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Research Article

Evaluation of antioxidant enzymes activity under drought stress in different genotype of sugar beet (*Beta vulgaris* L.)

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Abstract

To evaluate the response of the antioxidant defense system of three sugar beet genotypes to drought stress, a two-year field experiment was conducted at the Research Site of the Sugar Beet Seed Institute of Karaj, Iran (2018-2019). The irrigation treatments were arranged in the main plots during growing seasons and included 80 mm (control (I_1)), 130 mm (I_2), and 180 mm (I_3), evaporation from an A-class pan under surface irrigation method, 30 mm (I_4), 80 mm (I_5), 130 mm (I_6), and 180 mm (I_7: as severe drought); evaporation with 100% volume of water requirement under trickle irrigation (tape) method; and 30 mm (I_8) evaporation with 75% volume of water requirement under trickle irrigation (tape) method. The second factor was genotype types included: 7112 (G_1), BP-Karaj (G_2), and BP-Mashhad (G_3) were in the subplots. The results showed that drought stress increased the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPX) in sugar beet leaves. There were significant differences among genotypes for antioxidant enzyme activity. Also, irrigation × genotype interactions showed a significant difference in CAT and GPX activities. In addition, drought stress caused the production of reactive oxygen species (ROS), which results in greater membrane permeability, malondialdehyde (MDA) content, and oxidative stress in the plants. Moreover, genotypes having greater levels of antioxidants showed better resistance to drought stress.

Keywords: Catalase activity, Glutathione peroxidase, Reactive oxygen species, Sugar beet yield, Superoxide dismutase

Introduction

Sugar beet (*Beta vulgaris* L.) is an industrial crop after sugarcane; it is of particular importance among sugar products and has a significant share of sugar production worldwide. This product is exclusively used in the sugar industry. Under the influence of dehydration, the performance of this product decreases in terms of quantity and quality. Therefore, drought stress is a major factor that limits its production in the world. In some cases it reduces the yield of this plant by more than 40%, and compared to cereals, sugar beet shows better tolerance to drought stress (Islam *et al*., 2021; Wisniewska *et al*., 2019). It is very important to produce a favorable economic performance in a situation where the water shortage crisis is the main limiting factor in the cultivation and production of agricultural products, especially sugar beet (Pidgeon *et al*., 2006; Ebmeyera *et al*., 2021). Moreover, sugar beet yield is determined by genotype and environment (Hoffman and Kenter, 2018). It is also well recognized that drought stress is the main restrictive factor for sugar beet yield (Pidgeon *et al*., 2006). However, the response of sugar beet to drought stress has been insufficiently studied (Ebmeyera *et al*., 2021; Islam *et al*., 2021).

Environmental stresses, such as drought stress and high temperature, influence almost all aspects of plants physiology and biochemistry and considerably reduce plants yield (Ebmeyera *et al*., 2021). Drought is one of the most harmful stresses that threaten agricultural production. Water is very important for the growth and development of plants. Drought stress significantly restricts plant growth and development, and consequently crop productivity. However, in tolerant and/or adaptable plants, morphological and metabolic

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changes occur in response to drought stress, which contribute toward adaptation to these inevitable ecological limitations (Islam *et al*., 2021). Drought stresses are experienced by plants either due to insufficient water supply or due to a very high transpiration rate. Improving crop yield under drought stress is one of the most important goals of plant breeding (Cattivelli *et al*., 2012). When plants are subjected to different biotic stresses, some reactive oxygen species (ROS) are produced. These ROS may initiate destructive oxidative processes. Mechanisms of active oxygen species detoxification exist in all plants and include the activation of enzymatic defense systems. Moreover, the activities of antioxidant enzymes and the levels of elevated antioxidants under drought stress are very changeable among plant species and even between two cultivars of the same plant species. In drought stress, oxidative damage at the cellular level is a main damage in crops (Farooq *et al*., 2013). If there is a severe difference between the production of ROS and antioxidant defense in any cell, oxidative stress and damage occur. Wisniewska *et al*., (2019) reported that drought-tolerant/adaptable species enhanced their antioxidant enzyme activities and increased their antioxidant contents under drought stress conditions, but drought-sensitive species were unsuccessful in doing so. To overcome oxidative damage under drought stress conditions, plants must have an efficient antioxidant system.

On the other hand, it is possible to cultivate sugar beet with more confidence by obtaining genotypes tolerant to drought stress in areas where the possibility of water shortage during the growth period is considered an obstacle to the cultivation of this product. Therefore, one of the most effective ways to reduce production damage in drought stress conditions is to improve the production and plant cultivars that are less sensitive to drought and have less quantitative and qualitative yield loss in water shortage conditions. Thus, this research was carried out to study the effect of drought stress on quality and enzymatic defense systems in three sugar beet (*Beta vulgaris* L.) genotypes.

Material and methods

Experimental site: This experiment was conducted at the research site of the Sugar Beet Seed Institute, Kamal-Abad, in Karaj, Iran, during 2018-2019. This site is located at a latitude of 35º 59' N, longitude of 51º 6' E, and an altitude of 1300 m above mean sea level in a semi-arid climate (345 mm rainfall annually) in the center of Iran.

Soil sampling and analysis: A composite soil sample (from 24 points) was collected from 0-30 cm depth during both years of the study and was analyzed in the laboratory. Details of soil physical and chemical properties of the experimental site during both years (2018 and 2019) are given in Table 1. Also, climate temperature and rainfall from sowing to harvest during both years are presented in Fig. 1.

Field method: Eight treatments of irrigation were applied to the three genotypes using a split-plot experiment laid out in a randomized complete block design (RCBD) with four replications. Irrigation treatments arranged in main plots during growing seasons included 80 mm (I_1: as control), 130 mm (I_2) and 180 mm (I_3) evaporation from an A-class pan under the surface irrigation method; 30 mm (I_4), 80 mm (I_5), 130 mm (I_6) and 180 mm (I_7: as severe drought) evaporation with 100% volume of water requirement under the trickle irrigation (tape) method; and 30 mm (I_8) evaporation with 75% volume of water requirement under the trickle irrigation (tape) method. Genotypes included 7112 (G_1), BP-Karaj (G_2) and BP-Mashhad (G_3) were in subplots. Seeds of different genotypes were planted on April 22, 2018, and May 20, 2019. Recommended levels of urea (300 $kg.ha^{-1}$) in both years and triple superphosphate (50 $kg.ha^{-1}$) only in the first year of study were used. Pest and weed control performed according to general local practices and recommendations. Measured parameters included the amounts of SOD, CAT, and GPX (antioxidant enzymes).

Sample preparation for biochemical assay: In the 25-30 leaf stage, two leaves of each plant from each experimental unit were removed. Leaf samples were prepared as described by Lowry *et al.* (1951) method. Leaf samples were washed with distilled water and homogenized in 0.16 mol Tris buffer (pH = 7.5) at 4ºC. Then, 0.5 mL of total homogenized solution was used for protein determination. Based on the amount of protein per volume of homogenized solution, the following enzymes were assayed in the volume containing a known protein concentration in order to calculate the specific activities of the enzymes.

Malondialdehyde (MDA) content: The amount of MDA was determined by measuring the absorbance at 532 nm and correcting for non-specific turbidity by subtracting the absorbance at 600 nm. An extinction coefficient of 155 mM^{-1} cm^{-1} was applied to calculate the MDA, expressed in µmol g^{-1} fresh weight (Debona *et al*., 2012).

Proline content: The leaves were dried at a temperature of 70°C for 72 hours, and after grinding, 0.3 g of dry plant material was poured into a mortar, and 5 ml of 3% sulphosalicylic acid was added to it, then it was homogenized. The samples were centrifuged for 10 minutes at 4°C at 15000 rpm. 2 ml of ninhydrin acid were added to 2 ml of the resulting clarifier, and then they were mixed well, and solutions of 0, 4, 8, 12, 16, and 20 mg/l proline standards were used. Then 2 ml of ninhydrin acid and 2 ml of glacial acetic acid were added to them and mixed well. The samples were placed in a hot water bath (bain-marie) for 90 minutes at a temperature of 100°C, then placed in ice. 4 ml of toluene were added to the solutions and placed in the shaker for 30 minutes, and the absorbance was read using a spectrophotometer (PG Instruments Itd VIS/UV+T model) at a wavelength of 520 nm and

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Table 1. Soil physical and chemical properties of the experimental site (0-30 cm depth) in two experimental years.

Year	O.M (%)	EC ($ds.m^{-1}$)	K ($mg.kg^{-1}$)	P ($mg.kg^{-1}$)	N ($mg.kg^{-1}$)	pH	Soil texture
First year	2.61	1.20	385	13.36	5.58	7.64	Clay loam
Second year	2.02	1.35	372	14.01	5.13	7.65	loam

O.C: Organic Carbon, O.M: Organic matter, EC: Electrical Conductivity

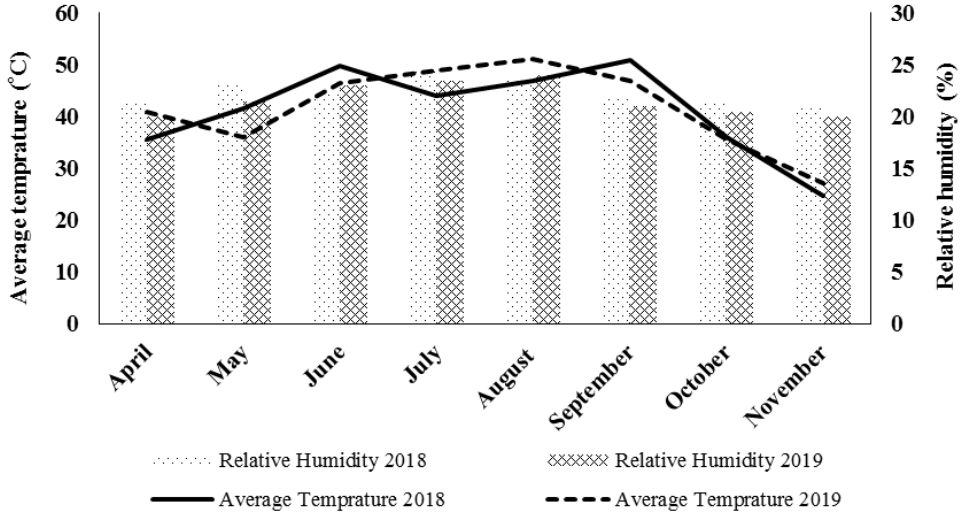

Figure 1. Mean monthly temperature and rainfall during crop growth in two experimental years

compared with a control sample (Bates *et al.*, 1973).

Superoxide dismutase (SOD) activity: SOD activity was determined as described by Misra and Fridovich (1972), with the reaction mixture containing 100 µL 1 µmol riboflavin, 100 µL 12 mmol L-methionine, 100 µL 0.1 mmol EDTA (pH 7.8), 100 µL 50 mmol Na_2CO_3 (pH 10.2), and 100 µL 75 µmol nitroblue tetrazolium (NBT) in 2300 µL 25 mmol sodium phosphate buffer (pH 6.8) and 200 µL crude enzyme extract in a final volume of 3 mL. SOD activity was assayed by measuring the ability of the enzyme extract to inhibit the photochemical reduction of NBT. Glass test tubes containing the mixture were illuminated with a fluorescent lamp (120 W); identical tubes that were not illuminated served as blanks. After illumination for 15 min, the absorbance was measured at 560 nm. One unit of SOD was defined as the amount of enzyme activity that was able to inhibit by 50% the photoreduction of NBT to blue formazan.

Catalase (CAT) activity: CAT activity was estimated by the method of Cakmak and Horst (1991). The reaction mixture contained 100 µL crude enzyme extract, 500 µL 10 mmol H_2O_2, and 1400 µL 25 mmol sodium phosphate buffer. The decrease in the absorbance at 240 nm was recorded for 1 min by spectrophotometer model Cintra 6 GBC (GBC Scientific Equipment, Dandenong, Victoria, Australia). Enzyme activity of the extract was expressed as enzyme units (µmol min^{-1} substrate) per milligram of protein.

Glutathione peroxidase (GPX) activity: GPX activity was measured by the Paglia and Valentine (1967) method, in which 0.56 mol (pH=7) phosphate buffer, 0.5 mol EDTA, 1 mmol $NaNO_3$, and 0.2 mmol NADPH were added to the extracted solution. GPX catalyzes the oxidation of glutathione (GSH) by cumene hydroperoxide. In the presence of glutathione reductase and NADPH, the oxidized glutathione is immediately converted to the reduced form with the concomitant oxidation of NADPH to NADP. The decrease in absorbance at 340 nm and 30ºC was measured with a spectrophotometer.

Relative water content (RWC): RWC was calculated through Equation 1 (Ferrat and Loval, 1999).

Equation 1: RWC = [Fw - DW]/[Sw - DW] × 100

Where, FW = leaf wet weight, DW = leaf dry weight, and SW = saturated leaf weight.

Photosynthetic pigments: The method of Ferus and Arkosiova (2001) was applied to measure the photosynthetic pigments of leaves, and finally the absorption value was read in the spectrophotometer at a wavelength of 663 nm for chlorophyll a, 647 nm for chlorophyll b, and 470 nm for carotenoid.

Statistical analysis: Statistical analyses were performed using SAS (Ver. 9.4) software (SAS Institute 2003). For all studied variables, a two-way analysis of variance (general linear model, GLM) was used to test the effects of irrigation, genotypes, and their interaction. The results of this test are shown as main effects and interactions. Treatment means were compared using Duncan's test at the 5% level ($P \leq 0.05$). A minimum of four replicates were used for each measurement.

Results

Root yield: ANOVA results presented that the main effects of irrigation, genotype, and the interaction of irrigation × genotype were significant ($P \leq 0.01$) for root yield (Table 2). The mean values showed that drought stress decreased sugar beet root yield, and among

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Table 2. Analysis of variance for root yield and antioxidant enzymes of sugar beet

S.O.V.	Df	Mean square					
		Root yield	Malondi aldehyde	Proline content	SOD enzyme	CAT enzyme	GPX enzyme
Year	1	35.43^{ns}	12.49^{ns}	0.035^{ns}	138782.52^{**}	6533.33^{**}	27^{ns}
Error	6	189.56	73.61	0.84	6322.3	111.32	256.54
Irrigation	7	375.69^{**}	543.75^{**}	2.52^{**}	3312181.78^{**}	26082.24^{**}	57500.09^{**}
Year × irrigation	7	21.56^{ns}	18.92^{ns}	0.76^{ns}	151224.02^{ns}	1611.33^{ns}	3833.57^{ns}
Error	42	27.89	75.76	0.83	201348.97	1511.23	4042.3
Genotypes	2	1356.11^{**}	638.94^{**}	3.81^{**}	9098469.00^{**}	42704.75^{**}	344745.94^{**}
Year × genotypes	2	934.68^{ns}	495.48^{ns}	0.786^{ns}	66116.02^{**}	419.08^{**}	1730.67^{ns}
Irrigation × genotypes	14	952.46^{**}	35.71^{ns}	0.814^{ns}	23225.31^{**}	858.33^{**}	3394.96^{**}
Year×irrigation×genotypes	14	75.21^{ns}	24.18^{ns}	0.916^{ns}	106.52^{ns}	89.33^{ns}	886.48^{ns}
Error	96	81.35	45.79	1.2	1047.71	71.22	689.2
C.V. (%)		13.46	10.95	6.52	6.03	5.38	7.52

ns= non-significant, ** = Significant at 0.01 probability level (MDA: Malondialdehyde, SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table 3. Means comparison for different irrigation treatments and sugar beet genotypes combination on root yield and antioxidant enzymes activity

Treatments		Root yield (ton ha^{-1})	SOD enzyme (µ mol min^{-1}/mg pr)	CAT enzyme (µ mol min^{-1}/mg pr)	GPX enzyme (µ mol min^{-1}/mg pr)
I_1	G_1	60.21^{b}	1121.13^{n}	111.85^{ij}	315.25^{h}
	G_2	60.35^{b}	1699.00^{hi}	133.04^{gh}	254.25^{l}
	G_3	62.58^{a}	1185.50^{n}	121.03^{hi}	360.13^{g}
I_2	G_1	59.05^{c}	1201.75^{n}	116.38^{ij}	362.75^{fg}
	G_2	58.96^{c}	1952.88^{ef}	159.65^{e}	255.50^{l}
	G_3	60.11^{b}	1420.64^{m}	154.44^{ef}	385.75^{fg}
I_3	G_1	54.38^{d}	1537.88^{kl}	142.10^{fg}	417.75^{cde}
	G_2	55.21^{d}	2265.75^{c}	201.86^{abc}	276.63^{jkl}
	G_3	57.76^{cd}	1841.13^{fg}	194.34^{bc}	426.75^{cde}
I_4	G_1	54.02^{de}	673.75^{p}	83.51^{k}	255.75^{l}
	G_2	54.16^{de}	1445.63^{lm}	104.86^{j}	203.25^{m}
	G_3	54.65^{de}	826.130^{o}	103.59^{jk}	309.50^{hi}
I_5	G_1	53.5^{e}	1443.50^{lm}	133.80^{gh}	399.63^{ef}
	G_2	53.44^{e}	2140.50^{d}	188.20^{cd}	279.88^{ijkl}
	G_3	53.81^{e}	1685.38^{ij}	183.65^{d}	404.38^{def}
I_6	G_1	48.25^{g}	1809.63^{gh}	145.63^{efg}	462.00^{ab}
	G_2	50.39^{f}	2576.75^{a}	210.13^{a}	301.25^{hij}
	G_3	51.94^{ef}	2007.75^{e}	207.54^{ab}	469.25^{a}
I_7	G_1	42.16^{j}	1585.75^{jk}	152.14^{ef}	434.25^{bcd}
	G_2	44.39^{i}	2450.88^{b}	206.79^{ab}	291.25^{hijk}
	G_3	46.11^{h}	1940.25^{ef}	201.40^{abc}	448.38^{abc}
I_8	G_1	39.51^{k}	1411.88^{m}	132.16^{gh}	404.50^{def}
	G_2	38.14^{l}	2151.88^{d}	188.93^{cd}	259.38^{kl}
	G_3	41.75^{j}	1693.50^{ij}	188.54^{cd}	402.88^{def}

Means in the same column with different letters differ significantly at the 0.05 probability level according to the Duncan test. (SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase). 80 mm (I_1: as control), 130 mm (I_2) and 180 mm (I_3) evaporation from an A-class pan under the surface irrigation method; 30 mm (I_4), 80 mm (I_5), 130 mm (I_6) and 180 mm (I_7: as severe drought) evaporation with 100% volume of water requirement under the trickle irrigation (tape) method; and 30 mm (I_8) evaporation with 75% volume of water requirement under the trickle irrigation (tape) method. Genotypes included 7112 (G_1), BP-Karaj (G_2) and BP-Mashhad (G_3).

genotypes, G_3 had a better function compared to other genotypes. The highest root yield (62.56 ton ha^{-1}) was seen in the interaction of $G_3 \times I_1$, and the lowest one (38.14 ton ha^{-1}) was related to the interaction of $G_2 \times I_8$ (Table 3).

Enzyme activities: Results of ANOVA showed significant differences in irrigation and genotype treatments ($P \leq 0.01$) for malondialdehyde, proline content, CAT, GPX, and SOD activities. Also, significant differences ($P \leq 0.01$) were observed for activities of CAT and GPX in irrigation × genotype interactions (Table 2). Overall, malondialdehyde, proline content, and the activities of all the antioxidant enzymes increased under drought stress in all the

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Table 4. Analysis variance for RWC and photosynthesis pigments of sugar beet

S.O.V.	Df	Mean square				
		RWC	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoid
Year	1	41.75^{ns}	9.73^{ns}	5.63^{ns}	10.61^{ns}	8.43^{ns}
Error	6	56.39	10.58	7.89	12.44	13.81
Irrigation	7	734.82**	42.69**	38.43**	81.59**	59.46**
Year × irrigation	7	81.25^{ns}	2.35^{ns}	5.75^{ns}	10.38^{ns}	9.34^{ns}
Error	42	76.34	11.73	13.61	34.61	13.59
Genotypes	2	659.46**	35.79**	41.28**	52.12**	75.11**
Year × genotypes	2	23.68^{ns}	5.33^{ns}	6.76^{ns}	7.83^{ns}	6.35^{ns}
Irrigation × genotypes	14	3561.25**	6.98^{ns}	7.81^{ns}	11.71^{ns}	8.49^{ns}
Year × irrigation × genotypes	14	28.49^{ns}	7.21^{ns}	7.53^{ns}	6.85^{ns}	10.12^{ns}
Error	96	35.73	10.25	13.89	12.95	18.41
C.V. (%)		12.56	13.96	11.17	9.43	7.96

ns= Non-significant. ** = Significant at 0.01 probability level (RWC: Relative water content)

Table 5. Means comparison for different irrigation treatments and sugar beet genotypes on malondialdehyde, proline content and photosynthesis pigments of sugar beet

treatments	Malondialdehyde (μ mol $g^{-1}FW^{-1}$)	Proline content (mg g^{-1} FW)	RWC (%)	Chlorophyll *a* (mg g^{-1} FW)	Chlorophyll *b* (mg g^{-1} FW)	Total chlorophyll (mg g^{-1} FW)	Carotenoid (mg g^{-1} FW)
Irrigation							
I_1	21.43^{e}	0.304^{e}	73.45^{a}	10.96^{a}	5.86^{a}	16.73^{a}	7.19^{a}
I_2	24.89^{d}	0.315^{d}	69.24^{b}	9.21^{b}	4.28^{b}	13.51^{b}	5.83^{b}
I_3	24.94^{d}	0.312^{d}	68.41^{b}	7.35^{c}	3.93^{c}	11.42^{c}	4.21^{c}
I_4	28.45^{c}	0.361^{c}	63.56^{c}	7.21^{c}	3.89^{c}	11.28^{c}	4.32^{c}
I_5	28.76^{c}	0.366^{c}	62.71^{c}	6.01^{d}	3.75^{c}	9.81^{d}	4.29^{c}
I_6	31.55^{b}	0.405^{b}	58.24^{d}	5.89^{e}	2.86^{cd}	8.73^{e}	3.98^{cd}
I_7	31.72^{b}	0.402^{b}	54.32^{e}	4.38^{f}	2.12^{d}	6.52^{f}	3.25^{d}
I_8	35.84^{a}	0.468^{a}	49.95^{f}	3.21^{g}	1.48^{e}	4.71^{g}	2.01^{e}
Genotypes							
G_1	29.73^{a}	0.462^{a}	56.41^{c}	5.48^{c}	2.69^{c}	8.17^{c}	3.55^{c}
G_2	27.45^{b}	0.405^{b}	65.73^{b}	7.61^{b}	3.71^{b}	11.32^{b}	5.23^{b}
G_3	23.12^{c}	0.342^{c}	70.28^{a}	9.53^{a}	5.02^{a}	14.58^{a}	7.16^{a}

Means in the same column with different letters differ significantly at the 0.05 probability level according to the Duncan test. 80 mm (I_1: as control), 130 mm (I_2) and 180 mm (I_3) evaporation from an A-class pan under the surface irrigation method; 30 mm (I_4), 80 mm (I_5), 130 mm (I_6) and 180 mm (I_7: as severe drought) evaporation with 100% volume of water requirement under the trickle irrigation (tape) method; and 30 mm (I_8) evaporation with 75% volume of water requirement under the trickle irrigation (tape) method. Genotypes included 7112 (G_1), BP-Karaj (G_2) and BP-Mashhad (G_3).

genotypes. The results of the means comparison indicated that in irrigation treatments I_8 (68.24%) increased malondialdehyde, and (53.94%) improved proline content compared to I_1. Also, in genotype treatments, G_3 (28.58%) promoted malondialdehyde, and 35.08%) increased proline content compared to G_1 (Table 5). The results of irrigation × genotype interactions showed that the interaction of $G_2 \times I_6$ increased (282.44%) SOD and improved (151.62%) CAT enzyme activity compared to $G_1 \times I_4$ (the lowest one). In addition, the interaction of $G_3 \times I_6$ increased (130.87%) GPX enzyme activity compared to $G_2 \times I_4$ (the lowest one) (Table 3).

Relative water content (RWC) and photosynthetic pigments: ANOVA results presented that the main effects of irrigation genotype were significant ($P \leq 0.01$) for RWC and photosynthetic pigments (Table 4). The results of mean comparison indicated that in irrigation treatments, I_1 (31.99%) increased RWC, (241.43%) improved chlorophyll *a*, (295.94%) improved chlorophyll *b*, (255.20%) raised total chlorophyll, and carotenoid (257.71%) compared to I_3. G_3 (24.58%) increased RWC, (73.90%) improved chlorophyll *a*, (86.61%) improved chlorophyll *b*, (78.45%) raised total chlorophyll, and carotenoid (101.69%) compared to G_1 (Table 5).

Discussion

In this study, drought stress reduced sugar beet yield. In drought stress conditions, the growth and development of the leaves was limited, and subsequently, with the reduction of the leaf area, the absorption of light by the plants was reduced. Finally, the total amount of photosynthesis in the plants reduced, and as a result, the plant yield decreased. Under drought stress, water absorption from the soil was limited, and the amount of water in the leaf cells and then the leaf surface decreased; These factors led to a decrease in photosynthesis, and finally, the dry weight of the plant and its yield components were reduced (Ebmeyera *et*

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al., 2021). Mahmoodi *et al*. (2012) indicated that drought stress decreased sugar beet root yield compared to the control (without stress). Also, Shafiq *et al*. (2021) confirmed that drought stress reduced sugar beet and maize yield compared to the control (normal condition).

In this study, under drought stress, proline content increased. It is well-known that proline accumulation, overexpression of MDA content, and CAT and SOD activities in sugar beet under drought display a defense mechanism against the negative impacts of drought; the abovementioned characters considerably increased in stressed sugar beet plants in comparison to control. The over-accumulation of MDA and proline is a response to drought. Our findings are in agreement with the results of other researchers; they reported that proline and MDA were considerably elevated under stress circumstances in sugar beet plants (Ghaffaria *et al*., 2021).

The mutual action of CAT and SOD converts the toxic O_2^- and H_2O_2 into water and molecular oxygen, preventing the cellular injure under drought stress. The highest CAT and SOD activities were found in G_2 and the highest GPX activity was found in G_3 genotype. The highest CAT activity in interaction treatments was found in G_2 and G_3 genotypes in drought stress treatments. The highest GPX activity in interaction treatments was found in G_3 genotype in drought stress treatments. In addition, the maximum antioxidant enzymes activities were found in water deficit stress conditions. In drought sensitive cultivars the decreased SOD activity was mostly observed and drought tolerance could be correlated with enzymatic defense. Activities of various antioxidant enzymes are known to increase in response to drought. However, CAT activities may increase, decrease or remain unchanged under drought stress. These results are in agreement with our findings. Different antioxidant enzymes activities in different genotypes could be related to different genetic behavior for tolerance to drought stress conditions. However, antioxidant enzymes such as SOD, CAT and GPX play a key role in scavenging those activated species (Wisniewska *et al*., 2019; Abdelaal *et al*., 2020).

The level of response to drought stress depends on the species, the developmental and metabolic state of the plant, and the duration and intensity of the drought stress. Many researchers have also suggested that drought tolerance is frequently associated with a more efficient anti oxidative system (Farooq *et al*., 2013). Moreover, Abdelaal *et al*. (2020) reported that ability for adaptation to drought stress depended on the maintenance of or increases in the capability to detoxify super oxide radical by antioxidant enzymes. Furthermore, SOD and CAT played a key role in protecting plants from oxidative stress by increasing their activities. Similarly, other researchers confirmed that drought stress increased the enzymes activity such as CAT, SOD, MDA in sugar beet (Islam *et al*., 2021).

In this study, RWC and photosynthetic pigments reduced due to drought in sugar beet compared with the control. It seems that this adverse effect on chlorophyll is because of the osmotic stress, decreasing water-holding capacity and stomatal movement, which limits CO_2 influx to leaves, decreasing photosynthesis, consequently reducing chlorophyll concentrations. Additionally, the decrease of chlorophyll concentration under drought might be due to the accumulation of ROS, resulting in chlorophyll degradation by the chlorophyllase enzyme, which increases the chlorophyll degradation and the destruction of chloroplasts. Also, drought led to a reduction in photosystem II activity and the rate of CO_2 assimilation in sugar beets. Similarly, other researchers recorded results in sugar beet plants (AlKahtani *et al*., 2021). The RWC of leaves is mentioned as a parameter to measure the water level of plants, and it shows the metabolic activities in the tissues. Decreasing leaves in RWC under drought conditions may be caused by the decrease in the amount of water absorbed by the plant, which is caused by the increase in osmotic potential, which causes it to collapse. The balance between the two processes of water absorption and transpiration is reached, and as a result, the RWC of the plant decreases, or it may be because the root systems are not able to compensate for the water lost by transpiration due to the reduction of the absorption level (AlKahtani *et al*., 2021).

Conclusion

The results of this study indicated that drought stress considerably reduced root yield, RWC, and photosynthesis of sugar beet, mainly due to oxidative stress. Drought stress significantly increased the reactive oxygen species and osmotic adjusting molecules in all sugar beet genotypes. Nevertheless, antioxidant enzyme activities were elevated under drought stress to induce the plant defense system. It can be concluded that drought stress increased enzymatic activity in sugar beet genotypes. Sugar beet might tolerate and protect itself from oxidative damage, such as lipid peroxidation, by increasing SOD, CAT, and GPX activities in leaves. Between genotypes, BP-Mashhad (G_3) was better than other genotypes because it has a high root yield and low enzyme activity compared to other genotypes. Thus, irrigation at a level of 80 mm (I_1: as control), evaporation from an A-class pan under the surface irrigation method, and the BP-Mashhad (G_3) genotype can be suggested.

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