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Antioxidant Enzyme Activities and Root Yield of Sugar Beet Genotypes under Drought Stress

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Abstract: The growth and development of plants are severely restricted by a variety of environmental stresses. Therefore, in order to evaluate the response of antioxidant defense system of three sugar beet genotypes to drought stress and improving soil water content management, a field experiment was conducted in a randomized complete block design under split plot arrangement with four replications at the Research Site of Sugar Beet Seed Institute in Karaj, Iran during 2012 and 2013. Irrigation treatments arranged in main plots included: 80 mm (I_1), 130 mm (I_2) and 180 mm (I_3) evaporation from A class pan under surface irrigation method, 30 mm (I_4) evaporation with 100% volume of water requirement under trickle irrigation (Tape) method, 80 mm (I_5), 130 mm (I_6), 180 mm (I_7) and 30 mm (I_8) evaporation with 75% volume of water requirement under trickle irrigation (Tape) method and genotypes included: 7112 (G_1), BP-Karaj (G_2) and BP-Mashhad (G_3) were in sub plots. Results of the study showed that drought stress decreased root yield (RY). Plants under water deficit stress indicated a significant increase in catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPX) activities in leaves. There were significant differences among genotypes for antioxidant enzyme activity except RY trait. Also, irrigation × genotype interactions showed significant difference on CAT and GPX activities. There was a negative correlation between enzymes activities and RY. It means that with increasing in enzymatic activity decrease RY. Our results suggested that drought stress leads to production of reactive oxygen species (ROSs), which results in increased membrane permeability, i.e. malondialdehyde (MDA) content and oxidative stress in the plants. Also, genotypes with higher levels of antioxidants showed higher resistance to drought stress.

Key words: Sugar beet · Antioxidant enzymes · Root yield · Drought stress · Iran

INTRODUCTION

Sugar beet yield are determined by genotype and environment [1]. Water is vital for plant growth and development [2]. Environmental stresses, including drought stress and temperature, affect nearly every aspect of the physiology and biochemistry of plants and significantly diminish yield [3]. It is well documented that drought is the major limiting factor for sugar beet yield [4]. By contrast, the sensitivity of sugar beet to water deficit has been poorly studied [5].

Drought stress significantly limits plant growth and crop productivity. However in certain tolerant-adaptable crop plants morphological and metabolic changes occur in response to drought, which contribute toward adaptation to such unavoidance environmental constraints [6]. Plants experience drought stresses either when the water supply to root becomes difficult or when the transpiration rate becomes very high [7]. Improving yield under drought is a major goal of plant breeding [8]. When plants are subjected to various a biotic stresses, some reactive oxygen species (ROS_s) such as superoxide radical (O_2^-), hydrogen Peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$) are produced [9]. These ROS_s may initiate destructive oxidative Processes such as Lipid oxidation, protein oxidation and damage to nucleic acids [10].

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The reaction of plants to water stress differ significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development [11]. Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic (superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione reductase) [12]. The degree to which the activities of antioxidant enzymes and the amount of antioxidants are elevated under drought stress is extremely variable among several plant species [13] and even between the two cultivars of the same species [6]. Much of the injury to plants exposed to stress is connected with oxidative damage at the cellular level [14]. If there is a serious imbalance in any cell compartment between the production of ROS_s and antioxidant defense, oxidative stress and damage occurs [15]. Foyer *et al.* [16] reported that drought-tolerant species increased their antioxidant enzyme activities and antioxidant contents in response to drought treatment, whereas drought-sensitive species failed to do so. To be able to endure oxidative damage under unfavorable conditions, plants must possess efficient antioxidant system [17].

Gunes *et al.* [18] and Manivannan *et al.* [19] reported that CAT and SOD activities of the Sunflower were increased by drought. Also, increase of SOD, CAT and GPX activities under water deficit in Canola was reported by Tohidi-Moghaddam *et al.* [20]. In response to drought, levels antioxidants showed increase, decrease or remained unchanged depending on crop, duration of drought and kind of antioxidants [21]. Therefore, the aim of the research was to investigate the effect of drought stress on enzymatic defense systems (SOD, CAT and GPX) and RY in sugar beet (*Beta vulgaris L.*) genotypes.

MATERIALS AND METHODS

Experimental Site: This experiment was conducted at the research site of Sugar Beet Seed Institute, Kamal-Abad, in Karaj, Iran during 2012-2013. This site is located at 35° 59' N latitude, 51° 6' E longitudes and an altitude of 1300 m above sea level. This region has a semi-arid climate (345 mm rainfall yearly).

Soil Sampling and Analysis: In order to determine soil physical and chemical properties of the experimental site, a composite soil sample 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 (2012 and 2013) are given in Table 1. Also, climate temperature and rainfall from sowing to harvest during both years (2012 and 2013) are presented in Table 2.

Field Methods: Eight treatments of irrigation were applied on the three genotypes using an experiment as split plot based on randomized complete block design (RCBD) with four replications. Irrigation treatments arranged in main plots included: 80 mm (I_1), 130 mm (I_2) and 180 mm (I_3) evaporation from A class pan under surface irrigation method, 30 mm (I_4) evaporation with 100% volume of water requirement under trickle irrigation (Tape) method, 80 mm (I_5), 130 mm (I_6), 180 mm (I_7) and 30 mm (I_8) evaporation with 75% volume of water requirement under trickle irrigation (Tape) method and genotypes included: 7112 (G_1), BP-Karaj (G_2) and BP-Mashhad (G_3) were in sub plots. Seed of different genotypes were planted on April 22, 2012 and May 20, 2013. Recommended levels of urea (300 kg ha^{-1}) in both years and triple super phosphate (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 were RY and the amounts of antioxidant enzymes (SOD, CAT and GPX). The harvested area for determination of RY was 6 square meter.

Sample Preparation for Biochemical Assay: After drought stress treatments (25-30 leaves stage), two leaves of each plant from each experimental unit were removed. Leaves sample were washed with distilled water and

Table 1: Soil physical and chemical properties of the experimental site (0-30 cm depth), 2012 and 2013

Date	Depth (cm)	pH	EC (dS m^{-1})	OC (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
2012	0-30	7.64	1.20	1.26	13.36	422	21.0	45.4	33.6	Clay loam
2013	0-30	7.65	1.35	1.11	40.01	771	25.7	49.2	25.1	Loam

Table 2: Mean temperature and monthly rainfall during crop growth (2012 and 2013)

Year		Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
2012	Temperature (°C)	22.8	20.8	24.9	28.0	27.2	24.3	18.3	7.40
	Rainfall (mm)	0.0	7.0	0.2	0.1	0.0	2.2	9.8	0.0
2013	Temperature (°C)	---	23.4	24.0	28.4	25.8	21.8	17.8	12.9
	Rainfall (mm)	---	1.3	6.8	0.0	1.6	10.3	7.9	26.5

homogenized in 0.16 mol Tries buffer (pH = 7.5) at 4 °C. Then, 0.5 mL of total homogenized solution was used for protein determination by the Lowry *et al.* method [22]. 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.

Superoxide Dismutase (SOD) Activity: SOD activity was determined with the reaction mixture contained 100 µL 1 µmol riboflavin, 100 µL 12 m mol L-methionine, 100 µL 0.1 m mol EDTA (pH 7.8), 100 µL 50 m mol Na_2CO_3 (pH 10.2) and 100 µL 75 µ mol nitroblue tetrazolium (NBT) in 2300 µL 25 m mol sodium phosphate buffer (pH 6.8), 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 photo reduction of NBT to blue formazan [23].

Catalase (CAT) Activity: CAT activity was estimated by the method of Cakmak and Horst [24]. The reaction mixture contained 100 crude enzyme extract, 500 µL 10 m mol H_2O_2 and 1400 µL 25 m mol 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 (µ mol min^{-1} substrate) per milligram of protein.

Glutathione Peroxidase (GPX) Activity: GPX activity was measured by the Paglia method [25] in which 0.56 mol (pH = 7) phosphate buffer, 0.5 mol EDTA, 1 m mol $NaNO_3$, 0.2 m mol NADPH were added to the extracted solution, GPX catalyses 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 30°C was measured with a spectrophotometer.

Statistical Analysis: All data were subjected to ANOVA using SAS statistical software. Means were separated by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

RESULTS AND DISCUSSION

Results of analysis of variance and the comparison of the means of irrigation, genotype and their interactions on different examine traits both years presented in Tables 3, 4, 5 and 6, respectively. There were significant differences among both years for SOD and CAT activities except GPX activity and RY (Table 3).

Root Yield (RY): Different irrigation treatments had a significant effect on RY of sugar beet during both years of study, but different genotypes and irrigation × genotype interaction treatments for the RY were not significant (Table 3). The highest RY (52.02 and 49.95 t ha^{-1}) observed in I_1 and I_2 treatments, respectively (Table 4). The lowest RY (28.16 t ha^{-1}) related to I_7 treatment (Table 4). Therefore, drought stress significantly reduced the RY of all the sugar beet

Table 3: Analysis of variance for root yield and antioxidant enzymes of sugar beet under different irrigation treatments (mean of 2013 and 2013)

		Mean square			
Source of variation	Df	RY	SOD enzyme	CAT enzyme	GPX enzyme
Year	1	211.37^{NS}	138782.52**	6533.33**	27^{NS}
Error	6	349.04	6322.3	111.32	256.54
Irrigation	7	1457.05**	3312181.78**	26082.24**	57500.09**
Year × Irrigation	7	112.67^{NS}	151224.02^{NS}	1611.33^{NS}	3833.57^{NS}
Error	42	77.85	201148.97	1511.23	4042.3
Genotype	2	129.3^{NS}	9098469.00**	42704.75**	344745.94**
Year × Genotype	2	41.47^{NS}	66116.02**	419.08**	1730.67^{NS}
Irrigation × Genotype	14	52.22^{NS}	23225.31^{NS}	858.33**	3394.96**
Year × Irrigation × Genotype	14	73.05^{NS}	10926.52^{NS}	189.33**	886.48^{NS}
Error	96	51.81	10147.71	71.22	689.2
C.V. (%)	---	17.52	6.03	5.38	7.52

NS = Non-significant

** = Significant at 0.01 probability level

(RY: root yield; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table 4: Means comparison for root yield and antioxidant enzymes between different irrigation treatments using DMRT at 5% (mean of 2012 and 2013)

Irrigation treatment	RY (t 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	52.02 a	1335.2 d	121.97 dc	309.88 d
I_2	49.95 ab	1525.1 dc	143.49 bc	334.67 dc
I_3	41.71 dc	1881.6 ab	179.44 a	373.71 abc
I_4	43.29 bc	993.80 e	97.320 d	256.17 e
I_5	41.76 dc	1756.5 bc	168.58 ab	361.29 bc
I_6	35.51 d	2131.4 a	187.76 a	410.83 a
I_7	28.16 e	1992.3 ab	186.78 a	391.29 ab
I_8	36.26 dc	1752.4 bc	169.88 ab	355.58 bc

Means in the same column with different letters differ significantly at 0.05 probability level according to DMRT.
(RY: root yield; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table 5: Means comparison for root yield and antioxidant enzymes between different sugar beet genotypes (mean of 2012 and 2013)

Sugar beet genotype	RY (t 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)
G_1	39.53 a	1348.16 c	127.21 b	381.48 a
G_2	41.91 a	2085.41 a	174.18 a	265.17 b
G_3	42.43 a	1579.53 b	169.31 a	400.87 a

Means in the same column with different letters differ significantly at 0.05 probability level according to DMRT.
(RY: root yield; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

genotypes. The decrease in RY in different sugar beet genotypes due to drought stress has been reported by other researchers [26, 27, 28].

Antioxidant Enzymes Activities: Results showed significant differences ($P \leq 0.01$) for CAT, GPX and SOD activities in irrigation and genotype treatments (Table 3). Significant differences ($P \leq 0.01$) observed for activity levels of CAT and GPX in irrigation × genotype interactions except SOD enzyme activity in both years (Table 3). Overall, activities of all the antioxidant enzymes increased under drought stress in all the genotypes. These results are in agreement with findings of Habibi *et al.* [29] and Tohidi-Moghaddam *et al.* [20]. The combined action of CAT and SOD converts the toxic O_2^- and H_2O_2 into water and molecular oxygen, averting the cellular damage under unfavorable conditions like water stress [30].

The highest CAT and SOD activities related to G_2 and then G_3 genotypes and about GPX activity related to G_3 genotype (Table 5). The highest CAT activity in interaction treatments related to G_2 and G_3 genotypes in drought stress treatments. The highest GPX activity in interaction treatments related to G_3 genotype in drought stress treatments. In addition, the maximum antioxidant enzymes activities related to water deficit stress conditions. In drought sensitive cultivars the decreased SOD activity was mostly observed and drought tolerance could be correlated with enzymatic defense [31]. Activities of various antioxidant enzymes are known to increase in response to drought [7, 32, 33]. Under drought, CAT activities can increase, decrease or remain unchanged [21]. Manivannan *et al.* [19] reported that CAT and SOD activities increased under drought stress in Helianthus annuus. Tohidi-Moghaddam *et al.* [20] reported that plants under water deficit stress showed a significant increase in SOD, CAT and GPX activities in leaves of Canola compared with control plants. 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 [34]. The increasing in resistance to drought stress in canola (Brassica napus L.) is associated with the antioxidant enzymes activities [20]. Simple correlation coefficients of final RY with other examined traits presented in Table 7.

Correlation coefficients between studied traits indicated that antioxidant enzymes activities had negative correlation with RY in different genotypes and irrigations treatments. It means that destructive of membrane (Lipid peroxidation) caused increase of MDA content and antioxidant enzyme activities. Finally, RY decreased due to consumption of energy for producing of antioxidant enzymes. The highest RY in different drought stress treatments related to G_2 and G_3 genotypes (Table 6). It means that these genotypes with increasing of antioxidant enzymes activities showed greater tolerance. The level of response to drought depends on the species, the developmental and metabolic state of the plant and the duration and intensity of the stress [35]. Several researchers have suggested that drought tolerance is

Table 6: Means comparison for different irrigation treatments and sugar beet genotypes combination on root yield and antioxidant enzymes using DMRT at 5% probability (mean of 2012 and 2013)

Irrigation × Genotypes		RY (t 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	46.53 abcdef	1121.13 n	111.85 ij	315.25 h
	G_2	55.27 a	1699.00 hi	133.04 gh	254.25 l
	G_3	54.24 a	1185.50 n	121.03 hi	360.13 g
I_2	G_1	49.84 abc	1201.75 n	116.38 ij	362.75 fg
	G_2	49.15 abcd	1952.88 ef	159.65 e	255.50 l
	G_3	50.85 ab	1420.64 m	154.44 ef	385.75 fg
I_3	G_1	40.32 defghi	1537.88 kl	142.10 fg	417.75 cde
	G_2	40.66 defghi	2265.75 c	201.86 abc	276.63 jkl
	G_3	44.15 bcdefg	1841.13 fg	194.34 bc	426.75 cde
I_4	G_1	41.55 cdefg	673.750 p	83.510 k	255.75 l
	G_2	43.10 cdefg	1445.63 lm	104.86 j	203.25 m
	G_3	45.23 bcdefg	826.130 o	103.59 jk	309.50 hi
I_5	G_1	39.56 efghi	1443.50 lm	133.80 gh	399.63 ef
	G_2	44.26 abcde	2140.50 d	188.20 cd	279.88 ijkl
	G_3	41.46 cdefgh	1685.38 ij	183.65 d	404.38 def
I_6	G_1	38.52 fghij	1809.63 gh	145.63 efg	462.00 ab
	G_2	32.66 hijk	2576.75 a	210.13 a	301.25 hij
	G_3	35.36 ghijk	2007.75 e	207.54 ab	469.25 a
I_7	G_1	27.50 k	1585.75 jk	152.14 ef	434.25 bcd
	G_2	29.79 jk	2450.88 b	206.79 ab	291.25 hijk
	G_3	27.20 k	1940.25 ef	201.40 abc	448.38 abc
I_8	G_1	32.32 ijk	1411.88 m	132.16 gh	404.50 def
	G_2	36.65 ghij	2151.88 d	188.93 cd	259.38 kl
	G_3	39.82 efghi	1693.50 ij	188.54 cd	402.88 def

Means in the same column with different letters differ significantly at 0.05 probability level according to DMRT.
(RY: root yield; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table 7: Pearson correlation coefficient between root yield and antioxidant enzymes of sugar beet

Trots	RY	SOD enzyme	CAT enzyme	GPX enzyme
RY	1	-0.293***	-0.29***	-0.241***
SOD enzyme	-0.293***	1	0.884***	0.116^{NS}
CAT enzyme	-0.29***	0.884***	1	0.33***
GPX enzyme	-0.241***	0.116^{NS}	0.33***	1

(RY: root yield; SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

often correlated with a more efficient antioxidative system [21, 36]. Jagtap and Bhargava [37] reported that SOD activity increased in drought-tolerant cultivars of maize (Zea mays L.). Fu and Huang [38] stated that capability for adaptation to drought stress related to the maintenance of or increases in the ability to detoxify superoxide radical by antioxidant enzymes. Particularly, SOD and CAT played a key role in protecting plants from oxidative stress by increasing their activities.

CONCLUSION

In this study, drought stress decreased RY and increased enzymatic activity in sugar beet genotypes. There was no difference between genotypes for RY trait. We found sugar beet tolerate and control drought stress with protecting itself from oxidative damage such as lipid peroxidation by increasing of SOD, CAT and GPX activities in leaves. Also, out come of this work indicated that G_2 and G_3 genotypes with irrigation amount of 80 mm evaporation of pan by surface method were better treatments. The most RY related to G_2 and G_3 genotypes when there was water stress.

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