The role of hydrogen peroxide and 24-epibrassinosteroid signaling on physiological traits of cumin (*Cuminum cyminum* L.) under drought stress

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Abstract

Two biochemical compounds of hydrogen peroxide and 24-epibrassinosteroid have significant biological effects on plant growth and development, including increased plant tolerance to environmental stresses. In the present study, the effects of drought and its interaction with H_2O_2 and 24-epibrassinosteroid on protein content, sugars, essential oil percentage, photosynthetic pigments, phenols, and flavonoids were investigated. A factorial pot experiment in a completely randomized design was conducted with three replications in the research greenhouse south of Kerman province and plants. In week 5 after germination, plants were treated under drought stress at three levels: 100%, 75%, and 50% field capacity. The plants were sprayed with hydrogen peroxide and 24-epibrassinosteroid at 0, 0.5, and 1 mM concentrations, sequentially at two steps: three days before stress and then, 15 days later. Results showed a significant effect of drought stress and spraying on the studied traits, so that with increasing stress level, the essential oil percentage, soluble sugars, carotenoids, phenolic compounds, and shoot flavonoids increased, and the shoot protein and chlorophyll contents decreased. With increasing levels of H_2O_2 and 24-epibrassinosteroid, the contents of chlorophyll a and b increased, so that spraying with 1 mM 24-epibrassinosteroid led to the highest level of chlorophyll (10.90 mg mL^{-1}). Spraying 24-epibrassinosteroid also increased the shoot Flavonoids content (1.58 mg/g DW) and decreased essential oil percentage (2.44%) under severe stress conditions. Results showed that hydrogen peroxide and 24-epibrassinosteroid played as signal molecules at optimum concentrations and allowed the cumin plant to adapt to drought conditions by reducing membrane peroxidation and inducing physiological and biochemical activities.

Keywords: Cumin; drought stress; epibrassinosteroid; hydrogen peroxide; protein; photosynthetic pigments

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Introduction

Cumin (*Cuminum cyminum* L.) is one of the most important herbs and medicinal plants that makes up the main part of medicinal plant export in Iran (Omidbeygi, 2007). It is the second most popular spice in the world after black pepper

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(Daniel and Maria, 2002) and is the most important domestic medicinal plant in Iran (Kafi, 2002).

Plants are exposed to a wide range of abiotic stresses throughout their lives, causing significant damage to the economy and production cycle of countries each year (Lotfallahi et al., 2016). Drought stress is one of the major factors that limits plant production all over the world and has adverse effects on plant growth and development and other metabolic processes (Lum et al., 2014). To avoid such conditions, plants have complex processes to respond appropriately to a variety of stress conditions (Krasensky and Jonak, 2012). Studied show that most of the areas under cumin cultivation in Iran belong to arid and semiarid regions, which makes it necessary to do research on proper use of water due to water scarcity in the agricultural sector of these areas (Kafi, 2003; Bahraminejad, 2011). The tolerance capacity of plants to salinity and drought conditions is characterized by assessing and measuring biomass production and determining the chlorophyll content (Bakhshi et al., 2018). Photosynthetic pigments are essential for crop production and energy supply and play an important role in photochemical reactions (Farooq et al., 2009). Chlorophyll is one of the main components of the chloroplast for photosynthesis and photosynthesis rate is directly and positively correlated with chlorophyll content (Anjum et al., 2011). The decrease in chlorophyll and protein contents under stress conditions is considered to be a prominent indicator of oxidative stress and may be the result of pigment photo-oxidation and chlorophyll degradation (Farooq et al., 2009). One of the key solutions of plants to cope with drought and reduce oxidative damage from the production of reactive oxygen species (ROS) is the production of signaling compounds such as the enzymatic and non-enzymatic compounds. Brassinosteroids (Br_s) are one of the signaling compounds that are naturally produced in plants at very low levels (ng) and increase plant resistance to environmental stresses (Behnamnia et al., 2009). Treating different plants such as grapes (Seif et al., 2014) under drought stress, rice (Wang et al., 2010) and green cucumber (Mao et al., 2007) under low temperature stress by brassinosteroids showed improved growth over the control plants. In plants under stress, brassinosteroids prevent the destruction of the nucleus and chloroplasts, thereby protecting the ultrastructure of leaf cells. Many studies have shown that the use of brassinosteroids alone or in combination with other signaling compounds increases crop yield and stress resistance in different plants (Peleg and Blumwald, 2011).

Hydrogen peroxide (H_2O_2) is one of the most stable types of ROS in plants, whose functions have been reported in basic plant processes such as development, metabolism, and adaptation to biotic and abiotic stresses (Bienert et al., 2006; Slesak et al., 2007). When the amount of hydrogen peroxide in the cell is naturally maintained by a number of antioxidant enzymes, it acts as a secondary messenger and along with other cellular signals protects plants against stresses and stimulates stress tolerance (Hung et al., 2011; Li et al., 2011). However, high concentrations of hydrogen peroxide lead to tissue destruction and ultimately plant death (Quan et al., 2008). Therefore, extensive research on different plants has shown that at low concentrations, it not only increases plant adaptation to stress conditions, but also activates physiological processes associated with plant growth. The results of the study done by Liu et al. (2010) showed that treatment of mint plants with H_2O_2 improved their resistance to osmotic stress by activating the antioxidant system.

Considering the nutritional and medicinal importance of cumin and geographical location of most areas of Iran as the arid and semi-arid regions, understanding the mechanisms of coping with drought stress and identifying the effects of drought stress on physiological and metabolic processes of cumin is very important. Therefore, the aim of the present study was to investigate the protective role of hydrogen peroxide and brassinosteroids and their interaction in coping with oxidative stress induced by drought stress in addition to investigating membrane damage caused by drought in cumin.

Materials and Methods

This study was conducted in the fall of 2016 in the Research Greenhouse of Agricultural Research, Education and Extension Organization

Table 1
Physical and chemical properties of the soil

soil texture	Electrical conductivity ($ds.m^{-1}$)	pH	Uptakable phosphorus	Uptakable potassium	Sodium	Calcium	Magnesium
					(mg/Kg)		
sandy clay	0.42	7.9	6.8	150	2.34	1365	105

(AREEO) in the south of Kerman province as a factorial experiment in a completely randomized design with three replications. The investigated treatments included three different levels of irrigation based on field capacity (FC): T1: 100% of FC (control), T2: 75% of FC (mild stress), and T3: 50% of FC (severe stress) (Safikhani et al., 2007). Drought stress was applied at the beginning of the plant flowering stage and based on the field capacity (FC) and amount of water required for plants. Three days before applying water deficit stress, 24-epibrassinosteroid (Sigma Aldrich) and hydrogen peroxide (H_2O_2: 30% Merck) were sprayed onto leaf surface at concentrations of (0, 0.5, and 1 mM) during two steps in two consecutive days in early morning. Then, after 15 days, all of the pots under stress were sprayed with 24-epibrassinosteroid and hydrogen peroxide.

The soil used in this study was sandy clay (Table 1). Due to the organic matter deficiency of the soil, all pots were appropriately fertilized with N, P, and K fertilizers, as well as Fe, Mn, and Zn fertilizers. After soil fertilization, high quality seeds of cumin with high level of viability, prepared from Pakan Bazr Esfahan Company, were separately disinfected by 15% sodium hypochlorite and 10% ethanol for two minutes and then, sterilized three times with distilled water and, were placed inside water for 24 hours for better growth. Thus, a large amount of seed phenols that prevent germination, were washed away. Then, the sterilized seeds were planted in the plastic pots (pots had drainage holes in the base) at a height of 30 cm and the volume of four kg soil. Ten seeds per pot were planted at a depth of 1-2 cm and after seven days, thinning of seedlings was performed and finally, four plants remained in each pot. The minimum and maximum day and night temperatures were set at 16 and 28 °C in the greenhouse, respectively. Given that research into cumin has showed that at high temperatures, a number of flowers are formed lacking an ovary (male flower), the greenhouse temperature was maintained using a ventilation and cooling system (Rahimian, 1990). Until the fifth week (the beginning of the reproductive stage), irrigation of all plants was carried out with water and according to the needs of the plants. During the experiment and at different stages of the plant growth, the biochemical and physiological characteristics such as protein content of the shoot, soluble and insoluble sugar content of the shoot, chlorophyll a and b, carotenoids, phenol, flavonoids, and essential oil percentage were measured.

Protein assays were performed by the method of Bradford (1976). For this purpose, one g fresh tissue of the shoot that was at 0 – 4 °C was homogenized with five mL 0.05 M HCl-Tris buffer (pH 7.5) for 30 minutes and then transferred to Ependorf tubes and centrifuged for 20 minutes at 1300 rpm. One hundred (100) mL of the extract was mixed with 5 mL Bradford solution, and the absorbance was measured with a spectrophotometer (Spekol 2000) at 595 nm and the protein concentration was expressed as mg/L.

The phenol-sulfuric method by Kochert (1978) was used to assay sugars. For this purpose, 0.1 g of the shoot tissue was mixed with ten mL ethanol 80% after drying in an oven at 75 °C for one week after which, the supernatant was utilized to measure soluble sugar contents and the remaining sediments for the assay of insoluble sugars. Two mL of the supernatant was mixed with one mL of phenol 5%, and five mL of sulfuric acid was also added to the solution. After 30 minutes, the absorbance was measured with a spectrophotometer (Spekol 2000) at 485 nm. After drying in an oven, the sediments were mixed with ten mL distilled water and placed in a boiling water bath for 15 minutes. After filtration, two mL of the solution was removed and mixed with one mL phenol and five mL sulfuric acid and the absorbance was measured at 485 nm and

Table 2
Analysis of variance of effects of drought stress, hydrogen peroxide and brassinosteroids on traits tested

Source of variation	Protein	Soluble sugar mg/L FW	Insoluble sugar	Chlorophyll a	Chlorophyll b mg/mL FW	Carotenoids	Phenol mg/g	Flavonoids absorbance g	Essential oil percentage
Drought									
100% FC	11.19^{a}	10.49^{c}	4.31^{c}	10.89^{a}	6.51^{a}	7.64^{a}	0.77^{b}	0.73^{c}	1.92^{c}
75% FC	11.16^{a}	10.78^{b}	4.50^{b}	9.65^{a}	5.43^{b}	9.52^{b}	0.78^{b}	1.17^{b}	2.71^{b}
50% FC	10.96^{b}	11.69^{a}	5.14^{a}	7.89^{b}	4.79^{c}	13.28^{a}	0.95^{a}	1.58^{a}	4.38^{a}
H_2O_2									
0 mM	11.07^{a}	10.98^{b}	4.64^{a}	9.38^{c}	5.34^{ab}	9.81^{b}	0.82^{a}	1.15^{b}	2.91^{b}
0.5 mM	11.09^{a}	10.99^{a}	4.65^{a}	9.55^{ab}	5.52^{a}	10.11^{b}	0.83^{a}	1.15^{ab}	3.02^{a}
1 mM	11.15^{a}	11.00^{a}	4.65^{a}	9.92^{a}	5.86^{b}	10.52^{a}	0.84^{a}	1.18^{a}	3.07^{a}
Br									
0 mM	10.87^{c}	10.89^{c}	4.47^{c}	8.31^{c}	4.09^{c}	8.62^{a}	0.77^{c}	1.02^{c}	3.66^{a}
0.5 mM	11.13^{b}	10.97^{b}	5.34^{ab}	10.06^{b}	5.52^{b}	10.01^{b}	0.83^{b}	1.17^{b}	2.91^{b}
1 mM	11.31^{a}	11.10^{a}	5.36^{a}	10.90^{a}	7.11^{a}	11.81^{a}	0.89^{a}	1.29^{a}	2.44^{c}

FW: Fresh weight, FC: Field capacity, H_2O_2: Hydrogen peroxide, Br: brassinosteroids. The same letters in each column indicate that there is no significant difference at 5% level.

expressed as mg L^{-1}. The sugar content was assayed by standard diagrams.

The method of Lichtenthaler (1992) was used to assay photosynthetic pigments, including chlorophyll a and b and carotenoids. For this purpose, 0.2 g fresh tissue of the developed shoots of the plant along with ten mL acetone 80% were pulverized with a porcelain mortar, and the extract was centrifuged for 10 minutes at 5,000 rpm. The final volume of the extract was then made to 20 mL with ten mL acetone 80%. The absorbance wavelength of the supernatant was measured with a spectrophotometer (Jenway Genova) at 633 and 645 nm. Finally, the amounts of chlorophyll a and b and carotenoids were measured in mg/L of solution and according to the fresh weight of the samples.

The method by Matta and Giai (1969) was used to assay phenol. Fresh samples were prepared from the shoot and were boiled after placing in ten mL ethanol 80%. Then, the samples were centrifuged and diluted Folin and saturated sodium carbonate were added, and the samples were re-centrifuged at 640 nm compared to the control. The standard curve was made using catechol and the amount of phenolic compounds was measured in mg/g fresh weight.

One gram of the fresh leaf tissue was homogenized in ten mL acidic methanol (containing 99.5% methylic alcohol and pure hydrochloric acid at a ratio of 99/1) and centrifuged to assay the amount of flavonoid. The absorbance of the supernatant was determined at 300 nm for flavonoids with a spectrophotometer, and the results were compared as the absorbance/g of fresh weight (Nogues and Baker, 2000).

For the extraction of the essential oil, 40 g of dried plant seeds were placed in the shade (taking into account the moisture content in the seeds) and under air flow, along with one liter of distilled water in a balloon of a Clevenger apparatus. The essential oil was extracted by heating the balloon for three hours after grinding the samples and the percentage of essential oil in each sample was determined.

Statistical Analysis

Data were analyzed using SAS 9.1. Comparison of the means were made by Duncan's post hoc test at 5% level and the diagrams were drawn using Excel 2016.

Results

The analysis of the shoot protein

The results of analysis of variance showed that there were significant differences at 5% level between different levels of drought, hydrogen peroxide, and 24-epibrassinosteroid (Table 2). The

Table 3
Comparison of the mean simple effects of drought stress, hydrogen peroxide, and brassinosteroids on traits tested

		Mean squares								
Source of variation	df	Protein content	Shoot soluble sugar	Shoot insoluble sugar	Chl a	Chl b	Carotenoids	Shoot Phenol	Shoot Fla.	Essential oil percentage
Drought	2	0.42**	5.25**	5.153**	44.74**	20.48**	222.07**	0.2780**	4.944**	42.59**
Br	2	1.35**	0.30**	1.649**	65.22**	61.90**	68.68**	0.1002**	0.497**	10.15**
H_2O_2	2	0.05	0.00**	0.001	2.05*	1.89	3.44**	0.0034	0.009	0.18*
Drought × Br	4	0.16**	0.04**	0.137**	1.27	0.40	6.79**	0.0084**	0.034**	1.420.18**
Drought × H_2O_2	4	0.03	0.001	0.001	0.21	0.73	0.23	0.0004	0.000	0.031
Br × H_2O_2	4	0.02	0.001	0.000	0.02	0.45	0.09	0.0004	0.001	0.092
Drought × Br × H_2O_2	8	0.03	0.001	0.001	0.15	0.21	0.14	0.0013	0.002	0.08*
Error	54	0.02	0.0005	0.0009	0.66	0.71	0.56	0.001	0.003	0.04
CV	-	1.30	0.21	0.66	8.84	11.11	7.40	4.32	4.51	6.66

Chl a: chlorophyll a, Chl b: Chlorophyll b, Fla: Flavonoid, Br: Brassinosteroids; ns, *, ** are non-significant and significant at 5% and 1% levels, respectively.

Table 4
Comparison of the mean triple interaction of drought, hydrogen peroxide, and 24-epibrassinosteroid on traits tested

	Protein content	Soluble sugar	Insoluble sugar	Chl a	Chl b	Carotenoids	Phenol	Flavonoids	Essential o
				mg/mL FW			mg/g	absorbance g	%
100% of FC × H_2O_2 (1mM) × Br (1mM)	11.55^{a}	10.78^{c}	5.41^{a}	11.81^{a}	8.46^{a}	9.63^{c}	0.96^{a}	0.97^{c}	1.30^{c}
75% of FC × H_2O_2 (1mM) × Br (1mM)	11.39^{b}	10.93^{b}	4.73^{b}	11.60^{a}	6.86^{b}	13.90^{b}	0.87^{b}	1.20^{b}	2.33^{b}
50% of FC × H_2O_2 (1mM) × Br (1mM)	11.19^{c}	11.16^{a}	4.63^{c}	11.05^{a}	5.93^{c}	16.36^{a}	0.86^{b}	1.82^{a}	3.16^{a}

The same letters in each column indicate that there is no significant difference at 5% level.

simple effects and triple interactions of experimental factors on the shoot protein content were also significant (Tables 3 and 4). Under drought stress and with increasing drought stress, the shoot protein content was decreased so that under drought stress it was significantly decreased from 11.19 mg/L fresh weight under normal conditions to 10.96 (mg/L) under severe stress conditions (50% of field capacity) (Table 3). Spraying with hydrogen peroxide at 0.5 and 1 mM levels had no significant effect on the shoot protein levels under drought stress (Table 3). However, the highest protein content (average 11.31 mg/L) was obtained for the treatment under stress sprayed with 1 mM 24-epibrassinosteroid and the lowest (average 10.87 mg/L) was obtained from the treatment involving not spraying with 24-epibrassinosteroid (Table 3). Moreover, according to the results, the interaction of hydrogen peroxide (1 mM) and 24-epibrassinosteroid (1 mM) in the 100% FC treatment (control) increased the shoot protein content (11.55 mg L^{-1}) (Table 4).

Level of soluble and insoluble sugars in the shoot

Results showed that there was a significant difference between different levels of drought stress, hydrogen peroxide, and 24-epibrassinosteroid at 5% level (Table 2). The interaction of drought, brassinosteroids, and hydrogen peroxide on soluble and insoluble sugars of the shoot was also significant at 5% (Table 4). The highest and lowest soluble sugars of the shoots were obtained as an average of 11.69 mg/L and 10.49 mg/L under severe stress (50% of FC) and non-stress (control) conditions, respectively, which increased by approximately 12% (Table 3). Furthermore, under severe stress (50% of FC) and spraying with 1 mM hydrogen peroxide, the level of this trait reached 11 mg/L while there was no significant difference between the levels of insoluble sugar of the shoots in treatments sprayed with hydrogen peroxide (H_2O_2) under similar conditions (Table 3).

The triple interaction of drought stress, brassinosteroids, and hydrogen peroxide on the

soluble and insoluble sugars of the shoot was also significant (Table 4). The highest level of soluble sugar (11.16 mg/L) was related to the simultaneous use of hydrogen peroxide (1 mM) and epibrassinosteroid (1 mM) and under high stress level (50% FC) (Table 4). On the contrary, the results of the interaction on the level of soluble sugar suggested that the highest level of insoluble sugar of the shoot (average 5.41 mg/L) was related to the control (100% FC), spraying with peroxide hydrogen (1 mM), and epibrassinosteroid (1 mM) (Table 4).

Chlorophyll a

Results showed that the interaction of drought stress, brassinosteroids, and hydrogen peroxide had significant effects on the level of chlorophyll a at 5% and 1% levels (Table 2). However, the triple interaction of drought, brassinosteroids, and peroxide hydrogen on the level of chlorophyll a was not significant (Table 4). The level of Chlorophyll a decreased with increasing stress level, so that the highest and lowest mean chlorophyll a were 10.90 and 7.89 (mg/mL), respectively, for the control treatment (100% of FC) and 50% FC while at all levels of drought stress, the highest level of chlorophyll a was obtained with 24-epibrassinosteroid (1 mM) and hydrogen peroxide (1 mM) at 10.90 and 9.92 (mg mL-1 FW), respectively. This indicates that spraying cumin with brassinosteroids and hydrogen peroxide was able to greatly reduce the effects of drought stress.

Chlorophyll b

The results of analysis of variance showed that the simple effects of different levels of drought, 24-epibrassinosteroid, and hydrogen peroxide on the level of chlorophyll b were significant (Table 2). The interactions of drought stress-brassinosteroids, drought stress-hydrogen peroxide, and drought stress-hydrogen peroxide-brassinosteroids on the levels of chlorophyll b were also significant (Tables 3 and 4). Comparison of the mean interactions of the experimental treatments showed that water-deficit stress decreased chlorophyll b but increased chlorophyll content due to the application of brassinosteroids and hydrogen peroxide compared to the control and stress conditions. The highest level of chlorophyll b (7.11 mg/mL FW) was observed under stress and 1 mM 24-epibrassinosteroid. The lowest level of chlorophyll b was attributed to stress conditions and the non-use of 24-epibrassinosteroid (Table 3).

Carotenoid content analysis

According to the results of analysis of variance, carotenoid content was affected by drought stress, spraying with brassinosteroids, peroxide hydrogen, as well as the interaction of drought stress and brassinosteroids (Table 2). The results of the comparison of the mean data showed that the highest mean of carotenoids (13.28 mg/mL FW) was related to 50% FC, that is, the carotenoid content also increased with increasing drought stress (Table 3). Spraying with 24-epibrassinosteroid increased the carotenoid content by 11.81 mg mL^{-1} FW. Spraying plants under severe drought stress (50% FC) with hydrogen peroxide (1 mM) had the highest carotenoid content at a rate of 10.52 (mg/mL FW), which showed a significant increase compared to the treatment under mild stress (75% FC) and the control treatment (Table 3). The results of this study showed that the use of brassinosteroids increased the activity of photosynthetic pigments such as carotenoids in the control as well as under both mild stress (75% FC) and severe (50% FC) conditions (Table 3). The triple interactions of the experimental factors also showed that the highest content of carotenoids was related to severe stress (50% FC) and the simultaneous application of hydrogen peroxide (1 mM) and 24-epibrassinosteroid (1 mM) at an average of 16.36 mg/mL FW, which increased by about 70% compared to the control.

Phenol and flavonoid contents of the shoot

The findings of the study confirmed that there was a significant difference at 5% level for different levels of drought stress, peroxide hydrogen, and 24-epibrassinosteroid (Table 2). According to the results of a comparison of the mean data, the highest flavonoid content of the shoot (1.58 Obsarbandce gFW-1) was related to

the severe stress level (50% of FC). As the level of stress increased, the flavonoid and phenol contents of the shoot increased (Table 3). Spraying with 24-epibrassinosteroid and hydrogen peroxide had a significant effect on the flavonoid and phenol contents at 1% level (Table 4). Severe drought stress (50% FC) and non-spraying resulted in the highest flavonoid and phenol contents of the shoot at an average of 1.58 and 0.95 mg/g DW, respectively (Table 3). Except for the interaction of drought and hydrogen peroxide, the other dual and triple interactions of the experimental factors were significant at 5% level, so that under severe stress conditions (50% FC) and simultaneous use of 24-epibrassinosteroid (1 mM) and hydrogen peroxide (1 mM), the phenolic compounds of the shoot were reduced to the lowest content (0.86 mg g FW^{-1}) (Table 4). Results showed that under the same conditions of drought stress and spraying with hydrogen peroxide, the phenol content of the shoot was not significant (Table 3).

Essential oil percentage

The results of analysis of variance showed that the simple effects of drought stress, 24-epibrassinosteroid, and hydrogen peroxide, the dual interaction of drought stress-brassinosteroids as well as the triple interactions of drought stress-24-epibrassinosteroid-peroxide on the essential oil percentage were significant at 1% and 5% levels (Table 2). According to the results of the comparison of the mean data, the highest essential oil percentage of cumin (4.38%) was related to the severe drought stress (50% FC) and non-spraying with 24-epibrassinosteroid. Spraying with 24-epibrassinosteroid (1 mM) reduced the essential oil percentage under severe stress conditions (50%) (Table 3). Comparison of the means showed that under the triple interaction of the studied traits (Table 4), the highest essential oil percentage (3.16%) was related to 50% FC, 1 mM hydrogen peroxide, and 1 mM 24-epibrassinosteroid.

Discussion

Environmental stresses affect a variety of photosynthetic pigments, protein content, compounds of the electron transfer system, activity of enzymes involved in photosynthesis, and essential oil percentage (Bakhsi et al., 2018). The results of this study showed that the use of growth-signaling compounds, including spraying with 24-epibrassinosteroid (1 mM) and hydrogen peroxide (0.5 mM) under drought stress improved the plant growth process. Hydrogen peroxide is one of the reactive oxygen species which unlike other reactive oxygen species, is non-radical and is known as a signaling molecule in plants. Due to its stability and diffusion through the membrane and as a secondary messenger, it results in the flow of Ca^{+2}, protein alteration, and gene expression (Bienert et al., 2006). In addition, it has been observed that hydrogen peroxide is also involved in many plant processes such as resistance to plant stresses and the development of plants (Ślesak et al., 2007). Oxygen free radicals break down photosynthetic pigments and proteins under stress conditions. It is possible that hydrogen peroxide and 24-epibrassinosteroid improve the chlorophyll status of plant cells by the scavenging effects of reactive oxygen species (ROS) produced under stress (Laspina et al., 2005). Results showed that drought stress decreased protein content. Under the influence of abiotic stresses, such as drought stress, the stress-induced free radicals destroyed the leaf protein, which was in line with studies done by Baghizadeh et al. (2009) on okras, Shah (2007) on rapeseeds, Shahin et al. (2003) on apples and Abd El-Monem (2009) on beans. Numerous studies have reported that in plants treated with brassinosteroids a significant increase is observed in protein, which is consistent with the results of the present study, so that the interaction of 24-epibrassinosteroid and drought stress in cumin increased the protein content of the shoot. For instance, this was consistent with the results of the study by Nobakht et al. (2019) on mints. Pustovoitova et al. (2000) stated that 24-epibrassinosteroid led to an increase in the content of HSP proteins under drought stress that increased plant resistance to stress.

Water-deficit stress by increasing peroxidation in chloroplast and thylakoid membranes as well as damaging photosystem-bound proteins to a large extent reduces chlorophyll a and b levels and photosynthetic capacity (Lawlor, 2002). Changes in chlorophyll content under stress conditions can be due to the

impaired biosynthesis or accelerated pigment degradation (Bakhshi et al., 2018). Molecular degradation of chlorophyll in stressed plants was due to the removal of the phytol chain from the porphyrin chain by the production of oxygen free radicals or chlorophyllase activity (Parvaiz and Satyawati, 2008) and increased ethylene content. A study by Izadi et al. (2010) on mint plants showed that drought stress reduced the level of pigments especially chlorophyll a. Such results have also been reported by Rassam et al. (2015) on the hyssop plant. The decrease in photosynthetic pigments affected by stress is probably due to the increased production of reactive oxygen species. In the present study, it was found that drought stress severely reduced the level of photosynthetic pigments, which is consistent with the results of studies by Massacci et al. (2008) and Jaleel et al. (2008).

Spraying plants with 24-epibrassinosteroid and hydrogen peroxide increased biosynthesis of photosynthetic pigments and protection of photosynthetic pigments under stress conditions, which is in agreement with the results of the study by Ali et al. (2007b). The results of the study by Ahmadimousavi et al. (2005), in accordance with the results of the present study, showed an increase in chlorophyll content with the use of brassinosteroids under drought stress. The reason for the increase in chlorophylls due to the use of brassinosteroids seems to be an increase in resistance to oxidative stress because brassinosteroids have antioxidant properties and can prevent chlorophyll degradation. In agreement with the results of the present study, Saffari et al. (2013) also suggested that brassinosteroids can prevent damage to plant membranes and macromolecules against environmental stresses.

Spraying plants under stress increases stomatal conductance and carbon dioxide content in the stomatal space, thereby increasing plant photosynthesis and subsequently increasing plant yield (Ali et al., 2007a).

Drought stress, in addition to reducing plant growth, may also alter the course of some metabolic processes. These changes can make the plant resistant to stress (Bartels and Sourer, 2004). Increased levels of carotenoids under stress conditions are expected due to their role in the antioxidant defense system to protect photosynthetic pigments (chlorophyll) (Navabpour et al., 2016). In fact, carotenoids are pigments that prevent the degradation of chlorophylls under oxidative stress. Increased carotenoids content under drought stress in the study by Rassam et al. (2015) on hyssop plants and the study by Lotfallahi et al. (2016) on chamomile have been reported to be in agreement with the results of the present study, indicating the role of carotenoids in modulating the level of reactive oxygen radicals (Navabpour et al., 2016). Brassinosteroids inhibit a variety of reactive oxygen species by increasing the synthesis of carotenoids (Khan et al., 2012). Spraying plants with 24-epibrassinosteroid also increased carotenoid levels by 11.81 (mg mL^{-1} FW), thereby inhibiting reactive oxygen and plant resistance. The use of brassinosteroids increased the activity of photosynthetic pigments such as carotenoids under both control and mild (75% FC) and severe (50% FC) conditions (Table 3). Increased carotenoid levels and subsequently, inhibition of chlorophyll degradation by brassinosteroids in mustard (Fariduddin et al., 2011) and chickpea (Ali et al., 2007b) are consistent with the results of this study.

The results of this study showed that the flavonoid content of the cumin shoots significantly increased with increasing stress level. These metabolites protected the plant against oxidative stress by scavenging free radicals (Debnath et al., 2019). The results in Table 4 show that the level of phenolic compounds decreased under severe drought (50% FC) and with the simultaneous application of hydrogen peroxide (1 mM) and 24-epibrassinosteroid (1 mM). This can be due to the synthesis of these compounds to other phenolic compounds, including lignin. Research has shown that under stress conditions, wall-bound phenols are more affected than soluble phenols (Matsouka et al., 2011). Phenolic compounds, both as antioxidant agents and as metal chelating agents, play important roles in inducing plant tolerance to abiotic stresses (Gohari et al., 2011).

The increase in soluble sugar contents under drought stress is probably due to increased amylase activity, hydrolysis of starch to simpler sugars, and decreased transfer of sugars from

leaves to other parts of the plant (Zhang et al., 2010). Under drought stress, soluble sugars act as osmotic and osmotic protecting agents (Bohnert et al., 1995). Soluble sugars help to continue water absorption and maintain turgidity by reducing the cell's osmotic potential. The role of the osmotic protection of sugars in the membrane and protein stability through formation of hydrogen bonds with polar polypeptide sequences (Crowe et al., 1992) and phosphate groups of phospholipid of membranes has been reported (Strauss and Hauser, 1986). According to the results, soluble and insoluble sugar contents in cumin under drought stress increased, which is in line with the results of studies by Baghizadeh et al. (2009) on okra, Salimi et al. (2016) on chamomile, and Tasgin et al. (2006) on wheat. The application of brassinosteroids under stress has a direct positive relationship with resistance with non-environmental stress conditions with increasing levels of osmolytes such as proline and soluble sugars (Debnath et al., 2019).

The essential oil percentage of cumin showed a significant increase (5%) with reduction of irrigation to 75% and 50% FC: 41% and 128% increase in the essential oil percentage compared to the control, respectively. Kheira et al. (2015) stated that some factors such as environmental conditions, growth season, and harvest season influence the content of secondary metabolites such as essential oil and could increase or decrease it. The highest essential oil percentage of cumin (4.38%) was obtained under severe drought stress (50% FC) and non-spraying with 24-epibrassinosteroid while spraying with 24-epibrassinosteroid (1 mM) under severe stress conditions (50%) reduced the essential oil percentage. There are no confirmed reasons for the response of secondary metabolites of medicinal plants to drought and plant growth regulators. However, the decline in the Calvin cycle function, leaf area depletion due to environmental factors such as water stress can reduce the biosynthesis of fatty acids and essential oils. The results of the study by Aliabadi et al. (2008) showed that water-deficit stress had significant effects on the flowering shoot yield, essential oil yield of the flowering shoot, and essential oil percentage of coriander. The highest essential oil and dry matter yield were obtained in complete irrigation and the highest essential oil percentage of the flowering shoot was observed under the severe stress treatment.

Conclusion

Understanding environmental factors such as drought stress is an important factor in the success of cultivation of medicinal plants. Meanwhile, the identification of growth signaling materials that can be adapted to increase plant resistance to stress in the arid and semi-arid regions and have favorable effects on plant quantitative and qualitative indices is necessary. Based on the results of the study, it can be concluded that although the plant growth and yield are affected by a water consumption decrease and consequently drought stress, the application of both signaling compounds of brassinosteroids and hydrogen peroxide can improve the plant growth process. Drought stress caused oxidative stress in cumin plants, in which spraying with brassinosteroids and hydrogen peroxide could play an important role in drought resistance by eliminating ROS. Using these compounds has had positive effects on the biochemical traits of cumin under drought stress through improvement of morphological traits, photosynthetic traits, and reduction of oxygen free radicals. In sum, our study revealed that spraying with brassinosteroids was more effective than hydrogen peroxide in reducing damage caused by drought stress in cumin and could save the plant under critical conditions.

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