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Effects of Foliar Silicon on Physiological, Biochemical Traits and Yield of Safflower under Different Irrigation Regimes

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ABSTRACT

This study was conducted to investigate the effects of silicon foliar application on the yield, photosynthetic pigments, biochemical traits, and antioxidant capacity of three Iranian safflower cultivars (Faraman, Goldasht, and Golmehr) under different moisture conditions during the 2019 and 2020 growing seasons in the hot and arid climate of Fars Province, Iran. The experiment was laid out as a factorial split-plot arrangement based on a randomized complete block design (RCBD) with three replications. The treatments included four irrigation regimes (full irrigation as control, irrigation withholding at stem elongation, flowering, and seed filling stages) assigned to the main plots, and the combination of three silicon concentrations (100, 150, and 200 mg L^{-1}) with three safflower cultivars allocated to subplots. Results showed that the highest leaf area index (3.5) and chlorophyll *a* content (4 mg g^{-1} fresh weight) were obtained under full irrigation (supplying 100% of the crop water requirement). In contrast, the highest proline content (5 mg g^{-1} fresh weight) and peroxidase activity (1.55 U mg^{-1} protein) were observed when irrigation was withheld during the flowering stage. Moreover, silicon application, particularly at 200 mg L^{-1}, significantly enhanced photosynthetic pigments, biochemical compounds, and seed yield. Among the cultivars, Faraman exhibited superior levels of pigments, biochemical constituents, and antioxidant activity, as well as higher yield and greater drought tolerance than the others. Overall, flowering was identified as the most drought-sensitive growth stage in safflower, and silicon foliar application especially at 200 mg L^{-1} proved to be an effective approach for mitigating drought stress effects and improving yield.

1. INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is a multipurpose oilseed crop known for its high adaptability to arid and semi-arid environments, making it an ideal candidate for cultivation across large parts of Iran. In addition to its resilience against abiotic stresses such as drought and salinity (Paseban Eslam et al., 2021), safflower is valued for its oil rich in unsaturated fatty acids and its high vitamin A, iron, phosphorus, and calcium content. Beyond its nutritional value, the crop has growing importance in the food, pharmaceutical, and animal feed industries (FAO, 2024), underlining its economic potential in marginal lands.

Despite these advantages, safflower remains underutilized in Iran. According to FAO data, the average safflower seed yield in Iran in 2022 was approximately 485 kg ha^{-1}, substantially lower than the global average of 995.5 kg ha^{-1}. This considerable yield gap emphasizes the need to adopt improved agronomic and physiological management practices to enhance productivity and resource use efficiency (Köstereli & Turgut, 2025).

Foliar application of silicon has been widely reported to improve plant tolerance to drought stress through multiple mechanisms, including enhancing water-use efficiency, improving leaf water retention, strengthening the cuticle and epidermal cell walls, reducing transpiration rate, maintaining chlorophyll stability, and upregulating antioxidant enzyme activities such as CAT, POD, and SOD (Efe et al., 2025). Silicon also contributes to membrane stabilization by reducing lipid peroxidation under oxidative stress conditions. These beneficial effects have been demonstrated in several crops including oat, wheat, maize, and rice, suggesting the potential of silicon as an effective foliar amendment for improving drought resilience (Ali et al., 2023; Khalid et al., 2022; El-Beltagi et al., 2024; Ullah et al., 2016).

In recent decades, abiotic stresses, particularly water deficit, salinity, and climate warming—have emerged as critical threats to sustainable crop production (Delijani et al., 2022; Masheva et al., 2022). Drought stress, as one of the most prevalent environmental constraints in Iran (Nouri et al., 2020), adversely affects key physiological functions, including photosynthesis, stomatal conductance, cell expansion, and osmotic balance. These disruptions lead to declines in both vegetative vigor and reproductive development (Matinizadeh et al., 2024; Trejo-Paniagua et al., 2025).

To counteract such stress-induced damage, plants activate various defense mechanisms, such as the accumulation of compatible solutes (e.g., proline), modulation of phytohormone levels, and induction of enzymatic and non-enzymatic antioxidant systems (Moshki et al., 2024). Key antioxidant enzymes such as catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) play a central role in detoxifying reactive oxygen species (ROS), thereby maintaining cellular homeostasis and integrity under stress conditions (Alagoz et al., 2023; Valizadeh et al., 2022).

Among exogenous treatments proposed to mitigate the effects of drought, foliar application of silicon has gained attention due to its beneficial effects on plant performance under environmental stress (Boztaş & Bayram, 2025). Although silicon is not classified as an essential nutrient, it is the second most abundant element in the Earth's crust and has been shown to confer multiple benefits to plants (Ullah et al., 2016). These include improvements in photosynthetic efficiency, water retention capacity, structural reinforcement of tissues, and upregulation of antioxidant defenses (Neeru et al., 2019; Rousta et al., 2023; Ali et al., 2023).

Despite extensive evidence demonstrating the positive effects of silicon on drought tolerance in cereals and horticultural crops, there is limited information regarding foliar silicon application in safflower. Previous studies on safflower have mainly focused on irrigation scheduling, drought physiology, and nutrient management, while research on silicon especially in foliar form remains scarce. Moreover, the interactive effects of silicon, irrigation regimes, and cultivar-specific responses under field conditions have not been systematically evaluated. Therefore, a clear knowledge gap exists in understanding how foliar silicon application modulates physiological, biochemical, and yield-related traits in safflower grown in water-limited environments (Paseban Eslam et al., 2021; Zafari et al., 2017; Mahdavi et al., 2014; Alabdulwahed & Huthily, 2023).

Given the genotypic differences among safflower cultivars in their responses to drought and nutrient treatments, this study was conducted to explain the mitigating effects of foliar silicon application at three concentration levels and irrigation regimes on the yield by changing physiological, and biochemical traits of three Iranian safflower cultivars (*Faraman*, *Goldasht*, and *Golmehr*) under field conditions in the hot and arid climate of Fars Province, Iran.

2. MATERIALS AND METHODS

2.1. Experimental Site and Climatic Conditions

This experiment was conducted during the 2019 and 2020 cropping seasons in Gerash County, Fars Province, Iran (27°40′ N, 54°08′ E, elevation 914 m above sea level). Given the region's saline soil and low precipitation, safflower (*Carthamus tinctorius* L.), known for its drought and salinity tolerance, was selected as a suitable crop for cultivation. Prior to the experiment, composite soil samples were collected from 0–30 cm depth and analyzed for selected physico-chemical properties (Table 1). Climatic data for the plant growing period were also recorded (Table 2).

Table 1 . Selected physical and chemical properties of the soil prior to planting

Sand (%)	Silt (%)	Clay (%)	K (PPM)	P (PPM)	N (%)	pH	EC (ds.m^{-1})
20	44	36	272	5	0.11	7.5	1.6

Table 2. Mean climatic characteristics of the plant growth periods in 2019 and 2020

2019	Min. Temperature (C°)	Max. Temperature (C°)	Min. Humidity (%)	Max. Humidity (%)	Precipitation (mm)	Sunny hour
Jan.	1.8	20.5	13	62	22	277.1
Feb.	6.4	22.9	21	72	23.8	240.1
Mar.	10.2	29.8	10	49	103.9	306
Apr	14.8	32.9	11	46	20.5	303.6
May	20	39.8	6	26	0	463
2020						
Jan.	1.8	16.7	26	86	12.3	267.7
Feb.	6	23	20	76	10.7	256
Mar.	7.8	29.1	8	46	1.6	294.6
Apr	15	32.6	9	37	0	304.1
May	19.6	38.2	6	25	0	360.5

2.2. Plant Material and Experimental Design

Three Iranian safflower cultivars (Faraman, Goldasht, and Golmehr) were placed in the subplot level together with the silicon treatments, whereas the four irrigation regimes were allocated to the main plots in a factorial split-plot arrangement based on a randomized complete block design (RCBD) with three replications. The main plots consisted of four irrigation regimes: Full irrigation (control: Full irrigation was defined as supplying 100% of the crop water requirement at each irrigation event, calculated based on local climatic data and soil moisture conditions), Irrigation withholding at the stem elongation stage, Irrigation withholding at the flowering stage, and Irrigation withholding at the seed filling stage. The subplots included combinations of three silicon concentrations and the three cultivars. Silicon was applied as potassium silicate at concentrations of 100, 150, and 200 mg L^{-1}. Foliar spraying was performed twice: at stem elongation and early flowering. A non-ionic surfactant (0.1% v/v) was added to improve leaf absorption. Climatic data are presented only for the crop growth period (March–June), which corresponds to the entire active growing season of safflower in the study region.

Soil moisture was monitored using a gravimetric method at 0–30 cm depth at the beginning and end of each stress period. Although safflower roots can penetrate deeper layers, soil profile of the study area showed minimal moisture contribution below 30 cm due to low rainfall and high evapotranspiration; therefore, deeper soil water was considered negligible.

2.3. Field Preparation and Crop Management

Before sowing, the field was prepared by plowing and leveling. Fertilizers were applied based on soil test results: 50 kg ha^{-1} of triple superphosphate and 100 kg ha^{-1} of potassium sulfate at planting. Urea (250 kg ha^{-1}) was applied in three splits: at sowing, early spring growth, and flowering stages. Each plot measured 12 m² and contained five planting rows spaced 50 cm apart, with 1 m between adjacent plots and 2 m between replications (Yari et al., 2015; Paseban Eslam et al., 2021). Sowing was carried out on March 15 in both years. Although safflower is relatively drought tolerant, irrigation every 8 days was maintained prior to stress treatments to ensure uniform establishment and avoid confounding effects related to uneven early growth. Irrigation withholding at stem elongation, flowering, and seed filling corresponded to approximately mid-April, early May, and late May, respectively.

2.4. Weed Control and Maintenance

Broadleaf weeds were manually removed during the early vegetative growth period and continued as necessary throughout the season. Narrow-leaved weeds were controlled by applying Super Gallant at the safflower 4–6 leaf stage in accordance with the manufacturer's recommendations.

2.5. Yield Measurements

Biological yield (B.Y) and Grain yield (G.Y) were measured after physiological maturity (early May), when the safflower capitula turned yellow and seeds separated easily. The final harvest was performed in early June from the central rows of each plot, excluding 0.5 m from both ends to avoid edge effects. The harvest index (HI) was calculated using the following formula (Karam et al., 2007) where grain yield and biological yield were measured on a per-plot basis:

$$HI = (\text{Grain yield} / \text{Biological yield}) \times 100 \quad (1)$$

Leaf area index (LAI) was measured using the destructive sampling method. At flowering, five representative plants were collected from each plot, and their leaf areas were measured using a leaf area meter. LAI was calculated by dividing the total leaf area per ground surface area.

2.6. Photosynthetic Pigments

Chlorophyll a, b, and total chlorophyll contents were determined according to Porra (2002), and carotenoid content using the method of Lichtenthaler and Wellburn (1983). Fresh leaf samples (500 mg) were homogenized in 5 mL of 80% acetone, centrifuged, and absorbance was read at 663.6, 646.6, and 470 nm with a spectrophotometer. Pigment concentrations were calculated using standard equations.

$$\text{Chlorophyll a} = 12.25\,(A_{663.6}) - 2.55\,(A_{646.6}) \quad (2)$$

$$\text{Chlorophyll b} = 20.31\,(A_{646.6}) - 4.91\,(A_{663.6}) \quad (3)$$

$$\text{Total Chlorophyll} = 17.76\,(A_{646.6}) + 7.34\,(A_{663.6}) \quad (4)$$

$$\text{Carotenoids} = (1000A_{470} - 3.27[\text{chl a}] - 104[\text{chl b}]) \quad (5)$$

2.7. Biochemical Traits

Malondialdehyde (MDA) content was measured based on Boominathan and Doran (2002) by reacting fresh tissue with thiobarbituric acid (TBA) after trichloroacetic acid (TCA) extraction and heating at 95°C. Absorbance at 532 nm was used to calculate MDA concentration against a standard curve.

Proline content was assessed using the method of Bates (1973). Fresh leaf samples (500 mg) were extracted in 3% sulfosalicylic acid, reacted with ninhydrin and acetic acid, heated, and the chromophore extracted in toluene. Absorbance was measured at 520 nm.

2.8. Antioxidant Enzymes

Catalase (CAT) and peroxidase (POX) activities were measured in fresh tissues ground in liquid nitrogen and extracted in phosphate buffer. CAT activity was assayed by monitoring the decrease in absorbance at 240 nm (Chance & Maehly, 1955), and POX activity by the increase in absorbance at 470 nm (Obinger et al., 1997).

2.9. Statistical Analysis

Data were tested for normality and homogeneity across years. Combined analysis of variance (ANOVA) was conducted using SAS v9.1. Mean comparisons were performed using Duncan's Multiple Range Test at the 5% level. Principal Component Analysis (PCA) was performed in R (version 3.5.2), and graphs were generated using Microsoft Excel.

3. RESULTS AND DISCUSSION

This is the first field-based study evaluating multiple physiological, biochemical, and agronomic traits of safflower cultivars under foliar silicon application combined with controlled drought at specific growth stages.

3.1. Main Effects and Interactions

According to the combined analysis of variance, the effect of the experimental year was significant for leaf area index, chlorophyll *a*, chlorophyll *b*, total chlorophyll (*a*+*b*), carotenoids, malondialdehyde (MDA), proline, activities of catalase and peroxidase enzymes, grain yield, biological yield, and harvest index. The main effects of irrigation levels, silicon foliar application, and cultivar were also highly significant for all measured traits.

The interaction between irrigation × silicon was highly significant only for catalase activity and grain yield. The interaction between irrigation × cultivar significantly affected carotenoid content, catalase activity, grain yield, biological yield, and harvest index at the 1% probability level, and chlorophyll *b*, total chlorophyll, and MDA content at the 5% level. The silicon × cultivar interaction also had a significant effect on MDA content, catalase activity, grain yield, and harvest index. Moreover, the three-way interaction of irrigation × silicon × cultivar showed a highly significant effect solely on grain yield and catalase activity (Table 3).

Table 3 . Combined Analysis of Variance for Experimental Treatments on Studied Traits in Three Safflower Cultivars during 2019 and 2020

S.O.V	d.f	LAI	Chlo. a	Chlo. b	Chlo. a+b	Car	MDA	Pro	Cat	Prx	B.Y	G.Y	H.I
Year (Y)	1	2.4*	9**	5**	28**	14.6**	526**	12.7**	0.21**	0.03**	3905583.3**	972681.6**	112.6**
R(Y)	4	2.6	1.4	0.5	1.2	1.7	155.2	0.5	0.02	0.3	36823032.7	670162.2	57.4
Irrigation (I)	3	18.3**	20.8**	4.4**	44.3**	49.7**	3714.3**	22.5**	0.4**	0.7**	39623327.5**	7460246**	714.7**
Y*I	3	0.002^{ns}	0.1^{ns}	0.05^{ns}	0.3^{ns}	0.04^{ns}	7^{ns}	0.01^{ns}	0.01**	0.01*	27270^{ns}	41322.4*	4.2^{ns}
Error a	12	0.27	0.3	0.06	0.5	0.2	38.2	0.3	0.001	0.01	787556	27050.5	14.4
Foliar application (F)	2	8.5**	13.3**	3**	28.7**	20**	871.5**	20.7**	0.26**	0.17**	6006881**	1879981.3**	238**
Y*F	2	0.003^{ns}	0.1^{ns}	0.06^{ns}	0.4^{ns}	0.003^{ns}	0.04^{ns}	0.03^{ns}	0.006**	0.0006^{ns}	40296.5^{ns}	41075.7*	8.2^{ns}
I*F	6	0.05^{ns}	0.2^{ns}	0.09^{ns}	0.6^{ns}	0.1^{ns}	15.4^{ns}	0.09^{ns}	0.01**	0.002^{ns}	37926.3^{ns}	53213.3**	6.6^{ns}
Y*I*F	6	0.006^{ns}	0.02^{ns}	0.02^{ns}	0.07^{ns}	0.008^{ns}	1.7^{ns}	0.01^{ns}	0.003^{ns}	0.001^{ns}	31457.7^{ns}	5325^{ns}	4.6^{ns}
Variety (V)	2	54**	20.2**	2.5**	23**	84.4**	1967.4**	11**	0.74**	0.7**	144541017.7**	5590869.6**	55.8**
Y*V	2	0.0009^{ns}	0.1^{ns}	0.3^{ns}	0.4^{ns}	0.01^{ns}	9.2^{ns}	0.1^{ns}	0.009*	0.004^{ns}	19062^{ns}	11262.5^{ns}	0.03^{ns}
I*V	6	0.8^{ns}	0.4^{ns}	0.16*	0.9*	0.75**	26*	0.3^{ns}	0.02**	0.008^{ns}	1081427.2**	188423**	90.8**
Y*I*V	6	0.005^{ns}	0.04^{ns}	0.006^{ns}	0.04^{ns}	0.01^{ns}	1^{ns}	0.02^{ns}	0.004*	0.006^{ns}	42198.4^{ns}	1786.7^{ns}	0.9^{ns}
F*V	4	0.04^{ns}	0.1^{ns}	0.09^{ns}	0.4^{ns}	0.02^{ns}	37*	0.04^{ns}	0.01**	0.002^{ns}	28781.7^{ns}	75343**	32.3**
Y*F*V	4	0.001^{ns}	0.05^{ns}	0.01^{ns}	0.1^{ns}	0.03^{ns}	1^{ns}	0.03^{ns}	0.003^{ns}	0.0008^{ns}	9235.7^{ns}	3573^{ns}	2.6^{ns}
I*F*V	12	0.02^{ns}	0.06^{ns}	0.02^{ns}	0.07^{ns}	0.1^{ns}	3^{ns}	0.05^{ns}	0.008**	0.0009^{ns}	41774.5^{ns}	30533.3*	10.8^{ns}
Y*I*F*V	12	0.005^{ns}	0.02^{ns}	0.009^{ns}	0.02^{ns}	0.02^{ns}	0.4^{ns}	0.03^{ns}	0.001^{ns}	0.001^{ns}	45156.8^{ns}	2385.5^{ns}	2^{ns}
Error	124	0.4	0.2	0.07	0.3	0.1	12	0.2	0.002	0.005	223358.7	13812.4	8
CV (%)	-	22.8	16.4	21	13.4	6	13	10.3	3.8	5	8.6	11.4	15.2

**, * and ns indicate significance at the 1% and 5% levels, and non-significance, respectively. (LAI: Leaf Area Index, Chlo.a: Chlorophyll a, Chlo. b: Chlorophyll b, Chlo a+b: Chlorophyll a+b, Car: Carotenoid, MDA: Malondialdehyde, Por: Proline, Cat: Catalase, Prx: peroxidase, B.Y: Biological Yield, G.Y: Grain Yield, H.I: Harvest Index)

3.2. Chlorophyll a, b, and Total Chlorophyll Content

Comparison between the two study years revealed that 2019, due to higher precipitation, exhibited greater levels of photosynthetic pigments compared to 2020. In the second year, chlorophyll *a*, *b*, and total chlorophyll decreased by 9%, 21.4%, and 14.8%, respectively, compared to the first year (Table 4). The highest chlorophyll *a* content was observed under full irrigation, while water deficit at stem elongation, flowering, and seed filling stages led to reductions of 20%, 37.5%, and 30%, respectively (Table 5).

Table 4. Comparison of the mean effects of experimental years on physiological, biochemical, antioxidant enzyme activities, and yield traits of three safflower cultivars.

Year	Chlo. a (mg.g⁻¹)	Chlo. b (mg.g⁻¹)	Chlo. a+b (mg.g⁻¹)	Car (mg.g⁻¹)	Pro (mg. g⁻¹)	MDA (µg.g⁻¹)	Cat (mg.prot⁻¹)	Prx (mg.prot⁻¹)	B.Y (kg ha⁻¹)	G.Y (kg ha⁻¹)	H.I (%)
2019	3.3a	1.4a	4.7a	6.0b	4.0b	25.3b	1.1b	1.41b	5623.0a	1097.3a	19.2a
2020	3.0b	1.1b	4.0b	6.6a	4.6a	28.4a	1.2a	1.44a	5354.2b	963.0b	17.7b

Values followed by the same letter within each column are (Chlo.a: Chlorophyll a, Chlo. b: Chlorophyll b, Chlo a+b: Chlorophyll a+b, Car: Carotenoid, MDA: Malondialdehyde, Por: Proline, Cat: Catalase, Prx: peroxidase, B.Y: Biological Yield, G.Y: Grain Yield, H.I: Harvest Index) not significantly different according to the multiple comparison test.

Table 5 . Effects of different irrigation levels on leaf area index, chlorophyll a, proline content, and peroxidase activity in three safflower genotypes

Irrigation Level	LAI	Chlo.a (mg.g⁻¹)	Pro (mg.g⁻¹)	Prx (mg.prot⁻¹)
I1	3.5ᵃ	4.0ᵃ	3.6ᵈ	1.3ᵈ
I2	3.0ᵇ	3.2ᵇ	4.0ᶜ	1.37ᶜ
I3	2.2ᵈ	2.0ᵈ	5ᵃ	1.55ᵃ
I4	2.5ᶜ	2.8ᶜ	4.5ᵇ	1.5ᵇ

Values followed by the same letter within each column are not significantly different according to the multiple comparison test. (LAI: Leaf Area Index, Chlo.a: Chlorophyll a, Por: ProlinePrx: peroxidase). I1 to I4 indicate irrigation treatments: full irrigation, withholding irrigation at stem elongation, flowering, and grain filling stages, respectively.

Foliar application of silicon at 200 mg L⁻¹ resulted in the highest levels of chlorophyll *a*, *b*, and total chlorophyll. In contrast, applications at 150 and 100 mg L⁻¹ caused significant decreases of 16.6% and 25% in chlorophyll *a*, and 14.2% and 40% in chlorophyll *b*, respectively (Table 6).

Table 6 . Mean comparison of silicon foliar spray effects on physiological and yield traits in three safflower cultivars

Foliar application	LAI	Chlo.a (mg.g⁻¹)	Chlo.b (mg.g⁻¹)	Chlo.a+b (mg.g⁻¹)	Car (mg.g⁻¹)	Pro (mg.g⁻¹)	Prx (mg.prot⁻¹)	B.Y (kg ha⁻¹)
F1	2.5ᵇ	2.7ᶜ	1ᶜ	3.8ᶜ	5.8ᶜ	3.8ᶜ	1.38ᶜ	5189.2ᶜ
F2	2.8ᵇ	3ᵇ	1.2ᵇ	4.3ᵇ	6.3ᵇ	4.3ᵇ	1.42ᵇ	5511ᵇ
F3	3.2ᵃ	3.6ᵃ	1.4ᵃ	5ᵃ	7ᵃ	4.8ᵃ	1.48ᵃ	5765.6ᵃ

Values followed by the same letter within each column are not significantly different according to the multiple comparison test. (LAI: Leaf Area Index, Chlo.a: Chlorophyll a, Chlo. b: Chlorophyll b, Chlo a+b: Chlorophyll a+b, Car: Carotenoid, Por: Proline, Prx: peroxidase, B.Y: Biological Yield). F1 to F4 represent foliar silicon applications at 100, 150, and 200 mg L⁻¹, respectively

Among the cultivars, Faraman exhibited the highest chlorophyll *a* content, which was 23.3% and 37% greater than that of Goldasht and Golmehr, respectively (Table 7). Moreover, Faraman consistently maintained the highest pigment concentrations across all irrigation levels. Drought stress at the flowering stage, the chlorophyll *b* and total chlorophyll content in Golmehr decreased by 63.8% and 55.8%, respectively, compared to the control treatment (Table 8). Similar findings were reported by Oluwole et al. (2023) in basil, where drought stress led to reduced chlorophyll content. Conversely, silicon application was shown to protect pigments by mitigating oxidative damage and improving stomatal conductance (Ali et al., 2023). Zafari et al. (2017) also reported that the Faraman cultivar had the highest chlorophyll content among safflower genotypes.

Table 7 . Comparison of the mean traits of three safflower cultivars (LAI: Leaf Area Index; Chlo. a: Chlorophyll a; Por: Proline; Prx: Peroxidase)

Variety	LAI	Chlo.a ($mg.g^{-1}$)	Pro ($mg.g^{-1}$)	Prx ($mg.prot^{-1}$)
V1	3.8^{a}	3.7^{a}	4.6^{a}	1.5^{a}
V2	2.5^{b}	3^{b}	4.5^{b}	1.4^{b}
V3	2.2^{c}	2.7^{c}	4^{c}	1.3^{c}

Values followed by the same letter within each column are not significantly different according to the multiple comparison test. V1 to V3 denote the cultivars Farman, Goldasht, and Golmehr, respectively.

Table 8 . Mean comparison of the interaction effects of irrigation levels × cultivar on studied traits of three safflower cultivars (Chlo. b: Chlorophyll b; Chlo a+b: Chlorophyll a+b; Car: Carotenoid; MDA: Malondialdehyde; B.Y: Biological Yield; H.I: Harvest Index)

Irrigation Level	Variety	Chlo.b ($mg.g^{-1}$)	Chlo.a+b ($mg.g^{-1}$)	Car ($mg.g^{-1}$)	MDA ($\mu g.g^{-1}$)	B.Y ($Kg.ha^{-1}$)	H.I (%)
I1	V1	2.1^{a}	6.8^{a}	6.4^{e}	15.5^{g}	7772.5^{a}	23.8^{a}
	V2	1.5^{c}	5.3^{c}	4.8^{j}	17.2^{g}	6416^{cd}	24.2^{a}
	V3	1.1^{de}	4.4^{e}	4.4^{k}	24^{e}	5323^{f}	22.8^{a}
I2	V1	1.8^{b}	5.7^{b}	7.2^{c}	17^{g}	7068.7^{b}	20.8^{b}
	V2	1.3^{d}	4.4^{e}	5.8^{g}	20^{f}	5735^{e}	20^{b}
	V3	0.96^{fg}	3.8^{f}	5^{i}	27.5^{d}	4606.1^{h}	14.7^{de}
I3	V1	1.2^{d}	4.2^{e}	8.6^{a}	32^{c}	6366^{d}	13.4^{e}
	V2	0.83^{gh}	3.2^{g}	7.2^{c}	37.5^{b}	4213.5^{a}	16.2^{cd}
	V3	0.76^{h}	3^{g}	6.4^{e}	41.7^{a}	2968.3^{k}	18^{c}
I4	V1	1.6^{c}	4.8^{d}	7.8^{b}	24^{e}	6692.3^{c}	15.2^{d}
	V2	1.02^{ef}	3.7^{f}	7^{d}	29.3^{d}	4987.4^{g}	17.3^{cd}
	V3	0.81^{gh}	3.2^{g}	5.6^{h}	36.6^{b}	3719.5^{j}	15.2^{d}

Values followed by the same letter within each column are not significantly different according to the multiple comparison test. I1 to I4 indicate irrigation treatments: control, withholding irrigation at stem elongation, flowering, and grain filling stages, respectively. V1 to V3 denote the cultivars Farman, Goldasht, and Golmehr, respectively.

3.3. Carotenoid Content

Carotenoid content in 2020 was 10% higher than in 2019 (Table 4), which was likely due to increased drought stress during the second year. Foliar application of silicon at higher concentrations enhanced carotenoid levels, with the highest value recorded at 200 mg L^{-1}. In contrast, silicon concentrations of 150 and 100 mg L^{-1} led to decreases of 10% and 17%, respectively (Table 6).

In the irrigation × cultivar interaction, drought stress unlike its effect on chlorophyll led to an increase in carotenoid content, particularly in the Faraman cultivar at the flowering stage, where carotenoid levels were 34.3% higher than the control (Table 8). The lowest carotenoid content was observed in the Golmehr cultivar under full irrigation.

These findings are consistent with those of Mahdavi et al. (2014) and Khalid et al. (2022), who reported increased carotenoid content under stress conditions and positive effects of silicon application. The enhancement of carotenoid levels due to silicon foliar spraying may be attributed to higher antioxidant activity, which improves plant tolerance to reactive oxygen species (ROS) and consequently enhances photosynthetic pigment stability.

3.4. Leaf Area Index (LAI)

Full irrigation resulted in the highest LAI, while drought stress particularly at the flowering stage significantly reduced LAI by 52.8% (Table 5). Specifically, irrigation cessation at stem elongation, grain filling, and flowering stages decreased LAI by 14.2%, 37%, and 52.8%, respectively, compared to the control. Foliar application of silicon at 200 mg L^{-1} produced the highest LAI, while lower concentrations did not differ significantly from one another (Table 6). Among the cultivars, Faraman exhibited the highest LAI, whereas LAI values for Goldasht and Golmehr were 34% and 42% lower, respectively (Table 7). The reduction in LAI under drought stress was associated with chlorophyll degradation and premature leaf senescence (Ali et al., 2023). Silicon application contributed to the maintenance of LAI by preserving cell turgor. Yari et al. (2015) also reported the lowest reduction in LAI for the Faraman cultivar under water deficit conditions.

Drought-induced reductions in photosynthetic activity and accelerated leaf senescence can lead to a decline in LAI (Ali et al., 2023). In the present study, reductions in photosynthetic pigments (chlorophyll a, b, and total) under drought stress likely contributed to the observed decreases in LAI. Silicon foliar spraying under drought conditions may mitigate LAI loss by maintaining cell expansion through improved cell turgor (Ali et al., 2023). The higher LAI in the Faraman cultivar compared to the other two cultivars may be attributed to its greater drought tolerance and its ability to sustain higher chlorophyll and carotenoid contents under stress conditions. Similarly, Yari et al. (2015), in a study on the effects of water stress on the growth and yield of spring safflower cultivars in Iran, reported that Faraman experienced the least reduction in LAI (46%) under drought stress among the tested cultivars.

3.5. Malondialdehyde (MDA) and Proline Content

According to mean comparisons, MDA and proline contents were 12.2% and 15% higher, respectively, in the second year compared to the first year, likely due to reduced rainfall (Table 4). Based on the interaction of irrigation levels × cultivar, reduced water availability led to increased MDA content in all three cultivars, with the highest value recorded under irrigation cessation at the flowering stage. Among the cultivars, Golmehr exhibited the highest MDA content, followed by Goldasht and Faraman. In particular, Golmehr under irrigation cutoff at flowering showed a 73.7% increase in MDA compared to its respective well-watered control. The lowest MDA content was recorded in Faraman under full irrigation (Table 8).

The interaction between silicon concentration × cultivar indicated a decrease in MDA with increasing silicon levels. Among cultivars, the highest MDA was observed in Golmehr, followed by Goldasht and Faraman. The highest MDA value was recorded in Golmehr treated with 100 mg L^{-1} silicon, which was 34.2% higher than that of the treatment with 200 mg L^{-1} silicon. The lowest MDA content was found in Faraman treated with 200 mg L^{-1} silicon (Table 9).

Mean comparisons of irrigation levels also showed that increasing drought stress elevated proline content. The highest proline content was observed under irrigation cessation at the flowering stage, which was 38.8% higher than the fully irrigated control (Table 5). Foliar application of 200 mg L^{-1} silicon led to the highest proline accumulation, resulting in 11.6% and 26.3% higher proline levels than the 150 and 100 mg L^{-1} concentrations, respectively (Table 6). Among the cultivars, Faraman recorded the highest proline content, with 2.2% and 15% increases compared to Goldasht and Golmehr, respectively (Table 7).

These findings are consistent with El-Beltagi et al. (2024), who reported that drought stress in maize led to significant increases in MDA, H_2O_2, and electrolyte leakage, while foliar application of putrescine, silicon, or their combination decreased lipid peroxidation indices. The authors noted that silicon alone significantly reduced MDA, H_2O_2, and membrane leakage, indicating enhanced oxidative stress tolerance and reduced MDA accumulation under drought conditions.

Proline is recognized as a crucial metabolite in plant stress tolerance mechanisms, capable of reducing abiotic stress damage and enhancing plant growth and productivity under such conditions (Trejo-Paniagua et al., 2025). During drought stress, proline acts as a key signaling molecule that enhances mitochondrial performance and activates genes essential for mitigating stress damage, thereby promoting cell proliferation (Masheva et al., 2022). Hayat et al. (2012) reported that proline overaccumulation under environmental stress enhances tolerance by maintaining cell turgor pressure, stabilizing membranes, and reducing reactive oxygen species (ROS) to normal levels. Similarly, Ali et al. (2023) reported silicon application improved proline synthesis in oats under drought stress, contributing to enhanced cell pressure, osmotic adjustment, and overall stress tolerance.

Table 9. Mean comparison of the interaction effects of silicon foliar application × cultivar on studied traits of three safflower cultivars (MDA: Malondialdehyde; H.I: Harvest Index)

Foliar application	Variety	MDA ($\mu g.g^{-1}$)	H.I (%)
F1	V1	25^{e}	17^{d}
	V2	29^{c}	16.3^{d}
	V3	37.6^{a}	17^{d}
F2	V1	21.7^{f}	18^{cd}
	V2	26.2^{de}	19.6^{b}
	V3	31.7^{b}	17.4^{cd}
F3	V1	19.6^{g}	20^{b}
	V2	23^{f}	22.4^{a}
	V3	28^{cd}	18.6^{bc}

Values followed by the same letter within each column are not significantly different according to the multiple comparison test. F1 to F4 represent foliar silicon applications at 100, 150, and 200 mg L^{-1}, respectively. V1 to V3 denote the cultivars Farman, Goldasht, and Golmehr, respectively.

3.6. Catalase and Peroxidase Enzyme Activity

According to the mean comparisons, the activities of catalase and peroxidase enzymes in 2020 were 9% and 2% higher, respectively, than in 2019 (Table 4). The interaction between irrigation level × silicon foliar application × cultivar showed that catalase activity increased with both drought severity and higher silicon concentrations. Moreover, the Faraman cultivar exhibited the highest catalase activity across all treatment combinations. The highest catalase activity was observed under irrigation cessation at the flowering stage in Faraman treated with 200 mg L^{-1} silicon, which was 15.4% higher than the same treatment under full irrigation. The lowest catalase activity was recorded in Golmehr under full irrigation and 100 mg L^{-1} silicon application (Figure 1).

Irrigation level comparisons showed that irrigation cessation at flowering stage resulted in the highest peroxidase activity. In contrast, cessation at grain filling stage, stem elongation, and full irrigation led to 3.2%, 11.6%, and 16% lower peroxidase activity, respectively, compared to drought stress imposed at flowering (Table 5). As shown in Table 6, foliar application of silicon at 200 mg L^{-1} resulted in 4.2% and 7.2% increases in peroxidase activity compared to the 150 and 100 mg L^{-1} treatments, respectively. Among the cultivars, Faraman exhibited the highest peroxidase activity, with 7% and 15.3% higher activity compared to Goldasht and Golmehr, respectively (Table 7).

Studies have shown a correlation between drought tolerance and the antioxidant defense capacity of plants (Valizade et al., 2022). Ning et al. (2023) reported that drought stress during vegetative, pollination, and grain filling stages in wheat reduced relative water content, photosynthetic rate, stomatal conductance, and transpiration rate, while increasing antioxidant enzyme activity. They stated that foliar application of silicon improved antioxidant enzyme function and soluble sugar accumulation, resulting in reduced reactive oxygen species (ROS). Furthermore, El-Beltagi et al. (2024) demonstrated that drought stress combined with foliar application of putrescine, silicon, or both significantly enhanced the activities of key antioxidant enzymes in maize (catalase, superoxide dismutase, peroxidase, and ascorbate peroxidase), leading to reductions in malondialdehyde, hydrogen peroxide, and electrolyte leakage.

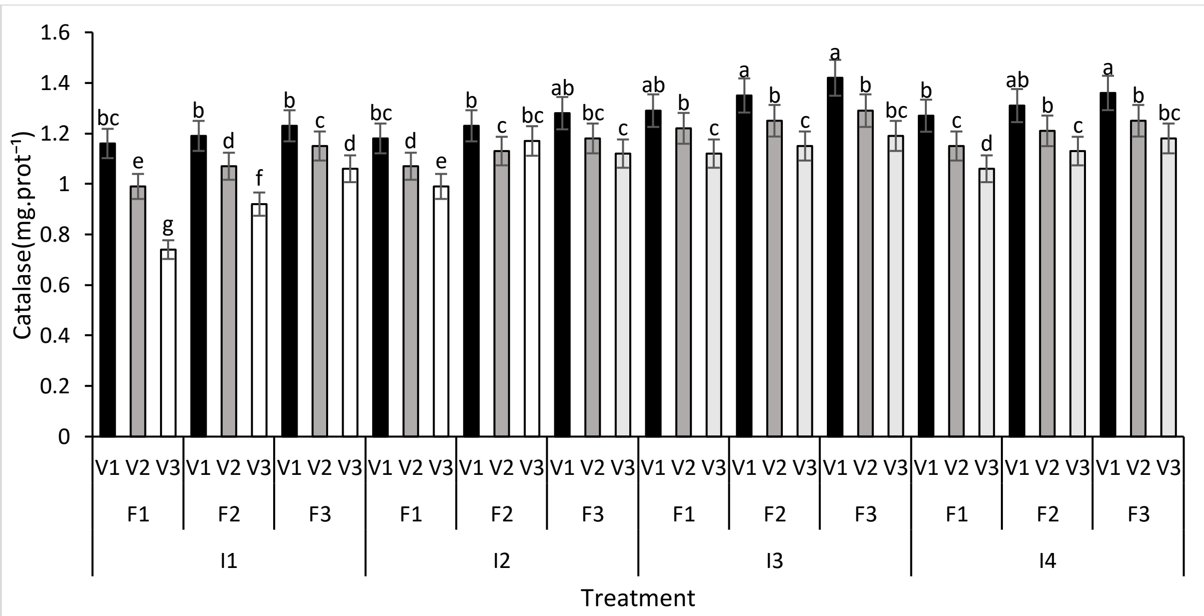

Figure 1. Mean comparison of the interaction effects of irrigation × silicon foliar application × cultivar on catalase activity in three safflower genotypes. I1 to I4 indicate irrigation treatments: full irrigation, withholding irrigation at stem elongation, flowering, and grain filling stages, respectively; F1 to F4 represent foliar silicon applications at 100, 150, and 200 mg L^{-1}, respectively; and V1 to V3 denote the cultivars Farman, Goldasht, and Golmehr, respectively. Means sharing the same letter are not significantly different at $p < 0.05$ according to the multiple comparison test.

3.7. Biological Yield, Grain Yield, and Harvest Index

According to mean comparisons, safflower produced 5%, 14%, and 8.4% higher biomass, grain yield, and harvest index, respectively, in 2019 compared to 2020 (Table 4). The effects of silicon foliar application on biomass indicated the highest value at 200 mg L^{-1}, which was 4.6% and 11% greater than the 150 and 100 mg L^{-1} treatments, respectively (Table 6). Based on the interaction between irrigation levels and cultivars (Table 8), the highest biomass was recorded for the Faraman cultivar under full irrigation, which was 10%, 22%, and 16% higher than the biomass under drought stress imposed at stem elongation, flowering, and grain filling stages in the same cultivar, respectively. The lowest biomass was observed in the Golmehr cultivar under drought stress at flowering, being 44.2%, 35.4%, and 20% lower than full irrigation, drought at stem elongation, and grain filling stages, respectively (Table 8).

The three-way interaction among irrigation, silicon foliar application, and cultivar revealed that grain yield decreased under drought stress and lower silicon concentrations in all cultivars. However, the Faraman cultivar consistently outperformed Goldasht and Golmehr across all treatments. The highest grain yield was observed in Faraman under full irrigation combined with 200 mg L^{-1} silicon foliar application. Under drought stress at flowering and 100 mg L^{-1} silicon, grain yield in Faraman decreased by 3.62%. The lowest grain yield was recorded in Golmehr under drought stress at flowering with 100 mg L^{-1} silicon application (Figure 2).

Mean comparisons of irrigation × cultivar interaction (Table 8) showed that all three cultivars had their highest harvest index under full irrigation without significant differences among them. However, drought stress caused a decline in harvest index across cultivars. Faraman, Goldasht, and Golmehr exhibited reductions of 43.6%, 33%, and 21% in harvest index under drought stress at flowering compared to full irrigation, respectively. Based on the silicon foliar application × cultivar interaction, the highest harvest index under silicon treatment at 200 mg L^{-1} was observed in Goldasht, while all cultivars had their lowest harvest index under 100 mg L^{-1} silicon application, without significant differences among them (Table 9).

Reduction of plant biomass under drought stress has been documented in previous studies. Consistent with the present results, Trejo-Paniagua et al. (2025) reported a decline in pepper biomass, and Pino et al. (2013) observed reduced growth in two wild potato species (*Solanum commersonii* and *Solanum tuberosum*) due to water deficit. This reduction is attributed to cellular damage caused by increased reactive oxygen species (ROS). It has been reported that increased proline accumulation along with silicon foliar application under drought stress can improve biomass, as proline activates specific stress-responsive genes that facilitate cell proliferation and mitigate stress damage (Masheva et al., 2022).

Crop yield is influenced by multiple interacting factors, including water, nutrients, light, and carbon dioxide availability throughout growth stages. Drought stress induces oxidative stress that disrupts chloroplast efficiency, decreases chlorophyll content, and consequently reduces photosynthetic activity and grain yield (Ponakala et al., 2023).

Silicon application has been shown to significantly increase grain yield in safflower. For instance, Alabdulwahed and Huthily (2023) reported yield improvements with silicon concentrations increasing from zero to 2 mM. The positive effect of silicon on plant growth is linked to improved leaf erectness, enhanced water use efficiency, reduced cuticular transpiration, increased light use efficiency, and better nutrient uptake (Sheykhzadeh et al., 2021). In the present study, Faraman exhibited the highest grain yield, likely due to its greater drought tolerance, higher photosynthetic pigment content, leaf area index, and antioxidant enzyme activity. Hasanvad et al. (2025) similarly reported the highest grain yield in Faraman under 100% and 60% irrigation levels, followed by Goldasht. They also noted that post-flowering irrigation cessation significantly reduced grain yield in Iranian safflower cultivars, consistent with the findings of this study.

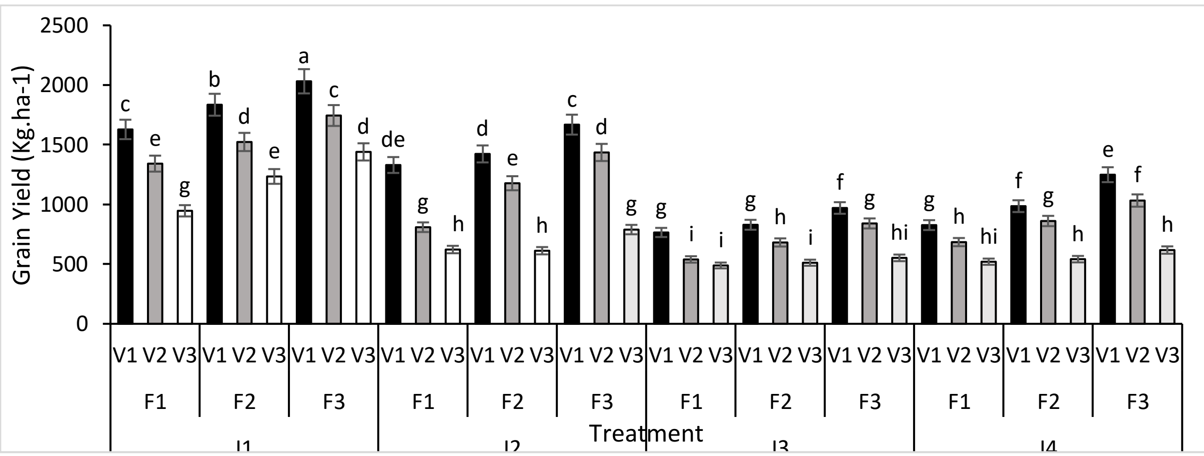

Figure 2. Mean comparison of the interaction effects of irrigation × silicon foliar application × cultivar on Catalase in three safflower genotypes. I1 to I4 indicate irrigation treatments: control, withholding irrigation at stem elongation, flowering, and grain filling stages, respectively; F1 to F4 represent foliar silicon applications at 100, 150, and 200 mg L^{-1}, respectively; and V1 to V3 denote the cultivars Farman, Goldasht, and Golmehr, respectively. Means sharing the same letter re not significantly different at $p < 0.05$ according to the multiple comparison test.

3.8. Interpretation of the Correlation Matrix Plot among Traits

The correlation matrix illustrates the complex relationships among agronomic, physiological, and biochemical traits in three safflower cultivars subjected to varying irrigation regimes (Figure 3). Grain yield (G.Y) exhibited significant positive correlations with biological yield (B.Y) (r = 0.80), harvest index (H.I) (r = 0.70), chlorophyll a (Chlo.a) (r = 0.75), chlorophyll b (Chlo.b) (r = 0.82), total chlorophyll (Chlo a+b) (r = 0.83), carotenoids (Car) (r = 0.76), and catalase activity (Cat) (r = 0.76). These positive associations underscore the critical role of enhanced photosynthetic pigment content, efficient biomass allocation, and antioxidant defense mechanisms in improving grain yield, particularly under water-limited conditions. Conversely, a strong negative correlation between G.Y and malondialdehyde (MDA) (r = –0.76) indicates that increased oxidative stress reduces yield performance. Similarly, proline (Por), a known osmoprotectant and stress indicator, showed a negative correlation with G.Y (r = –0.59), suggesting that higher proline accumulation may be a response to drought stress but not necessarily linked to improved yield. Peroxidase (Prx) also exhibited a moderate negative correlation with G.Y (r = –0.64), possibly reflecting cultivar-specific differences in antioxidant strategies under different irrigation levels. Moreover, leaf area index (LAI) was positively associated with yield and pigment traits, emphasizing the importance of canopy development in photosynthetic efficiency and overall plant productivity. The significant interaction between silicon concentration and irrigation regime indicates that silicon effectiveness depends on drought intensity. Silicon at 200 mg L^{-1} most strongly improved chlorophyll content, antioxidant activity, and grain yield under flowering-stage drought, which was the most critical stress period. The consistency of strong positive correlations between yield components and pigment-related traits across cultivars and irrigation treatments highlights the adaptive significance of maintaining chlorophyll integrity and ROS-scavenging capacity under drought stress. Overall, the correlation structure suggests that under the interactive influence of irrigation levels and genotype, safflower yield is strongly influenced by the plant's ability to maintain photosynthetic activity and mitigate oxidative damage, highlighting key traits for selecting drought-resilient cultivars.

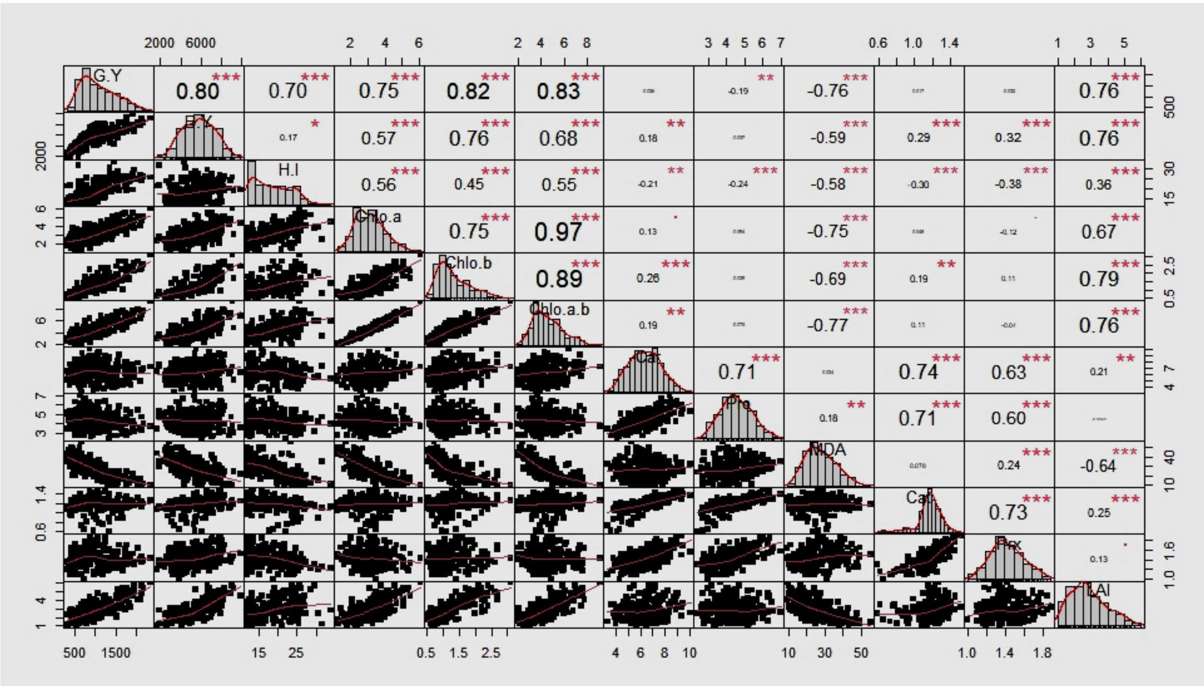

Figure 3 . Correlation matrix among physiological, biochemical, and agronomic traits including grain yield (G.Y), biological yield (B.Y), harvest index (H.I), leaf area index (LAI), chlorophyll a (Chlo.a), chlorophyll b (Chlo.b), total chlorophyll (Chlo a+b), carotenoids (Car), malondialdehyde (MDA), proline (Por), catalase (Cat), and peroxidase (Prx). Values indicate Pearson's correlation coefficients. Histograms on the diagonal show the distribution of each variable. Asterisks indicate significance levels ($p < 0.05$, *$p < 0.01$, **$p < 0.001$).

4. CONCLUSION

The results of this study demonstrated that despite the relative drought tolerance of safflower, the genetic, physiological, and biochemical traits of different cultivars significantly influence their resistance to drought stress. Additionally, the cultivars showed significant differences in growth and yield traits. An 85% increase in rainfall in 2019 improved the physiological traits, antioxidant capacity, and overall performance of safflower compared to

2020. According to the results, water stress during the flowering stage caused the greatest reductions in photosynthetic pigments (chlorophyll a, b, and total), leaf area index, biological yield, seed yield, and harvest index. Specifically, irrigation cutoff at flowering reduced the leaf area index by 37% and chlorophyll a content by 50%, while it increased proline content by approximately 39% and peroxidase enzyme activity by 19%. Therefore, the flowering stage is the most sensitive growth phase to drought stress, and to achieve maximum seed yield and water use efficiency, drought stress should be avoided during reproductive stages—especially flowering—while deficit irrigation can be applied during vegetative and late growth stages. Furthermore, foliar application of silicon at different concentrations improved all studied traits. Foliar application at 200 mg L^{-1} compared to 100 mg L^{-1} increased leaf area index by 28%, chlorophyll a by 33%, chlorophyll b by 40%, total chlorophyll (a+b) by 31%, carotenoid content by 12%, proline content by 26%, peroxidase activity by 7%, and biological yield by 11%. Among the cultivars tested, Farman exhibited the highest drought tolerance, followed by Goldasht and Golmehr. Farman had the highest leaf area index, photosynthetic pigments, proline content, and antioxidant enzyme activities, which enabled it to allocate more photosynthetic products to the capitula and seeds, resulting in the highest biological and seed yields.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interest or personal relationships.

STATEMENTS AND DECLARATIONS

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