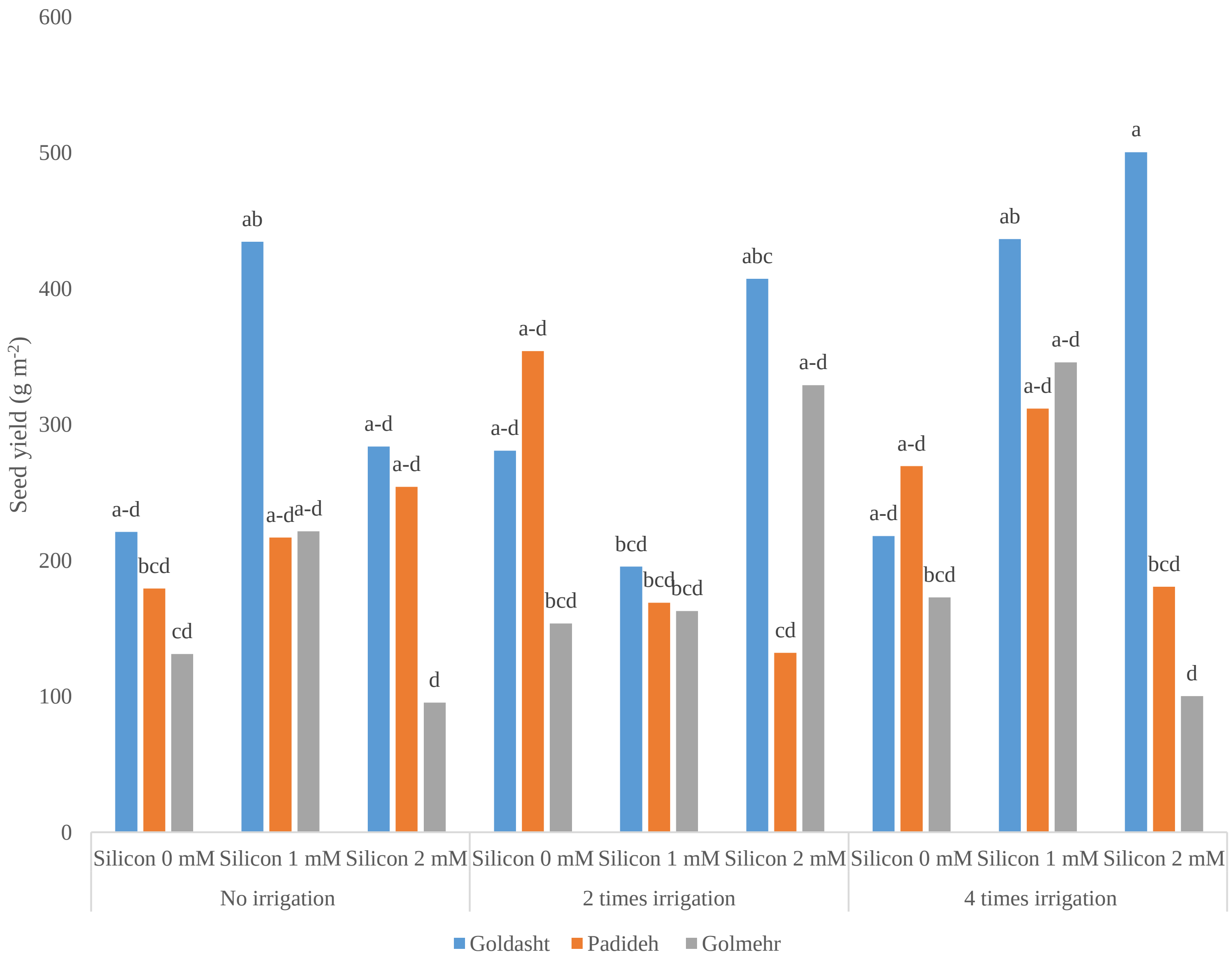

Figure 1. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on seed yield. Mean values with the same letters are not significantly different at $P \leq 0.05$ according to duncan test.

Drought stress leads to reduce in seed yield either by limiting the amount of water soluble nutrient during grain filling, or by number of decreasing the grains per plant [21]. Furthermore, drought stress leads to stomatal closure and a restriction in carbon dioxide uptake which is related to a reduction in photosynthesis. These events, subsequently, cause a decline in seed yield [22]. This finding was in line with those obtained by some researchers [23, 24]. It has been demonstrated that foliar sprays of silicon increased the seed by directly effect on plant biomass, grain set and 1000 grain weight. In addition, silicon can lead to delay in leaf senescence and sustain the leaf photosynthetic activities through grain filling [25].

Malondialdehyde Content (MDA)

In the present study, MDA content (as a lipid peroxidation marker) was mainly increased by drought stress. The highest MDA content was observed in severe drought stress (Table 3). MDA content was also increased in all cultivars exposed to drought stress (Fig. 2). MDA content was decreased by applying 1 mM and 2 mM of silicon (Table 3). Regarding three ways interaction among drought, silicon and cultivar, the highest level of MDA content was detected in Goldasht cultivar exposed to drought stress and non-application of silicon. Moreover, the lowest MDA content was reported in Golmehr cultivar which treated by 2mM of silicon under normal condition. (Fig. 2).

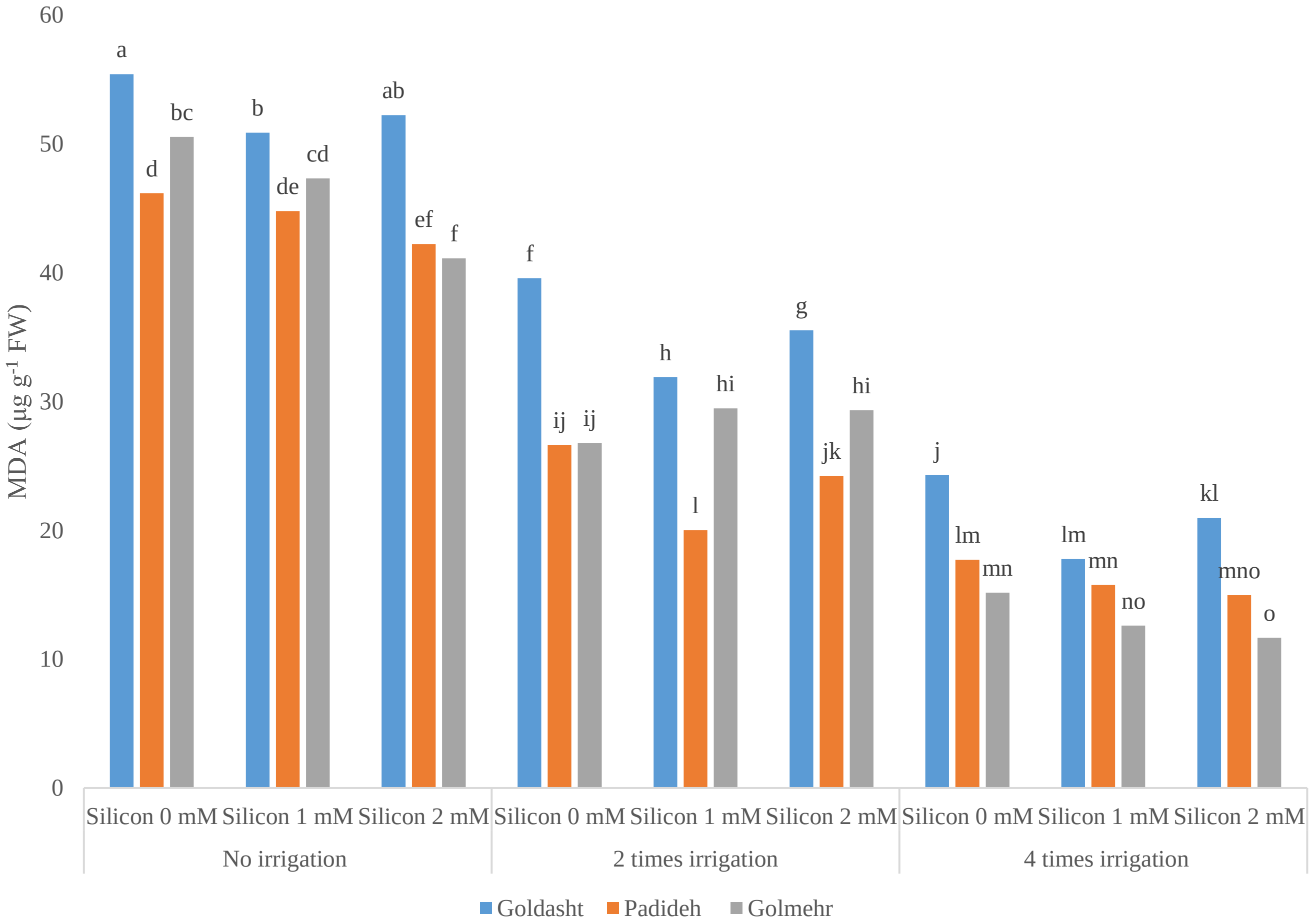

Figure 2. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on Malondialdehyde content (MDA). Mean values with the same letters are not significantly different at $P \leq 0.05$ according to duncan test.

MDA content is usually applied for evaluation of crops sensitivity to oxidative stress. Enhancing MDA content by drought stress could be the result of ROS aggregation and lipid peroxidation. [26]. Alleviating the levels of MDA content in groups exposed to drought using exogenously application of silicon may be due to a reducing in the detrimental effects of this stress by ROS scavenging and antioxidative defense of cell membranes stability and photosynthetic system [27]. This finding was in line with those obtained by Yao et al. (2011), who showed that silicon decreases the MDA content in wheat crop exposed to drought stress [28].

PPO

PPO activity was enhanced by application of 2mM silicon and drought stress condition (Table 3). The lowest PPO activity belonged to Goldasht cultivar under normal irrigation condition without silicon treatment, however, no significant differences were observed among the other treatments when compared in this irrigation condition (Fig 3).

Table 3. Main effects comparison in studied traits

		Seed yield	**MDA**	**PPO**	**APX**	**SOD**	**Oil yield**	**Oil content**
irrigation	0 time during growth	226.46 c	47.85 a	2.75 a	11.35 a	3.69 a	63.19 c	28.34 b
	2 times during growth	242.74 b	29.26 b	2.18 b	9.84 b	2.80 b	71.15 b	29.48 b
	4 times during growth	281.83 a	16.77 c	1.62 c	8.22 c	2.17 c	86.40 a	31.25 a
silicon	0 mM	220.09 c	33.59 a	2.01 b	10.80 a	2.65 b	65.76 b	30.00 a
	1 mM	277.19 b	30.05 b	2.23 a	9.26 b	3.01 a	77.68 a	28.29 b
	2 mM	253.75 a	30.24 b	2.31 a	9.35 b	3.00 a	77.30 a	30.79 a
cultivars	Goldasht	330.98 a	36.50 a	2.10 b	10.42 a	3.12 a	95.05 a	28.93 b
	Padideh	229.74 b	28.05 c	2.26 a	9.36 b	2.72 b	69.08 b	30.14 a
	Golmehr	190.31 c	29.33 b	2.20 ab	9.63 b	2.82 b	56.60 c	30.00 ab

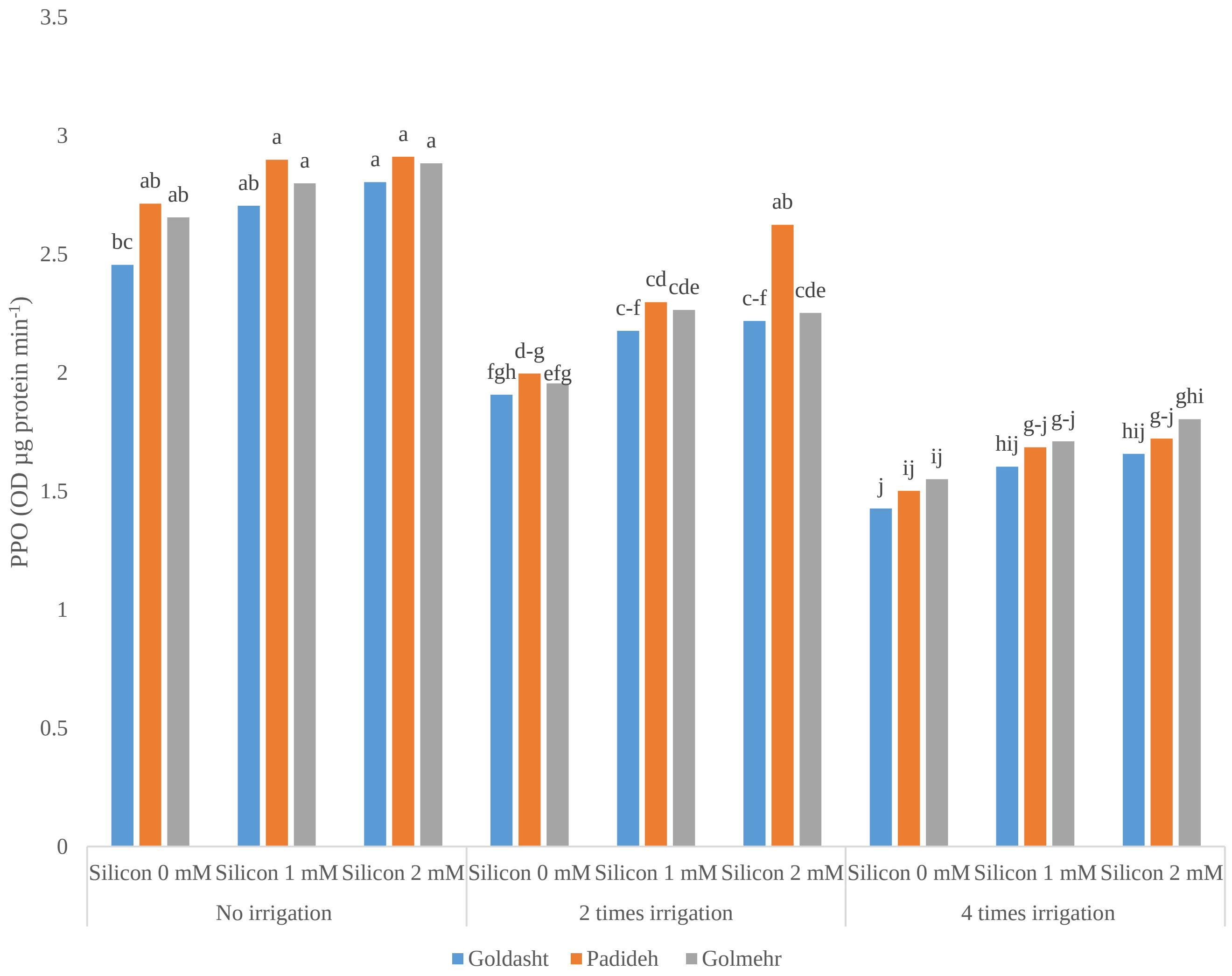

Figure 3. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on polyphenol oxidase (PPO). Mean values with the same letters are not significantly different at P ≤ 0.05 according to duncan test

A cascade of events occurs in plants exposed to drought stress. First, generation of ROS will be increased, second, levels of antioxidant systems and antioxidants will be promoted, and then improves ROS scavenging capacity, and tolerance to drought stress [12]. Although in drought stress condition, oxidative damage will be increased by the generation of ROS, antioxidant enzymes play pivotal role to protect the plant against ROS. PPO enzyme is a part of protective mechanism to alleviate oxidative damage. Augmenting activity of PPO enzyme under drought stress condition could be the result of increased ROS generation. It has been demonstrated that activity of SOD and PPO were increased in wheat exposed to drought stress [29] which is consistent with our results.

Silicon nanoparticles are capable to increase the activities of PPO as a reaction to drought stress. Silicon by inducing the activity of antioxidant enzymes involved in defense mechanism, improves the reaction of plants to biotic and abiotic stresses [30].

SOD

The maximum SOD activity was obtained in crops exposed to severe drought stress during growth. Also, application of silicon in both dosages, had significantly highest SOD activity over the control group. In addition, SOD at Goldasht cultivar was highest activity over the other groups (Table 3). The highest SOD activity was recorded in Goldasht cultivar exposed to drought stress and treated by 1 or 2 mM silicon (Fig. 4).

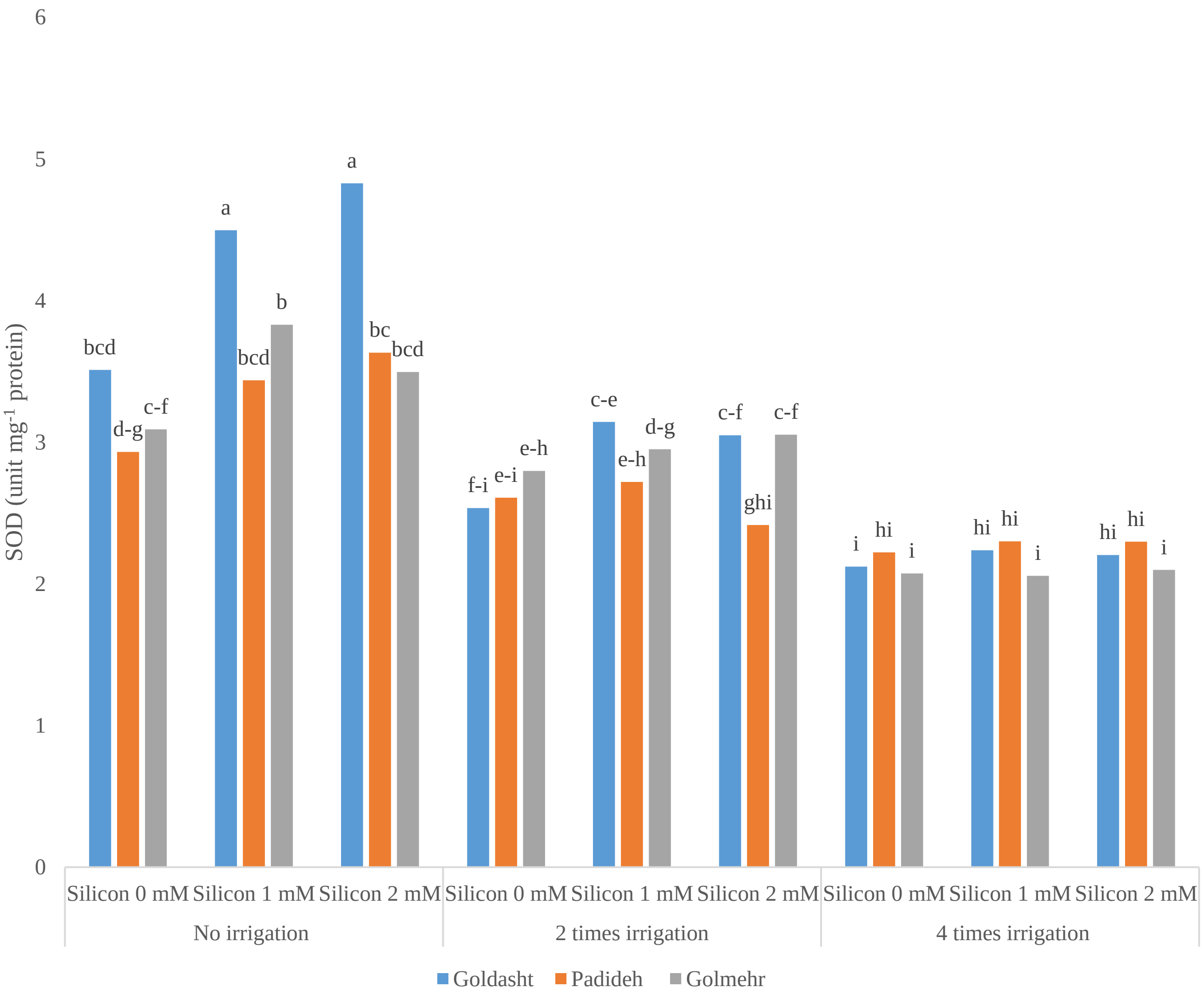

Figure 4. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on superoxide dismutase (SOD). Mean values with the same letters are not significantly different at P ≤ 0.05 according to duncan test

When plants are subjected to biotic and abiotic stresses, free radicals such as superoxide and peroxide, which are harmful to plant growth will be increased. SOD as an antioxidant enzyme is the first line of defense and quenches superoxide radicals to hydrogen peroxide in various cell compartments [31]. Drought stress mainly increased the SOD activity in leaves to prevent oxidative damage; hence, a concurrent increase in activity of antioxidant enzymes helps to an alleviation of detrimental influences of hydrogen peroxide under drought stress. These results are in line with study of Soleimanzadeh et al. (2010) in sunflower [32].

Accumulating evidence suggests that silicon mediated a rise in the SOD activity and antioxidant defense capacity, ameliorated chloroplast ultrastructure and membrane integrity, which leads to preserve the contents of photosynthetic pigments under drought stress and decrease of oxidative damage [12].

APX

Data showed that APX was significantly enhanced by drought stress (Table 3). Moreover, the difference among cultivars was significant for APX activity. The Goldasht cultivar had higher APX activity when compared to other cultivars (Table 3). While, application of silicon in three cultivars, decreased the APX activity in comparison to non-silicon-treated cultivars. Among the non-silicon-treated cultivars, Goldasht showed a higher APX activity in both normal and stressed conditions (Fig. 5).

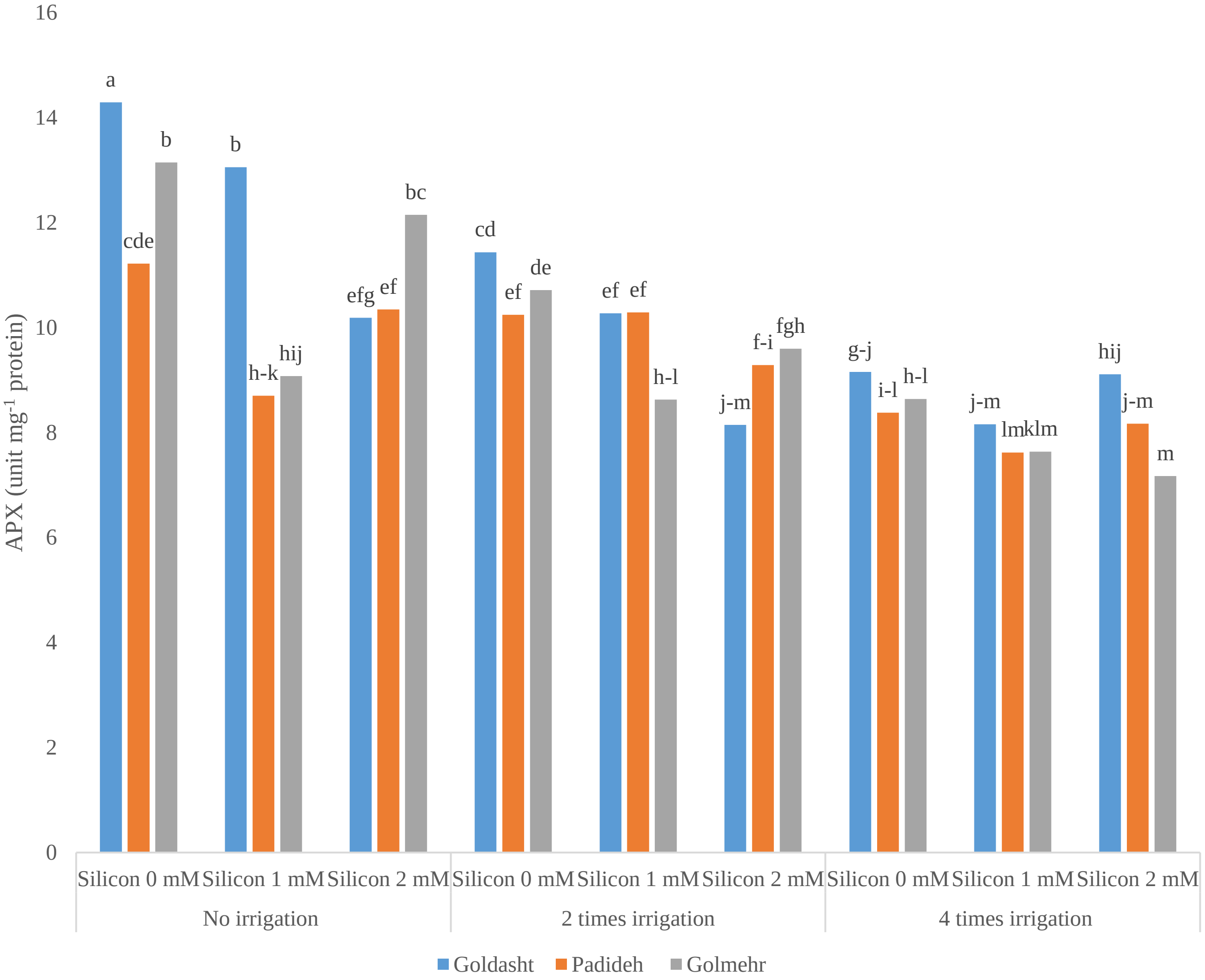

Figure 5. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on ascorbate peroxidase (APX). Mean values with the same letters are not significantly different at $P \leq 0.05$ according to duncan test

Although the activity of antioxidant enzymes enhances during drought stress condition [33], Gong et al. (2005) reported a reduction in antioxidant enzymes in wheat exposed to drought stress [12]. It seems that impacts of drought stress on the antioxidant enzymes are related to the stage and time of water deficit, species of plant, and cultivars [34]. In this study, APX was enhanced under drought stress condition in both years. this result was in line with previous findings by Cheng et al. (2018) [35].

APX scavenges cellular hydrogen peroxide through the ascorbate-glutathione cycle [36]. Thus, in the drought tolerant crops, increase in APX activity may be an adaptive system to overcome the oxidative damages.

Oil Content and Oil Yield

A decrease in oil content and yield of safflower was observed in gropes under moderate and severe drought stress. Oil yield was enhanced in safflower seeds by application of silicon. Results showed that the effect of irrigation on oil content was not significant. The lowest oil content was obtained from Goldasht cultivar. While, this cultivar had the highest oil yield in comparison two other cultivars (Table 3). Goldasht and Padideh cultivars by application 1mM silicon and Golmehr cultivar by application 2mM silicon at drought stress condition had lowest Oil content (Fig. 6). The highest oil yield in full irrigation was obtained by application of 2mM silicon in Goldasht cultivar (Fig. 7).

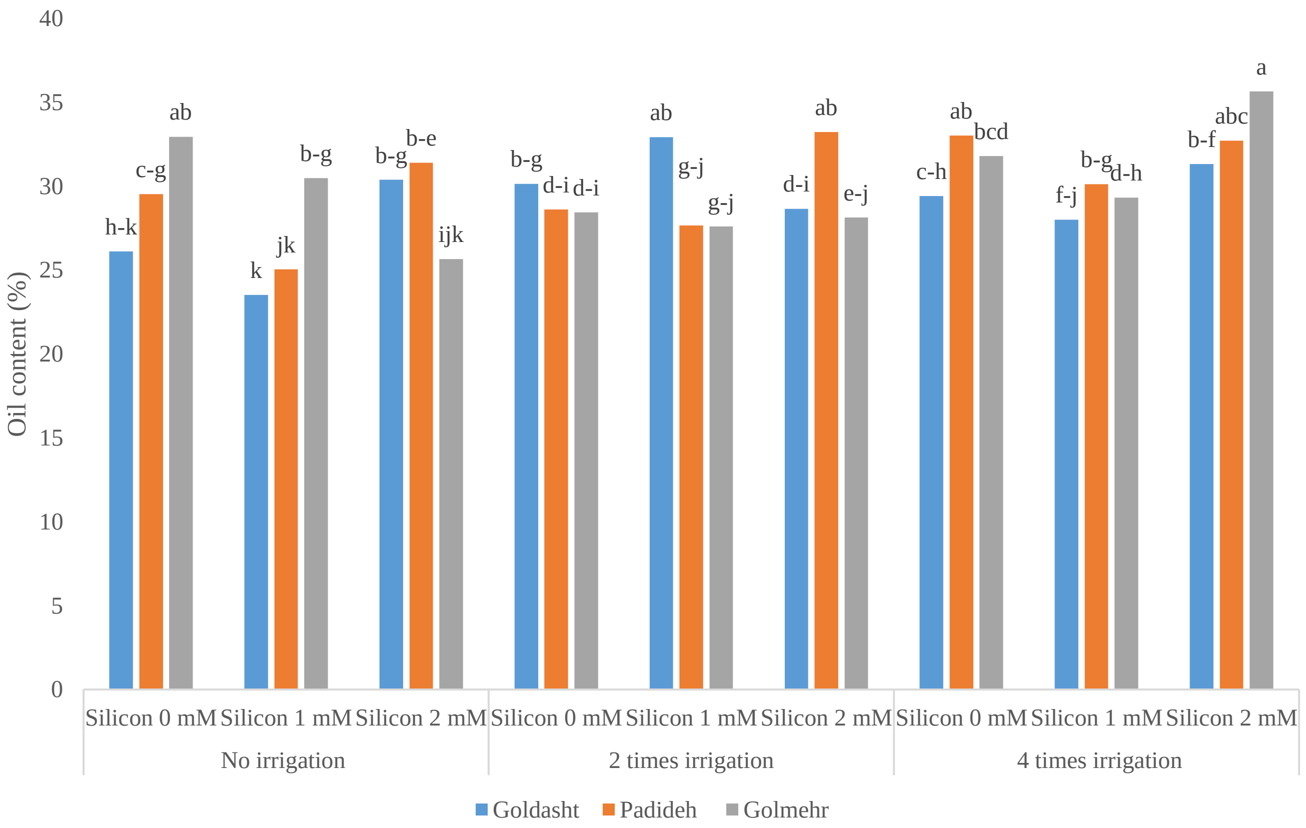

Figure 6. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on oil content. Mean values with the same letters are not significantly different at P ≤ 0.05 according to duncan test

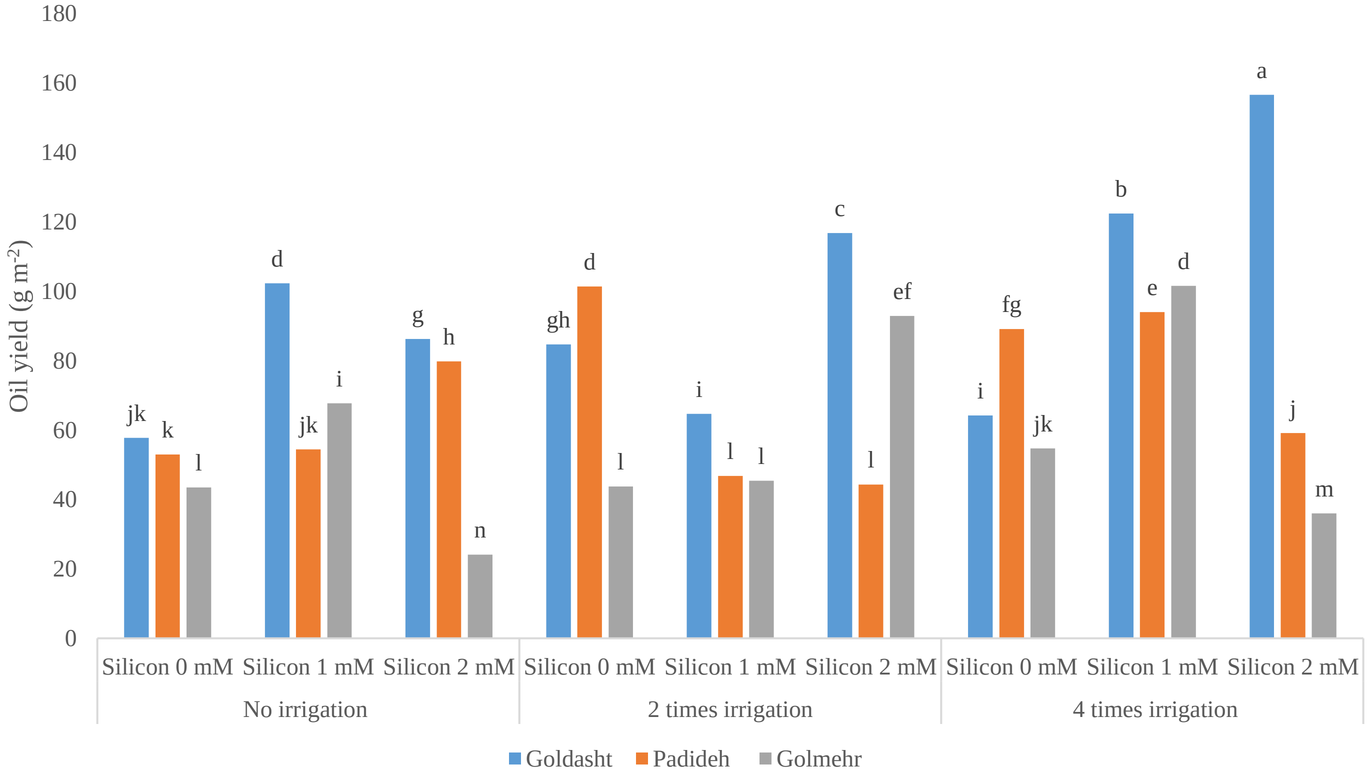

Figure 7. Interaction effect of different irrigation regimes, silicon foliar and safflower cultivars on oil yield. Mean values with the same letters are not significantly different at P ≤ 0.05 according to duncan test

Reduction in oil content under these levels of drought stress may be related to the decline in seed capacity for aggregation of oil, or increase oil oxidation such as some polyunsaturated fatty acids in seeds under drought stress after physiological maturity [37, 38]. This decline could be also associated with a limitation in accessibility of carbohydrates for oil synthesis in crops exposed to drought stress. The decrement in oil yield under drought stress was the consequence of the decrease in seed yield [39].

Conclusions

Our examination revealed that drought stress drastically decreased the seed and oil yield, and increased MDA, PPO, APX and SOD. Results showed that silicon can decline the deleterious effect of drought stress and increased amount of seed yield, oil content, oil yield, PPO and SOD. The best performance for almost all evaluated traits, was obtained under full irrigation with applying 2 mM of silicon foliar. Our study suggests that application of 1 mM of silicon foliar can improve the performance of safflower exposed to drought stress.

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