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Effect of Exogenous Application of 24-Epibrassinosteroids and Hydrogen Peroxide on Some Biochemical Characteristics of *Cuminum cyminum* L. grown under Drought Stress

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ABSTRACT

To evaluate the responses of cumin plant to different levels of drought stress with exogenous application of either 24-epibrassinolide (24-EBL) or hydrogen peroxide (H_2O_2) and a mixture of them, a factorial experiment was conducted, in a complete randomized design with three replicas, at the experimental greenhouse. Treatments included three levels of drought stress (100%, 75%, and 50% of FC) in which at fifth week after germination (beginning of reproductive growth) application of H_2O_2 (0, 0.5 and 1mM) and 24-epibrassinosteroid (0, 0.5 and 1mM) at two stages, first 3 days before applying the then, 15 days after. The results showed that with increasing stress intensity, the amounts of proline, malondialdehyde and superoxide dismutase (SOD) enzyme increased, but decreased the total protein and catalase enzyme in the root. The results of interaction between drought stress and exogenous application of 24-EBL showed the highest total protein content (11.30 mg/l) at 1mM 24-EBL under moderate stress of 75% of FC. Also, the highest of proline content of the root and shoot were obtained (10.15 and 10.91 mMol gFW^{-1}, respectively) under severe stress (50% FC) and spraying with 1mM 24-EBL, respectively. However, a decrease in MDA content, with the application of 24-EBL e and H_2O_2, was recorded. In general, it can report that drought stress reduced the efficiency of photosynthesis and plant production. Moreover, application of 24-EBL and H_2O_2 was able to improve the photosynthetic system and resistance of the cumin plant to stress.

Keywords: Antioxidant enzymes, Cumin (*Cuminum cyminum* L.), Drought stress, Malondialdehyde, 24-epibrassinosteroid.

INTRODUCTION

Medicinal plants are very valuable resources in the wide areas of natural resources in Iran, in case of which scientific knowledge, domestication, cultivation, development and optimal utilization can play an important role in health, employment and export. Cumin (*Cuminum cyminum* L.) is an annual plant belonging to the family Apiaceous, which is one of the most important and widely used medicinal plants. The family is mainly cultivated for various industries such as pharmaceuticals, food, cosmetics and sanitation (Omidbeigi, 2007). One of the most important research fields for medicinal plants is the study of environmental conditions affecting the qualitative and quantitative performance of these plants. Drought stress is one of the most important limiting factors for plant growth and in sequence affects many metabolic path-ways such as photosynthesis, respiration, absorption and transfer of water, mineral elements, enzyme activities, and the transfer and accumulation of organic matter (Suzuki *et al*., 2012). Therefore, the degradation effect of drought stress, especially in areas with low water reserves or poor rainfall distribution, is more than any other abiotic stress (Einizade *et al*., 2016). In drought tolerant- plants to environmental abiotic stresses, some morphological and metabolic changes occurred in response to stress that result in plant adaptation to the limiting environmental conditions (Xocon-ostle-Cazares *et al*., 2012). The cultivation of medicinal plants under water deficit conditions needs to be considered for their ability to produce in a wide area, and to assess their resistance to adverse environmental conditions, including drought. In studying the effect of different levels of drought stress (30, 60 and 100% of field capacity) on the activity of some antioxidant enzymes, photosynthetic pigments, proline and yield of Borago officinalis, Gholi Nejad *et al*., (2014) concluded that with increasing drought stress, the yield and photosynthetic pigments decreased and proline content and antioxidant enzymes increased. Since drought stress is one of the biggest challenges in the production of crops in the arid and semi-arid areas, identifying the different reactions of medicinal plants to water deficit is important (Moghaddam *et al.*, 2014). Under abiotic stress conditions, drought stress has led to a 45% reduction in crop yields in different parts of the world (Kafi *et al*., 2009) and causes similar cell damage along with salinity stress, temperature stress and oxidative stress (Lisar *et al*., 2012; Daghino *et al*., 2016). Among the physiological and metabolic changes that may occur in response to the drought and salinity stresses, reactive oxygen species (ROS), such as superoxide radicals (O^2) and a single oxygen (O^{1-}), hydroxide radicals (OH^-) and along with them the formation of H_2O_2 increase under stress conditions in cell organelles in plants (Sharma *et al*., 2012).

Plants have an antioxidant system that controls the excess production of (reactive oxygen species) ROS under stress conditions and thus protects them against

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the harmful effects of ROS, and, on the other hand, provides a managed level of ROS for growth and signal transmission pathways (Ishibashi *et al*., 2012). This antioxidant defense system includes antioxidants such as β-carotene, ascorbic acid, α-tocopherol and reduced glutathione (GSH), as well as antioxidant enzymes including catalase, ascorbate peroxidase, glutathione reductase and guaiacol peroxidase (Benhmimou *et al*., 2018). Under stress conditions, ROS caused by the biotic and abiotic stresses stimulate the degrading processes of membrane fatty acids, and thus, malondialdehyde increases under drought stress conditions, which indicates the membrane lipid peroxidation. Investigating the concentration of malondialdehyde in plant tissues can indicate the degree of destruction of the cell membrane because it is released by the degradation and peroxidation of the cell membrane (Bhattacharjee, 2012).

ROS-scavenging enzymes neutralize the toxic effects of ROS, which may be the result of continuous and simultaneous action of a number of antioxidant enzymes including catalase (CAT), peroxidase (POD), superoxide dismutase (SOD) and ascorbate peroxidase (APX). Plant cells are equipped with a free radical scavenging system to protect against oxidative damage (Hussein and Alva, 2014).

Hydrogen peroxide (H_2O_2) works as a regulator in various physiological processes, including photosynthesis (Noctor *et al*., 2017), aging (Gao *et al*., 2010), stomatal movement, cell growth and develo-pment (Suzuki *et al*., 2012). When the amount of hydrogen peroxide in the cell is maintained at a natural level by a series of antioxidant enzymes, it works as a secondary messenger along with other cellular signals to protect the plants from stress and stimulate stress tolerance (Qiao *et al*., 2014; Choudhury *et al*., 2017; Caverzan *et al*., 2016). Shan *et al*., (2018) stated that the treatment of plants with exogenous H_2O_2 prevents from increasing oxi-dative stress and tolerance of plants to salinity stress by increasing the production of the antioxidant enzyme and non-enzymatic compounds that can modulate ROS and lipid peroxidation.

Brassinosteroids are also one of the cellular signaling compounds that can increase plant resistance to the environmental stresses (Siddiqui *et al*., 2018). For example, the treatment of various plants such as grapes (Seif *et al*., 2014) under drought stress conditions, rice (Lisar *et al*., 2012) and cucumber (Fariduddin *et al*., 2014) under low temperature stress conditions by brassinosteroids showed better growth than the control plants.

According to the previous research, most areas cultivated with cumin in Iran are related to the arid and semiarid areas. Due to water deficit in the agricultural sector in these areas, research into the proper use of water in this sector is necessary (Kafi *et al*., 2006; Bahraminejad, 2011).

Therefore, the purpose of this study was to study the protective role of hydrogen peroxide and brassinosteroids and there interaction with oxidative stress caused by drought stress.

MATERIALS AND METHODS

This study was conducted in the fall of 2016 in a research greenhouse of Agricultural Research, Education and Extension Organization (AREEO) in the south of Kerman Province. A factorial experiment, in a completely randomized design with three replications, was carried out. The investigated factors included: three different levels of irrigation based on field capacity (FC) (50, 75 and 100% FC) (Benhmimou *et al*., 2018). With the beginning of flowering period, drought stress was applied; in the fifth week after cultivation, based on FC, mild stress: 75% of FC and severe stress: 50% of FC, based on the amount of water needed for each pot was conducted. Three days before applying drought stress, the 24-epibrassinosteroid (Br: Sigma Aldrich) hormone at (0, 0.5 and 1 mM) and hydrogen peroxide (H_2O_2: 30% Merck- 0, 0.5 and 1 mM) as foliar spray-ing during two steps in two consecutive days in early the morning. Then, after 15 days, all of the stressed pots were sprayed with 24-EBL and H_2O_2.

The soil used in this study was sandy clay soil. Other features are given in Table 1. Due to the organic matter deficiency of the soil, all pots were fertilized with N, P, K fertilizers, as well as Fe, Mn, Zn fertilizers. After soil fertilization, seeds of cumin were disinfected separately by 15% sodium hypochlorite and 10% ethanol for 2 min, then, they were rinsed three times with distilled water. For faster germination, sterilized washed seeds were soaked in water for 24 hrs. Thus, a large amount of seed phenols that prevent germination, were washed away. Then, sterilized seeds were planted in the drainage plastic pots of 30 cm in height and three kg soil. For each pot, ten seeds per pot were planted at a depth of 1-2 cm and after emergence of seedlings; four plants remained to apply the different treatments under greenhouse condition with day and night temperatures at 16 and 28 °C in the greenhouse, respectively. Given that research into cumin has showed that at high temperatures, a number of flowers lacking an ovary (male flower) are formed, the greenhouse temperature was maintained using 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 for irrigation. During the experiment and at different stages of the plant growth, the biochemical and physiological characteristics such as proline content, malondialdehyde, total protein concentration and the antioxidant enzymes of catalase, superoxide dismutase and ascorbate peroxidase were measured in the shoot and root.

Antioxidant Enzymes Activity Assay

Enzyme Extractions and Measurements Catalase, Ascorbate peroxidase and Super oxide dismutase.

To extract antioxidant enzymes, five g of fresh root tissue and five g of fresh leaves from each treatment were weighed, and in a porcelain mortar containing 5ml of 0.05 M HCl-Tris buffer (pH 7.5), were completely pulverized in the ice bath for 30 min. The

homogeneous mixture was centrifuged for 20 min at 10,000 pm, and the surfactant was used at 4 °C for enzyme assay (Zelinova *et al*., 2013). The catalase activity (CAT; EC 1.11.1.6) was assayed by absorption reduction at 240 nm via catabolization of H_2O_2. The activity of ascorbate peroxidase enzyme was also assayed by observing the ascorbate oxidation rate at 290 nm using the method by Kaya *et al.*, (2019) and the activity of the superoxide dismutase enzyme using the method by Beauchamp and Fridovich (1976), and the changes in the absorbance of the reaction solution compared to the control were measured by the spectrophotometer (Spekol2000) at 560 nm and the activity of the superoxide dismutase enzyme was expressed as U mg per protein.

Method of proline extraction and assay

Proline was assayed according to the method by Bates *et al*., (1973) in which 0.1 g of the fresh root tissue and shoot were separately homogenate by 10 ml of 3% sulfosalicylic acid solution and filtered by filtered by filter paper after 48 h, followed by one mL of the solution was removed and, after mixing with two mL of glacial acetic acid was placed in a Bain Marie at 100 °C for one hour and immediately cooled. Then, four mL of toluene were added. After 30 min, the absorption at 520 nm was measured. The concentration of proline in each sample measured was calculated based on standard curve, in terms of mM/g fresh weigh.

Concentration of malondialdehyde (MDA) in leaves

To determine the concentration of malondialdehyde in the leaves, 0.5 g of the fresh leaves thoroughly powdered was mixed with 20% of trichloroacetic acid 3 (TCA) solution containing 0.5% of thiobarbituric acid 4, and then, this mixture was heated in the Bain Marie bath at 95 °C for 25 min. The mixture was then cooled in the ice bath and, according to the method of Valentovic *et al*., (2006); the concentration of malondialdehyde was measured at 532 nm.

Protein Concentration

Protein assay were performed by the method by Bradford (1976). For this purpose, 1 g of the shoot fresh tissue that was exposed to 0-4 °C, homogenated with 5 mL of 0.05 M HCl-Tris buffer (pH 7.5) for 30 min, and after transferring to Eppendorf tube, it was centrifuged at 1300 rpm at 4 °C for 20 min. One hundred (100) ml of the extract were mixed with five mL of Bradford's solution and the absorption rate was measured by spectrophotometer (Spekol2000) at 595 nm and the protein concentration rate was expressed in mg/l.

Statistical Analysis

Data were statistically analyzed using SAS software. One-way ANOVA analysis followed by Duncan's multiple range tests at 5% level was performed.

Table (1): Physical and chemical properties of soil used.

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

RESULTS

Proline Variations in the Shoot

The results of analysis of variance showed that the effect of drought stress, 24-epibrassinosteroid, as well as the interaction of drought stress and 24-brassinosteroids (24-EBL) on the mean proline content in the shoot was significant different (Table 2). According to a comparison of the mean data, the highest mean proline in the shoot (10.33 mMol gFW^{-1}) was related to spraying of 24-epibrassionsteroids, which showed a 12% increase compared to the optimum irrigation treatment (Table3). The results of effect of 24-epibrassinosteroid on plant growth under drought stress showed that the highest proline content in the root (10.08 mMol gFW^{-1}) was obtained from the 1 mM 24-epibrassinosteroid and 50% FC of drought stress (Table3).

Proline Variations in the Root

The proline content of root tissues showed significant differences among the different levels of drought stress as responses of exogenous application of 24-epibrassinosteroid (Br) and hydrogen peroxide (H_2O_2) at 1% level (Table 2). The highest proline content in the root tissues was obtained under severe stress (50% of FC) at a rate of 9.95 mMol gFW^{-1} and the lowest (9.52 mMol gFW-1) was related to optimal irrigation (100% FC). Among the different concentrations of 24-epibrassinosteroid, the highest proline content of the root (10.08 mMol gFW^{-1}) was related to the concentration of 1mM 24-EBL. Moreover, the concentration of 1mM of H_2O_2 also increased the mean proline content of the root at a rate of 9.84mML^{-1} FW (Table 3). The results of a comparison of the mean interactions of applying 24-epibrassinosteroid under drought stress conditions indicated that the highest proline content in the root with 9.84 mMol gFW^{-1} was related to the concentration of 1mM 24-epibrassinosteroid and severe drought stress (50% FC) (Table 4).

Catalase Activity (CAT) Variations in the Shoot and Root

According to the results obtained, the effect 24-epibrassinosteroid hormone and hydrogen peroxide, on the root catalase, as well as the interaction of both 24-epibrassinosteroid and hydrogen peroxide on the activity of shoot catalase was recorded table 2. The data showed significant ($p \leq 0.05$) effect in which with increasing drought stress, the root catalase was

reduced, while spraying with 24-epibrassinosteroid at 1mM in under control conditions, mild stress (75% FC) and severe stress (50% FC) increased the root catalase was detected table 4. The catalase enzyme of the shoot was also affected by drought stress and spraying with either brassinosteroids or hydrogen peroxide was highly affected (Table 2). The highest activity of this enzyme was obtained at severe stress (50% FC) condition and a concentration of 1 mM 24-epibrassino-steroid and the lowest was related to the desirable irrigation (100% FC) and no spraying with brassino-steroids table 3. The results of interactions of drought stress, 24-epibrassinosteroid × hydrogen peroxide showed the highest content of the shoot catalase (1.31mM H_2O_2 min^{-1}) concentration of 0.5 mM hydrogen peroxide and 1mM 24-epibrassino-steroid at 50% of FC (Figure 1).

Ascorbate Peroxidase (APX) Activity in the Shoot and Root

Variations in PAX activity, in response to drought stress, spraying with hydrogen peroxide or 24-epibrassinosteroid hormone were significant differe-nces on the shoot and root tissues (Table 2). The highest activity of the ascorbate peroxidase enzyme was recorded in the root (0.70 mM H_2O_2 min^{-1}) under the third level of drought stress (50% FC), which showed an increased by 35% compared to the control (100% FC) (Table 3).

Furthermore, the highest level of APX in the shoot (0.80) was recorded at the third level of drought stress (50% FC), which showed an increase by 30% compared to the control (100% FC) (Table 3). However, the different levels of 24-epibrassinosteroid recorded the highest APX in the shoot (0.83 mM H_2O_2 min^{-1}) at 1mM 24-EBL when applied exogenous. The highest activity of APX in the shoot (0.72 mM H_2O_2 min^{-1}) was detected when plants treated with 1 mM hydrogen peroxide. At this H_2O_2 level, APX was the highest when compared to the plants treated with 24-epibrassinosteroid (Table 3).

Superoxide Dismutase (SOD) Activity in the Shoot and Root

SOD, in response to different treatment under drought stress conditions, confirms the effects of drought stress, and spraying with either (Table 2). According to our study, the highest activity of SOD in the root (0.48 umg $protein^{-1}$) was detected under severe drought stress (50% FC), and represented by 37% increase compared to the control (100% FC). Moreover, the highest amount of SOD in the root (0.47) was obtained when 1 mM hydrogen peroxide was applied. In the evaluation of the activity of this enzyme in the shoot, the highest value recorded was 0.8 umg $protein^{-1}$ at 1 mM 24-epibrassinosteroid (Table 3). In meantime, the application of brassinosteroids showed that the highest SOD value in the root tissue (0.64 umg $protein^{-1}$) was recorded under severe drought stress and 1 mM brassinosteroids (Table 4). In add-ition, the interaction of drought stress and 24-epibra-ssinosteroid on the activity of superoxide dismutase in the shoot showed that the highest mean activity of this enzyme (0.91 µmg $protein^{-1}$) was related to 50% FC and one mM 24-epibrassinosteroid (Table 4).

Level of Malondialdehyde (MDA) In the Shoot

The exogenous application of 24-epibrassinosteroid, as well as both combination of brassinosteroids and hydrogen peroxide on MDA in the shoot was significantly different (Table 2). Moreover, for shoot tissue combination of 24-epibrassinosteroid and hydrogen peroxide under severe drought stress on MDA level was also significantly different at 5% level (Table 2). In general, drought stress significantly increased the malondialdehyde content in the leaves compared to the control application of 24-epibrass-inosteroid (Table 3). According to our data obtained for shoot tissues the highest MDA (0.45) was recorded under 50% of FC and 0.5mM brassinosteroids and 1 mM hydrogen peroxide (Figure 2).

Total Protein assay

The effects of exogenous hormonal treatment and H_2O_2 on total protein content of cumin plant, grown under drought stress, were significant different ($p \leq$ 0.05) were significant (Table 2). Drought stress showed a decreased in protein content of cumin. The highest protein content was observed for the control plant (100% FC) and those treated with 1 mM 24-epibrassinosteroid hormone (11.40 mg L^{-1}) (Table 3)

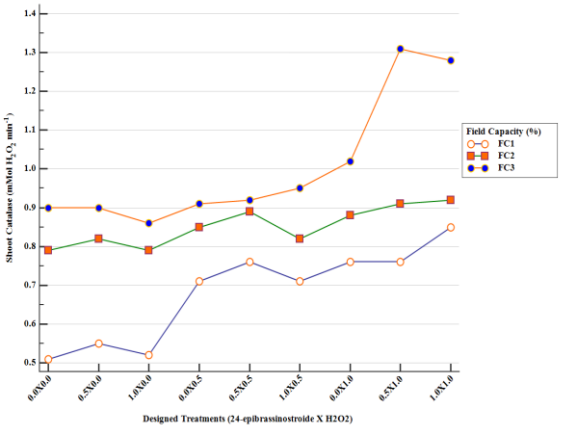

Figure (1): Interaction of drought stress, 24-epibrassinosteroid and H_2O_2 on the shoot catalase; FC1, 100%; FC2, 75% and FC3, 50%.

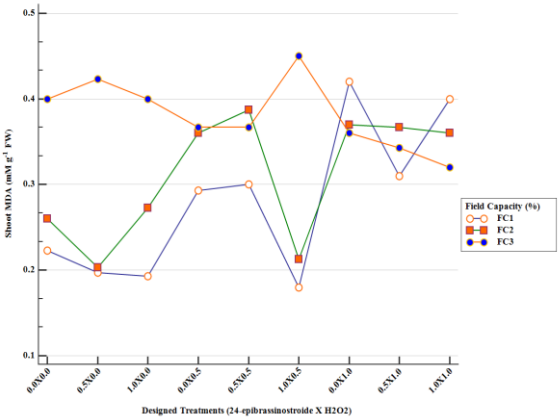

Figure (2): Interaction of drought stress, 24-epibrassinosteroid and H_2O_2 on the shoot MDA; FC1, 100%; FC2, 75% and FC3, 50%.

Table (2): Analysis variance of drought stress, 24-epibrassinolide and hydrogen peroxide on experimented traits.

Different treatments	df	Proline Content (mMol gFW^{-1})		CAT Activity in	APX activity		SOD activity		Leaf MDA content ($mM\ gFW^{-1}$)	Soluble protein content
		Shoot	Root	Root	Shoot	Root	Shoot	Root		
Drought (D)	2	12.32^{**}	1.0^{**}	0.57^{**}	0.37^{**}	0.48^{**}	0.26^{**}	0.22^{**}	0.06^{**}	0.42^{**}
H_2O_2	2	0.15^{**}	0.32^{**}	0.03^{*}	0.03^{**}	0.003^{ns}	0.009^{*}	0.01^{**}	0.011^{*}	0.047^{ns}
24-EBL	2	1.69^{**}	2.97^{**}	0.29^{**}	0.53^{**}	0.33^{**}	0.45^{**}	0.14^{**}	0.48^{**}	1.35^{**}
D × 24-EBL	4	0.32^{**}	0.28^{**}	0.04^{**}	0.009^{ns}	0.004^{ns}	0.013^{**}	0.028^{**}	0.04^{**}	0.15^{**}
D × H_2O_2	4	0.012^{ns}	0.029^{ns}	0.001^{ns}	0.006^{ns}	0.009^{ns}	0.001^{ns}	0.0004^{ns}	0.004^{ns}	0.03^{ns}
24-EBL × H_2O_2	4	0.012^{ns}	0.047^{ns}	0.0006^{ns}	0.001^{ns}	0.01^{ns}	0.0006^{ns}	0.0008^{ns}	0.008^{*}	0.023^{ns}
D×24-EBL×H_2O_2	8	0.021^{ns}	0.021^{ns}	0.001^{ns}	0.0008^{ns}	0.015^{ns}	0.0022^{ns}	0.0003^{ns}	0.007^{*}	0.029^{ns}
Error	54	0.008	0.052	0.0068	0.004	0.01	0.0022	0.001	0.003	0.021
CV (%)	-	0.93	2.35	10.43	10.11	16.76	7.01	8.43	18.02	1.30

ns, *, **, non-significance and significant at the 5% and 1% level of probability, respectively.

Table (3): The effects of drought stress, 24-epibrassinosteroid (24-EBL) and hydrogen peroxide (H_2O_2) on measured experimented traits (Ascorbate peroxidase (APX), Proline content, Superoxide dismutase (SOD), MDA.

Treatments	Applied Conditions	Root APX ($mM\ H_2O_2\ min^{-1}$)	Shoot APX ($mM\ H_2O_2\ min^{-1}$)	Root proline ($mMol\ gFW^{-1}$)	Shoot proline	Root SOD ($Umg\ protein^{-1}$)	Shoot SOD	Shoot MDA ($mM\ gr^{-1}\ FW$)	Total protein ($mg\ g^{-1}$)
Drought stress (FC %)	100	0.45^{b}	0.56^{c}	9.52^{c}	9.51^{c}	0.30^{c}	0.58^{c}	0.27^{c}	11.19^{a}
	75	0.67^{a}	0.71^{b}	9.69^{b}	9.84^{b}	0.36^{b}	0.66^{b}	0.31^{b}	11.16^{a}
	50	0.70^{a}	0.80^{a}	9.95^{a}	10.18^{a}	0.48^{a}	0.77^{a}	0.37^{a}	10.96^{b}
24-EBL concentration (mMol)	0 .0	0.50^{c}	0.55^{c}	9.42^{c}	9.83^{c}	0.31^{c}	0.54^{c}	0.37^{a}	10.87^{c}
	0.5	0.60^{b}	0.68^{b}	9.67^{b}	10.00^{b}	0.36^{b}	0.67^{b}	0.32^{b}	11.13^{b}
	1.0	0.72^{a}	0.83^{a}	10.08^{a}	10.33^{a}	0.46^{a}	0.80^{a}	0.28^{c}	11.31^{a}
H_2O_2 concentration	0.0	0.60^{a}	0.65^{b}	9.63^{b}	10.00^{a}	0.36^{b}	0.65^{b}	0.34^{a}	11.07^{a}
	0.5	0.62^{a}	0.69^{ab}	9.69^{b}	10.02^{a}	0.38^{b}	0.68^{a}	0.32^{ab}	11.09^{a}
	1.0	0.60^{a}	0.72^{a}	9.84^{a}	10.14^{a}	0.47^{a}	0.68^{a}	0.31^{b}	11.15^{a}

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range tests in 5% level of probability.

Table (4): Interaction effect of drought and 24- epibrassinosteroide on proline content, root catalase, supper oxide dismutase (SOD) and total protein content of cumin plant.

24-EBL Concentration (mM)	Applied Drought Conditions (FC %)	Shoot proline ($mMol\ gFW^{-1}$)	Root proline ($mMol\ gFW^{-1}$)	Root catalase ($mM\ H_2O_2\ min^{-1}$)	SOD ($\mu mg\ protein\text{-}1^{-1}$) Root	SOD ($\mu mg\ protein\text{-}1^{-1}$) Shoot	Total protein ($mg\ g^{-1}$)
0.0	100	9.13^{c}	9.03^{c}	0.50^{c}	0.27^{b}	0.44^{c}	10.94^{b}
	75	9.63^{b}	9.41^{b}	0.74^{b}	0.32^{ab}	0.57^{b}	11.08^{a}
	50	10.76^{a}	9.84^{a}	0.81^{a}	0.37^{a}	0.64^{a}	10.58^{c}
0.5	100	9.42^{c}	9.48^{c}	0.69^{c}	0.30^{b}	0.55^{c}	11.23^{a}
	75	9.82^{b}	9.65^{b}	0.81^{b}	0.36^{b}	0.66^{b}	11.10^{b}
	50	10.77^{a}	9.89^{a}	0.90^{a}	0.44^{a}	0.79^{a}	11.07^{b}
1.0	100	10.01^{c}	10.06^{b}	0.75^{c}	0.35^{c}	0.76^{b}	11.40^{a}
	75	10.07^{b}	10.04^{b}	0.82^{b}	0.41^{b}	0.76^{b}	11.30^{b}
	50	10.91^{a}	10.15^{a}	1.10^{a}	0.64^{a}	0.91^{a}	11.24^{c}

Similar letters in each column shows non-significant difference according to Duncan's Multiple Range tests in 5% level of probability

DISCUSSION

Currently, due to the expansion of the arid and semi-arid areas as well as the limited availability of water resources, identifying and selecting resistant cultivars to water-deficit stress is necessary to minimize the future problems of the world to provide foods. In the present study, the concentrations of two signaling compounds of H_2O_2 and brassinosteroid in the treated plants under drought stress conditions were used to reduce oxidative damage caused by water deficit and adaptation to stress conditions. The results of this study showed that drought stress (control, 75% FC, 50% FC), especially severe stress (50% FC), increased the proline content in the shoot and root (Tables 2 and 3). Cumin under drought stress conditions by storing the osmotic regulating agents such as proline confronted with drought stress (Safari et al., 2015). The results of table 2 support this claim. The proline accumulation in the cell is influenced by the use of 24-epibrassinosteroid, due to the effect of increasing this phytohormone on the enzymes of delta 1-pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR). Plant hormones contain chemical messages in response to environmental factors, and it can be said that during drought stress, the accumulation of metabolites such as proline and osmotic regulators is influenced by plant hormones (Mousavi *et al*., 2010). The results were consistent with the results by Neveen *et al*., (2012) and Sekmen *et al*., (2014). They also observed that with decreasing irrigation water and applying stress, the proline content in plant tissues increased.

The destruction of cell membranes is known as one of the direct consequences of water deficit. A large amount of toxic substance, including MDA, is produced in plants under drought stress conditions. The reason for this phenomenon is that drought stress eliminates the permeability balance of the plasma membrane of plants (Iqra and Naveela, 2013). In the present study, reducing the accumulation of malondialdehyde in plants treated with 24-epibrassinosteroid and H_2O_2 along with stress is likely to indicate a decrease in the lipid peroxidation and a greater survival of the membrane under stress conditions. Increasing MDA in water-deficit stress in plants such as olive (Petridisa *et al*., 2012), corn (Valentovič *et al*., 2006), alfalfa (Antolín *et al*., 2010) and wheat (Keyvan, 2010; Moaveni, 2011) have been reported, which support the results of this study. The plants treated with epibrassinosteroid under stress conditions efficiently eliminate oxygen free radicals compared to the same plants without the 24-epibrassinosteroid. Therefore, cell damage is reduced by 24-epibrassinosteroid and hydrogen peroxide in cumin (Table 3).

Under stress conditions, reducing protein concentrations is a common symptom of oxidative stress, which is often seen in the plants treated with drought stress (Duran *et al*., 2017). In the present study, with increasing drought stress, the total protein content was reduced (Table 3). Reducing the total protein content of the plant treated with drought stress is in fact due to free radicals produced under stress conditions, which is consistent with the findings of studies by Raza *et al*., (2013) on okra plant, Iqbal and Ashraf (2013) on rapeseed plant, Shahin *et al*., (2010) on apple fruit, and Asim Masood *et al*., (2016) on beans. The interaction of hydrogen peroxide and drought stress in cumin plant increased the protein content of the leaves, which is consistent with the results by Laxa *et al*., (2019) and Mohamed *et al*., (2012). They suggested that drought stress initially produced ABA, which increased the production of reactive oxygen species and, which by decreasing the protein content, promoted the expression of antioxidant system and their activity.

Barazesh *et al*., (2017) reported that the treatment of spermine and epibrassinolid hormones significantly increased the activities of DNA and RNA polymerase and resulted in the formation of DNA and RNA in pistachio tree. Increased protein synthesis has been observed in various plants treated with brassinosteroids. Neveen *et al*., (2015) also argued that 24-epibrassinosteroid resulted in an increase in the HSP protein contents under water-deficit stress, which increased the resistance of plants to stress. These findings are consistent with the results of this study. The results of this study suggest that the use of brassinosteroids and its repetition during growth can be recommended in cumin plants. However, it is obvious that further research is needed to clarify the molecular mechanism of the activity of brassinosteroids under stress conditions.

The protective and antioxidant role of brassinosteroids is well proven against some abiotic stresses (Vardhini, 2013; Peres *et al*., 2019). In this study, it was found that the use of 24-epibrassinosteroid by reducing the stress effects resulted in a significant increase in proline, the enzymes of SOD, APX and CAT in the shoot and root, and total protein content under severe stress conditions (%50 FC) and mild stress (75% FC) (Table 2). By increasing the level of antioxidants (SOD, APX and CAT) in response to drought stress in cumin plants treated with brassinosteroids and hydrogen peroxide to improve the growth process, it may be suggested that this antioxidant system, at least in part, be responsible for the resistance of cumin plants to drought stress.

Antioxidant enzymes play an important role against the oxidative stress caused by adverse environmental conditions. In the present study, with increasing drought stress, the CAT activity of the root had a significant decrease compared to the control treatment. However, the spraying of plants with brassinosteroids and hydrogen peroxide could increase the level of this enzyme and thereby reducing the harmful effects of reactive oxygen species (ROS), so that the activity of this enzyme in the root tissue under severe stress conditions and spraying with one mM brassinosteroid reached at a rate of 1.10 mM H_2O_2 min^{-1} (Table 4). A decrease in and deactivation of the CAT enzyme under osmotic stress conditions may be due to inhibiting the enzyme synthesis or inactivation of the enzyme by the radical, peroxide and hydroxyl oxygen (Hosseini

Boldaji *et al*., 2012). The SOD enzyme also plays a major role in protecting plant cells against oxidative stress, because the enzyme can convert the superoxide radical into hydrogen peroxide and molecular oxygen.

In this study, the highest level of the root superoxide dismutase was related to the severe drought stress treatment (50% FC) (0.48 Umg protein^{-1}), which showed a 37% increase compared to the control treatment. Increasing the SOD activity under drought stress can be due to an increase in the superoxide radical or a defense mechanism against oxidative stress in plants (Mirzaee et al., 2013).

With the exogenous application of H_2O_2 at low doses (0.5 and 1 mM), along with the application of 24-EBL during the experiment, the antioxidant activity of enzymes of catalase, ascorbate peroxidase and superoxide dismutase increased under drought stress conditions in the shoot and root (Table 2). Such a conclusion has been reported by other researchers (Debnath et al., 2019; Mirzaee et al., 2013; Marcin ska et al., 2013).

Considering the importance of antioxidant enzymes in scavenging free oxygen radicals and preventing oxidative stress caused by water deficit, it seems that increased proline activity, accompanied by increasing the activity of superoxide dismutase in the shoot and root, caused to reduce the negative effects of oxidative stress caused by free oxygen radicals, and thus cumin showed greater resistance to drought stress. The lower increase of malondialdehyde in the shoot during the experiment also supported this conclusion (Table 3).

CONCLUSION

According to the results of the present study, drought stress had adverse effects on the physiological characteristics of Cumin plant. Plants; however, the use of 24-epibrassinosteroids and hydrogen peroxide could improve these adverse effects and increase tolerance of this plant to drought stress conditions. Improvement of drought tolerance under the conditions of this experiment could be due to (a) improvement of antioxidant enzyme activity and protection against oxidative stress, (b) reduction MDA content and proline accumulation. Therefore, to offset as least some of the harmful effects of drought stress on cumin, spraying of hydrogen peroxide and 24- epibrassin-osteroids before the onset of stress and its recurrence within two weeks thereafter can be recommended as a a management tool to improve the growth and stress tolerance of cumin in arid and semi-arid regions.

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