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Exogenous application of gamma-aminobutyric acid (GABA) alleviates the effect of water deficit stress in black cumin (*Nigella sativa* L.)

Esmaeil Rezaei-Chiyaneh[a,*], Seyyed Mohammad Seyyedi[b], Elnaz Ebrahimian[c], Sina Siavash Moghaddam[a], Christos A. Damalas[d,*]

[a] *Department of Agronomy, Faculty of Agriculture, Urmia University, Urmia, Iran*
[b] *Department of Plant Production, Faculty of Agriculture & Natural Resources, University of Torbat Heydarieh, P.O. Box 95161-68595, Torbat Heydarieh, Iran*
[c] *Department of Agronomy, Faculty of Agriculture, Ferdowsi University of Mashhad, P.O. Box 91779-48974, Mashhad, Iran*
[d] *Department of Agricultural Development, Democritus University of Thrace, Orestiada, Greece*

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ABSTRACT

Water deficit is an abiotic stress factor that negatively affects black cumin (*Nigella sativa* L.) production. Gamma aminobutyric acid (GABA), an endogenous signaling molecule and metabolite, has high physiological and molecular activity in plant cells, which can promote tolerance to water deficit stress, but little information is available on the effect of exogenous application on growth of black cumin. A field experiment over two years was carried out at a farm located in Naqadeh-Urmia, West Azerbaijan, Iran to evaluate the effects of GABA on some agronomic and biochemical attributes of black cumin under water deficit stress conditions. Three irrigation treatments (irrigation after 50, 100, and 150 mm evaporation based on evaporation from class A pan) and four levels of GABA application (0, 0.5, 1.0, and 2.0 mg L^{-1}) were tested. Irrespective of GABA application, the severe water deficit treatment (i.e., irrigation after 150 mm evaporation) provided the lowest seed number per follicle, 1000-seed weight, and seed yield. Increasing water deficit, significantly reduced chlorophyll *a* by 8.2 to 15.8% and chlorophyll *b* by 18.4 to 41.5%, whereas GABA application significantly improved these traits. The application of 2.0 mg L^{-1} GABA increased chlorophyll *a* content by 6.2% and chlorophyll *b* content by 19.2% compared with control. In addition, GABA application showed a positive and significant effect on soluble sugars content, proline accumulation, and catalase (CAT) activity. The maximum values of these variables were obtained with the application of GABA at 2.0 mg L^{-1}. CAT, peroxidase (POX), and superoxide dismutase (SOD) activity increased with decreasing chlorophyll *a* and chlorophyll *b* contents, whereas soluble sugars and proline content increased with increasing activity of those antioxidant enzymes. Overall, in addition to cellular mechanisms, such as osmoregulation and antioxidant defense, GABA application can improve growth and productivity of black cumin under water deficit stress conditions.

1. Introduction

Black cumin (*Nigella sativa* L.) or simply nigella is an annual medicinal plant of the family Ranunculaceae (D'Antuono et al., 2002). This plant is native to south and southwest Asia and is cultivated in arid and semi-arid regions of Iran (Mozaffari et al., 2000; Ghamarnia et al., 2010). The seeds contain 0.5 to 1.6% essential oil (Ramadan, 2007) and also are rich in a wide range of compounds, such as fixed (non-volatile) oil, mucilage, alkaloids, essential amino acids, tannins, resins, crude fiber, saponins, minerals, and vitamins (Al-Jassir, 1992; Takruri and Dameh, 1998; Ashraf et al., 2006; Ramadan, 2007). Due to several beneficial properties and health effects (e.g., antibacterial, antidiabetic, anti-inflammatory, and others) (Khan, 1999; Burits and Bucar, 2000; Ali and Blunden, 2003; Singh et al., 2005; Ait Mbarek et al., 2007; Majdalawieh et al., 2010; Jrah Harzallah et al., 2011), black cumin has been used in traditional medicine in Iran for years (Nickavar et al., 2003) and emerges as a miracle herb with a rich historical and religious background due to a wide spectrum of pharmacological potential (Ahmad et al., 2013).

Effective utilization of *N. sativa* for therapeutic purposes as well as for trade will vastly depend upon yield and essential oil production and quality (Datta et al., 2012). However, research on cultivation practices of black cumin is rather limited. Although black cumin is considered a drought tolerant species cultivated in semi-arid regions of the world, water deficit stress still threatens growth and productivity of this plant (Bannayan et al., 2008; Al-Kayssi et al., 2011). Previous research

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* Corresponding authors.
E-mail addresses: e.rezaeichiyaneh@urmia.ac.ir (E. Rezaei-Chiyaneh), chris.damalas@yahoo.gr (C.A. Damalas).

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showed that black cumin could tolerate water deficit, except when irrigation was terminated at seed formation (Bannayan et al., 2008). In the same study, the minimum seed yield was obtained when irrigation was stopped at the blooming stage, with the number of seeds per plant being the main yield component affected. Drought stress, which is a common phenomenon in arid and semi-arid regions, is a major abiotic factor that can dramatically impact black cumin seedling establishment, plant growth, and seed yield (Ahmadpour Dehkordi and Balouchi, 2012; Haj Seyed Hadi et al., 2016). Hence, water deficit stress can be a serious limiting factor determining black cumin yield.

Generally, water deficit stress occurs when there is a low water potential in plant tissues as a result of low water potential in the soil, high evaporative demand, and a substantial resistance to water flow through the plant (Gao et al., 2002, Tanguilig et al., 1987). The detrimental effects of water deficit stress on growth and seed production of black cumin are mainly due to reduced water flow through the plant, which disturbs cellular metabolic pathways (Ghamarnia et al., 2010; Goswami, 2011; Haj Seyed Hadi et al., 2016). Plant organisms have enzymatic and non-enzymatic mechanisms that compose a fundamental detoxification apparatus for overcoming oxidative damage induced by abiotic stress (Ashraf et al., 2002; Hasanuzzaman et al., 2010). Several antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), maintain the balance between generating and quenching of reactive oxygen species (ROS) in the plant cells within the ascorbate-glutathione (AsA-GSH) cycle that regulates the redox reaction of AsA and GSH (Mittler, 2002; Li et al., 2010; Lotfi et al., 2015).

According to the abovementioned information, the use of compounds that can improve tolerance to water deficit stress through cellular signaling can be beneficial under drought stress conditions. In this regard, GABA (γ-aminobutyric acid), an endogenous signaling molecule involved in plant regulation (Li et al., 2016a), can play an important role in improving growth and productivity of crops under stressful conditions (Kinnersley and Turano, 2000; Hu et al., 2016; Soleimani Aghdam et al., 2016). GABA improved tolerance of winter wheat to high temperature by regulating the endogenous hormone system, protecting membrane stability, increasing the activity of antioxidant enzymes, removing active oxygen species, modulating the balance of C/N metabolism, and lessening yield losses brought by high temperature (Wang et al., 2009). Moreover, GABA application could increase heat tolerance in bentgrass (*Agrostis stolonifera* L.) through accumulating sugars, organic acids, and adjusting osmotic pressure (Li et al., 2016b). Improved drought tolerance with exogenous application of GABA has been reported also in perennial ryegrass (*Lolium perenne* L.) (Krishnan et al., 2013). However, the effects of GABA application on nigella growth and productivity and its involvement in metabolic events of this crop have not been studied. Therefore, the objective of the current study was to examine the effects of GABA application on physiological parameters of growth and yield as well as on essential oil content and antioxidant enzyme activity of black cumin under water deficit stress conditions. Furthermore, the relationships between antioxidant enzymes activity, proline accumulation, soluble sugars, and chlorophyll content were investigated. The research question of this study was set as: does exogenous application of gamma-aminobutyric acid (GABA) improve the response of black cumin to water deficit stress and, if so, by which mechanism(s)?

2. Material and methods

2.1. Field experiments

Two field experiments were conducted in 2014 and 2015 at a farm located in Naqadeh-Urmia, West Azerbaijan, Iran (long. 45° 25′, lat. 36° 57′, altitude 1307 m) at a location with an annual rainfall of 334 mm and a silty clay soil (Table 1). Some meteorological data of the experimental site during the growing seasons are given in Table 2. The experiments were established in a randomized complete block design (RCBD) with a split plot arrangement and three replicates. Three irrigation levels (irrigation after 50, 100, and 150 mm evaporation based on evaporation from class A pan) were considered the main plots and four levels of GABA application (0, 0.5, 1.0, and 2.0 mg L^{-1}) were considered the sub-plots.

Table 1
Soil physico-chemical characteristics of the experimental site.

Soil analysis	2014	2015
Physical		
Clay (%)	42	47
Silt (%)	41	31
Sand (%)	17	22
Chemical		
OC (%)	1.27	1.14
Available N (mg kg^{-1})	0.16	0.12
Olsen-P (mg kg^{-1})	14.1	10.66
Available K (mg kg^{-1})	482.12	450.82
pH	7.4	7.6
EC (dS m^{-1})	0.31	0.38

All plots consisted of eight crop rows 4 m in length with plant spacing on the row 0.3 m. Plots and blocks were separated by a buffer space of 1.5 m and 3 m, respectively. The seeds were sown on March 22 in 2014 and 2015 in furrows 7 cm apart. The seedlings were thinned at the four-leaf stage to reach a final planting density of about 45 plants per m^2. GABA foliar application was performed twice in each growing season, once at seedling establishment and once three weeks later. Tween® 20 (a polysorbate-type nonionic surfactant) was added to the spray solution to reduce the surface tension and enhance foliage wetting. Control plants were sprayed using distilled water. Fertilizers were applied according to soil analysis as 150 kg ha^{-1} urea (soil-incorporated before sowing and broadcast applied at stem elongation), 150 kg ha^{-1} triple superphosphate and 250 kg ha^{-1} sulfur (soil-incorporated before sowing). Since the soil analysis showed sufficient level of potassium, no potassium fertilizer was applied. Weeds were manually controlled during growing seasons.

At the end of each growing season (August 6 in 2014 and 2015), when the plants started to turn yellow, but before the opening of the follicles (138 days after sowing), ten plants from each plot were randomly harvested and examined for plant height, follicle number per plant, seed number per follicle, and 1000-seed weight. To determine seed yield and biomass yield, all plants per plot were harvested after ignoring border plants. Harvesting was performed when seed moisture content was 15%. To determine other traits, three separate samples were taken from each plot and an average was calculated from the raw data.

2.2. Extraction of essential oil

Black cumin seeds were shade-dried at room temperature and crushed using a grinder to extract the essential oil. The essential oil (v/w) was isolated from 10 g of the seeds with 100 mL distilled water with conventional hydro-distillation for 4 h, using a Clevenger-type apparatus (Ashraf et al., 2006).

2.3. Soluble sugars content

Tissue of fresh leaves was homogenized with 10 mL distilled water to determine the soluble sugars content. The extracts were stirred and left at room temperature for 1 h. Then, the extracts were centrifuged at 4500 rpm for 20 min. The soluble sugars content was determined according to Bai and Tang (1993) and measured using a glucose standard (expressed as mg g^{-1}).

Table 2
Monthly meteorological data of the experimental site during 2014 and 2015 growing seasons.

Month	Minimum temperature (°C)		Maximum temperature (°C)		Precipitation (mm)		Evaporation (mm)	
	2014	2015	2014	2015	2014	2015	2014	2015
March	2.8	1.4	14.8	13.1	66.4	51.6	0.0	0.0
April	6.1	5.9	20.5	19.4	27.5	26.9	151.4	179.6
May	9.8	10.5	25.5	24.9	32.4	21.7	200.4	220.5
June	12.7	13.3	30.1	31.7	1.5	0.0	238.5	284.2
July	16.0	16.4	33.0	34.3	28.2	0.4	249.6	281.2
August	15.6	15.5	34.2	33.5	0.8	0.0	282.0	259.5
Average	10.5	10.5	26.3	26.2	–	–	–	–
Total	–	–	–	–	156.8	100.6	1121.9	1225.0

Table 3
Effects of irrigation regimes and GABA application on yield and yield components of black cumin.

Treatment	df	Plant height	Follicles per plant	Seeds per follicle	1000-seed weight	Grain yield	Dry biomass	Essential oil
Year (Y)	1	NS	NS	NS	NS	NS	**	*
Irrigation (I)	2	**	**	**	**	**	**	**
Y × I	2	NS	NS	NS	NS	NS	NS	NS
GABA (G)	3	**	**	**	**	**	**	**
Y × G	3	NS	NS	NS	NS	NS	NS	NS
I × G	6	**	*	**	*	**	*	*
Y × I × G	6	NS	NS	NS	NS	NS	NS	NS

* Significant at $P < 0.05$.
*Significant at $P < 0.01$.
NS: non-significant.

2.4. Proline content

Tissue of the youngest fully expanded leaves were used to determined proline accumulation. The proline content was determined using acid-ninhydrin procedure in filtered extracts (Bates et al., 1973).

2.5. Chlorophyll a and chlorophyll b

Chlorophyll *a* and chlorophyll *b* were determined according to Arnon (1949). At flowering stage, fresh leaves were taken and extracted in 80% acetone. The absorbance of the extracts was recorded at 663 and 645 nm.

2.6. Antioxidant enzymes activity

Young leaf samples (0.5 g) were homogenized in ice-cold 0.1 mol L^{-1} phosphate buffer (pH 7.5) containing 0.5 mmol L^{-1} EDTA, with a pre-chilled mortar and pestle placed on ice. Homogenates were transferred to centrifuge tubes and centrifuged at 4 °C at 15000 x *g* for 15 min. The supernatant was used to assay antioxidant enzymes activity. Catalase (CAT) activity was determined following Aebi (1984). The reaction mixture contained 30 mmol L^{-1} H_2O_2 in a 50 mmol L^{-1} phosphate buffer (pH 7.0), and 0.1 mL enzyme extract in a total volume of 3 mL. CAT activity was determined by measuring the degradation of H_2O_2 at 240 nm. Peroxidase (POX) activity was determined in a reaction mixture containing 25 mmol L^{-1} phosphate buffer (pH 7.0), 0.05% guaiacol, 10 mmol L^{-1} H_2O_2, and 0.1 mL enzyme extract. Activity was determined by monitoring increase in absorbance at 470 nm due to guaiacol oxidation ($\varepsilon = 26.6\ m\,M^{-1}\,cm^{-1}$) (Hemeda and Klein, 1990). Superoxide dismutase (SOD) activity was determined according to Dhindsa et al. (1980). One unit (U) of SOD was considered as the amount of enzyme that causes 50% inhibition of nitro blue tetrazolium (NBT) reduction in the presence of light. Finally, antioxidant enzymes activity was expressed in units per mg of protein (Cakmak and Horst, 1991).

2.7. Statistical analysis

All data were subjected to analysis of variance (ANOVA) using SAS software version 9.3 (SAS, 2011). A split-plot ANOVA (three irrigations by four GABA treatments) with three replicates over two years was used. Means were compared with Duncan's Multiple Range Test (DMRT) at 5% probability level ($P < 0.05$). Simple correlation was used to examine the relationship between two variables.

3. Results

3.1. Yield and yield components

Weather conditions were quite similar in both growing seasons, except from rainfall, which was higher in 2014 due to more rainy March, May, and August, compared with 2015 (Table 2). There was no significant interaction between year (Y) and irrigation (I) or between Y and GABA application (G) concerning nigella seed yield and yield components as well as essential oil content (Table 3). However, there was a significant interaction between I and G concerning these variables.

Plant height decreased by 34.6% with mild water deficit stress (i.e., irrigation after 100 mm evaporation) and by 55.5% with severe water deficit stress (i.e., irrigation after 150 mm evaporation), so that the shortest plants were related to severe water deficit stress treatment (Table 4). The application of GABA at 1.0 or 2.0 mg L^{-1} significantly increased plant height at the mild and severe water deficit stress levels compared with control (Table 4). The same trend was also observed for follicle number, seed number per follicle, and 1000-seed weight. Total dry biomass and grain yield were decreased by 15.4 and 31.1%, respectively, with the mild water deficit stress and by 53.5 and 50.2%, respectively, with the severe water deficit stress. In both cases, the application of GABA at 1.0 or 2.0 mg L^{-1} significantly increased these variables at the mild and severe water deficit stress levels (Table 4). By contrast, the essential oil content was increased significantly at the mild and severe water deficit stress levels. Irrespective of GABA levels, the maximum essential oil percentage was related to severe water deficit

Table 4
Interaction effects of irrigation regimes and GABA application on yield and yield components of black cumin.

Irrigation after evaporation (mm)	GABA (mg L^{-1})	Plant height (cm)		Follicles per plant		Seeds per follicle		1000-seeds weight (g)		Grain yield (kg ha^{-1})		Biomass yield (kg ha^{-1})		Essential oil (%)	
50	0	52.21	a	26.62	abc	47.81	ab	2.85	abc	735.7	a	2163.9	abc	0.81	d
	0.5	49.04	ab	25.00	bcd	51.31	ab	2.83	abc	802.2	a	2180.9	ab	0.85	d
	1.0	46.46	abc	27.59	ab	53.06	a	2.94	ab	757.4	a	2304.2	a	0.90	d
	2.0	51.09	a	28.55	a	49.88	ab	3.10	a	717.1	ab	2314.9	a	0.82	d
100	0	34.17	d	18.54	ef	33.96	c	2.46	def	506.6	e	1830.9	bcd	1.28	c
	0.5	34.83	d	18.24	ef	34.23	c	2.80	abc	610.3	cd	1882.5	bcd	1.22	c
	1.0	43.17	c	23.50	d	45.71	b	2.72	bcd	712.1	ab	1979.4	abc	1.44	ab
	2.0	43.29	bc	24.64	cd	52.71	a	2.63	cde	640.3	bc	1930.4	bc	1.48	ab
150	0	23.21	e	10.69	g	21.89	e	1.91	g	366.1	f	1006.7	f	1.39	b
	0.5	19.75	e	12.88	g	24.14	de	2.25	f	520.1	de	1301.4	ef	1.44	ab
	1.0	32.63	d	18.79	e	32.63	c	2.36	ef	575.1	cde	1802.7	dc	1.48	ab
	2.0	30.50	d	15.97	f	30.29	cd	2.42	def	633.1	bc	1539.7	de	1.50	a

Means within each column followed by at least one letter in common are not significantly different at $P < 0.05$ (DMRT).

stress, whereas GABA at 1.0 and 2.0 mg L^{-1} further improved the essential oil content. The maximum essential oil percentage was obtained with GABA application at 2 mg L^{-1} in plants under severe water deficit stress (Table 4).

3.2. *Chlorophyll, proline, soluble sugars, and antioxidant enzymes*

There was no significant interaction between year (Y) and irrigation (I) or between Y and GABA application (G) concerning chlorophyll, proline, soluble sugars, and antioxidant enzymes (Table 5). However, there was a significant interaction between I and G concerning most variables. The effect of water deficit stress was significant on chlorophyll content, soluble sugars content, proline content, and antioxidant enzymes activity (Table 5). In addition, the effect of GABA application was significant on chlorophyll content, soluble sugars content, proline content, and CAT activity.

Increase in water deficit stress severity significantly decreased chlorophyll *a* and chlorophyll *b* content (Fig. 1). Nevertheless, GABA application mitigated the adverse effect of water deficit stress on chlorophyll content. For instance, application of GABA at 2 mg L^{-1} under severe water deficit stress (i.e., irrigation after 150 mm evaporation) increased chlorophyll *a* content by 6.2% and chlorophyll *b* content by 19.2%, compared with control (Fig. 1). On the other hand, soluble sugars content, proline accumulation, and antioxidant enzymes activity increased with increasing water deficit stress. When GABA at 1.0 or 2.0 mg L^{-1} was applied, soluble sugars content increased by an average of 14.5 and 19.9% over irrigation regimes compared with control (no GABA application) (Fig. 1). The same trends were observed with proline content and CAT activity when GABA at 1.0 or 2.0 mg L^{-1} was applied. Irrespective of irrigation regimes, application of GABA (mainly at 1.0 or 2.0 mg L^{-1}) increased chlorophyll *a* and chlorophyll *b* content, soluble sugars comtent, and CAT activity, compared with control (no GABA application). The maximum values were obtained when GABA at 2 mg L^{-1} was applied. Under severe water deficit stress, in comparison with control, application of 2 mg L^{-1} GABA increased soluble sugars content and CAT activity by 14.1 and 10.9%, respectively (Fig. 1).

Chlorophyll *a* and chlorophyll *b* content showed a negative correlation with CAT, POX, or SOD activity (Figs. 2 and 3). However, soluble sugars showed a positive correlation with CAT ($r = 0.962$, $P < 0.01$), POX ($r = 0.890$, $P < 0.01$), and SOD ($r = 0.909$, $P < 0.01$) activity (Fig. 4). A similar response was found between proline content and antioxidant enzymes activity (CAT: $r = 0.957$, POX: $r = 0.915$, SOD: $r = 0.946$) (Fig. 5).

4. Discussion

This study provides novel evidence on the role of gamma-aminobutyric acid (GABA) in alleviating water deficit stress in black cumin, for which experimental data are scarce in the literature. Although black cumin is cultivated in arid and semi-arid regions in Iran and elsewhere (D'Antuono et al., 2002; Seyyedi et al., 2015, 2016), reductions in plant height, follicle number, seed weight, and finally seed yield suggest that black cumin is susceptible to water deficit stress. In other words, although black cumin can adjust to harsh conditions in arid and semi-arid regions, water deficit stress can largely reduce growth and seed production in this plant, i.e., the adjustment or tolerance of the plant to unfavorable conditions is at the detriment of the plant, in terms of overall productivity. This finding is in line with previous reports with respect to black cumin growth and yield under adverse conditions (Ghamarnia et al., 2010; Ghamarnia and Jalili, 2013; Haj Seyed Hadi et al., 2016). Although seed yield decreased due to water deficit stress, the essential oil percentage significantly increased. Increase in essential oil percentage on account of water deficit stress might be considered as

Table 5
Effects of irrigation regimes and GABA application on chlorophyll a, chlorophyll *b*, soluble sugars, proline content, and antioxidant enzymes activity of black cumin.

Treatment	df	Chlorophyll *a*	Chlorophyll *b*	Soluble sugars content	Proline	CAT	POX	SOD
Year (Y)	1	NS	NS	NS	NS	NS	NS	NS
Irrigation (I)	2	**	**	**	**	**	**	**
Y × I	2	NS	NS	NS	NS	NS	NS	NS
GABA (G)	3	**	**	**	**	**	NS	NS
Y × G	3	NS	NS	NS	NS	NS	NS	NS
I × G	6	*	*	*	NS	*	NS	NS
Y × I × G	6	NS	NS	*	NS	NS	NS	NS

CAT: catalase, POX: peroxidase, SOD: superoxide dismutase.
*Significant at $P < 0.05$.
** Significant at $P < 0.01$.
NS: non-significant.

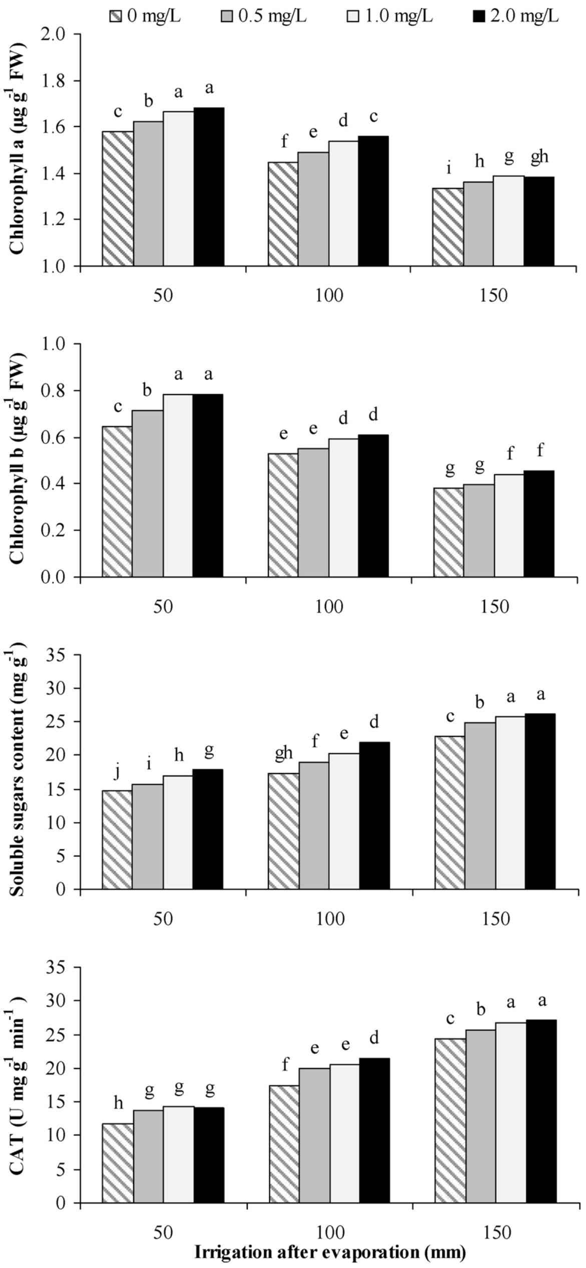

Fig. 1. Interaction effects of irrigation regimes (based on evaporation from class A pan) and GABA application on chlorophyll *a*, chlorophyll *b*, soluble sugars content, and catalase (CAT) activity in black cumin. Values followed by the same letter are not significantly different at $P < 0.05$ (DMRT).

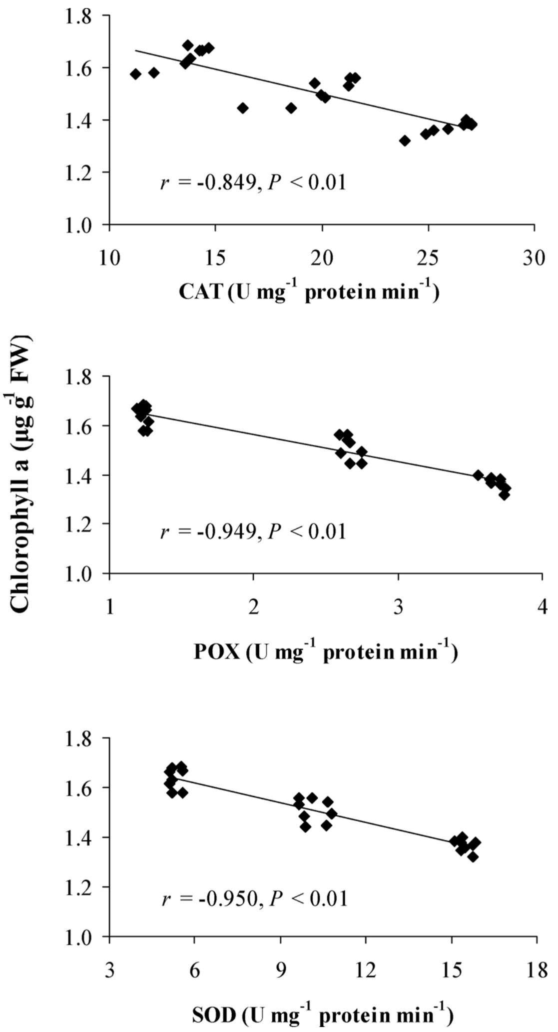

Fig. 2. Correlation between chlorophyll *a* with CAT: catalase, POX: peroxidase, and SOD: superoxide dismutase.

an adaptation mechanism of black cumin to drought conditions. It appears that increase in essential oil synthesis during seed filling improves plant tolerance against water deficit stress and probably the relevant mechanisms that are involved in assimilates remobilization increase. The increase in essential oil could be attributed to a decrease in cell size and general reduction in plant mass, which at constant oil production rate caused an increase in oil concentration. A similar response of essential oil percentage of black cumin to irrigation intervals has been reported (Mozaffari et al., 2000).

Increasing water deficit stress caused a significant reduction in chlorophyll *a* and chlorophyll *b*, but GABA application significantly improved these traits. The maximum values were obtained with the application of GABA at 2.0 mg L^{-1}. Foliage applied osmoprotectants improved the stay green trait of wheat plants under drought stress, which was visible through improvement in chlorophyll contents (Wang et al., 2009). The same response was also observed in the current study. This better stay green trait might be due to activation of enzymes, protection of the machinery of photosynthesis, and metabolic regulation during the drought stress (Farooq et al., 2017). Plant species possessing a better stay green character have a better grain filling and can cope with the drought stress conditions (Nawaz et al., 2013). It appears that this was the case also for black cumin examined in this study. Moreover, better translocation of photo-assimilates from leaves to seed as a result of longer duration of photosynthesis due to a better stay green trait under stress might have resulted in better grain yield under drought stress conditions (Khakwani et al., 2011). The exact role of GABA involvement in increasing plant growth and development is not clearly known. However, increase in plant height, follicle number, seed and essential oil yield in black cumin on account of GABA application might be due to its properties in stimulating growth and remobilizing assimilates to the seeds. Increase in seed number per plant due to GABA application at 2 mg L^{-1} has been reported in bitter gourd (*Momordica charantia* L.) (Ashrafuzzaman et al., 2010) and similar trends have been found in soybean (Islam et al., 2010) and sesame (Zubair et al., 2010).

Generally, increase in respiration, reduction in photosynthesis, and cell membrane damage are known as the main results of water deficit stress in higher plants (Gamble and Burke, 1984; Türkan et al., 2005; Wang et al., 2009). In addition, ROS generation due to water shortage reduces chlorophyll and carotenoids content (Kiani et al., 2008; Chéour et al., 2014). According to the results of this study, the reduction in

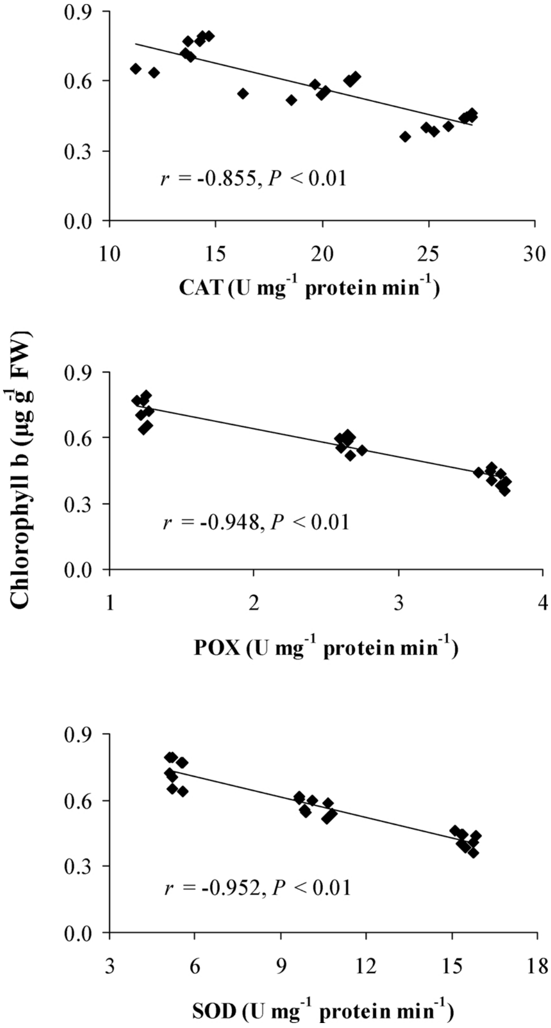

Fig. 3. Correlation between chlorophyll *b* and CAT: catalase, POX: peroxidase, and SOD: superoxide dismutase.

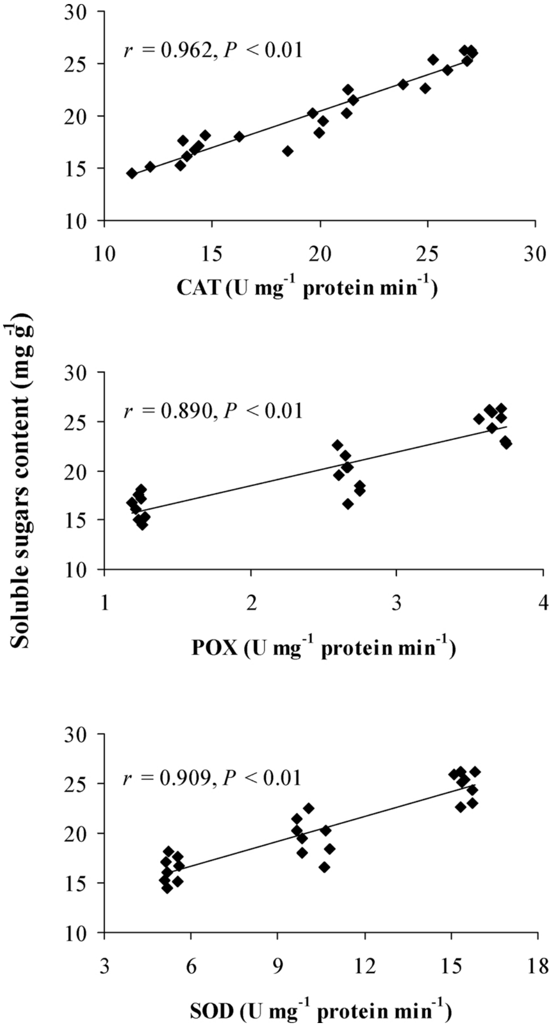

Fig. 4. Correlation between soluble sugars content and CAT: catalase, POX: peroxidase, and SOD: superoxide dismutase.

chlorophyll *a* and chlorophyll *b* content in black cumin could be attributed to the negative effects of water deficit stress. Reduction of chlorophyll content is a common consequence of water deficit stress in sunflower (Ebrahimian and Bybordi, 2012). Besides, low water availability limits water uptake by the plants. In addition, salt accumulation around the root area leads to osmotic stress and ions toxicity, which increase the risk of injury to plants (Ebrahimian and Bybordi, 2011; Martínez et al., 2007). It seems that an increase in osmolytes level, such as soluble sugars and proline, is a mechanism in black cumin for balancing water potential in the plant under water deficit stress. Increase of soluble sugars content as a mechanism of adjustment of the osmotic potential may occur under water deficit stress (Ebrahimian and Bybordi, 2012). In general, it can be concluded that osmotic adjustment through accumulating sugars or amino acids plays a vital role in the resistance or tolerance of plants under water deficit stress conditions. In addition to soluble sugars, amino acids, such as proline, have a substantial effect on drought resistance or tolerance in plants. Proline is among the most abundant amino acids acting as a compatible solute under stressful conditions (Hayat et al., 2012). Therefore, it is not surprising that proline accumulation would increase under water deficit stress conditions. Moreover, it has been reported that although thyme (*Thymus vulgaris* L.) growth decreased with increasing water deficit stress, thymol and proline accumulation significantly increased (Babaee et al., 2010), which is in line with findings of this study for black cumin. It should be noted, however, that the increase in soluble sugars and proline may be a manifestation of water stress and not a mechanism of protection against further water stress. Thus, without any measurements of plant water relations, the importance of soluble sugars and proline to osmoregulation cannot be definitely determined by this study.

Increase of proline accumulation and CAT and POX activity in black cumin under water deficit stress has been reported (Ahmadpour Dehkordi and Balouchi, 2012). On the other hand, considering the negative correlation between CAT, POX, or SOD activity and chlorophyll content, an increase in antioxidant enzymes activity may be considered as a mechanism for retaining the efficiency of the photosynthesis under water deficit stress conditions. In addition, the positive correlations between antioxidant enzymes activity and soluble sugars or proline accumulation suggest that antioxidant enzymes activity and osmotic substances act on the target to minimize the adverse effects of oxidative stress caused by water deficit stress.

Exogenous application of GABA improved the response of black cumin to water deficit stress, but the exact role of GABA in increasing chlorophyll, soluble sugars, and proline as well as CAT activity remains unknown in this plant. Based on the findings of this study, it appears that foliar application of GABA improves black cumin tolerance to water deficit stress through increasing water absorption, chlorophyll content, and efficiency of the photosynthesis. The beneficial effects of GABA application on plant response to water deficit stress have been reported also in other crops under different growth conditions. For

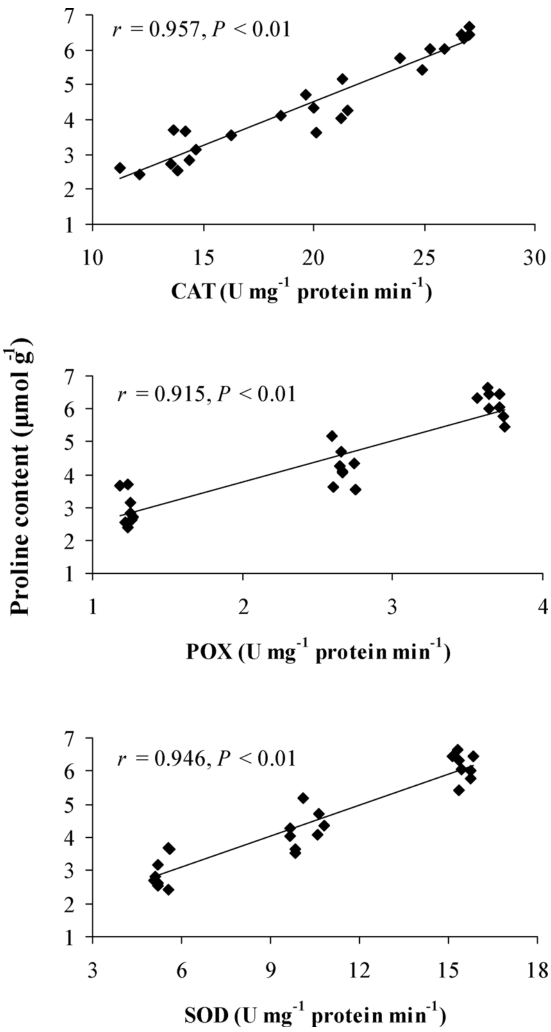

Fig. 5. Correlation between proline and CAT: catalase, POX: peroxidase, and SOD: superoxide dismutase.

example, Wang et al. (2009) found that GABA could improve wheat tolerance to heat stress through scavenging ROS with antioxidant enzymes, enhancing membrane stability, and adjusting nitrogen as well as carbon remobilization. Furthermore, Li et al. (2016b) reported that under heat stress conditions, GABA application could increase sugars, amino acids, and organic acids accumulation in creeping bentgrass (*Agrostis stolonifera* L.). Increase in seedling growth and net photosynthesis on account of GABA application has been reported in maize (Li et al., 2016a). In summary, the findings of the current study keep in line with literature reports, confirming that exogenous application of GABA could be considered as a potential managerial practice for maintaining black cumin growth and productivity under drought conditions.

5. Conclusion

According to the results of this study, reduced growth and development as well as disruption in photosynthesis are the main negative consequences of water deficit stress on black cumin. Furthermore, the results suggest that soluble sugars, proline, and antioxidant enzymes possibly play a role in the tolerance of black cumin to water deficit. Moreover, we found that foliar application of GABA could reduce the adverse effects of water deficit stress on black cumin, which was reflected in increased seed yield. At the farm level, foliar application of GABA could possibly be a cost-effective and value for money option for growing black cumin successfully under limited water supply. Nevertheless, further research is still required to unravel the involvement of GABA-mediated regulations in plant growth and its associated biochemical mechanisms at molecular and genetic levels.

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