Journal of Plant Physiology and Breeding

University of Tabriz

Print ISSN: 2008-5168 Online ISSN: 2821-0174

2025, 15(2): 193-215

Mitigating the effects of drought stress by applying levels of zeolite on yield, pigments, and some physiological traits of sesame cultivars

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Article Info

Article type:
Research article

Article history:
Received: August 27, 2025
Revised: September 27, 2025
Accepted: October 8, 2025
Published online: December 31, 2025

Keywords:
Darab,
Dashtestan,
Halil,
Grain yield,
Oil percentage,
Proline.

Abstract

Objective: Drought stress is a significant environmental factor that impacts the yield and quality of the sesame crop. This study was conducted to examine the effects of drought stress and zeolite on the yield, quantity, and some physiological traits of sesame cultivars.

Methods: The experiment followed a split-plot factorial design based on a randomized complete block design with three replications. It was conducted at two research locations: the Agricultural and Natural Resources Research Center of Sistan, Zabol, Iran, and the Agricultural and Natural Resources Research Center of Baluchestan, Iranshahr, Iran. The main plots consisted of three drought-stress conditions: full irrigation (according to the irrigation scheme of the area), irrigation cut-off at 50% flowering, and irrigation cut-off at 50% seed filling. The sub-factors included factorial combination of three sesame cultivars (Halil, Dashtestan, and Darab) and four zeolite levels (0, 3, 6, and 9 tons per hectare). Zeolite was mixed with the soil before planting. The traits measured included grain yield, biomass, oil percentage, pigments, soluble carbohydrates, proline, and protein.

Results: The amount of chlorophyll a and chlorophyll b decreased with increasing drought stress intensity; however, the application of zeolite mitigated the adverse effects of drought stress. Under irrigation cut-off at 50% flowering, total soluble carbohydrates significantly increased compared to the non-stress conditions. Also, leaf proline increased in both locations with increasing drought severity; however, the application of zeolite generally reduced the proline content. Under the 50% irrigation cut-off during flowering, the protein level decreased compared to full irrigation. The highest protein content and grain yield were obtained in the Halil cultivar at the Iranshahr location under non-stress conditions and with the application of nine tons/ha of zeolite. However, in Zabol, the highest grain yield was obtained by the Darab cultivar at all irrigation conditions, with the application of nine tons of zeolite. Generally, grain yield and oil content declined with increasing drought stress, but zeolite application mitigated the drought effects.

Conclusion: The use of zeolite in the sesame production was beneficial due to its positive impact on reducing the adverse effects of drought stress. Also, the Darab cultivar may be suggested for the sesame production in Zabol, while the Halil cultivar seemed more suitable for the Iranshahr location.

Cite this article: Narouei M, Sirousmehr A, Dahmardeh M, Seyedabadi E. 2025. Mitigating the effects of drought stress by applying levels of zeolite on yield, pigments, and some physiological traits of sesame cultivars. J Plant Physiol Breed. 15(2): 193-215. https://doi.org/10.22034/jppb.2025.68868.1374

Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oilseed crops, valued for its adaptability to arid conditions and highly nutritious seeds due to their oil content and nutrient profile. Despite its high potential, sesame has low yield due to poor agronomic management, biotic and abiotic environmental stresses, low input consumption, and the lack of compatible and high-yielding improved cultivars (Pham *et al*. 2010). Sesame seeds have nutritional, cosmetic, and health properties, and have high nutritional value. Additionally, its oil exhibits extremely high stability due to the presence of strong antioxidants such as sesamulin, sesamol, and sesamin (Sabannava and Lakshman, 2008). Its seeds are rich in oil (>50%), containing high levels of unsaturated essential fatty acids and low levels of saturated fatty acids. It also has high carbohydrates (> 20%) and protein (> 20%) (Dravie *et al*. 2020). Although sesame is relatively tolerant to drought, its yield and quality are reduced under high levels of water-deficit stress (Mensah *et al.* 2006; Ebrahimian *et al.* 2019; Kouighat *et al*. 2022).

The growing world population and food demand make agricultural production essential for ensuring food security. On the other hand, climate change challenges crop production, especially in arid and semi-arid regions (Desoky *et al*. 2023). Drought stress negatively impacts photosynthesis, yield, and quality (Kamara *et al*. 2021). Plants respond to drought stress by accumulating osmolytes, which help regulate cellular osmotic potential. These osmolytes, including proline, methylamines, polyamines, sugars, and polyols, stabilize protein structure across various organisms (Ozturk *et al*. 2021; Singh *et al*. 2021). Among these, proline is essential as both a structural unit of proteins and a protective osmolyte for plants under stress (Alvarez *et al*. 2022; Spoormann *et al*. 2023). Proline accumulation during stress results from increased synthesis and decreased degradation, protecting cellular membranes, proteins, and enzymes from oxidative stress and scavenging free radicals (Alvarez *et al*. 2022). However, proline accumulation alone is insufficient for drought tolerance; other pathways and mechanisms also play a role (Chun *et al*. 2018; Ozturk *et al*. 2021).

Plants reduce the adverse effects of water deficit by various molecular and physiological mechanisms, including activating stress-responsive genes, enhancing antioxidant defenses, stabilizing membrane structure, and accumulating osmoprotective compounds like sugars, proline, and proteins (Chaves *et al*. 2003; El-Sharkawy, 2004; Ksouri *et al*. 2016; Sharma *et al*. 2017; Mahmood *et al*. 2020; Jia *et al*. 2021).

Zeolites, (Na, K, Ca)2-3Al3(Al, Si)2Si13O36•12H2O, are hydrated alumino silicates with oxygen atoms linked in a three-dimensional SiO4 and AlO4 network. These structures contain cavities and channels filled with water molecules and cations that balance the negative charge from Si4+ and Al3+ substitutions and can exchange with other cations (Mastinu *et al*. 2019). By selectively absorbing and releasing cations, zeolites enhance nutrient availability, improving plant growth and development (Gholamhoseini *et al*. 2012; Murphy *et al*. 2023). Also, Zeolites are beneficial under deficit irrigation conditions due to their water-holding capacity (GhassemiSahebi *et al*. 2020; Sun *et al*. 2023). Zeolites are non-toxic and safe for humans, and therefore, are useful for crop production (Cerri *et al*. 2002).

Given the importance of sesame and the strategic nature of this plant and water scarcity in Iran, expanding its cultivation is of great importance, especially under drought conditions. Therefore, this research aimed to examine the effects of drought stress through different irrigation levels and zeolite treatments, and to identify high-performing sesame cultivars.

Materials and Methods

This study was conducted in two locations: the Agricultural and Natural Resources Research Center of Sistan, Zabol, Iran, and the Agricultural and Natural Resources Research Center of Baluchestan, Iranshahr, Iran. Zabol is located at 61° 29' East longitude and 31° 13' North latitude, at an altitude of 498.2 meters above sea level. Iranshahr is located 350 km from Zahedan, at 27° 12' North latitude and 60° 24' East longitude. The experiment was conducted as a split-plot factorial design, based on a randomized complete block design with three replications. Table 1 shows the climatic characteristics of the study areas during the study period.

In each site, the soil was tilled to a depth of 30 cm, disked, and leveled. Soil samples were taken before planting, with the soil characteristics summarized in Table 2. Plot dimensions were set to 2.5 × 2.5 meters, with 1.5 meters between main plots and 45 centimeters between subplots. The planting depth was 2-3 cm, with rows spaced 45 cm apart and plants spaced 10 cm within rows. Planting dates were April 15, 2020, for Zabol and August 5, 2020, for Iranshahr. The main plots consisted of three irrigation levels: full irrigation as the control, irrigation cut off at 50% flowering, and irrigation cut off at 50% seed filling. The subplots included the factorial combinations of sesame cultivars (Halil, Dashtestan, and Darab) and four levels of zeolite application (0, 3, 6, and 9 tons per hectare). The

Table 1. Climatic characteristics of the studied areas.

Zabol				Iranshahr			
Month	**Average temperature (Celsius)**	**Average rainfall (mm)**	**Humidity (%)**	**Month**	**Average temperature (Celsius)**	**Average rainfall (mm)**	**Humidity (%)**
April	23.8	7.5	35	August	37	0	19
May	28.1	0	23	September	31.3	0	20
June	33.5	0	20	October	22.2	0	18
July	37.7	0	16	November	13.2	0	32
August	38	0	18	December	10.4	0	35.5
September	31.1	0	20	-	-	-	-

Table 2. Physicochemical properties of soil in the studied areas.

Location	Depth	EC (ds/m)	pH	Organic C (ppm)	Total N (%)	Available P (ppm)	Available K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
Zabol	0-30	4.3	8.4	0.35	0.031	6.2	145	15	37	48	Clay
Iranshahr	0-30	3.9	7.9	0.54	0.033	12	160	11	40	49	Silty clay

Halil cultivar is drought- and wilt-resistant, suitable for the warm, arid south of Iran. Dashtestan is relatively drought- and wilt-resistant, suited for the hot south, and Darab is a large-capsule cultivar adapted to the semi-southern regions (SPII, 2015). The zeolite used in this study was clinoptilolite from the Semnan zeolite mine (Afrazmand Mineral Co.), Iran. Zeolite, with particle sizes of less than 2 mm, was incorporated to a depth of 15-20 cm before sowing. The chemical properties of the zeolite are shown in Table 3.

Table 3. Chemical analysis of zeolite used in this study.

Aluminum oxide	Phosphorus pentoxide	Manganese oxide	Iron oxide	Silicon dioxide (SiO2)	Potassium oxide	Sodium oxide	Magnesium oxide	Calcium oxide	Titanium oxide
10.5-11.75	0.026	0.03	0.5	66-68	4-4.5	3-3.5	0.58	0.2-0.6	0.8

Numbers are in percentage.

Leaf pigments were measured after drought stress treatment using the method of Rangana (1977). The method of Bates *et al*. (1973) was used to measure proline in plant leaves. The soluble carbohydrate content in leaves was determined based on the phenol-sulfuric acid method (Irigoyen *et* *al*. 1992). Seed oil percentage was determined using a Soxhlet apparatus at the agricultural research laboratory of Zabol University, Iran.

The plants were harvested manually on September 16, 2020, in Zabol and on November 5, 2020, in Iranshahr. Harvesting was done from two central rows of each plot, excluding border effects. Then, grain yield and biomass were measured.

The homogeneity of experimental errors (Bartlett's test) was verified before the combined analysis of variance (ANOVA), and mean comparisons were performed using the least significant difference (LSD) test at the 5% probability level. Data analysis was performed using SAS 9.2, with graphs created in Excel.

Results and Discussion

Chlorophyll a

The combined ANOVA results showed significant effects of location, drought stress, zeolite, cultivar, and the interactions of drought stress × zeolite, drought stress × cultivar × location, and some other interactions on chlorophyll a (Table 4). The highest chlorophyll a (14.5 mg/g fresh weight) was observed under non-stress conditions for 9 tons of zeolite per hectare (Table 5). In general, the amount of chlorophyll a decreased with increasing stress intensity. Also, at each level of drought stress, chlorophyll a increased with increasing zeolite application, and the lowest amount of chlorophyll a was observed in the absence of zeolite application.

According to Table 6, comparing the means of combinations of location, drought stress, and cultivar for chlorophyll a showed that the highest chlorophyll a (18.76 mg/g fresh weight) was obtained from the Iranshahr location and under control irrigation conditions in the Dashtestan cultivar. In the Zabol location, the Dashtestan cultivar also had a higher chlorophyll a level under full irrigation conditions. In general, the amount of chlorophyll a was higher in Iranshahr than in Zabol under full irrigation and irrigation cut-off at 50% seed filling, but was lower than in Zabol at irrigation cut-off at 50% flowering. Also, in two locations, the highest amount of chlorophyll a was observed at the control irrigation, and under deficit irrigation, the amount of chlorophyll a decreased. Overall, the Dashtestan cultivar had a higher chlorophyll a content in two locations under both drought stresses.

Chlorophyll b

The combined ANOVA (Table 4) indicated significant effects of location, drought stress, cultivar, zeolite, cultivar × location, cultivar × zeolite, cultivar × drought stress, and location × drought stress

× zeolite interactions on chlorophyll b at the 1% probability level. The highest chlorophyll b (7.97 mg/g fresh weight) was recorded in Iranshahr under non-stress conditions with 9 tons of zeolite per hectare (Table 7). In general, in both locations, the amount of chlorophyll b decreased with increasing stress intensity (irrigation interruption); however, the amount of chlorophyll b was increased by using zeolite. Overall, the amount of chlorophyll b in Iranshahr was higher than in Zabol.

Table 4. Combined analysis of variance of sesame traits under the influence of cultivar, zeolite, and drought stress at two locations.

SOV	df	Mean squares			
		Chlorophyll a	Chlorophyll b	Carotenoids	Soluble carbohydrates
Location	1	14.14**	9.89**	3.64**	220.42**
Replication (Location)	4	4.3	0.01	0.008	47.71
Drought stress	2	240.88**	27.66**	12.02**	7481.18**
Drought stress × Location	2	122.86**	10.92**	3.6**	462.39**
Error 1	8	0.87	0.07	0.011	5.6
Zeolite	3	102.43**	30.57**	11.56**	6861.21**
Zeolite × Location	3	30.57**	8.21**	3.16**	206.29**
Drought stress × Zeolite	6	1.65**	2.13**	0.07**	192.25**
Drought stress × Zeolite × Location	6	0.89^{ns}	1.35**	0.18**	186.07**
Cultivar	2	19.06**	1.59**	0.16**	94.98**
Cultivar × Location	2	78.59**	8.39**	18.1**	278.24**
Drought stress × Cultivar	4	3.41**	0.24**	0.12**	14.008^{ns}
Drought stress × Cultivar × Location	4	2.66**	0.05ns	0.16**	0.26^{ns}
Zeolite × Cultivar	6	1.98*	0.32**	0.12**	88.18**
Zeolite × Cultivar × Location	6	0.9^{ns}	0.04^{ns}	0.19**	12.33^{ns}
Drought stress × Zeolite × Cultivar	12	0.18^{ns}	0.01^{ns}	0.019^{ns}	2.67^{ns}
Drought stress × Zeolite × Cultivar × Location	12	0.149^{ns}	0.04^{ns}	0.018^{ns}	21.95*
Error	60	0.39	0.043	0.013	5.16
Location	-	8.13	7.58	4.97	6.38

ns, *, and **: Not significant and significant at the 5 and 1 percent probability levels, respectively.

Table 4 continued

SOV	df	Mean squares				
		Proline	Protein	Grain yield	Biomass	Oil %
Location	1	1.75**	0.69**	2610000**	1940000**	2072.64**
Replication (Location)	4	0.00001	0.006	0.000	50000	1.18
Drought stress	2	0.18**	0.46**	920000**	8820000**	379.17**
Drought stress × Location	2	0.04**	0.048**	0.000^{ns}	0.000^{ns}	0.000^{ns}
Error 1	8	0.00004	0.003	3000	2000	0.94
Zeolite	3	0.17**	0.0508**	1270000**	7570000**	632.95**
Zeolite × Location	3	0.06**	0.025**	0.000^{ns}	0.000^{ns}	0.000^{ns}
Drought stress × Zeolite	6	0.0027**	0.002*	13000**	92000**	12.79**
Drought stress × Zeolite × Location	6	0.0023**	0.009**	0.000**	0.000^{ns}	0.000^{ns}
Cultivar	2	0.098**	0.058**	333000^{ns}	401000**	0.000^{ns}
Cultivar × Location	2	0.17**	0.104**	1390000**	3820000**	57.17**
Drought stress × Cultivar	4	0.012**	0.0003^{ns}	2000^{ns}	11000^{ns}	0.000^{ns}
Drought stress × Cultivar × Location	4	0.012**	0.0002^{ns}	500^{ns}	20000**	0.09^{ns}
Zeolite × Cultivar	6	0.025*	0.004*	1000^{ns}	7000^{ns}	0.000^{ns}
Zeolite × Cultivar × Location	6	0.028**	0.005*	800^{ns}	6000^{ns}	3.08*
Drought stress × Zeolite × Cultivar	12	0.0009**	0.004*	2000^{ns}	16000**	0.000^{ns}
Drought stress × Zeolite × Cultivar × Location	12	0.001**	0.003*	6000**	42000**	3.04**
Error	60	0.00013	0.0009	1500	2700	0.504
CV (%)	-	4.46	4.23	4.16	2.39	1.96

Table 5. Chlorophyll a content of sesame for different amounts of zeolite under three irrigation regimes.

Drought stress	Control (Full irrigation)				Irrigation cut-off at 50% seed filling				Irrigation cut-off at 50% flowering			
Zeolite (t/ha)	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**
Chlorophyll a (mg/g FW)	8.03	10.3	11.5	14.5	5.49	7.76	7.83	10.21	3.82	4.45	5.78	8.11
LSD 5%	0.4065											

Table 6. Means for sesame chlorophyll a and carotenoids under the influence of drought stress and cultivar in two locations.

Location	Drought stress	Cultivar	Chlorophyll a (mg/g FW)	Carotenoids (mg/g FW)
Zabol	Control (Full irrigation)	Halil	7.52	1.58
		Dashtestan	8.64	2.72
		Darab	8.61	2.80
	Irrigation cut-off at 50% seed filling	Halil	6.68	1.57
		Dashtestan	7.52	2.49
		Darab	7.62	2.44
	Irrigation cut-off at 50% flowering	Halil	6.70	1.31
		Dashtestan	6.93	2.25
		Darab	7.33	2.22
Iranshahr	Control (Full irrigation)	Halil	12.92	3.66
		Dashtestan	18.76	4.98
		Darab	10.29	2.77
	Irrigation cut-off at 50% seed filling	Halil	8.21	2.69
		Dashtestan	10.45	3.09
		Darab	4.15	1.60
	Irrigation cut-off at 50% flowering	Halil	3.60	1.69
		Dashtestan	5.60	2.38
		Darab	2.11	0.90
LSD 5%			0.3606	0.0658

Table 7. Means for sesame chlorophyll b and carotenoids under the influence of drought stress and zeolite rates in two locations.

Location	Drought stress	Zeolite (t/ha)	Chlorophyll b (mg/g FW)	Carotenoids (mg/g FW)
Zabol	Control (Full irrigation)	0	2.19	2.25
		3	2.83	2.38
		6	3.18	2.58
		9	3.50	2.90
	Irrigation cut-off at 50% seed filling	0	1.93	1.86
		3	2.28	2.21
		6	2.64	2.35
		9	3.05	2.61
	Irrigation cut-off at 50% flowering	0	1.74	1.58
		3	2.16	1.89
		6	2.40	2.08
		9	2.77	2.30
Iranshahr	Control (Full irrigation)	0	1.97	2.29
		3	4.26	3.43
		6	5.45	3.98
		9	7.97	4.92
	Irrigation cut-off at 50% seed filling	0	1.60	1.63
		3	2.56	2.17
		6	3.08	3.29
		9	5.21	3.48
	Irrigation cut-off at 50% flowering	0	0.91	0.73
		3	1.44	1.29
		6	1.64	2.07
		9	3.74	2.83
LSD 5%			0.1382	0.0843

According to Table 8, the chlorophyll b content of Halil and Dashtestan cultivars was higher in Iranshahr than in Zabol. Overall, the Dashtestan cultivar had higher chlorophyll b levels at the two test locations. According to Table 9, the amount of chlorophyll b in the sesame cultivars decreased with increasing drought stress intensity, but among the cultivars, Dashtestan showed higher levels of chlorophyll b under all irrigation conditions. Based on Table 10, the amount of chlorophyll b increased with increasing the amount of zeolite in all sesame cultivars. Dashtestan and Halil cultivars showed higher chlorophyll b content than Darab at all zeolite levels.

Carotenoids

Carotenoids were significantly affected by location, drought, zeolite, cultivar, and several interactions (Table 4). The highest carotenoid content (4.92 mg/g fresh weight) was found in Iranshahr with the application of 9 tons/hectare of zeolite under non-stress conditions (Table 7). Also, the highest carotenoid content (4.98 mg/g fresh weight) was obtained from the Dashtestan cultivar in the Iranshahr location under no drought stress conditions (Table 6). In general, the Dashtestan cultivar showed higher carotenoid content than other sesame cultivars, especially in Iranshahr. Also, according to the interaction of location × zeolite × cultivar (Table 11), the highest carotenoid content (3.08 mg/g fresh weight) was obtained from the I ranshahr location, application of 9 tons/hectare of

Table 8. Comparison of the sesame cultivars in Zabol and Iranshahr locations in terms of chlorophyll b.

Location	**Zabol**			**Iranshahr**		
Cultivar	**Halil**	**Dashtestan**	**Darab**	**Halil**	**Dashtestan**	**Darab**
Chlorophyll b (mg/g FW)	2.16	2.77	2.54	2.94	4.2	1.96
LSD 5%	0.0691					

Table 9. Comparison of the sesame cultivars in different irrigation regimes in terms of chlorophyll b.

Drought stress	**Control (Full irrigation)**			**Irrigation cut-off at 50% seed filling**			**Irrigation cut-off at 50% flowering**		
Cultivars	**Halil**	**Dashtestan**	**Darab**	**Halil**	**Dashtestan**	**Darab**	**Halil**	**Dashtestan**	**Darab**
Chlorophyll b (mg/g FW)	3.46	4.65	2.94	2.39	3.28	2.24	1.78	2.53	1.57
LSD 5%	0.0847								

Table 10. Comparison of the sesame cultivars at different zeolite levels in terms of chlorophyll b.

Cultivar	**Halil**				**Dashtestan**				**Darab**			
Zeolite (t/ha)	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**	**0**	**3**	**6**	**9**
Chlorophyll b (mg/g FW)	1.81	2.62	3.28	4.39	1.94	2.59	3.26	4.38	1.65	2.39	2.85	3.66
LSD 5%	0.0978											

zeolite, and the Darab; however, this amount was not significantly different from that of the Dashtestan cultivar.

The amount of chlorophyll in living plants is one of the important factors in maintaining photosynthetic capacity. In this study, the amount of chlorophyll a, b, and carotenoids decreased due to drought stress. It seems that the decrease in chlorophyll content due to drought stress is due to the increased production of oxygen radicals, which cause peroxidation (Wise and Naylor 1989). As as a result, it leads to the breakdown of photosynthetic pigments (Schutz and Fangmeir 2001). It can also be stated that the decrease in chlorophyll content under drought stress conditions may be due to the increase in chlorophyllase activity (Gross 1991). In general, leaf chlorophyll is highly dependent on chloroplasts, and factors that cause damage to the chloroplast membrane contribute to the reduction of chlorophyll levels (Lawlor and Cornic 2002).

Zeolite increases water availability and results in the improvement of plant growth. Zeolite also stimulates the synthesis of chlorophylls and carotenoids (Mohamad Naseri and Tadayon 2024). In our experiment, zeolite had a positive effect on chlorophyll and carotenoid content under lower water-deficit stress (irrigation cut-off at 50% seed filling), in line with the findings of Gholam Hosseini *et al*. (2013) and Shirani Rad and Eyni-Nargeseh (2024). On the other hand, under drought stress and subsequent use of zeolite to adjust the stress, the photosynthetic pigments (chlorophylls) and auxiliary pigments (carotenoids) were increased. Carotenoids not only are essential to photosynthesis, but also have antioxidant properties under drought-stress conditions (Hazrati *et al.* 2016).

The higher chlorophyll a and carotenoid content in Iranshahr could be due to better climatic and soil conditions (Tables 1 and 2) during the plant growth period compared to Zabol, which may have led to greater plant access to water and soil nutrients.

Soluble carbohydrates

According to the results of the combined ANOVA, the effect of location, drought stress, zeolite, cultivar, and the interaction of location × drought stress × zeolite × cultivar, and several other interactions on soluble carbohydrates were significant (Table 4). The highest soluble carbohydrates (75.41, 72.5, and 71.07 mg/g fresh weight) were observed in Zabol for the Halil, Darab, and Dashtestan cultivars, respectively, under irrigation cut-off at 50% flowering with no zeolite application (Table 12). Under stress conditions of irrigation cut-off at 50% flowering, total soluble carbohydrates significantly increased compared to the non-stress conditions. Under drought stress conditions, photosynthesis decreases as the amount of available water decreases, and consequently, plant dry matter production also decreases. The decrease in sucrose in leaves under drought stress

Table 11. Means for sesame carotenoids under the influence of cultivars and zeolite rates in two locations.

Location	Zeolite (t/ha)	Cultivar	Carotenoids (mg/g FW)
Zabol	0	Halil	1.37
		Dashtestan	1.80
		Darab	1.67
	3	Halil	2.08
		Dashtestan	2.14
		Darab	2.25
	6	Halil	2.28
		Dashtestan	2.54
		Darab	2.50
	9	Halil	2.72
		Dashtestan	2.71
		Darab	2.97
Iranshahr	0	Halil	2.40
		Dashtestan	2.39
		Darab	2.30
	3	Halil	2.30
		Dashtestan	2.39
		Darab	2.29
	6	Halil	2.40
		Dashtestan	2.57
		Darab	2.56
	9	Halil	2.62
		Dashtestan	2.66
		Darab	3.08
LSD 5%			0.076

conditions indicates that the rate of sucrose synthesis has decreased, and its distribution has also been disrupted. However, these events depend on the plant species, duration of stress, developmental stage of drought-stress application, and stress intensity (Farooq *et al*. 2009). Under drought stress

Table 12. Means for effects of location × stress × zeolite × cultivar combination on some sesame traits.

Location	Drought stress	Zeolite (ton/ha)	Cultivar	Soluble Car (mg/g wet weight)	Proline (mg/g wet weight)	Protein (mg/g)	Grain yield (t /ha)	Biomass (t/ha)	Oil percent
Zabol	Control (Full irrigation)	0	Halil	21.78	0.351	0.6	703.3	2610	30.76
		0	Dashtestan	22.45	0.43	0.6	713.3	2753	29.3
		0	Darab	23.3	0.515	0.611	999.3	3036	28.5
		3	Halil	17.85	0.25	0.633	812.7	2926	35.73
		3	Dashtestan	25.84	0.27	0.658	820.0	2993	35.06
		3	Darab	21.53	0.311	0.667	1144.9	3500	34.52
		6	Halil	16.86	0.205	0.743	991.0	3396	37.65
		6	Dashtestan	17.28	0.243	0.757	1026.7	3556	36.71
		6	Darab	17.96	0.248	0.773	1202.6	3763	36.39
		9	Halil	14.72	0.2	0.829	1073.6	3736	44.8
		9	Dashtestan	15.21	0.203	0.866	1087.0	3756	40.32
		9	Darab	16.57	0.23	0.905	1316.0	3926	41.32
	Irrigation cut-off at 50% seed filling stage	0	Halil	44.29	0.37	0.515	604.6	2410	28.41
		0	Dashtestan	45.26	0.488	0.53	616.9	2493	27.3
		0	Darab	46.43	0.567	0.54	866.2	2866	27.07
		3	Halil	41.57	0.282	0.639	704.3	2616	32.43
		3	Dashtestan	43.47	0.329	0.621	721.3	2670	31.81
		3	Darab	44.17	0.41	0.636	962.7	2953	31.17
		6	Halil	24.66	0.265	0.703	753.3	2890	34.24
		6	Dashtestan	27.8	0.303	0.712	865.7	3121	33.67
		6	Darab	28.81	0.343	0.722	1089.3	3436	32.23
		9	Halil	18.52	0.222	0.811	965.0	3106	38.47
		9	Dashtestan	19.76	0.258	0.818	966.7	3208	36.84
		9	Darab	22.33	0.321	0.857	1204.0	3673	37.54
	Irrigation cut-off at 50% flowering stage	0	Halil	75.41	0.461	0.461	507.8	2146	25.06
		0	Dashtestan	71.07	0.657	0.472	510.4	2120	23.23
		0	Darab	72.5	0.848	0.48	729.3	2500	22.07
		3	Halil	60.3	0.374	0.479	655.3	2276	28.83
		3	Dashtestan	64.98	0.53	0.492	658.0	2456	25.28
		3	Darab	65.77	0.611	0.499	896.7	2703	26.54
		6	Halil	40.93	0.324	0.583	657.0	2540	32.37
		6	Dashtestan	43.03	0.436	0.592	673.3	2633	31.52
		6	Darab	44.74	0.486	0.594	989.0	3113	31.54
		9	Halil	29.27	0.327	0.65	840.3	2750	35.55
		9	Dashtestan	36.79	0.366	0.685	861.3	2856	33.98
		9	Darab	38.25	0.405	0.717	1202.0	3463	34.21
Iranshahr	Control (Full irrigation)	0	Halil	47.04	0.124	0.879	1219.0	2846	37.31
		0	Dashtestan	45.92	0.113	0.734	933.3	2563	37.84
		0	Darab	34.49	0.106	0.767	923.3	2420	39.57
		3	Halil	22.75	0.105	0.992	1365.0	3310	43.34
		3	Dashtestan	21.53	0.093	0.915	1040.0	2803	43.87
		3	Darab	21.31	0.086	0.899	1031.3	2736	44.55
		6	Halil	12.6	0.092	1.108	1422.0	3573	45.2
		6	Dashtestan	11.89	0.079	0.982	1246.0	3366	45.52
		6	Darab	10.7	0.064	0.986	1211.0	3207	46.46
		9	Halil	8.29	0.051	1.123	1536.0	3736	50.13
		9	Dashtestan	7.57	0.043	1.0805	1307.0	3566	49.13
		9	Darab	7.28	0.038	1.097	1293.0	3546	53.62
	Irrigation cut-off at 50% seed filling	0	Halil	57.91	0.174	0.712	1086.2	2676	35.89
		0	Dashtestan	56.79	0.157	0.638	836.9	2303	36.11
		0	Darab	51.72	0.146	0.664	824.6	2220	37.22
		3	Halil	39.77	0.148	0.955	1182.6	2763	39.99
		3	Dashtestan	39.69	0.153	0.694	941.3	2480	40.62
		3	Darab	35.17	0.122	0.687	924.3	2426	41.24
		6	Halil	26.11	0.12	0.999	1309.3	3246	41.4
		6	Dashtestan	23.82	0.112	0.775	1085.7	2931	42.48
		6	Darab	23.71	0.099	0.77	973.3	2700	43.05

	9	Halil	19.69	0.093	0.977	1424.0	3483	46.36
	9	Dashtestan	17.16	0.079	0.877	1186.6	3018	45.66
	9	Darab	16.64	0.057	0.865	1185.0	2916	47.29
Irrigation cut-off at 50% flowering	0	Halil	63.8	0.215	0.521	949.3	2310	30.88
	0	Dashtestan	59.96	0.205	0.482	730.4	1930	32.04
	0	Darab	54.74	0.185	0.382	727.8	1956	33.87
	3	Halil	54.89	0.197	0.81	1116.6	2513	35.36
	3	Dashtestan	54.64	0.174	0.673	878.0	2266	34.1
	3	Darab	52.69	0.162	0.659	875.3	2086	37.65
	6	Halil	37.99	0.158	0.866	1209.0	2923	40.35
	6	Dashtestan	36.5	0.15	0.71	893.3	2443	40.34
	6	Darab	32.18	0.142	0.718	877.0	2350	41.19
	9	Halil	27.04	0.123	0.943	1422.0	3273	43.03
	9	Dashtestan	24.8	0.111	0.779	1081.3	2666	42.79
	9	Darab	23.9	0.093	0.797	1060.3	2560	44.38
L.S.D(%5)			2.6230	0.0132	0.0346	44.72	60.00	0.8198

Soluble Car: Soluble carbohydrates

conditions, increasing water-soluble carbohydrates maintains leaf cell turgor, protects cell membranes, and inhibits protein degradation; it also prevents the plant from certain death by providing it with the energy it needs (Xue *et al*. 2008). In such conditions, zeolites may help maintain water reserves in the soil, reduce the effects of drought stress, and reduce carbohydrate accumulation.

Proline

The effect of location, drought stress, zeolite, cultivar, and the interaction of location × drought stress × zeolite × cultivar, and several other interactions on proline were significant (Table 4). The highest proline (0.848 and 0.657 mg/g fresh weight) was recorded under severe drought in the Darab and Dashtestan cultivars with no zeolite application in the Zabol location (Table 12). Overall, with increasing drought severity, leaf proline increased in both locations, although zeolite application reduced the drought stress effects. The higher proline levels in Zabol compared to Iranshahr may be attributed to growth timing and regional planting cycles. The results indicate differences among cultivars in their ability to accumulate proline under stress. Proline accumulation improves the tolerance of crops to water-deficit conditions through osmotic regulation (Forlani *et al*. 2019). Under drought stress conditions, proline plays a crucial role in eliminating free radicals and reactive oxygen species, maintaining osmotic potential and stability of proteins and cell membranes against oxidative damage, protecting macromolecules from denaturation, and stabilizing cellular pH (Forlani *et al*. 2019). Additionally, proline serves as a nitrogen and carbon source for plants under severe stress, enhancing their stress tolerance (Amini *et al*. 2015).

Regarding the increase in proline under drought stress conditions, it has been stated that its accumulation is mainly due to the increased de novo synthesis rather than reduced catabolism or elevated protein degradation (Hildebrandt 2018; Forlani *et al*. 2019). It has been proposed that starch degradation plays a role in proline synthesis under drought-stress conditions (Zanella *et al*. 2016). In the experiment of Sarvari *et al*. (2017), the amount of proline in resistant sunflower varieties increased under water deficit stress conditions.

Seed protein content

The combined ANOVA (Table 4) revealed significant effects of location, drought stress, zeolite, cultivar, and the 4-way interaction of location × drought stress × zeolite × cultivar on protein content. The Halil cultivar produced the highest protein content (1.123 and 1.108 mg/g) with the application of 9 and 6 tons per hectare of zeolite, respectively, at Iranshahr, under full irrigation conditions, followed by Darab and Dashtestan (1.097 and 1.081, respectively) under full irrigation conditions, with the application of 9 tons per hectare of zeolite (Table 12). These findings suggest the existence of genetic variation among cultivars and responses to agronomic practices such as zeolite application and irrigation levels on protein content. Researchers have reported that varietal differences in protein concentration relate to the nitrogen conversion efficiency into amino acids and proteins (Ahmad *et al*. 2004). Additionally, research has shown that zeolite application in wheat increases protein content (Tsadilas and Argyropoulos 2010). In general, under the 50% irrigation cut-off during flowering, protein levels decreased compared to full irrigation. Overall, full irrigation resulted in improved seed protein. In an experiment on sesame in Birjand, Iran, with two levels of normal irrigation and deficit irrigation and the use of zeolite and super-absorbent, it was observed that seed protein content was reduced under deficit irrigation compared to normal irrigation; however, the use of zeolite and super-absorbent separately or together increased the amount of seed protein at both irrigation levels (KhasheiSiuki *et al*. 2023). Contrary to our results, Amini *et al*. (2015) stated that under water-deficit stress, reduced seed size results in higher protein density compared to non-stress conditions, with water stress increasing seed protein content.

Grain yield

The combined ANOVA (Table 4) showed significant effects of location, stress, zeolite, and the 4-way interaction of location × drought stress × zeolite × cultivar on grain yield at 1% probability level. The highest grain yield (1530, 1424, and 1422 kg per hectare) was recorded for the Halil cultivar in Iranshahr under full irrigation, irrigation cut-off at 50% seed filling, and irrigation cut-off at 50% flowering, respectively, with the application of 9 tons of zeolite per hectare. However, in Zabol, the highest yield was achieved by the Darab cultivar at all irrigation conditions, with the application of 9 tons of zeolite (Table 12). Generally, grain yield decreased with increased drought stress, but at each stress level, higher zeolite application mitigated drought effects and increased yield. Comparing yields between Iranshahr and Zabol, Iranshahr showed a higher yield, likely due to planting timing and the sesame plant's (C3 photosynthetic type) growth phases coinciding with cooler temperatures than in Zabol (Table 1). Other reasons for the decrease in yield in the Zabol location compared to the Iranshahr location include the higher soil acidity (pH) and electrical conductivity of soil (EC) in the Zabol location (Table 2). Also, the reduction of pigments, especially chlorophyll a, at the drought-stress levels (Tables 5 and 6) causes a decrease in sunlight absorption and photosynthesis, which leads to a decrease in yield.

In an experiment studying the effect of drought stress and zeolite on camelina (*Camelina sativa* L.), it was found that increasing the application of zeolite under stress and non-stress conditions produced higher grain yield (Shirani Rad and Eyni-Nargeseh 2024). Drought stress during reproductive phases is more devastating, and drought at the flowering stage generally leads to barrenness. Drought stress can reduce plant yield by closing stomata, reducing CO2 intake, lowering relative leaf water content, and limiting photosynthesis (Li *et al*. 2021). Thus, impacts plant growth and productivity significantly (Zandalinas *et al*. 2016). Reproductive stages, particularly flowering, are very sensitive to drought stress, as it disrupts pollination, reduces pollen viability and ovule function, and slows pollen growth (Devi *et al*. 2022). Drought stress also reduces leaf area, causes premature leaf senescence, limits the source through reduced photosynthetic material transfer due to decreased turgor potential, and shortens the reproductive phase, while also increasing embryo abortion and reducing pod size and number, which collectively diminish final yield (Din *et al*. 2011). Increased grain yield under zeolite application may be due to reduced stress impact, with zeolite's positive effects on soil moisture retention and nutrient availability being a key factor (Polat *et al*. 2004).

Biomass

Combined ANOVA indicated significant effects of location, drought stress, zeolite, cultivar, and the location × drought stress × zeolite × cultivar interaction on biomass at the 1% probability level (Table 4). The highest biomass (3920 kg per hectare) was observed for the Darab cultivar in Zabol under full irrigation, with the application of 9 tons of zeolite per hectare (Table 12). Also, Darab produced the highest biomass in Zabol under both drought-stress conditions. Plant growth depends on the availability of water. Water-deficit stress reduces water absorption, lowering plant green coverage, reducing sunlight absorption, and subsequently decreasing photosynthesis, resulting in less dry matter and lower biomass. Under drought stress, early senescence of photosynthetic organs and the reduction of photosynthesis, decreases biomass (Angadi and Entz 2002). Other studies have reported a significant reduction in biomass under drought stress, likely due to decreased leaf area index, reduced crop growth rate, and consequently lower dry matter accumulation (Sanjari Pireivatlou *et al*. 2010). Reduction in biomass under water-deficit stress conditions can be attributed to the reduction in cell division and elongation, and the reduction in translocation of assimilates (Sehgal *et al*. 2018). On the other hand, zeolite absorbs and retains water and nutrients for a long time, which results in an increase in biomass of plants under water-deficit-stress conditions (Hazrati *et al*. 2022). Pourghasemian *et al*. (2020) stated that the disruption in growth of sesame under water-deficit stress is due to the decrease in stomata conductivity, chlorophyll pigments, and photosynthesis. In an experiment on safflower, it was found that increasing water-deficit stress reduces the amount of plant biomass (Farajzadeh-Memari-Tabrizi and Babashpour-Asl 2022).

In this study, applying nine tons of zeolite per hectare improved water retention, released moisture gradually, and reduced plant damage severity. Zeolite's positive impact on soil moisture and nutrient retention is a major factor in increased yield, aligning with results from other studies (Gholamhoseini *et al*. 2013; Shirani Rad and Eyni-Nargeseh 2024).

Oil content

Analysis of combined data (Table 4) revealed significant effects of location, stress, zeolite, and the location × drought stress × zeolite × cultivar interaction on sesame oil content at the 1% probability level. The highest oil content (53.62%) was observed in Iranshahr, under no drought stress, with 9 tons of zeolite per hectare, and for the Darab cultivar (Table 12). In general, all three cultivars, Darab, Dashtestan, and Halil, produced the highest oil content in both locations under all irrigation conditions with the application of 9 tons of zeolite per hectare. Drought stress reduced sesame oil content, possibly due to the disruption in metabolic processes in seeds and impaired assimilate transfer (Figueiredo *et al*. 2008; Eyni Nargeseh *et al*. 2020; Mostafa *et al*. 2021). Also, the reason for the decrease in seed oil content under drought-stress conditions has been attributed to increased oxidation of fatty acids (Singh and Sinha 2005). In addition, drought stress reduces the capacity of seeds to absorb photosynthetic materials and convert them into oil (Elferjani and Soolanayakanahally 2018). Sangtarash *et al*. (2009) and Bijani *et al*. (2023) also noted reduced oil content with the irrigation cut during flowering.

Zeolites can absorb water up to 70% of their volume due to their high porosity and crystalline structure, enhancing soil permeability and moisture retention, even under steep slopes (Conversa *et al*. 2024). Consequently, zeolite application reduces drought-stress effects, thus increasing the sesame oil content. Additionally, zeolite improves water and nutrient absorption and reduces leaching of nitrogen and phosphorus, enhancing soil quality and nutrient availability (Gholamhoseini *et al*. 2013; Motaghi *et al*. 2014 Shirani; Rad and Eyni-Nargeseh 2024).

Conclusion

The results of this study indicated that the time of drought stress plays a significant role in the accumulation of soluble carbohydrates and proline. Applying nine tons of zeolite per hectare, due to its water retention and nutrient-availability enhancing properties, mitigated the adverse effects of water-deficit stress on sesame, and so that grain yield showed a smaller decrease compared to other rates of zeolite application. Zeolite application seems to be a promising method to reduce water-deficiency impacts on crop production, particularly the sesame crop. Grain yield results differed between Iranshahr and Zabol, with the highest yields in Iranshahr for the Halil cultivar under all irrigation conditions, with the application of nine tons of zeolite per hectare. However, in Zabol, the Darab cultivar produced the highest grain yield under similar conditions. Therefore, Darab may be suggested for the sesame production in Zabol, while Halil seems more suitable for Iranshahr.

Funding

This research was partially supported by a research grant (IR-UOZ-GR-2904) provided by the University of Zabol, Iran.

Conflict of Interest

The authors declare that they have no conflict of interest with any organization concerning the subject of the manuscript.

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