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Original Article

Quantification of Individual and Interactive Effects of Some Antioxidants on Drought Tolerance in *Cuminum cyminum* L.

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ABSTRACT

The growth and metabolism of plants are significantly affected by primary abiotic stresses such as heat and drought. The interplay between antioxidants and plant drought tolerance is documented excellently. However, conventional techniques, including correlation analysis, are insufficient for elucidating the relationship between antioxidants and biomass content. This study aimed to assess the individual and interactive impacts of six antioxidants on cumin biomass content through non-linear multiple regression analysis. Field experiments were carried out at two sites in Iran in 2020. The experimental treatments included a factorial split-plot arrangement of magnetic water (conventional and magnetized waters), deficit irrigation at 100%, 70%, and 40% of water requirement, and superabsorbent at 0, 100, and 200 kg/ha, resulting in diverse trait variations. The findings indicate the harmful effects of superoxide dismutase (SOD) and catalase (CAT) on biomass content. However, anthocyanins partially mitigated their adverse impacts, showing a synergistic promotion of biomass content when combined with anthocyanins, SOD, and CAT. Carotenoids did not individually favor biomass content; however, their interactions with SOD and anthocyanins prompted biomass content. Regarding influencing biomass content, the antioxidants demonstrated the following impact in descending order: peroxidase (POD), anthocyanins, carotenoids, proline, superoxide dismutase (SOD), and catalase (CAT). The estimated optimal activities or concentrations of antioxidants, derived from maximizing the regression function, resulted in a 12% increase in biomass content, offering potential applications in breeding programs.

INTRODUCTION

Cumin (*Cuminum cyminum* L.) is widely esteemed as a medicinal seed spice [1], prized for its potent and distinctive aroma, and is extensively utilized as a culinary spice and seasoning in diverse cultural cuisines worldwide [2]. Its seed oil finds widespread application in cooking soups, stews, meats, sausages, pickles, chutneys, cheeses, and curries. This plant is notably acknowledged for its therapeutic properties, encompassing antifungal, antibacterial, antioxidant, lipid-lowering, antidiabetic, immunomodulatory, anti-osteoporotic, anti-asthmatic, anti-inflammatory, antitussive, anti-cancer, anti-sterility, anti-stress, antipyretic, antihypertensive, anti-platelet coagulation, and analgesic effects [3, 4]. Water scarcity poses a significant abiotic stress to global agriculture, including Iran. If this stress persists for a certain time, reactive oxygen species (ROS) are excessively produced, causing oxidative damage to plants. ROS includes free radicals such as superoxide ($O_2^{\bullet-}$) and hydroxyl ($OH^{\bullet}$), and non-radicals like singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2) [5]. Plants possess effective enzymatic and non-enzymatic antioxidants to shield themselves from ROS. Enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) work alongside non-enzymatic antioxidants such as plastoquinone/ubiquinone, ascorbic acid, reduced glutathione, flavonoids, α-tocopherol, and carotenoids to mitigate oxidative damage.

In the context of ROS scavenging, there are both commonalities and differences among antioxidants. For instance, $O_2^{\bullet-}$ is scavenged through the participation of flavonoids, ascorbate, and SOD [6]. CAT, APX, guaiacol peroxidase, peroxiredoxins, glutathione, and ascorbate catalyze the breakdown of H_2O_2 [7]. Singlet oxygen is scavenged by α-tocopherol and carotenoids. While CAT demonstrates a rapid turnover rate, its affinity for

H_2O_2 is considerably lower than that of APX and peroxiredoxin, characterized by KM values below 100 μM [8]. In addition to these similarities and differences between antioxidants, it is interesting to note that a decrease in the activity of one antioxidant can be compensated by an increase in the activity of another; this finding was derived from a comparison between mutant (catalase-deficient) and wild-type varieties for peroxidase activity [9]. Hydroxyl radical ($OH^•$) is scavenged by proline [6]. The conversion of $O_2^{•-}$ into O_2 and H_2O_2 is catalyzed by SOD; therefore, this enzyme (indirectly) diminishes the formation of $OH^•$ through the Haber–Weiss reaction ($O_2^{•-} + H_2O_2 \rightarrow OH^- + OH^• + O_2$) [10].

The mentioned issues prove the existence of complex inter- and intra-relations between antioxidants and plant drought tolerance (biomass; biomass content); this is also confirmed by the existence of a non-linear (parabolic) relationship between POD and biomass content in recent studies [11]. It is clear that the commonly used data mining techniques, including correlation analysis and path coefficient analysis, are insufficient to describe the relationship between antioxidants and biomass content; these techniques are only appropriate for describing non-interactive and linear relationships between variables. The present field experiments, conducted at two sites, aimed to quantify the individual and interactive effects of six enzymatic and non-enzymatic antioxidants on cumin biomass content using a non-linear multiple regression. The regression function was also maximized (optimized) to find the best activities/concentrations of antioxidants for higher biomass content. These findings can potentially inform the development of breeding programs aimed at enhancing cumin biomass content.

MATERIALS AND METHODS

Field experiments were conducted in a randomized complete block design with three replicates at two sites in Rahanjan (36.2564°N, 54.7649°E) and Garman (36.6107° N, 55.0268° E), Iran, in 2020. The cumin seeds utilized in the experiments were procured from Mahan Mehr Daris Company, IRAN. The plant materials employed in the study had received approval from the Department of Botany, Shahrood Faculty of Agriculture. The experimental treatments consisted of a factorial split plot design, with magnetic water (conventional water as control and magnetically treated water) and deficit irrigation (100% as control, 70%, and 40% of the plant's water requirements) as the main plot factors, and superabsorbent application (0, 100, and 200 kg/ha) as the subplot factors.

CROPWAT 8.0 software, developed by FAO [12], was utilized to determine irrigation schedules for the specified levels of deficit irrigation, using climate parameters obtained from the nearest weather stations. The superabsorbent polymer, Stockosorb, characterized by a pH of 7.7, density of 650 g/l, CEC of 400 meq 100/g, and durability of 5 years, was sourced from Balure-Ab Production Company in Iran. During sowing, the designated quantities of superabsorbent were applied in soil strips at a depth of 5 cm around the plants.

Table 1 Geographical and climatic characteristics of experiment locations.

Region	Elevation	Longitude	latitude	Annual Temperate (ºC)	Maximum temperature (ºC)	Minimum temperature (ºC)	EC (Ds/m)	Climate
Rahanjan	1125	54.46.17	36.15.41	13.1	38	-11.8	3.62	Cold temperate
Garman	1434	55.3.38.	36.37.11.3	15.2	27	3.1	2.21	Temperate

Table 2 Physical and chemical soil properties.

location	Clay %	Sand %	Silt %	pH	EC (dS/m)	O.C	T.N.V	P (ppm)	K (ppm)
Rahanjan	20	18	62	7.61	3.62	0.54	16.21	5.03	379
Garman	28	19	53	7.83	2.21	0.81	30.22	3.36	483

Table 3 The chemical composition of water before and after crossing the magnetic field.

location	Treatment	Ec(dS/m)	pH	Na	Mg+Ca	Totalcations	Cl	HCO3	CO3	Totalanions
	Normal	0.99	8.15	6.95	4.8	20.98	3.76	2.85	0.28	6.89
Rahanjan	Magnetized	0.99	7.8	6.36	4.96	19.45	2.97	2.33	0.27	5.57
Garman	Normal	3.38	8.32	24.33	11.28	47.31	24.68	3.47	0.38	28.53
	Magnetized	3.21	7.99	22.27	11.65	43.5	19.49	2.20	0.36	22.05

Equal amounts of conventional water were provided to the plants until they reached the two-leaf stage. Afterward, they were subjected to the prescribed levels of deficit irrigation and magnetized water. At the grain dough stage, plant and leaf samples (fully developed upper leaves) were collected to measure the oven-dried biomass of the plant and the following antioxidants, respectively.

Carotenoids Assay

The method developed was applied to measure the non-enzymatic antioxidant concentration by Arnon [13]. First, 0.25 grams of freshly collected leaves were ground and placed in a falcon tube containing 80% acetone. The tissue was then fully bleached on a shaker at 25 °C. Finally, absorbance readings were recorded at 470 nm, 645 nm, and 663 nm using a UV-VIS spectrometer S2000. The carotenoid content was determined using the equations provided by Arnon [13].

Anthocyanins Assay

Anthocyanin concentration was measured following the method described by Mita *et al*. [14]. A fresh leaf sample of 20 mg was homogenized with 3 ml of 1% (v/v) hydrochloric acid in methanol, and the extract was kept in the dark at 4 °C for 24 hours. After centrifugation of the mixture at 10,000 g for 15 minutes, the absorbance of the supernatant was recorded at 530 nm and 657 nm. One anthocyanin unit is equivalent to one absorbance unit [A530 - (0.25 × A657)] per ml of extract.

Superoxide Dismutase (SOD) Assay

SOD determination proceeded following the method described by Beauchamp and Fridovich [15]. This method utilizes riboflavin and methionine to produce superoxide in the presence of light. The reduction of nitroblue tetrazolium and the formation of purple formazan are triggered by superoxide. The reaction mixture was incubated under three fluorescent lamps (15 W) for 10 minutes to initiate the reaction. Ultimately, the absorbance was measured at 560 nm.

Proline Assay

The ninhydrin method [16], was employed to quantify the proline content in the leaves. Initially, 4 ml of 3% sulfuric acid was introduced to 0.05 g of fresh leaf tissue. After centrifugation, the resulting supernatant was combined with 2 ml of glacial acetic acid and 2 ml of ninhydrin solution. The mixture underwent heating at 90 °C for 30 minutes. Following this, 4 ml of toluene was added to each cooled tube to extract the formed chromophore. After removing the supernatant, the spectrophotometric absorbance was measured at 520 nm. Proline content was determined using a standard curve prepared with proline concentrations of 1, 2, 3, 4, 5, and 6 μg/ml.

Catalase (CAT) Assay

The activity of CAT was quantified using the spectrophotometric method proposed by Aebi [17]. This method is based on the disappearance of hydrogen peroxide. First, 2.5 ml of potassium phosphate buffer (pH = 7) and 0.3 ml of hydrogen peroxide 3% percent were mixed in an ice bath and then immediately added to 0.2 ml of the enzyme extract. The change in absorbance at 240 nm was recorded for 60 seconds. Enzyme activity was determined by calculating the amount of hydrogen peroxide decomposed.

Peroxidase (POD) Assay

The activity of POD was determined using the method proposed by Maehly and Chance [18]. This method is based on POD-catalyzed oxidation of pyrogallol to purpurogallin substance. The three ml reaction mixture consisted of 0.1 ml enzyme extract, 50 mM potassium phosphate buffer (pH=7), 40 mM H2O2, and 20 mM guaiacol. The reaction was initiated by the addition of the enzyme extract. For both the sample and blank, the absorbance was taken at 470 nm for 5 minutes.

The SAS software was used to perform a non-linear multiple regression analysis to determine the relative contributions of six antioxidants (CAT, SOD, POD, anthocyanins, proline, and carotenoids) as independent variables to the biomass (biomass content) as the dependent variable. The dataset had one dependent variable and 53 regressors, including the 6 above-mentioned antioxidants (main regressors) and their combinations. Part of these combinations included double, triple, quadruple, quintuple, and sextuple interactions between the named six regressors; the rest consisted of the 1st, 2nd, and 3rd power of each of the mentioned 6 regressors. The 1st, 2nd, and 3rd powers were applied to quantify the effects of low, medium, and high concentrations/activities of each regressor on biomass content, respectively; for better understanding, these effects were described graphically and mathematically in Fig. 1, using hypothetical data.

In this investigation, data related to the supply of 40% of the water required by the plant was used for data analysis; because we intended to quantify the effects of regressors on biomass content under very severe water scarcity conditions. All data have been normalized to facilitate comparison of the regressors based on their respective contribution values in determining biomass content. The stepwise selection was applied to remove regressors that do not contribute to determining biomass content. The regression function was then optimized to obtain the best combination of the tested regressors to achieve the maximum possible biomass content. Optimization was performed by the MATLAB software according to the constraints which included the minimum and maximum observed values of the regressors.

RESULTS

Biomass Content

Table 4 shows the significant impact of all treatments on the studied traits of Cumin. The FC 75% with ordinary water and unused SA application resulted in the highest biomass content (17.55 gr/plant). Conversely, the lowest biomass content at 7.41 gr /plant was observed of FC 100 % + magnetic water, along with control SA application (SA non-utilization) (Fig. 1).

Prolin Content

According to a meane comparison, the triple interaction of water requirement × magnetic water × superabsorbent had significant effects on proline content. The highest concentration of proline at 3.99 µmol/mg was observed in the control treatment and the lowest concentration at 2.52 µmol/mg in the treatment of 200 kg/ha of super absorbent, showed a decrease of 36.84% compared to the control treatment (Fig. 2).

Some of the descriptive statistical properties of the studied traits are given in Table 7. The largest and second-largest range of changes were obtained at SOD 464% and POD 461%, respectively; thus, the synthesis of these antioxidants was strongly stimulated in response to water scarcity.

Table 4 Results of analysis of variance (mean squares) of Biomass and antioxidant characteristics of Qumin in two location

Sours	DF	Biomass	Proline	Carotenoids	Anthocyanins	SOD	CAT	POD
Location (L)	1	1.27 ns	25.05 **	0.0004 ns	0.0001 **	0.001 ns	0.0005 *	0.003 **
Error	4	0.35	0.06	0.0005	0.000004	0.0004	0.0001	0.0001
Deficit Irrigation (DI)	2	24.22 *	0.37 *	0.005 **	0.0001 **	0.001 ns	0.01 **	0.005 **
Magnetic Water (MW)	1	10.87 *	0.43 *	0.01**	0.0001 **	0.0005 ns	0.03 **	0.001 **
DI * MW	2	4.86 ns	2.77 **	0.005 **	0.0003 **	0.0008 ns	0.0008 **	0.002 *
DI * L	2	3.91 ns	0.09 ns	0.0002 ns	0.00003 **	0.0007 ns	0.000009 ns	0.0001 ns
MW * L	1	0.02 ns	0.02 ns	0.002 *	0.00001 ns	0.00001 ns	0.001 **	0.0002 *
DI * MW*L	2	8.33 *	1.59 **	0.001 ns	0.00001 ns	0.001 ns	0.0001 ns	0.0001 ns
Main Error	20	4.73	0.05	0.0006	0.000009	0.0007	0.00008	0.00004
Super Absorbent (SA)	2	1.00 ns	1.33**	0.003 **	0.0001 **	0.001 ns	0.001 **	0.0007 **
SA*L	2	0.85 ns	0.01ns	0.0002 ns	0.00001 ns	0.0007 ns	0.0002 *	0.000004 ns
DI * SA	4	7.86 **	0.72 **	0.007 **	0.00005 **	0.001 ns	0.007 **	0.0009 **
DI * SA*L	4	0.47 ns	0.31 **	0.0002 ns	0.00002 **	0.001 ns	0.001 **	0.0001 *
MW *SA	2	72.17 **	5.97 **	0.02 **	0.0001**	0.0004 ns	0.0005 **	0.002 **
SA * MW*L	2	3.04 ns	1.09 **	0.0001 ns	0.000003 ns	0.0007 ns	0.0001 ns	0.00003 ns
DI * MW*SA	4	34.86 **	1.96 **	0.03 **	0.0002 **	0.001 ns	0.005 **	0.001 **
L*DI*MW*SA	4	4.71 ns	0.09 ns	0.003 **	0.00001 *	0.0006 ns	0.001 **	0.0004 **
Residual Error	48	1.94	0.07	0.0005	0.000005	0.0007	0.00008	0.00005
CV%		8.24	8.71	9.98	0.0003	47.99	11.77	9.87

* and ** Significantly at the probability level of %5 and %1, respectively. ns= Not significantly

Table 5 Interaction mean comparison of Location × water requirement × Magnetic water × Superabsorbent on cumin characters in the Rahanjan location

water requirement	water	Super absorbent (kg/ha)	Carotenoid (mg/g FW)	Anthocyanin (µmol/g FW)	Peroxidase (U/g FW)	Catalase (µMol/g FW. min)
100% FC	Ordinary	control	021 b	0.034 a	0.084 b	0.068 a
		100	0.32 a	0.037 a	0.103 a	0.049 b
		200	0.2 1 b	0.035 a	0.088 ab	0.050 b
	Magnetic	control	0.29 a	0.046 a	0.091 a	0.101 a
		100	0.2 0 b	0.046 a	0.08 a	0.122 a
		200	0.2 6 a	0.047 a	0.093 a	0.05 b
75% FC	Ordinary	control	0.13 b	0.031 c	0.058 c	0.022 c
		100	0.26 a	0.047 a	0.110 a	0.051 b
		200	0.2 a	0.037 b	0.081 b	0.044 b
	Magnetic	control	0.29 a	0.047 a	0.108 a	0.079 a
		100	0.28 a	0.037 b	0.080 a	0.105 a
		200	0.18 b	0.029 c	0.04 b	0.064 a
50% FC	Ordinary	control	0.27 a	0.046 a	0.094 a	0.125 a
		100	0.21 b	0.043 a	0.088 ab	0.052 b
		200	0.21 b	0.041 b	0.084 b	0.051 b
	Magnetic	control	0.20 b	0.041 b	0.058 c	0.135 a
		100	0.22 b	0.045 a	0.067 b	0.044 c
		200	0.27 a	0.040 b	0.086 a	0.190 a

Means followed by the same letter in a column are not significantly different according to LSD test (P<0.0)

Table 6 Interaction mean comparison of Location × Water requirement × Magnetic water × Superabsorbent on cumin characters in the Garman location.

water requirement	Water	Super absorbent (kg/ha)	Carotenoid (mg/g FW)	Anthocyanin (µmol/g FW)	Peroxidase (U/g FW)	Catalase (µMol/g FW. min)
100% FC	Ordinary	control	0.22 ab	0.033 a	0.073 b	0.071 a
		100	0.27 a	0.040 a	0.088 a	0.066 a
		200	0.21 b	0.035 a	0.066 b	0.053 b
	Magnetic	control	0.29 a	0.045 a	0.074 a	0.074 b
		100	0.27 a	0.04 1b	0.072 a	0.083 a
		200	0.30 a	0.044 a	0.068 a	0.072 b
75% FC	Ordinary	control	0.18 c	0.028 c	0.049 b	0.024 b
		100	0.29 a	0.048 a	0.099 a	0.044 a
		200	0.23 b	0.039 b	0.063 b	0.043 a
	Magnetic	control	0.34 a	0.038 a	0.089 a	0.077 b
		100	0.26 b	0.037 a	0.064 b	0.090 a
		200	0.18 c	0.029 b	0.061 b	0.059 c
50% FC	Ordinary	control	0.26 a	0.043 a	0.073 a	0.097 a
		100	0.22 b	0.037 b	0.073 a	0.068 b
		200	0.21 b	0.034 b	0.075 a	0.064 b
	Magnetic	control	0.23 b	0.035 a	0.069 b	0.125 a
		100	0.20 b	0.039 a	0.060 c	0.074 b
		200	0.27 a	0.038 a	0.074 a	0.135 a

Means followed by the same letter in a column are not significantly different according to LSD test ($P<0.05$).

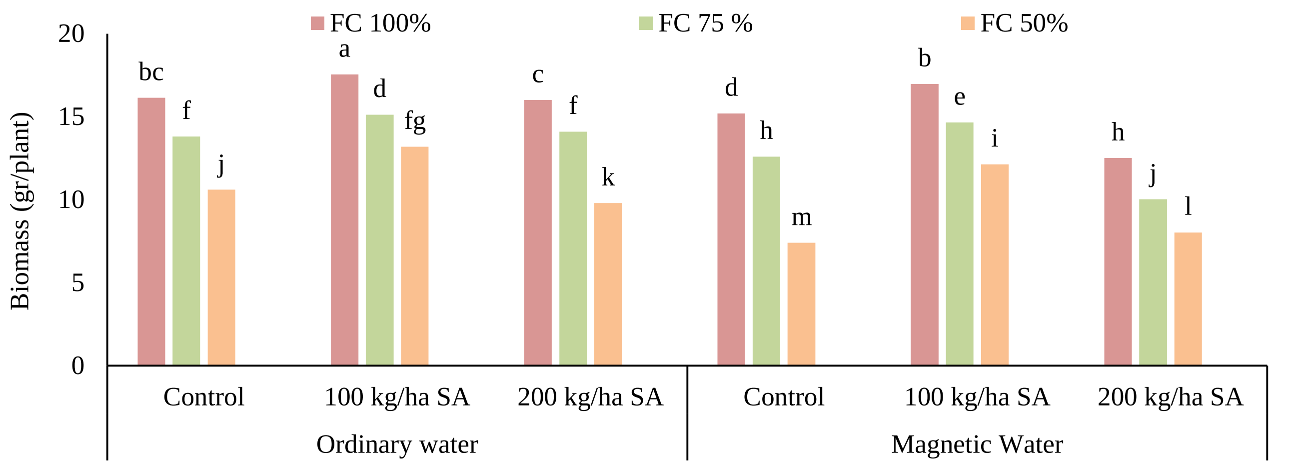

Fig. 1 Interaction mean comparison of water requirement × Magnetic water × Superabsorbent on biomass content of cumin

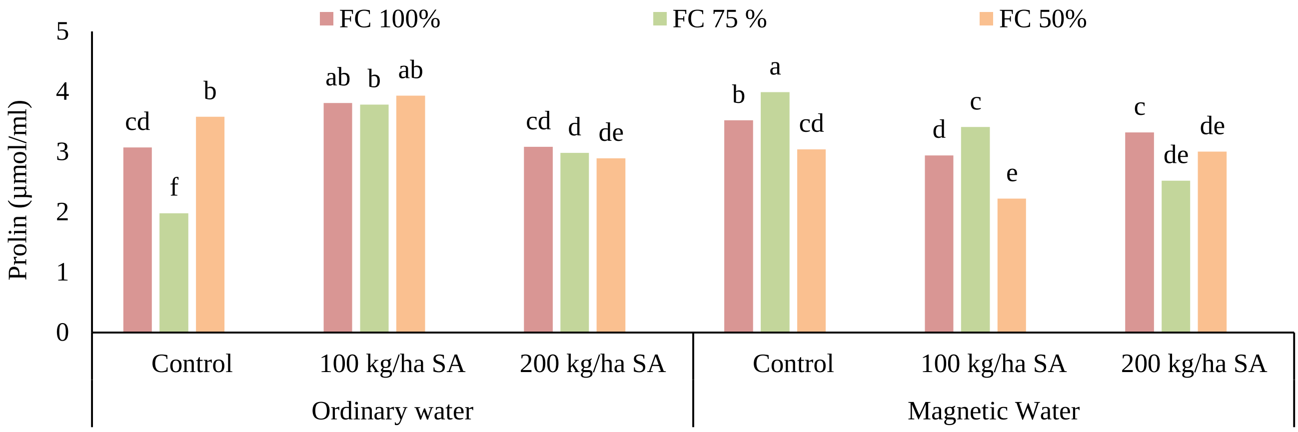

Fig. 2 Interaction mean comparison of water requirement × Magnetic water × Superabsorbent on prolin content of cumin

Table 7 The descriptive statistical characteristics of the independent variables and biomass (drought tolerance indicator; biomass content) were examined under severe deficit irrigation conditions (providing 40% of the plant's water requirement), as well as the optimized value of regressors after maximizing the regression function for higher biomass content.

Statistical properties	Biomass (g /plant)	Proline (mg/g FW)	Carotenoids (mg/g FW)	Anthocyanins (µmol /g FW)	SOD (µmol /g FW)	CAT (µmol /g FW /min)	POD (U/g FW)
Maximum	17.557	4.870	0.323	0.048	0.205	0.098	0.227
Minimum	7.415	1.947	0.183	0.032	0.036	0.053	0.040
Range	10.142	2.923	0.140	0.016	0.168	0.045	0.187
Percent	136.787	150.171	76.364	50.678	463.636	85.795	461.120
Average	12.323	3.117	0.235	0.041	0.058	0.076	0.097
Optimized	19.141	3.661	0.192	0.044	0.039	0.077	0.194

Anthocyanins showed the lowest range of change, at 51%. Aboveground biomass, taken here as an indicator of biomass content, ranged from 7.41 to 17.56 g per plant, representing a 137% variation.

A stepwise selection from 53 regressors resulted in the finding of 17 effective regressors, listed in Table 8. The general effects, shown as "Model" in Table 8, and the individual and interactive effects of these 17 regressors on biomass content were highly significant. The medium and higher SOD activities (SOD2 and SOD3, respectively; the relative effects shown in Table 8) resulted in decreased biomass content; in other words, over a range of medium and higher SOD activities, biomass content showed a decreasing trend with increasing SOD activity.

Table 8 Analysis of variance results and the effect of regressors on drought tolerance (biomass value; biomass content) of cumin.

Source of Variation	DF	Sum of Squares	Mean Square	F Value	Pr>F
Model	17	2.278	1.3E-01	4730	<0.01
Error	90	0.003	2.8E-05		
Corrected Total	107	2.280			
#Regressor	*Regressor Effect	Standar Error	@Relative Effect	F Value	Pr>F
Intercept	0.631	0.235	0.631	7.2	<0.01
Anthocyanins	1.604	0.179	1.604	80.4	<0.01
CAT	-3.024	0.778	-3.024	15.1	<0.01
CAT^3	-2.468	0.425	-1.351	33.8	<0.01
$Proline^2$	0.721	0.004	0.849	31.2	<0.01
SOD^2	-0.473	0.125	-0.688	14.4	<0.01
SOD^3	-0.523	0.048	-0.806	12.0	<0.01
POD^2	5.241	0.972	2.289	29.1	<0.01
POD^3	-0.117	0.015	-0.489	58.6	<0.01
$Carotenoids^3$	-0.217	0.050	-0.601	18.6	<0.01
Proline_POD	-0.201	0.050	-0.448	16.2	<0.01
Carotenoids_SOD	0.849	0.190	0.921	20.1	<0.01
Anthocyanins_SOD	-2.495	0.301	-1.580	68.9	<0.01
Anthocyanins_CAT	-1.547	0.164	-1.244	89.3	<0.01
Proline_Carotenoids	-0.479	0.099	-0.692	23.3	<0.01
Anthocyanins_SOD_CAT	2.646	0.284	1.383	86.6	<0.01
Carotenoids_Anthocyanins_POD	0.302	0.071	0.671	18.4	<0.01
Proline_Carotenoids_Anthocyanins	0.739	0.101	0.904	53.2	<0.01

#: The regressor to the power of two (e.g. CAT2) is related to the effect of medium concentrations/activities of the regressor on biomass; the regressor to the power of three (e.g. CAT3) is related to the impact of high concentrations/activities of the regressor on biomass. Further mathematical and graphical descriptions are presented in Fig. 3.

*: It is the same as the parameter estimate in the SAS software output.

@: For comparison purposes, the square root of the regressor2 effect, and the dual interactive influences (absolute value) were calculated; regarding the regressor3 effect and the triple interactive effects, the cube root of the values was presented and used in the text.

Interestingly, the decreasing effect of higher activities of SOD on biomass content was slightly lower compared to medium activities. The low level of SOD activities (SOD to the power of one) did not significantly affect biomass content, as it was removed from the model during the stepwise selection process. In terms of promoting biomass content, the results showed a significant superiority at 2.289 of medium activities of POD over all other regressors. Higher values of POD activities negatively affected biomass content, while its lower activities had no significant impact on biomass content.

Lower and higher values of CAT activity appeared to have a decreasing effect on biomass content; however, the diminishing effect of the higher values was less than twice that of the lower values. The impact of medium values of this enzyme was statistically negligible and was thus removed from the final model. Biomass content was favored linearly by anthocyanins, as the effect of 2nd and 3rd power of this antioxidant appeared to be statistically negligible. Results showed that only the medium concentrations of proline affected biomass content; its effect was 0.849, slightly lower than the average effect of the 17 regressors (averaged absolute values = 1.15). The higher concentrations of carotenoid effect on biomass content were found to be considerable at -0.601; however, the influence of lower and medium concentrations was statistically negligible. This antioxidant appeared to interact negatively with proline in affecting biomass content; on the other hand, it interacted positively with some other antioxidants (carotenoids_SOD, carotenoids_anthocyanins POD, and carotenoids_anthocyanins_proline) in promoting biomass content. Biomass content was negatively affected by anthocyanins_SOD and anthocyanins_CAT, but positively by anthocyanins_SOD and CAT interactions.

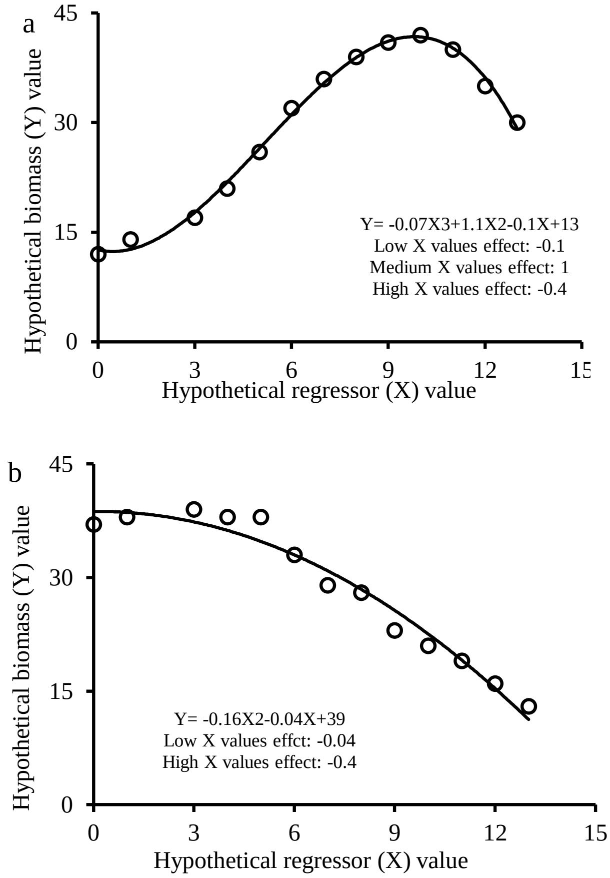

Fig. 3 The hypothetical data was used to draw these figures. The insignificant effect of low regressor values (X coefficient in the equation), significant positive effect of medium regressor values (X2 coefficient), and significant negative effect of high regressor values (X3 coefficient) on biomass (a). The insignificant impact of low regressor values (X coefficient) and the significant negative effect of high regressor values (X2 coefficient) on biomass (b). As presented in the figure, the cube root of the X3 coefficient, the square root of the X2 coefficient, and the X coefficient were used to compare the effect of high, medium, and low values of X on Y, respectively.

DISCUSSIONS

In this experiment, a wide range of changes was obtained in the value of all the examined cumin traits, grown under severe deficit irrigation conditions (supplying 40% of water requirement) (Table 4). These changes were caused by treatments (magnetic water, superabsorbent) and site-related climatic and edaphic conditions. Such a large variation is a prerequisite for a valid regression analysis result. The stepwise selection procedure was applied to remove ineffective regressors. This procedure made it possible to detect 17 regressors with a high level of significance ($P < 0.01$) of the contribution to the determination of biomass content (Table 5), and the attainment of the highest accuracy ($R2 = 0.99$). The analysis revealed that there was no significant quadruple, quintuple, or sextuple interactions among the main regressors. Only the double and triple interactions were found to have statistical significance.

Among the main regressors, only anthocyanins appeared to have a positive linear relationship with biomass content; in other words, across all observed concentrations of this antioxidant (0.032–0.048 μmol /g FW), biomass content showed a steady upward trend with increasing concentrations in plant leaves. The main reason for the linear relationship is likely to be the narrow range of change (51%) for anthocyanins compared to other main regressors (Table 1). Based on the mentioned relationship between anthocyanins and biomass content, we expected the optimal value of anthocyanins, obtained from the optimization of the mathematical function, to be equal to the maximum observed value; however, the optimal anthocyanins value was 0.044 μmol/g FW, approximately 8% lower than the maximum observed value. This was due to five different double and triple interactions of anthocyanins with other regressors (Table 5). The overall effect of anthocyanins, I.e. the sum of individual and interactive effects, on biomass content was positive (1.738); therefore, this antioxidant, with an overall impact higher than the average effects of 17 regressors, is known to be beneficial for biomass content. To date, no reports similar to our results have been published. Therefore, our results cannot be compared exactly with the results of other researchers. However, from a general perspective, our results are in agreement with several other reports regarding anthocyanins benefits, reviewed by Kaur *et al* [19]. Part of the positive effect of this antioxidant on biomass content is attributable to its role in protecting the plant from strong sunlight, especially due to the creation of a reddish color, and in controlling water loss by reducing the stomatal aperture [20].

Proline, a distinctive low molecular weight osmolyte, is known as a non-enzymatic antioxidant and osmoprotectant, protecting phospholipids, plasmalemma, mitochondria, and plastid membranes from damage caused by drought stress [21]. In this study, the range of changes in proline concentrations was nearly wide 150% but only a narrow range of medium concentrations appeared to have a slight positive effect (lower than average) on biomass content. Similarly, it had no synergistic interaction with other antioxidants. Therefore, genetic manipulation of cumin for higher proline concentrations is expected to have a slight stimulatory effect on biomass content; this is quantitatively confirmed by the low overall effect of proline on biomass content at 0.613.

The optimization results showed that the optimal concentration of proline is 3.661 mg/g FW. Proline plays a crucial role in the recovery process by relieving stress on DNA, various cell barriers, and protein complexes once drought stress is removed [22]. The weak proline effect in this study might be due to the continued water stress that was not permanently lessened or removed throughout the growing season.

Carotenoids are lipophilic antioxidants that scavenge different ROS [23]. Here, they were found to benefit biomass content (overall effect = 1.203) by activating/stimulating other regressors rather than directly affecting biomass content. Because low and medium concentrations of carotenoids did not significantly affect biomass content; furthermore, their higher concentrations had a negative effect on biomass content. They had favorable interactions with other regressors, particularly an impressive synergistic interaction with SOD (Table 3). Optimization results revealed that the positive interactions of this antioxidant peaked at a concentration of 0.192 mg/g FW. The promoting effect of carotenoids on biomass content can be attributed to their capability to scavenge lipid peroxy-radicals and singlet oxygen, thereby preventing lipid peroxidation [24]. Carotenoids are also able to quench the triplet sensitizer, thereby protecting the photosynthetic apparatus in drought-stressed plants [12].

Table 9 The antagonistic and synergistic interactions of some antioxidants affecting drought tolerance (biomass value; biomass content) of cumin.

Trait	Relative interactive effects	Sum of relative individual effects	#Interaction type	*Intensity of interaction
Proline_POD	-0.448	1.749	Antagonism	-2.197
Carotenoids_SOD	0.921	-1.348	Synergism	2.269
Anthocyanins_SOD	-1.580	0.857	Antagonism	-2.437
Anthocyanins_CAT	-1.244	-0.584	Antagonism	-0.660
Proline_Carotenoids	-0.692	0.248	Antagonism	-0.940
Anthocyanins_SOD_CAT	1.383	-1.331	Synergism	2.714
Carotenoids_Anthocyanins_POD	0.671	1.903	Antagonism	-1.232
Anthocyanins_Proline_Carotenoids	0.904	1.852	Antagonism	-0.948

#: Antagonism refers to a situation in which the combined effects of individuals are greater than the interactive effects. Conversely, synergism occurs when the interactive effects exceed the sum of the individual effects.

*: Subtraction of the relative interactive effects from the sum of the relative individual effects; a larger difference indicates a higher intensity and vice versa.

POD has been identified as a conductive antioxidant for determining the stress status in wheat [11]. The biomass content was greatly favored by moderate levels of POD activity. On the other hand, biomass content was unresponsive to lower levels of POD activity; over higher activities, biomass content was slightly decreased. A non-linear (parabolic) relation of POD with biomass content has also been reported in wheat [28]. The negative interaction of POD with proline in affecting biomass content -0.448 was completely counteracted by the interaction between POD, carotenoids, and anthocyanins 0.671. This can be attributed to the stronger antagonistic impact observed in the Proline_POD interaction compared to the POD_carotenoids_anthocyanins interaction (Table 3). Optimization results showed that the beneficial effects of POD peaked at an activity of 0.194 U/g FW. Concerning the overall impact on biomass content, POD demonstrated the most favorable result with a value of 2.267. In drought-stressed wheat, POD has also been the best as it has exhibited the highest relationship R2 = 0.79 with net photosynthesis [11]. Similarly, its importance for promoting biomass content has also been witnessed in other plants, including tomato [25] and rice [26].

The medium and higher levels of SOD activity had a diminishing effect on biomass content (Table 2). SOD also had a harmful antagonistic interaction with anthocyanins on biomass content. On the other hand, the diminishing effects of SOD were slightly alleviated by the synergistic interaction of anthocyanins_SOD_CAT and carotenoids_SOD; the magnitude of the synergy for the former interaction was approximately 20% higher than that of the latter interaction (Table 3). The overall effect of SOD was negative (-1.75), suggesting that increasing SOD activity is not consistent with promoting biomass content in cumin. This was also confirmed by the optimization results, which showed that 0.039 μmol g-1 FW activity, about 8% higher than the observed minimum activity (Table 1), was the optimal activity. Razzaq *et al*. [27] have also found that in response to water shortage, the activity of SOD remains unchanged in the drought-stressed *Daucus carota* L.; the same has been true for a drought-sensitive cultivar of *Cerasus humilis* (Bg.) Sok. [28].

Considering our results, it seems that CAT does not appear to be an effective antioxidant for enhancing biomass content in cumin. This was due to (1) biomass content was unresponsive to medium activities of CAT (Table 2), (2) biomass content exhibited strong negative reactions to both low and high levels of this enzyme, and (3) Although CAT had a positive effect on biomass content when interacting with anthocyanins and SOD (1.383), this positive effect was nearly nullified by its negative interaction with anthocyanins (-1.244). The overall effect of CAT was highly diminishing (-2.048). The optimal activity of this antioxidant was 0.077 μmol g-1 FW, which is close to medium activity (Table 1); this finding is consistent with the fact that CAT is an integral part of the defense system in metabolizing peroxides, such as H2O2 [29]. The ineffectiveness of CAT in promoting the biomass content of cumin might be due to its very low affinity for H2O2 compared to other antioxidants such as APX and peroxiredoxin [8]. Snider *et al*. Based on a study by [30], despite the increased activity of catalase (CAT) in heat-stressed cotton, it has proven ineffective in protecting cells from oxidative damage.

According to the data presented in Table 1, the Rahanjan site exhibited a higher electrical conductivity (EC) of 3.62 d/Sm in the soil compared to the Garman location, which had an EC of 2.21 dSm-1. Furthermore, it was observed that cumin cultivation at the Rahanjan site occurred at lower temperatures compared to the Garman location, as indicated in Table 1. The Rahanjan location experienced comparatively lower minimum and average annual temperatures while registering higher maximum temperatures. The findings from Canonical Correspondence Analysis (CCA) revealed that both the average and maximum temperatures exerted a significant influence on the growth characteristics of asparagus [31].

CONCLUSION

In summary, the opposing interactions observed among the antioxidants analyzed can be broadly classified into two categories:

(1) Non-intensive antagonistic interactions: The interactive effect on biomass content remained positive, even though the sum of the individual effects exceeded the interactive effects. Carotenoids-anthocyanins-POD and anthocyanins_proline_carotenoids interactions are categorized under this class (Table 3).

(2) Intensive antagonistic interactions: The interactive effect on biomass content became negative, such as the anthocyanins_SOD and anthocyanins_CAT interactions.

In terms of affecting biomass content, the antioxidants were ranked from highest to lowest as POD (overall effect, i.e. sum of individual and interactive effects = 2.267), anthocyanins (1.738), carotenoids (1.203), proline (0.613), SOD (-1.75), CAT (-2.048). Anthocyanins appeared to be very important as they interacted synergistically with SOD and CAT, enhancing biomass content. In other words, anthocyanins could slightly alleviate the negative impacts of SOD and CAT on biomass content. By genetically manipulating for optimal concentrations/activities of the antioxidants presented in Table 1, biomass content could be promoted by 12%. In forthcoming research endeavors, a more comprehensive assessment should be conducted by including additional antioxidants and biochemicals aimed at influencing biomass content.

Abbreviations

Biomass content = Drought tolerance, SOD= Superoxide dismutase, POD= Peroxidase, CAT= Catalase, ROS=Reactive Oxygen Species, APX= Ascorbate Peroxidase, FAO= Food and Agriculture Organization of the United Nations.

Declaration

Ethics Approval and Consent to Participate

The following article does not include any studies involving human participants or animals conducted by the authors. The authors have no conflicts of interest to declare. Cumin seeds were procured from Mahan Mehr Daris Company, located in Iran. The plant materials utilized in this study were authorized by the Department of Botany at the Shahrood Faculty of Agriculture. All plant samples were housed at the Department of Botany, Shahrood Faculty of Agriculture, Iran, for the duration of this study.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analysed during this study are included in this published article.

Competing Interests

The authors declare that they have no competing interests

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Authors' contributions

All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by A. Ashori and M. Gholipoor. The first draft of the manuscript was written by A. Ashori. Methodology: H. Abbasdokht and A. Gholami; Formal analysis and investigation: M. Gholipoor and A. Gholami, Writing - original draft preparation: A. Ashori; Writing - review and editing: H. Abbasdokht, A. Gholami, Resources: A. Ashori, Supervision: M. Gholipoor. All authors commented on previous versions of the manuscript. All authors participated in reviewing previous manuscript versions and approved the final manuscript.

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