Morpho-physiological responses of cumin (*Cuminum cyminum* L.) to the application of growth regulators under drought stress

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Abstract

A field experiment was conducted as split plots based on a randomized complete block design with three replications to assess the responses of cumin (*Cuminum cyminum* L.) to foliar fertilization with growth regulators under drought stress. The study factors included three levels of irrigation regime according to the evapotranspiration percentage of reference plant (supply of 100%, 70%, and 40% water requirement of the plant (WR) as the main plot and 5 foliar treatments with salicylic acid (SA), jasmonic acid (JA), paclobutrazol (PBZ), chitosan (CS), and control (no foliar nutrition).Based on the results, drought stress negatively affected photosynthetic pigments, yield, and yield components while morpho-physiological traits improved under both drought stress and normal conditions following the foliar fertilization. Moreover, foliar nutrition, especially of JA, PBZ, and SA, resulted in synthesizing more proline and photosynthetic pigments, increasing antioxidant enzyme activity, and consequently improving yield components. The most positive effect of increasing chlorophyll content was related to the use of JA, which resulted in 27.9%, 29.4%, and 28.4% increase in chlorophyll a, b, and total, respectively. The highest SOD activity (3.89 U/mg protein) was related to the non-foliar nutrition under non-stress treatment. Additionally, SA, JA, and CS applications under severe drought stress showed the highest SOD activity. The effects of SA and JA were superior on RWC. PBZ application under non-stress conditions showed the highest seed yield (698.9 kg/ha). The lowest seed yield (103.4 kg/ha) was related to non-foliar nutrition under severe drought stress. The highest essential oil content (2.4%) was related to the JA application under severe drought stress (40% WR).

Keywords: antioxidant enzymes, drought stress, growth regulator, paclobutrazol, photosynthetic pigment

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Introduction

Cumin (*Cuminum cyminum* L.) is an herb belonging to the Apiaceae family with culinary and medicinal applications. Beside their application as seasoning and food flavor, cumin seeds with their phenolic compounds and essential oils have been widely used as medicine for treating toothache, diarrhea,

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epilepsy, jaundice, and dyspepsia (Alinian et al., 2016; Archangi et al., 2019). Also, the vegetative organs of cumin are a source of natural antioxidants such as anthocyanin, flavonoids, and phenolic compounds (Razmjoo and Alinian, 2017).

Cumin is cultivated in Asia, Middle East, and North Africa, and India and Iran are the two main producers and exporters of cumin seeds and products (Archangi et al., 2019). Cumin cultivation area in Iran is around 0.04 million hectares with an average seed yield of 500–1500 kg/ha in the rain-fed and irrigated conditions, respectively (Ghasemi Pirbalouti, 2010; Archangi et al., 2019).

Production, survival, and distribution of plants are limited by drought as it suppresses plant growth and development, thereby reducing average yield by as much as 50% in most crops (Bayati et al., 2020). Low rainfall, climate change, agricultural malpractices, and increasing population are responsible for the world-wide water shortage that has adversely affected crops' morpho-physiological and growth characteristics (Zarrinabadi et al., 2019; Bayati et al., 2020). Cultivation of medicinal and aromatic plant species is of high economic value, particularly in drought conditions where the concentration and quality of essential oils and secondary metabolites are enhanced in these plants. One approach for mitigating the harmful effects of drought on crop growth is using foliar spraying with growth regulators and micronutrients to increase plant tolerance to water shortage (Shahverdi et al., 2020).

Paclobutrazol (PBZ) is a member of the triazole family with growth-regulating properties (Afshari et al., 2020). PBZ improves membrane stability index and plant water relation, enhances plant photosynthetic pigments, induces antioxidant activities, changes the level of plant growth hormones, and increases the level of proline in plants (Soumya et al*.,* 2017; Afshari et al., 2020).

Jasmonic acid (JA) is a phytohormone considered as a key regulator of various growth processes in plants (Thakur and Kumar, 2020). JA regulates plants' physiological and biochemical processes and thereby enhances their responses to many biotic and abiotic stresses (Sheteiwy et al., 2020). There are many reports on the positive effects of exogenous application of phytohormones such as IJ on crops (Sheteiwy et al., 2020). Foliar spray of JA on different plants reduced the harmful effects of salt stress and improved plant growth and yield through scavenging of ROS by antioxidant enzymes and ion absorption (Kang et al., 2005; Qiu et al., 2014). JA at low concentrations was reported to enhance plant tolerance to abiotic stresses, including salt, osmotic stress, and low temperature (Cheong and Do Choi, 2003; Sheteiwy et al., 2020), and drought (Alam et al., 2014). Its application also improves pest protection responses in plants.

Chitosan is a biodegradable compound with important biological and physiological properties. Studies show that chitosan stimulates growth indexes in plants (Shams Peykani and Farzami Sepehr, 2018; Zayed et al., 2017; Mandal, 2010; Lee et al., 2005) improving their tolerance against various biotic as well as abiotic stresses.

A natural growth stimulant, salicylic acid is also known for its positive effects on various physiological and biochemical processes in plants. Bulk of studies point to the key role SA plays in improving the plants' tolerance to various stressors such as drought, heat, heavy metals, and osmotic pressure (Habibi, 2012; Liu et al., 2011; Kadioglu et al., 2011; Wang et al., 2010; Hayat et al., 2008).

An important strategy to mitigate the adverse effects of abiotic stresses in plants and improve their overall performance is applying biologic stimulants. As an aromatic plant, cumin is used for spice and contains valuable essential oils. While this important medicinal and industrial plant is adapted to dry conditions, there is limited research on the effects of JA, PBZ, chitosan (CS), and salicylic acid (SA) on morpho-physiological and yield attributes of cumin under drought stress conditions. The present study was an attempt to investigate the responses of cumin (*Cuminum cyminum* L.) to foliar application of JA, PBZ, CS, and SA under drought stress conditions.

Materials and Methods

Experimental design and treatments

Table 1
Climatic characteristics of the region in the year of the experiment

Months	Average Temperature (℃)	Average Rainfall (mm)	Average Relative Humidity (%)	Average Wind Speed (km/h)
December	12	7.4	52	5.8
January	9.2	27.1	59	7
February	11.9	15.9	51	8.6
March	16.9	0	35	11.4
April	19.9	23.7	50	7.8
May	27.9	23.3	34	3.9

Table 2
Results of physical and chemical analysis of soil at the test site

Soil Texture	Nitrogen (mg/kg)	Phosphorous (mg/kg)	Potassium (mg/kg)	Sodium (mg/L)	Magnesium (mg/L)	Calcium (mg/L)	Organic Carbon (%)	pH	EC (dS/m)	Sampling Depth (cm)
sandy-loam	0.17	9.2	125	6.8	9.4	2.6	0.44	7.9	1.20	0-30

The experiment was conducted in Sistan Agricultural and Natural Resources Research and Education Center (31˚40´N latitude, 61˚54´E longitude, and 496 m above sea level altitude) during 2019-2020. The average annual rainfall, evaporation rate, and annual temperature are 55 mm, between 4500 and 5000 mm, and 21.7 °C, respectively. According to the Gaussian climatic classification, Sistan has a desert climate with dry and hot weather particularly in summers. The meteorological status of the study period is reported in Table 1.

To determine the physical and chemical properties of the soil, sampling was performed before sowing operations from a depth of 0-30 cm from several points of the field, and after preparing a composite sample separately and sending it to the laboratory, the results were obtained as shown in Table 2.

This experiment was performed as split plots based on randomized complete block design (RCBD) with three replications. The studied factors included irrigation regime in three levels according to the evapotranspiration percentage of the reference plant (supply of 100% as control, 70%, and 40% water requirement of the plant) as the main plot and foliar treatment consisting of 5 levels (control, i.e. no foliar application, foliar application with SA, JA, PBZ, and CS) were assigned to subplots.

The dimensions of each plot were 4 × 2 m and 1 m distance was considered between the treatments to prevent the irrigation water from mixing with adjacent plots. Each main plot was set at a distance of 1.5 m from the others with six planting lines with a length of four m and planting line distances of 20 cm. The total number of experimental plots were 45.

In The beginning of the October, plowing was carried out, and then in late November, the soil was softened with disk twice. The plot borders of were prepared by the labor force to implement the experiment. Pre-planting fertilizer was added to the soil at the same time according to the soil analysis.

The cumin seeds (Birjand native seed cultivar) were prepared from Sistan Zabol Seed Company (Registration Number: 1974). The growth regulators used (SA, JA, PBZ, and CS) were obtained from SIGMA-ALDRICH, Germany.

After preparing the farm, cumin seeds were planted on December 11, 2019. The seeds were primed by soaking in water for about 12 h before sowing. Irrigation was performed once every seven days based on water requirements and recommended phenological and growth stages of the cumin plant. The seeds germinated in the field after 20 to 25 days in late December.

Irrigation treatments were applied at 3-4 leaf stage at intervals of 10 days. The required volume of water was provided in each plot according to the irrigation regimes.

Determining the water requirements of a plant is an important issue in determining the time and amount of irrigation (Howell et al., 2008; Piccinni et al., 2009). Water requirement was calculated using FAO method and evaporation statistics from Class A pan using Equations 1 and 2. Then, considering the efficiency of 80% for water distribution in the field, irrigation was performed (Howell et al., 2008).

$$ET_0 = K_{pan} \times E_p \qquad (1)$$

$$ET_c = K_c \times ET_0 \qquad (2)$$

where ET_0, K_{pan}, and E_p are evaporation and transpiration of the reference plant, pan coefficient, and evaporation from the pan, respectively (Piccinni et al., 2009). Kc was 0.65 at the beginning of growth, 0.92 at the development stage, 1.21 at the middle stage, and 0.85 at the end of the growth period (Reyhani and Khashei Siuki, 2015).

Concentrations of growth regulators included salicylic acid (300 mg/L), jasmonic acid (60 µmol/L), paclobutrazol (100 mg/L), and chitosan (50 mg/L) based on the laboratory experiments reported in the literature (Hasanuzzaman et al., 2019; Sheteiwy et al., 2020; Afshari et al., 2020; Afshari et al., 2022). During the growing season, the first and the second rounds of foliar spray were done at a distance before applying the drought stress and at the beginning of the plants' reproductive stage as per the plan for each randomly designated plot. During foliar application, the entire leaf surface was completely soaked with the designated solution. The control plants were sprayed with distilled water.

Morphological and yield attributes

The cumin harvest was done when the bushes turned yellow, and the umbrellas turned brown at the end of the growing season. After physiological maturity and just before full maturity, plants in lines with a length of 4 m equal to 1.2 m^2 were harvested keeping aside the plants in marginal lines. The samples were then placed in plastic bags and transferred to the laboratory to determine the growth and yield attributes.

Physiological parameters measurements

In the end of the experiment period, leaf samples from each plot were collected and immediately immersed in liquid nitrogen to freeze before they were stored in an ultra-low freezer at –80 ℃ for physiological analysis. For photosynthetic pigments measurement, the extract prepared with fresh leaf tissue (0.25 g) and 80% acetone (5 ml) was centrifuged at 11000 rpm for 10 min. The extract was then submitted to photometry and the optical density of the extract was recorded at 646.8, 663.2, and 470 nm wavelengths (Aghighi Shahverdi et al., 2017; Shahverdi et al., 2019; Afshari et al., 2020). Chlorophyll a, b, total, and also carotenoid contents were calculated using the following equations:

$$\text{Chlorophyll a } (\mu\text{g/g FW}) = 12.7\ (\text{O.D of } 663.2) - 2.69\ (\text{O.D of } 646.4) \times \frac{v}{w \times 1000} \qquad \text{(Eq. 1)}$$

$$\text{Chlorophyll b } (\mu\text{g/g FW}) = 22.9\ (\text{O.D of } 646.8) - 4.68\ (\text{O.D of } 663.2) \times \frac{v}{w \times 1000} \qquad \text{(Eq. 2)}$$

$$\text{Total chlorophyll } (\mu\text{g/g FW}) = 20.2\ (\text{O.D of } 646.8) + 8.02\ (\text{O.D of } 663.2) \times \frac{v}{w \times 1000} \qquad \text{(Eq. 3)}$$

$$\text{Carotenids } (\mu\text{g/g FW}) = 46.95\ (\text{O.D of } 440.6 - 0.268\ \times Chl\ a + Chl\ b) \qquad \text{(Eq. 4)}$$

Where W denotes the fresh weight by grams for extracted tissue, V represents the final size of the extract in 80% acetone, and O.D is the optical density at a specific wavelength.

Peroxidase (POD) activity was assayed based on the method explained by MacAdam et al. (1992). First, 0.2 g of fresh leaf was pulverized in a mortar with liquid nitrogen to which 1 ml of 0.05 M buffer Tris-HCl (pH = 7.5) was added. Then, the mixture was centrifuged at 13,000 rpm for 21 min and under 4 °C. The supernatant was submitted to photometry analysis and the enzyme activity was assayed in a reaction mixture consisting 28 mM

Table 3
Effects of different levels of irrigation regimes and foliar application of JA, SA, PBZ, and CS on photosynthetic pigments and enzymatic activities of cumin (*Cuminum cyminum* L.)

Treatments	Ch. a (µg/g FW)	Chl. b (µg/g FW)	Total Ch. (µg/g FW)	Carotenoids (µg/g FW)	CAT (U/mg protein.min)	POD (U/mg protein.min)	SOD (U/mg protein)
Irrigation regimes (% of WR)							
100% (control)	18.05+1.94 a	8.56+1.05 a	26.6+2.37 a	4.16+0.52 a	1.01+0.62 b	2.24+0.9 b	1.97+1.16 b
70%	16.42+2.64 a	7.0+1.5 b	23.4+3.78 b	3.09+0.97 b	1.26+0.33 b	3.76+0.9 a	2.02+0.68 b
40%	11.94+3.38 b	5.06+1.25 c	16.9+4.24 c	2.82+0.73 b	1.56+0.52 a	4.11+1.0 a	3.07+0.82 a
LSD ($p\le0.05$)	1.75	0.81	2.05	0.69	0.26	0.60	0.42
Foliar application							
Control	12.47+3.53 b	5.67+2.23 c	18.14+5.5 c	3.03+1.46 a	0.99+0.48 c	3.06+0.8ab	2.55+1.13a
SA	15.06+3.66 a	6.78+1.38b	21.84+4.7b	3.3+0.78 a	1.46+0.41 ab	3.56+0.8ab	2.49+0.76a
JA	17.31+2.52 a	8.04+1.5 a	25.35+3.6 a	3.67+0.83 a	1.61+0.77 a	3.85+1.7 a	2.21+1.29a
PBZ	17.08+3.49 a	7.13+1.8 ab	24.2+4.8 ab	3.33+0.81 a	1.24+0.39 bc	3.41+1.4ab	2.12+0.75a
CS	15.43+3.88 a	6.74+2.12 b	22.17+5.7 b	3.46+0.78 a	1.09+0.45 c	2.95+1.2b	2.39+1.24a
LSD ($p\le0.05$)	2.26	1.05	2.64	NS(0.89)	034	0.78	NS(0.54)
Irrigation regimes × foliar application							
	NS	NS	NS	NS	**	*	**

Means followed by the same letter in each column are not significantly different according to LSD test at 5 % level. NS: not significant; * and **: significant at α=0.05 and 0.01%.

guaiacol, 5 mM H_2O_2, 25 mM Na-phosphate buffer (pH 6.8).

Catalase (CAT) activity was assayed spectrophotometrically using the method described by Chance (1955). A reaction mixture (3 ml) was prepared by adding protein solution (30 µg) to 0.05 mM sodium phosphate buffer (2.5 ml, pH = 7); then, 30% H_2O_2 (30 µl) was added and the absorbance of the reaction mixture was recorded at 240 nm after 60 seconds under 25 ℃ and was recorded. A mixture of sodium phosphate buffer (2.5 ml) and protein (30 µg) served as the control. CAT activity was recorded in terms of alternations in the absorption per mg protein per min.

Superoxide dismutase (SOD) activity was assayed following Beauchamp and Fridovich (1971). A reaction mixture (3 ml) was prepared with enzyme extraction (0.05 ml), 200 mM methionine (0.1 ml), 100 mM potassium phosphate buffer (1.5 ml), 3 mM EDTA (0.1 ml), 2.25 mM nitro blue tetrazolium (0.01 ml), and distilled water (1 ml) in test tubes in duplicate from each enzyme sample. The reaction was discontinued by covering the tubes with black cloth to block the light. Absorbance was recorded spectrophotometrically at 560 nm.

Statistical Analysis

Statistical Analysis System software (SAS Institute, Cary, NC, USA, Version 9.2) was used for data analysis. The least significant difference test (LSD) was used to determine the significance of the differences among mean values for each attribute at p≤0.05. Also Pearson correlation between the attributes was analyzed using SAS 9.2 and Minitab 18 software.

Results

Photosynthetic pigments

Photosynthetic pigments, i.e. chlorophyll a, b, total, and carotenoids were affected by irrigation regimes and foliar application of all growth regulators, except for carotenoids. Moreover, the interaction of irrigation regimes and foliar application on these traits was not significant. The results showed that drought stress compared to normal irrigation (100% WR) led to a significant reduction in photosynthetic pigments. The reductions in chlorophyll a, b, total, and carotenoids were 33.8%, 40.8%, 36.4%, and 32.2%, respectively (Table 3). On the other hand, growth regulators, namely SA, JA, PBZ, and CS led to increased chlorophyll synthesis. Maximum increase in the chlorophyll content was related to

Table 4
Effect of different levels of irrigation regimes and foliar application of JA, SA, PBZ, and CS on proline, RWC, and morphological characteristics of cumin (*Cuminum cyminum* L.)

Treatments	Proline (µmol/g FW)	RWC (seed/day)	Number of umbellets per umbel	Number of umbel per m^2	Number of seed per umbel	Seed yield (Kg/ha)	Essential oil (%)
Irrigation regimes (% of WR)							
100% (control)	0.26+0.07 c	73.12+7.22 a	3.64+0.5 a	496.5+65.0a	29.19+2.7 a	532.0+120.7 a	0.86+0.3 c
70%	0.52+0.11 b	59.47+5.66 b	2.94+0.2 b	472.5+106.2a	26.76+3.5 b	418.6+152.2 b	1.73+0.29 b
40%	0.7+0.16 a	42.17+6.16 c	2.68+0.6 b	398.31+77.4b	21.66+4.7 c	224.5+82.5 c	2.21+0.24 a
LSD (*p*≤0.05)	0.10	5.06	0.31	40.65	1.98	55.17	0.17
Foliar application							
Control	0.56+0.3 a	53.42+14.7b	2.5+0.68c	359.2+94.8 b	21.52+5.64b	237.15+157.5b	1.49+0.68b
SA	0.49+0.21 a	61.66+15.3a	3.2+0.58ab	480.51+95.7a	26.21+4.39a	407.81+150.8a	1.66+0.68ab
JA	0.51+0.23 a	60.36+14.5 a	3.53+0.57a	456.04+96.8a	28.08+2.41a	460.2+154.7a	1.84+0.59a
PBZ	0.45+0.17 a	58.54+13.8ab	3.11+0.5b	507.48+62.1a	26.57+4.79a	446.5+209.9a	1.47+0.63b
CS	0.46+0.2 a	57.28+14.5ab	3.1+0.66b	475.56+37.8a	26.96+4.63a	407.04+130.2a	1.55+0.63b
LSD (*p*≤0.05)	0.13	6.54	0.40	52.49	2.56	71.23	0.22
Irrigation regimes × foliar application							
	NS	NS	NS	**	*	**	*

Means followed by the same letter in each column are not significantly different according to LSD test at 5 % level.
NS: not significant; * and **: significant at α=0.05 and 0.01%.

the use of JA, which resulted in 27.9, 29.4, and 28.4% increase in chlorophyll a, b, and total, respectively. In addition to JA, the use of PBZ also had positive effects on the photosynthetic pigments and was statistically in the same group with the superior treatment (Table 3).

Antioxidant enzymes activity

As shown in Table 3, the effect of irrigation regimes and interaction of irrigation regimes and foliar application were significant on the activity of CAT, POD, and SOD enzymes.

The effects of foliar application were significant only on the activity of CAT and POD enzymes. Drought stress due to irrigation water shortage led to a significant increase in the average activity of antioxidant enzymes. The highest activity of CAT (2.39 U/mg protein.min) was related to the use of JA under severe drought stress (40% WR). On the other hand, the lowest activity of this enzyme was recorded under normal irrigation without foliar application (100% WR) (Fig. I).

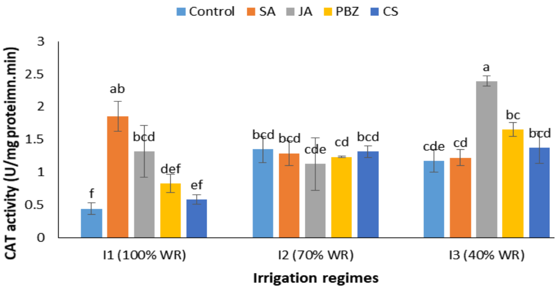


Fig. I. Catalase (CAT) activity of cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS). Different letters in each factor indicate significant differences at $p < 0.05$.

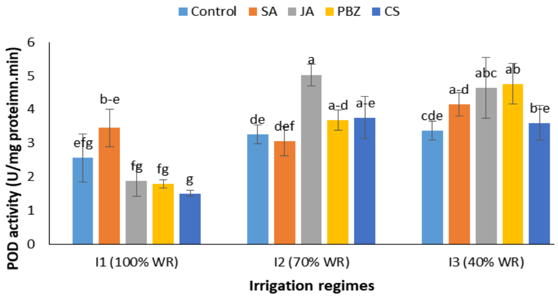


Fig. II. Peroxidase (POD) activity of cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS). Different letters in each factor indicate significant differences at $p < 0.05$.

Our obtained results revealed that irrigation water shortage led to a remarkable increase in POD activity compared to normal irrigation. The highest values of POD activity (5.03 U/mg protein.min) were obtained with JA application under mild drought stress (70% WR). Additionally, PBZ and CS under mild stress and SA, JA, and PBZ under 40% WR showed the highest POD activity. Moreover, CS application under non-stress conditions (100% WR) resulted in the lowest activity of this enzyme (1.50 U/mg protein.min) (Fig. II).

Drought stress due to irrigation water shortage led to remarkable increases in SOD activity. The presented results in Fig. (III) indicated that the highest SOD activity (3.89 U/mg protein) was related to the no foliar application under non stress treatment. Additionally, SA, JA, and CS application under severe water stress showed the highest SOD activity. Jasmonic acid application under normal irrigation had the lowest POD activity (0.59 U/mg protein).

Proline content

Irrigation regimes had significant effects on proline content. But the foliar application treatments and interaction effects of irrigation and foliar nutrition was not significant on this attribute. Increased drought stress levels led to an increase in proline content so that non-stress conditions resulted in the lowest (0.26 µmol/g FW) proline content while severe drought stress showed the highest proline content (0.7 µmol/g FW) (Table 4).

Relative water content (RWC)

The analysis of variance results showed a significant effect of irrigation regimes and foliar treatments of the study on RWC. Our findings in Table 4 suggest that RWC considerably decreased in cumin under severe drought stress compared with normal conditions (42.3%). Conversely, foliar application with all growth regulators caused a significant increase in RWC. The effect of SA and JA was superior to other compounds.

Yield and yield components

Yield and yield components such as the number of umbellets, number of umbels, number of seed, and seed yield were affected by irrigation regimes and foliar application. Moreover, interaction effects of irrigation regimes and foliar application were significant on the number of umbels, number of seeds, and seed yield (Table 4). Drought stress significantly decreased number of umbellets, number of umbels, number of seed, and seed yield compared with well water plants. Severe drought stress led to a decrease by 26.3% in the number of umbellets compared to the normal irrigation (Table 4). The highest number of umbellets was recorded in JA application (3.53 per umbel). Also, SA application showed the highest mean number of umbellets (Table 3).

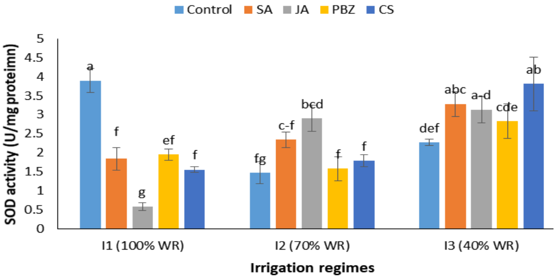


Fig. III. Superoxide dismutase (SOD) activity of cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS); different letters in each factor indicate significant differences at $p < 0.05$.

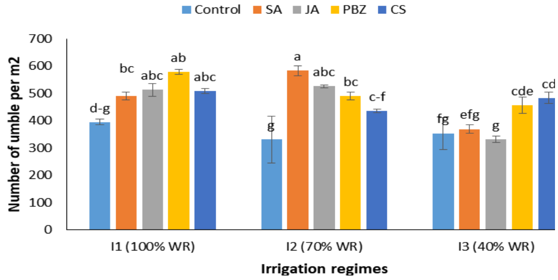


Fig. IV. Number of umbles in cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS); different letters in each factor indicate significant differences at $p < 0.05$.

Drought stress had a significantly negative effect on the number of umbels. As shown in Fig. (IV), the highest number of umbel was achieved under JA, PBZ, and CS treatments in normal irrigation (512.04, 557.8, and 508.0, respectively). This was followed by SA and JA application under 70% WR treatments (582.1 and 525.0, respectively), non-application of growth regulators under 70% WR (330.5), and JA application under severe water stress (331.0).

Number of seed per umble decreased as drought level increased. PBZ application under non-stress conditions (100% WR) resulted in the highest number of seeds per umbel (32.14). Also, the use of SA and CS under non-stress and SA, JA, and CS in 70% WR treatments led to the highest number of seeds per umble. Under severe drought stress (40% WR), JA application resulted in a 42.8% increase in the mean of this trait compared to the control. The lowest number of seeds per umble (15.7) was related to the non-foliar application of growth regulators under severe drought stress (Fig. V).

Drought stress, foliar application, and interaction of drought and foliar nutrition significantly affect ted seed yield (Table 4). Drought stress decreased grain yield, while growth regulators improved grain yield. PBZ application under non-stress conditions showed the highest seed yield (698.9 kg/ha). In contrast, the lowest seed yield (103.4 kg/ha) was related to the non-foliar nutrition under severe drought stress (40% WR) (Fig. VI).

Essential oil content

As shown in Table 4, the results indicated that the effects of irrigation regimes, foliar nutrition, and interaction irrigation and foliar nutrition were significant on essential oil contents. Drought stress increased the essential oil contents. The maximum essential oil content (2.4%) was related to the JA application under severe drought stress (40% WR). Additionally, other growth regulating treatments at this level of irrigation treatment had the highest mean essential oil. On the other hand, application of PBZ and CS under non-stress conditions showed the lowest essential oil contents (0.69 and 0.72%, respectively) (Fig. VII).

Correlation coefficients

There were significantly negative and positive correlations among physiological characteristics as well as among yield components. For example, the antioxidant enzymatic activities (CAT, POD, and SOD) were significantly and negatively correlated with photosynthetic pigments. However, the seed yield was negatively and significantly correlated with the enzymatic activities and proline content while positively and significantly correlated with photosynthetic pigments and yield components (Table 5).

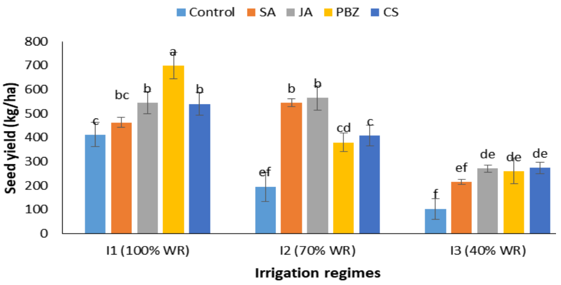


Fig. V. Number of seeds in cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS); different letters in each factor indicate significant differences at $p < 0.05$.

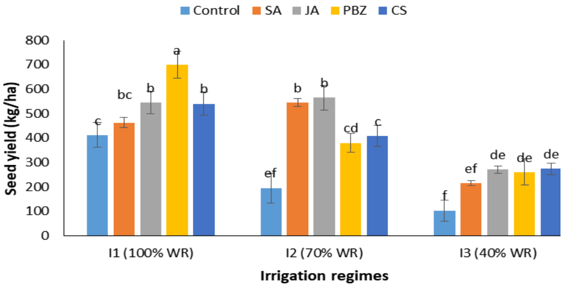


Fig. VI. Seed yield in cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS); different letters in each factor indicate significant differences at $p < 0.05$.

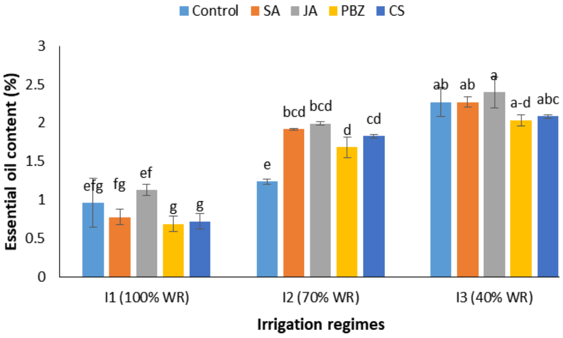
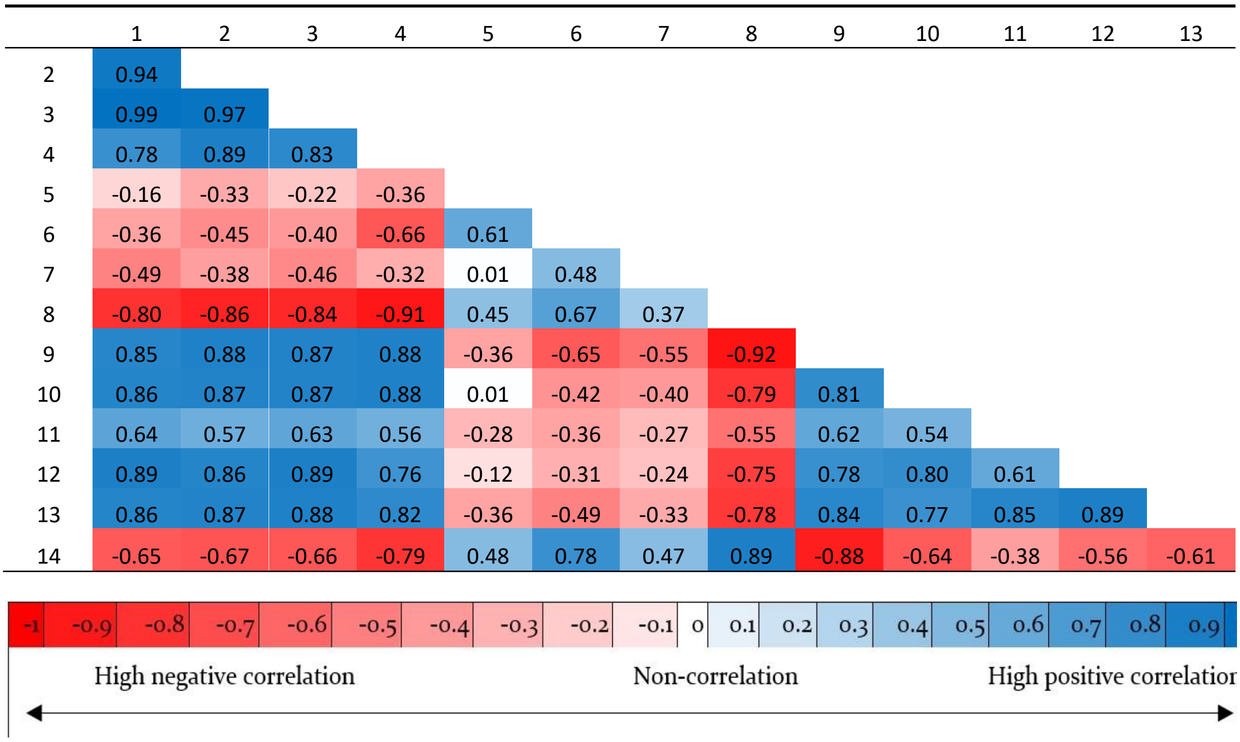


Fig. VII. Essential oil contents in cumin under different irrigation regimes (100%, 70%, and 40% WR) and foliar nutrition (control, SA, JA, PBZ, CS), different letters in each factor indicate significant differences at $p < 0.05$.

Discussion

The present study aimed to assess the morpho-physiological and yield responses of cumin to foliar fertilization with SA, JA, PBZ, and CS under different irrigation regimes. Findings showed that the photosynthetic pigments were affected by water shortage treatment. The chlorophyll a, b, and total, and also carotenoid contents significantly decreased with the increase in drought treatment (irrigation at 40% WR). On the other hand, a significant rise was observed in the level of these pigments after applying growth regulators such as JA, PBZ, and CS compared with non-foliar fertilization (control).

Table 5
Correlation coefficients among different physiological parameters and yield components in cumin



	1	2	3	4	5	6	7	8	9	10	11	12	13
2	0.94												
3	0.99	0.97											
4	0.78	0.89	0.83										
5	-0.16	-0.33	-0.22	-0.36									
6	-0.36	-0.45	-0.40	-0.66	0.61								
7	-0.49	-0.38	-0.46	-0.32	0.01	0.48							
8	-0.80	-0.86	-0.84	-0.91	0.45	0.67	0.37						
9	0.85	0.88	0.87	0.88	-0.36	-0.65	-0.55	-0.92					
10	0.86	0.87	0.87	0.88	0.01	-0.42	-0.40	-0.79	0.81				
11	0.64	0.57	0.63	0.56	-0.28	-0.36	-0.27	-0.55	0.62	0.54			
12	0.89	0.86	0.89	0.76	-0.12	-0.31	-0.24	-0.75	0.78	0.80	0.61		
13	0.86	0.87	0.88	0.82	-0.36	-0.49	-0.33	-0.78	0.84	0.77	0.85	0.89	
14	-0.65	-0.67	-0.66	-0.79	0.48	0.78	0.47	0.89	-0.88	-0.64	-0.38	-0.56	-0.61

1: Chlorophyll a; 2: chlorophyll b; 3: total chlorophyll; 4: carotenoids; 5: CAT activity; 6: POD activity; 7: SOD activity; 8: proline content; 9: RWC; 10: number of umbellets; 11: number of umbles; 12: number of seeds; 13: seed yield; 14: essential oil content

The lower water uptake by seeds under drought stress conditions (40% WR) and the concomitant rise in the levels of reactive oxygen species (ROS) among the possible explanations reason for reduced photosynthetic pigments and yield attributes under drought stress conditions (Afshari et al., 2022). Ghahremani et al. (2019) and Ahmad et al. (2020) argued that a decrease in chlorophyll a, b, and total content, increased activity of antioxidant enzymes such as CAT, POD, and SOD, and producing a greater level of osmoprotectants such as proline could be among the physiological reasons for decreased growth indices and yield attributes in plants under drought stress. Moreover, a decline in contents of photosynthetic pigments, namely chlorophyll a, b, and total is considered a typical symptom of oxidative stress in the stressed plant (Afshari, 2020). In the current study, an enhancement in the drought stress levels decreased the photosynthetic pigment contents significantly compared with the control conditions (100% WR). In fact, the decrease in chlorophyll and carotenoid contents of plants is a typical symptom of oxidative stress in drought stress conditions which might be a result of pigment photo-oxidation and chlorophyll degradation (Hazrati et al., 2016; Fathi and Tari, 2016). Chlorophyll loss is reported to be accompanied by the damage in mesophyll chloroplasts, resulting in a lower photosynthetic rate under stress (Afshari et al., 2022). This is also the case with carotenoids, which are play important roles in plants including energy dissipation, scavenging ROS, stabilizing photosynthetic complexes, and alleviating the adverse effects of abiotic stresses (Zafari et al., 2020).

Muscolo et al. (2014), Zafari et al. (2020), and Afshari et al. (2022) attributed the reduction in yield and yield components of plants under drought stress mainly to the alteration of some enzymes and hormones, as well as oxidative damage due to ROS production, and decrease in relative water content under stress, which reduces cell turgor pressure, and consequently inhibits cell enlargement and division, and plant growth.

Results showed increases in the yield and yield components of cumin due to application of growth regulators. Various biologic stimulants such as SA, PBZ, etc. have been reported to enhance plant growth and yield attributes upon foliar nutrition, and help the plants under abiotic stresses to tolerate the adverse abiotic conditions (Hajihashemi and Ehsanpour, 2013; Afshari et al., 2022; Gorzi et al., 2017). The mechanism of these favorable effects might be the role of these growth regulators in increasing cell expansion, cell division, and meristematic growth, which cause an increase in plant growth (Afshari et al., 2020). Afshari et al. (2020, 2022) found that SA and PBZ treatments had a significant stimulatory effect on the biosynthesis of chlorophyll and also mitigated the adverse effects of drought stress on the chlorophyll content of the plants under study. The PBZ application was also found to enhance photosynthetic pigment contents in stevia (Afshari et al., 2022), *Festuca arundinacea* and *Lolium perenne* (Shahrokhi et al*.,* 2011), and wheat cultivars (Aly and Latif, 2011).

ROS are a common product of metabolism processes in plant cells both under normal and stress conditions. However, their generation may exceed well beyond the normal levels under abiotic stress. This might lead to progressive oxidative damages in plant organs. A number of defensive mechanisms are adopted by plants in their attempt to overcome the oxidative stress, which fall within two broad category of enzymatic and non-enzymatic processes (Ghahremani et al., 2019).

In the current study, drought stress increased antioxidant activity such as CAT and POD in the cumin plants under study. These enzymes are described as two of the most important antioxidant enzymes protecting plants against cellular oxidative damage (scavenging of H_2O_2) caused by water shortage (Shahverdi et al., 2019). An increase was also observed in the proline contents of the cumin plants as a natural response to drought. It was also observed that SA application further increased the proline accumulation, improving induction of different antioxidant enzymes. Proline is an osmoprotectant actively involved in scavenging ROS, adjusting the osmotic pressure, stabilizing cell membranes and proteins, and storing nitrogen and carbon in plants subjected to biotic stresses (Afshari et al., 2020). Initially, drought stress may impair and decrease the activities of proline catabolic genes, which is a defensive strategy in the stressed plant. Then, the increased water shortage induces the expression of the genes responsible for proline biosynthesis, namely 1-pyrroline-5-carboxylase reductase, pyrroline-5-carboxylase synthase1, and 1-pyrroline-5-carboxylase synthase 2. Foliar spray of SA in this study seems to have stimulated the production of proline and eventually helped the plant under stress. Gorzi et al. (2017) argued that the SA treatment (at low concentration) reduces oxidative stress due to generation of ROS by improving the plants' antioxidant defense mechanism.

Ghassemi-Golezani and Farhangi-Abriz (2018) reported that the SA and JA treatments improved ATP content and H^+-ATPase activity of roots. Also, soybean's nutrient uptake and plant performance were improved by SA and JA. These findings are supported in this study, where a positive effect of JA was observed on the morpho-physiological traits of cumin. For instance, the highest photosynthetic pigments, grain yield, and yield components were related to the use of JA. Among the phytohormones, jasmonates, including JA and its derivative compounds, are naturally occurring plant hormones that can act as a critical mediator in the signaling pathway, triggering the expression of plant defense genes in response to various stressors. JA is also involved in stomatal conductance, photosynthetic process, cell division, and plant growth (Ghaffari et al., 2020).

In sum, this study suggests that water shortage due to the irrigation regimes has inhibitory effects on all growth, morphological, and yield attributes of cumin; nevertheless, foliar fertilization with SA, JA, PBZ, and CS effectively promotes photosynthetic pigment, the activity of antioxidant enzymes, growth, and yield characteristics under severe water stress. Jasmonic acid was the most effective compound among growth regulators that could positively affect most traits, including photosynthetic pigment content, antioxidant enzyme activity, seed yield, and yield components. Finally, this study recommends applying growth regulators such as JA and PBZ to moderate the negative effects of severe drought stress on cumin.

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