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Research paper

Biochemical responses of sugar beet plant to phytoprotectants and vermicompost under moisture stress

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Abstract
In recent years, with the spread of drought and increasing demand for water, the need for water management in irrigation of plants has become more apparent. Present investigation studied yield-related biochemical responses of sugar beet to vermicompost and phytoprotectants to mitigate drought stress based on a split-plot-factorial design with three replications. The main plots consisted of irrigation at 90%, 70%, 50%, and 30% field capacity (FC). The subplots subjected to treatments comprised a factorial combination of vermicompost (0 and 7 Mg/ha) and foliar application of phytoprotectants [distilled water as a control, zinc (5μM), silicon (4mM), glycine betaine (4mM) and ascorbic acid (0.5mM)]. The findings showed that concentration of ascorbate peroxidase, catalase, dehydroascorbate reductase, glutathione peroxidase, and superoxide dismutase, were significantly enhanced under stress conditions. Despite the higher sugar percentage, the lower root yield and biomass were recorded in the plants irrigated with 30 and 50% FC. Sugar content increased gradually in response to increasing in water deficit (from 70% to 30% FC). Root yield increased insignificantly with zink, glycine betaine, and ascorbic acid treatments. The highest root yield was obtained at 70% FC that followed by other water regimes (90, 50, and 30% FC, respectively). Malondialdehyde increased with increasing stress level but it decreased when phytoprotectants, especially glycine betaine, were applied. Vermicompost treatment had positive effect on the prevention of lipid peroxidation. It can be concluded that phytoprotectants and vermicompost protect sugar beet plants from drought-induced oxidative stress, and improve root and sugar yield by enhancing plant water-stress tolerance.

Keywords: Abscisic acid; Antioxidant; *Beta vulgaris*; Glycine betaine; Irrigation; Silicon

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Abbreviations
ABA: Abscisic acid
APX: Ascorbate peroxidase
AsA: Ascorbic acid
CAT: Catalase
DHAR: Dehydroascorbate reductase
FC: Field capacity
GB: Glycine betaine
GPX: Glutathione peroxidase
MDA: Malondialdehyde
RY: Root yield
SC: Sugar content
Si: Silicon
SOD: Superoxide dismutase
Zn: Zinc

Introduction

Sugar beet (*Beta vulgaris* L., family Chenopodiaceae) is a commercial plant for the production of beet sugar that grows in a wide range of climatic conditions (Hoffman *et al*. 2009). Irrigation water plays a major role in sugar beet cultivation, especially in arid and semiarid regions (Faberio *et al*. 2003; Hassanli *et al*. 2010). Therefore, optimal use of water resources is needed to have sustainable agriculture (Cribb 2017).

Under water deficit conditions, the plants'

metabolic activities can generate toxic levels of reactive oxygen species (ROS) by the mitochondrial, or chloroplast electron transfer chains (Gaspar *et al*. 2002) which leads to oxidative stress (Dewir *et al*. 2006). For scavenging ROS, the enzymatic antioxidant defense system [e.g. ascorbate peroxidase (APX), catalase (CAT), peroxidase, and superoxide dismutase (SOD)] is an efficient protective mechanism to minimize the concentration of H_2O_2 (Asada 2000). Plants also possess a non-enzymatic defense system, including α-tocopherol, ascorbate (ASC), carotenoids, flavonoids, and glutathione (GSH) (Gill and Tuteja 2010; Anjum *et al*. 2011), and other enzymes such as dehydroascorbate reductase (DHAR), glutathione reductase (GR), and monodehydroascorbate reductase (MDHAR) (Gill and Tuteja 2010; Kadioglu *et al*. 2011). Based on the previous studies, an effective approach to strengthen ROS-scavenging capacity is exogenous application of antioxidants (Chen and Murata 2011; Kobayakawa and Imai 2017). Malondialdehyde (MDA) is an organic compound that is widely used as a biomarker of oxidative stress in plants due to the membrane lipid damages by ROS (Davey *et al*. 2005). To survive from the drought stress (Lang 2007), plants increase the accumulation of osmoprotectants for better osmotic regulation (Hossain and Fujita 2010; Ranganayakulu *et al*. 2013).

Drought impairs water balance, absorption of mineral elements, and abscisic acid (ABA) accumulation in plants (Osakabe *et al*. 2014; Rostami *et al*. 2019; Khalilzadeh *et al*. 2020). The main role of micronutrients such as zinc (Zn) is to make plants tolerant against the drought stress. Foliar application of Zn provides better protection against water stress that improves yield components (Thalooth *et al*. 2006). In addition, the exogenous use of silicon (Si) improves the photosynthesis gas exchange parameters, net photosynthesis (Zuccarini 2008; Hasanuzzaman *et al*. 2014b; Tahmasebi *et al*. 2018), and physio-hormonal attributes contributing to the mitigation of the adverse effects of drought (Hamayun *et al*. 2010). Glycine betaine (GB), as a key osmoprotectant (Turkan and Demiral 2009), enhances the plant growth under different water supply conditions due to altering the level of MDA and ROS, and increases APX, CAT and SOD activities (Farooq *et al*. 2008).

The application of exogenous ascorbic acid (AsA) increases the endogenous AsA in plants at drought stress conditions (Farooq *et al*. 2013; Alam *et al*. 2014; Ghassemi *et al*. 2020). AsA as a redox buffer in plant cells, has significant roles in plant growth, metabolism and development (Alam *et al*. 2014; Anjum *et al*. 2014). It protects the plasma membrane against oxidative damage and helps to regenerate zeaxanthin and α-tocopherol (Smirnoff 1996).

Vermicompost is a natural eco-manure that has high porosity with proper ventilation and drainage. Therefore, it exhibits a high water storage capacity (Hosseinzadeh *et al*. 2015b). Application of vermicompost to soil boosts the soil water content through modifying soil physical properties and provides the necessary nutrients (Singh *et al*. 2010). Therefore, the present study

was aimed to determine the biochemical responses of the sugar beet plant to vermicompost and phytoprotectants (Zn, Si, GB, and AsA) under water stress.

Materials and Methods

Site description and the experimental design

This experiment was conducted at the research field of the Urmia University (latitude 36˚48'42"N, longitude 45˚14'08" E, altitude 1467 m), with the semi-arid climate during 2015 and 2016 growing seasons. The monthly precipitation, air temperatures, and relative humidity in 2015 and 2016 are given in Table 1.

The experiment was carried out as a split-plot-factorial design with three replications. Treatments were consisted of three factors. The main plots included the irrigation levels [irrigation at 90% field capacity (FC) (extra-watered), 70% FC (well-watered), 50% FC (moderate stress), and 30% FC (severe stress)], and the factorial combination of vermicompost (0 and 7 Mg/ha) and phytoprotectants [distilled water as a control, Zn (5µM), Si (4mM), GB (4mM), AsA (0.5 mM)] were assigned to sub-plots.

The foliar application of phytoprotectants was carried out two times; first at the vegetation growth stage (16-leaf stage), and the second two weeks after the drought stress treatment (at the yield formation stage, 24-leaf stage). Tween-20 (0.05%, v/v) was used as a surfactant. A backpack sprayer (10 L capacity, 1000 L ha^{-1} delivery) was used for spraying the phytoprotectants solutions.

The seeds (*Beta vulgaris* L. cv. Isabella; KWS company, Germany), were sown at 2.5 cm depths with 50 cm inter-row and 18 cm intra-row spaces on March 26, 2015 and 2016. Experimental plots had 6 m length and 10 m width with a 3-m space between them to minimize the water movement among treatments. The vermicompost was spread and mixed with 30 cm of soil before planting.

The required water for irrigation to bring the soil into different field capacities were 8250, 6171, 4680, and 2620 m3 ha^{-1} (in the first year), and 8625, 6732, 5616 and 3930 m^3 ha^{-1} (in the second year) for irrigation at 90% FC, 70% FC, 50% FC, and 30% FC, respectively.

In each plot, whole plants of sugar beet were harvested from 8 m^2 on 25 October in each year. The leaves and roots were weighed in the field and oven-dried at 80 °C until reaching the constant weight. Percentage of sugar content (SC) was measured with a polarimeter (p3000, KRUESS, Germany) after extraction of sugar from the pulp with lead acetate (ICUMSA 2007). Biomass included the total weight of leaves and roots.

MDA Content

Lipid peroxidation was determined by measuring MDA (Fu and Huang 2001). At first, 0.5 g of fresh leaves was homogenized in trichloroacetic acid (TCA) and then centrifuged. The reaction mixture contained 500 µl of the supernatant and 4 mL of 20% TCA with 0.5% thiobarbituric acid (TBA) and then centrifuged. The absorbance of samples was recorded at 532 and 600 nm, and the extinction coefficient for calculating the MDA content was 155 mM cm^{-1}. The lipid peroxidation

Table 1. Total rainfall, average monthly air temperature and relative humidity during two years in Urmia, Iran

	2014						2015					
	April	May	June	July	August	September	April	May	June	July	August	September
Rainfall (mm)	102	82	3	0	0	24	153	23	35	2	0	0
Temperature (°C)	10.4	15.8	22.2	26.9	27.8	22.9	9.6	17.1	20.3	25.3	27.1	24.4
Relative humidity (%)	62	53	42	37	37	49	64	53	47	46	43	43

was expressed in nmol MDA g^{-1} fresh weight.

Antioxidant enzymes assays

Leaf samples (0.5 g) were homogenized in an ice bath using 50 mmol/L sodium phosphate buffer (pH 6.8), which consisted of 1 mmol/L EDTA.Na_2 and 2% (w/v) polyvinylpolypyrrolidone. The extraction operation was performed at 0–4 °C. Homogenates were centrifuged at 13,000 g for 40 min, and supernatants were separated for measuring the enzyme activity. The protein assay was done according to Bradford (1976), in which the bovine serum albumin was used as the standard.

Activity of SOD was determined at 560 nm by measuring the inhibition of the photochemical reduction of nitroblue tetrazolium (Beauchamp and Fridovich 1971). Activity of APX was determined according to the method described by Nakano and Asada (1981). The oxidized ascorbate content was estimated by the extinction coefficient 2.8 mM^{-1}/cm. APX was expressed as 1 mmol/mL ascorbate oxidized per minute. Activity of glutathione peroxidase (GPX) was determined according to Paglia and Valentine (1967). For this purpose, 0.5 mol EDTA, 1 mmol $NaNO_3$, 0.56 mol (pH 7) phosphate buffer, 0.2 mmol NADPH were added to the extracted solution. The decrease in absorbance was measured at 340 nm with a spectrophotometer. The activity of DHAR was determined according to De Tullio *et al*. (1998). The reaction mixture contained 50 µg protein extract, 0.1 M KPO_4 (pH 6.2), and 2 mM GSH. The production of ascorbate was measured at 265 nm using the extinction coefficient of 14 $Mm^{-1}cm^{-1}$ and the changes in absorbance were followed for 1 minute. The rate of non-enzymatic dehydroascorbic acid reduction was corrected by subtracting the values obtained in the absence of enzyme extract. CAT activity was measured by calculating the initial rate of H_2O_2 disappearance (Bergmeyer 1970). The decrease in H_2O_2 was observed by the decline in optical density at 240 nm and CAT activity was recorded as mmol H_2O_2 consumed per minute. ABA was analyzed using HPLC (high performance liquid chromatography) and ELISA (enzyme linked immunosorbent assay) (Aroonrungsikul *et al*. 1997; Olivella *et al*. 1998). A volume of 100 µl from the extract was used for ABA by ELISA according to the procedure given by the manufacture of the assay kits (Agdia Inc. USA).

Statistical analysis

The analysis of variance (ANOVA) for the two-year data was performed using the GLM procedure (SAS 9.1.3, SAS Institute Inc., Cary, NC, USA) in the form of a split-plot-factorial design combined over years. Means were compared using the Least Significant Difference

(LSD) test ($p \leq 0.05$).

Results

Results of ANOVA showed the significant effects of irrigation on the RY (root yield), SOD, and APX, vermicompost on the RY, biomass, ABA, MDA, and CAT, phytoprotectants on RY and APX. The irrigation × vermicompost × phytoprotectants interaction was significant for the activity of DHAR and GPX. The effect of irrigation × phytoprotectants interaction on biomass, SC, CAT, ABA, and MDA and effect of vermicompost × phytoprotectants on SOD were also significant (Table 2).

The highest RY (1078.54 g/plant equal to 86.28 Mg/ha) was achieved at 70% FC, while the lowest RY (419.79 g/plant equal to 33.58 Mg/ha) was observed at 30% FC treatment. On the other hand, water-deficit stress (30 and 50% FC) and extra irrigation (90% FC) significantly reduced RY by 61, 27 and 17% respectively. Increasing in RY (3%) and biomass (3%) was observed in the vermicompost treatments. RY increased significantly when Zn, GB, and AsA was applied as compared to the control (Table 2). The sugar beet biomass was higher when plants were irrigated at 70% FC rather than at the stress conditions (extra irrigation and water deficit stress) and was even higher when phytoprotectants were added (Table 3).

Stress increased SC along with the lower irrigation water supply. Foliar-applied mineral phytoprotectants (Zn and Si) were not effective in the accumulation of sugar under water deficit-stress conditions, but organic phytoprotectants (GB and AsA) had positive effects at all irrigation levels, without superiority between GB and AsA (Table 3).

ABA was also increased by application of vermicompost (Table 2). The highest value of ABA was recorded at 30 and 50% FC. It was observed that the exogenous application of AsA increased the ABA content and raises its content to the highest value under water deficit stress conditions (Table 3).

The lipid peroxidation declined in the vermicompost treatments (Table 2). Regardless of the phytoprotectants application, MDA increased gradually in response to increasing water deficit stress (from 70 to 30% FC), and excessing the irrigation water (90% FC). The highest MDA (51.03 nmol/g FW) was obtained from the untreated control plants irrigated at the 30% FC, while the lowest MDA (34.66 nmol/g FW) was obtained at the 70% FC using GB (Table 3).

A significant decrease in CAT activity was observed by the use of vermicompost (Table 2). On the average of two years, CAT concentration was maximal when the crop was exposed to the severe stress conditions. However, when phytoprotectant was applied, CAT significantly decreased in the stressed plants (30% FC). At 70 and 90% FC, Si and AsA had no significant effect on the CAT activity compared to the untreated control plants (Table 3).

SOD content was increased with the exacerbation of drought, as the highest SOD was recorded in the stressed plants, especially at 30% FC (Table 2). SOD was strongly differed between control plants and sugar beet plants treated with

Table 2. Analysis of variance based on the effect of year, irrigation, phytoprotectant, and vermicompost on the sugar beet enzymes

Source	df	Mean Squares									
		RY	Biomass	SC	ABA	MDA	CAT	SOD	APX	DHAR	GPX
Year (Y)	1	189141.3*	22789*	5.18**	5.22^{ns}	2.93^{ns}	0.17^{ns}	1.89^{ns}	12.00^{ns}	2.38^{ns}	1.10^{ns}
Block / Year	4	21748.7	1135	0.08	16.42	16.7	0.05	2.02	3.00	0.50	0.30
Irrigation (I)	3	4634539**	395412**	152**	31.24**	2316.3**	29.5**	3915**	1481**	427**	133**
Y×I	3	83080**	54911**	4.92**	1.26^{ns}	9.38^{ns}	0.09^{ns}	2.07^{ns}	2.00^{ns}	1.06^{ns}	0.30^{ns}
Block (I×Y)	12	6120	345.4	2.42	0.54	13.81	0.12	1.40	3.00	0.97	0.20
Vermicompost (V)	1	27360**	3703**	0.41^{ns}	0.42*	1.76**	0.03**	1.51**	0.20^{ns}	0.16^{ns}	0.05^{ns}
Phytoprotectant (P)	4	18987**	2051**	7.04**	17.63**	38.66**	0.12**	24.02**	10.00**	1.29**	0.40**
V×P	4	190.4 ^{ns}	36.10^{ns}	0.08^{ns}	0.04^{ns}	0.10^{ns}	0.001^{ns}	0.39*	0.02^{ns}	2.39**	0.70**
I×V	3	3369^{ns}	252.1^{ns}	0.06^{ns}	0.01^{ns}	0.20^{ns}	0.001^{ns}	0.08^{ns}	0.04^{ns}	15.81**	4.90%**
I×P	12	2580^{ns}	198.7*	0.27*	0.66**	0.61**	0.007*	0.12^{ns}	0.10^{ns}	15.37**	4.80**
I×V×P	12	2430^{ns}	153.2^{ns}	0.04^{ns}	0.05^{ns}	0.08^{ns}	0.0005^{ns}	0.08^{ns}	0.006^{ns}	14.89**	4.60**
Y×V	1	2423^{ns}	691.9*	0.10^{ns}	0.04^{ns}	0.13^{ns}	0.002^{ns}	4.67**	0.10^{ns}	0.16^{ns}	0.07^{ns}
Y×P	4	5741**	370.4**	0.74**	1.56**	0.30^{ns}	0.07**	0.31^{ns}	0.40**	0.17*	0.05*
Y×I×V	3	219.4^{ns}	59.03^{ns}	0.02^{ns}	0.01^{ns}	0.08^{ns}	0.0008^{ns}	0.02^{ns}	0.008^{ns}	0.03^{ns}	0.007^{ns}
Y×I×P	12	2339^{ns}	103.6^{ns}	0.33**	0.30**	0.54**	0.006^{ns}	0.14^{ns}	0.60**	0.06^{ns}	0.02^{ns}
Y×V×P	4	1103^{ns}	94.57^{ns}	0.02^{ns}	0.13^{ns}	0.04^{ns}	0.001^{ns}	0.57**	0.007^{ns}	0.23*	0.07*
Y×I×V×P	12	2231^{ns}	149.4^{ns}	0.09^{ns}	0.10^{ns}	0.30^{ns}	0.001^{ns}	0.03^{ns}	0.009^{ns}	0.07^{ns}	0.02^{ns}
Error	144	1593	109	0.12	0.10	0.21	0.003	0.15	0.09	0.07	0.02
CV (%)		5.00	4.06	2.03	3.44	1.10	0.47	0.49	1.18	1.73	1.72
		(g/plant) (Mg/ha)	(g/plant)	(%)	(ppm)	(nmol/g FW)	(U/mg protein)	(U/mg protein)	(U/mg protein)	(U/mg protein)	(U/mg protein)
Year											
2015		824.90a 65.99a	266.78a	17.74a	9.53a	42.08a	1.274a	78.82a	2.57a	15.78a	0.885a
2016		768.75b 61.50b	247.29b	17.45b	9.23a	42.30a	1.280a	78.64a	2.52a	15.59a	0.871a
$LSD_{(0.05)}$		52.86 4.22	12.07	0.10	1.45	1.46	0.008	0.51	0.06	0.25	0.02
Irrigation											
90%% FC		897.71b 71.81b	288.26b	15.79c	8.93b	38.97c	1.23c	73.75c	2.33b	14.34c	0.803c
70% FC		1078.54a 86.28a	336.83a	17.42b	8.60c	35.57d	1.20d	71.46d	2.23c	13.82d	0.773d
50% FC		791.25c 63.30c	257.51c	17.50b	9.96a	44.58b	1.31b	80.20b	2.33b	14.96b	0.838b
30% FC		419.79d 33.58d	145.55d	19.67a	10.02a	49.66a	1.35a	89.50a	3.29a	19.62a	1.098a
$LSD_{(0.05)}$		31.12 2.4896	7.39	0.61	0.29	1.47	0.01	0.47	0.07	0.39	0.02
Vermicompost											
0		786.14b 62.89b	253.11b	17.56a	9.34b	42.28a	1.27a	78.81a	2.55a	15.66a	0.877a
7		807.50a 64.60a	260.96a	17.64a	9.42a	42.11b	1.27b	78.65b	2.54a	15.71a	0.880a
$LSD_{(0.05)}$		10.17 0.8143	2.66	0.09	0.08	0.11	0.001	0.09	0.007	0.06	0.003
Phytoprotectant											
Control		763.02b 61.04b	246.12c	17.22c	8.96c	43.64a	1.280a	77.77d	2.53b	15.88a	0.889a
Zn		805.99a 64.47a	259.23b	17.39b	9.01c	42.33b	1.272c	78.40c	2.52bc	15.58b	0.872b
Si		799.21a 63.93a	257.28b	17.36b	9.20b	41.55d	1.279b	79.43a	2.53bc	15.55b	0.871b
GB		815.10a 65.20a	263.61a	18.07a	9.29b	41.36e	1.272c	79.41a	2.52c	15.57b	0.871b
AsA		800.78a 64.06a	258.94b	17.94a	10.44a	42.09c	1.279b	78.64b	2.63a	15.85a	0.887a
$LSD_{(0.05)}$		16.09 1.2876	4.21	0.14	0.13	0.18	0.002	0.15	0.01	0.10	0.006

RY: root yield; SC: sugar content; ABA: abscisic acid; MDA: malondialdehyde; CAT: catalase; SOD; superoxide dismutase; APX: ascorbate peroxidase; DHAR: dehydroascorbate reductase; GPX: glutathione peroxidase

^{ns}: non-significant, *: significant at $p \leq 0.05$, **: significant at $p \leq 0.01$; Means with different letters within each factor are significantly different at $p \leq 0.05$.

phytoprotectants. Sprayed plants showed higher SOD in the order of GB, Si, AsA, Zn, and distilled water. However, vermicompost had no significant effect on the SOD content (Table 4).

Normal irrigation reduced the activity of APX (2.238 unit/mg protein) as compared to other irrigation conditions. Exogenous GB, Zn, and Si had no significant effect on the APX activity, but AsA showed a remarkable enhancement in the APX activity compared to the untreated plants (Table 2).

The higher levels of DHAR were observed in the plants under the severe stress conditions, so that both had significantly higher DHAR than those obtained with the normal irrigation. It is worth noting that DHAR concentration was stable under water deficit stress conditions (30 and 50% FC) either in the control or treated plants (except AsA at 30% FC). Whereas, it was decreased under other irrigation levels in the vermicompost treated plants as much as the plants with no vermicompost (Table 5).

Table 3. Means of some physiological traits of sugar beet plants affected by the irrigation × phytoprotectants interaction

Irrigatio (% FC)	Phytoprotectants	Biomass (g/plant)	SC (%)	ABA (ppm)	MDA (nmol/g FW)	CAT (U/mg protein)
90%	Control	279.65d	15.23h	8.48h	40.42h	1.240i
	Zn	288.29c	15.55g	8.60gh	39.10i	1.229k
	Si	290%.62c	15.52g	8.79g	38.20j	1.234j
	GB	291.02c	16.44f	8.77g	38.01j	1.223l
	AsA	291.68c	16.23f	10.02b	39.10i	1.237ij
70	Control	325.53b	16.90%e	8.55gh	37.44k	1.209m
	Zn	342.08a	17.33d	8.16i	35.58l	1.202n
	Si	335.17a	17.34d	8.59gh	34.94mn	1.210m
	GB	338.89a	17.77c	8.57gh	34.66n	1.203n
	AsA	342.50a	17.79c	9.14f	35.20m	1.208m
50	Control	248.52g	17.32d	9.39ef	45.65d	1.315e
	Zn	261.71ef	17.38d	9.67cd	44.86e	1.303h
	Si	257.90%ef	17.21d	9.72c	43.93g	1.310fg
	GB	264.00e	17.92c	9.79bc	44.00g	1.307gh
	AsA	255.41fg	17.67c	11.25a	44.46f	1.314ef
30	Control	130.78j	19.42b	9.44de	51.03a	1.367a
	Zn	144.84i	19.32b	9.61cde	49.80b	1.355cd
	Si	145.44i	19.39b	9.72c	49.13c	1.361b
	GB	145.54i	20.17a	10.01b	48.76c	1.352d
	AsA	146.16i	20.08a	11.35a	49.59b	1.359bc

Control: distilled water; ABA: abscisic acid; MDA: malondialdehyde; CAT: catalase; Bio: biomass; SC: sugar content
Means with different letters in each column are significantly different at $p \leq 0.05$.

Table 4. Means of some physiological traits of sugar beet plants affected by the phytoprotectants × vermicompost interaction.

Phytoprotectants	SOD (U/mg protein)	
	Non-vermicompost	Vermicompost
Control	77.77f	77.76f
Zn	78.50de	78.29e
Si	79.41ab	79.46a
GB	79.61a	79.20b
AsA	78.75c	78.54cd

Control: Distilled water; SOD: Super oxide dismutase
Means with different letters in each column are significantly different at $p \leq 0.05$.

Table 5. Means of DHAR and GPX in sugar beet plants affected by the irrigation × phytoprotectants × vermicompost interaction

Irrigation (% FC)	Phytoprotectants	DHAR (U/mg protein)		GPX (U/mg protein)	
		Non-vermicompost	Vermicompost	Non-vermicompost	Vermicompost
90%	Control	14.71c	14.28d	0.825c	0.800d
	Zn	14.22d	14.23d	0.795d	0.796d
	Si	14.17d	14.25d	0.793d	0.798d
	GB	14.24d	14.18d	0.800d	0.795d
	AsA	14.18d	14.92c	0.795d	0.836c
70	Control	14.74c	13.63e	0.826c	0.763e
	Zn	13.63e	13.63e	0.761e	0.763e
	Si	13.60e	13.58e	0.761e	0.760e
	GB	13.66e	13.70e	0.763e	0.766e
	AsA	13.65e	14.38d	0.763e	0.805d
50	Control	14.36d	14.30d	0.805d	0.801d
	Zn	14.31d	14.21d	0.801d	0.798d
	Si	14.28d	14.25d	0.801d	0.798d
	GB	14.22d	14.16d	0.796d	0.793d
	AsA	14.76c	14.35d	0.828c	0.803d
30	Control	20.20b	20.14b	1.128b	1.126b
	Zn	20.18b	20.10b	1.131b	1.126b
	Si	20.22b	20.18b	1.131b	1.128b
	GB	20.10b	19.96b	1.125b	1.116b
	AsA	20.82a	20.75a	1.165a	1.163a

Control: distilled water; DHAR: dehydroascorbate reductase; GPX: glutathione peroxidase
Means with different letters in each column are significantly different at $p \leq 0.05$.

The highest GPX concentration was observed at the severe water-deficit stress and it was the same in the plants treated with vermicompost and not treated with this compound. The GPX was decreased with increasing the consumption of irrigation water. The AsA content was reached to the maximum level by using the phytoprotectants at all irrigation levels (Table 5).

Discussion

Water stress affected the plant growth attributes (root, sugar yield and biomass) and the results clearly demonstrated the physiological and biochemical responses of sugar beet to water deficit and excess water.

Drought stress is accompanied by increasing the oxidative stress as a result of excessive accumulation of ROS, particularly hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) in mitochondria, chloroplasts and peroxisomes, which ultimately reduces the plant growth (Hajheidari *et al*. 2005; Sayfzadeh and Rashidi 2010). Our observations provide evidence that water deficit alters RY and biomass. A significant decrease (by 50%) was observed in the RY of plants irrigated at 30% FC (severe stress) compared to the well-irrigated plants (70% FC). Sugar beets plants exposed to the drought produce

more osmolytes like proline, GB, total soluble carbohydrate, total soluble sugar, total polyphenol, total flavonoid, and α, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging activity (Islam *et al.* 2020) that improve the plant tolerance to a wide range of water stresses by signal transduction, stimulating resistance genes, improving antioxidant and non-antioxidant defense system, protecting from the osmotic pressure, protecting cell membranes, as well as regulation of PSII activity (Hamani *et al.* 2021; Niazian *et al.* 2021). It was reported that water shortage increases the membrane injury, lipid peroxidation, and lipoxygenase activity, along with higher levels of O_2^- and H_2O_2 (Kubis *et al.* 2014). Higher MDA as a substrate for lipid peroxidation was produced by water stress (water deficit and excess irrigation water).

Our findings showed that SOD activity was increased in response to drought stress, and accumulation of APX, CAT, GPX, and DHAR in the leaves. The antioxidant enzymes, active in scavenging the stress-induced ROS, were increased more in the plants irrigated at 30, 50, and 90% FC. The increase in the CAT activity in the stressed plants is related to the necessity of counter effecting photorespiratory-induced H_2O_2. Gradual and prolonged water deprivation and increasing activities of POX and APX indicate the early stages of stress (Lee *et al*. 2007; Farzane *et al*. 2020). Also, production of H_2O_2 from superoxide could be due to the increase in APX and CAT activity (Asada 2000). It has also been suggested that CAT is a less susceptible scavenging enzyme than APX concerning oxidative stress (Cruz de Carvalho 2008).

The activity of APX, the first enzyme in the ascorbate-glutathione cycle, should be maintained continuously to ensure the plant's survival against oxidative stress due to its activity in converting the H_2O_2 into water (Foyer and Noctor 2005). SOD is considered as the first line of defense against ROS mediated oxidative stress (Gill and Tuteja 2010; Sayfzadeh and Rashidi 2010) which reduces the production of hydroxyl radical by the metal catalyzed Haber-Weiss reaction (Gill and Tuteja 2010).

Increasing the DHAR activity during drought may be attributed to the regeneration of ascorbate and enhancing APX, which is considered to be a crucial ROS detoxification machinery of ASH/GSH pathway (Gill and Tuteja 2010; Kusvuran *et al*. 2016). GPX aids to moderate the oxidative-stress effects by reducing GSH, through scavenging H_2O_2 and lipid hydroperoxides, and converting them to harmless products (Cruz de Carvalho 2008; Caverzan *et al*. 2016).

The higher levels of osmoprotectants protect the plant cells through facilitating the enzyme activity and increasing water uptake. Improvement of antioxidant defense system, sustainability in ion homeostasis (Wang *et al*. 2003; Ashraf and Foolad 2007; Ranganayakulu *et al*. 2013), and the impact of osmoprotectants on water regulation (Wang *et al*. 2003) are the drought adapting mechanisms.

Exogenous Si improves biochemical and hormonal attributes and also influences the mineral nutrition contributing to the mitigation of the adverse effects of drought (Hamayun *et al*.

2010; Tahmasebi *et al*. 2018). Similar to our results, Si may play a role in maintaining the integrity of cell membranes (Pei *et al*. 2010; Coskun *et al*. 2016) due to lower MDA content and hydrogen peroxide (Soylemezoglu *et al*. 2009; Pei *et al*. 2010). Also, Si alleviate effects of drought by improving nutrient uptake, translocation, and nutritional efficacy (Laane 2018; Artyszak *et al*. 2021).

In response to drought, GB increase the permeation of water into cells for sustaining the intracellular osmotic equilibrium (Kumar *et al*. 2003; Ranganayakulu *et al*. 2013) through Na^+/K^+ discrimination, inducing membrane consistency and defense enzymes (Ashraf and Foolad 2007). Moreover, exogenous GB can enhance the activities of mono- and di-hydro-ascorbate reductase, resulting in higher AsA levels (Hasanuzzaman *et al*. 2014a).

Khodadadi *et al.* (2020) showed that water deficit stress resulted in the significant reduction of RY, sugar percent, relative water content and leaf area index, and significant increase in enzyme activities. However, exogenously applied AsA caused the accumulation of enzymatic and non-enzymatic antioxidants as well as proline and GB. Based on Hasanuzzaman *et al*. (2012), the levels of the components of the AsA-GSH cycle are often correlated with the drought tolerance. Exogenous AsA scavenges generated excessive ROS from drought stress and protects the cell membrane stability (Xu *et al*. 2015; Billah *et al*. 2017; Wang *et al*. 2017), and also mitigates the reduction of biomass, carbohydrate content, and soluble protein content (Alam *et al*. 2014). Moreover, Si treatment increases ROS inhibitory antioxidants and decreases ROS production (Rios *et al*. 2017). In contrast, when sugar beet plants were treated with Si, a decrease in membrane damage was recorded as compared to untreated plants.

It has been noted that the use of Zn as micro-element increases the yield of sugar beet plants by improving the quality traits and saving the plants' needs from micronutrient and nitrogen fertilizers (Abbas *et al.* 2020; Zewail *et al.* 2020).

Vermicompost fertilizer contains micronutrients that act as a prosthetic group of CAT, peroxidase, and SOD (Atik 2013) and by which destroys ROS in the plant. Vermicompost also improves the availability of water and nutrients such as potassium and nitrogen, involving to regulate osmotic pressure. Vermicompost increases the content and stability of chlorophyll, thus helping to reduce the effects of water stress. The effect of vermicompost application on decreasing MDA and inhibiting lipid oxidation in the stressed plants was also exhibited by Amiri *et al*. (2017).

Conclusions

In this research, we investigated the variation in biochemical compounds and yield of the sugar beet plant under different irrigation levels, when subjected to vermicompost and phytoprotectants. It was observed that RY was significantly decreased with increasing the water-deficit stress, so that the highest RY was produced at 70% FC. Water-deficit stress lead to the increased antioxidant activity (CAT, SOD, APX, DHAR,

GPX) and MDA via diminishing the RY. The vermicompost and phytoprotectants had positive effects on plants, which can have implications on the potential use of exogenous biochemical compounds to improve the water stress tolerance. The effectiveness of organic phytoprotectants in reducing the effects of water stress was greater than inorganic phytoprotectants.

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Conflict of Interest

The authors declare that they have no conflict of interest with any people or organization concerning the subject of the manuscript.

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پاسخ های بیوشیمیایی گیاه چغندر قند به محافظ‌های گیاهی و ورمی کمپوست تحت تنش رطوبت

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چکیده

در سال‌های اخیر، با گسترش خشکسالی و همچنین افزایش تقاضا برای آب، نیاز به مدیریت آب در آبیاری گیاهان بیشتر نمایان شده است. در تحقیق حاضر پاسخ‌های بیوشیمیایی مربوط به عملکرد چغندرقند از طریق ورمی‌کمپوست و محافظ‌های گیاهی برای کاهش اثر تنش خشکی بر پایه طرح اسپلیت پلات فاکتوریل با سه تکرار مطالعه شد. کرت‌های اصلی شامل آبیاری در ۹۰٪، ۷۰٪، ۵۰٪ و ۳۰٪ درصد ظرفیت زراعی بود. کرت‌های فرعی شامل ترکیب فاکتوریل از ورمی‌کمپوست (۰ و ۷ مگاگرم در هکتار) و محلول‌پاشی محافظ‌های گیاهی (آب مقطر به عنوان شاهد، روی ۵ میکرو مولار، سیلیسیم ۴ میلی مولار، گلایسین بتائین ۴ میلی مولار و اسید اسکوربیک ۰٫۵ میلی مولار) بود. غلظت آنزیم‌های کاتالاز، سوپراکسید دیسموتاز، آسکوربات پراکسیداز، دهیدرواسکوربات ردوکتاز و گلوتاتیون پراکسیداز در شرایط تنش کم‌آبی به طور قابل توجهی افزایش یافت. با وجود درصد قند بالاتر، عملکرد ریشه و زیست‌توده کمتری در تیمارهای ۳۰ و ۵۰ درصد ظرفیت زراعی مشاهده شد. با افزایش کمبود آب (از ۷۰ به ۳۰ درصد ظرفیت زراعی) درصد قند در چغندرقند به تدریج افزایش یافت. مالون دی‌آلدئید با افزایش سطح تنش افزایش یافت ولی با کاربرد محافظ‌های گیاهی به ویژه گلایسین بتائین کاهش پیدا کرد. ورمی‌کمپوست در جلوگیری از پراکسیداسیون لیپید تأثیر مثبت داشت. می‌توان نتیجه گرفت که محافظ‌های گیاهی و ورمی کمپوست از گیاه چغندرقند در برابر تنش اکسیداتیو ناشی از خشکی محافظت می‌کنند و با افزایش تحمل به تنش آب عملکرد ریشه و قند را بهبود می‌بخشند.

واژه‌های کلیدی: آبسیزیک اسید؛ آبیاری؛ آنتی اکسیدان؛ چغندرقند؛ گلایسین بتائین؛ سیلیکون