

## Effect of Drought Stress on Antioxidant Enzyme Activities and Root Yield of Sugar Beet (*Beta vulgaris*)

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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 2008 and 2009 growing seasons. Irrigation treatments arranged in main plots during growing seasons included: 80 mm (I<sub>1</sub>: as control), 130 mm (I<sub>2</sub>) and 180 mm (I<sub>3</sub>) evaporation from A class pan under surface irrigation method, 30 mm (I<sub>4</sub>), 80 mm (I<sub>5</sub>), 130 mm (I<sub>6</sub>) and 180 mm (I<sub>7</sub>: as severe drought) evaporation with 100% volume of water requirement under trickle irrigation (Tape) method, and 30 mm (I<sub>8</sub>) evaporation with 75% volume of water requirement under trickle irrigation (Tape) method. Genotypes included: 7112 (G<sub>1</sub>), BP-Karaj (G<sub>2</sub>) and BP-Mashhad (G<sub>3</sub>) 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 decreased RY. The obtained results suggested that drought stress leads to production of reactive oxygen species (ROS), which resulted in an 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 • Enzymatic defense system • Peroxidation lipid • Root yield • Water deficit

### INTRODUCTION

Sugar beet yield is 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 unavoidable 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) such as superoxide

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radical ( $O_2^-$ ), hydrogen Peroxide ( $H_2O_2$ ), hydroxyl radical (OH) and singlet oxygen ( $^1O_2$ ) are produced [9]. These ROS<sub>s</sub> may initiate destructive oxidative Processes such as Lipid oxidation, protein oxidation and damage to nucleic acids [10].

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<sub>s</sub> 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 study 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 study site of Sugar Beet Seed Institute, Kamal-Abad, in Karaj, Iran during 2008-2009. 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 (2008 and 2009) are given in Table 1. Also, climate temperature and rainfall from sowing to harvest during both years (2008 and 2009) are presented in Table 2.

**Field Method:** 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 during growing seasons included: 80 mm ( $I_1$ : as control), 130 mm ( $I_2$ ) and 180 mm ( $I_3$ ) evaporation from 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. Genotypes included: 7112 ( $G_1$ ), BP-Karaj ( $G_2$ ) and BP-Mashhad ( $G_3$ ) were in sub plots. Seed of different

Table 1: Soil physical and chemical properties of the experimental site (0-30 cm depth), 2008 and 2009

Date	Depth (cm)	pH	EC (dS m <sup>-1</sup> )	OC (%)	P (ppm)	K (ppm)	Sand (%)	Silt (%)	Clay (%)	Soil texture
2008	0-30	7.64	1.20	1.26	13.36	422	21.0	45.4	33.6	Clay loam
2009	0-30	7.65	1.35	1.11	40.01	771	25.7	49.2	25.1	Loam

Table 2: Mean monthly temperature and rainfall during crop growth, 2008 and 2009

Year		Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.
2008	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	0.00
2009	Temperature (°C)	---	23.4	24.0	28.4	25.8	21.8	17.8	12.90
	Rainfall (mm)	---	1.3	6.8	0.0	1.6	10.3	7.9	26.50

genotypes were planted on April 22, 2008 and May 20, 2009. Recommended levels of urea ( $300 \text{ kg ha}^{-1}$ ) in both years and triple super phosphate ( $50 \text{ kg ha}^{-1}$ ) only in the first year of study were used. Pest and weed control performed according to general local practices and recommendations. Measured parameters were root yield (RY) and the amounts of antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (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 homogenized in 0.16 mol Tris buffer (pH = 7.5) at  $4^{\circ}\text{C}$ . Then, 0.5 mL of total homogenized solution was used for protein determination according to the method described by Lowry *et al.* [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  $\mu\text{L}$  1  $\mu\text{mol}$  riboflavin, 100  $\mu\text{L}$  12 m mol L-methionine, 100  $\mu\text{L}$  0.1 m mol EDTA (pH 7.8), 100  $\mu\text{L}$  50 m mol  $\text{Na}_2\text{CO}_3$  (pH 10.2) and 100  $\mu\text{L}$  75  $\mu\text{mol}$  nitroblue tetrazolium (NBT) in 2300  $\mu\text{L}$  25 m mol sodium phosphate buffer (pH 6.8), 200  $\mu\text{L}$  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  $\mu\text{L}$  10 m mol  $\text{H}_2\text{O}_2$  and 1400  $\mu\text{L}$  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 ( $\mu\text{mol min}^{-1}$  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  $\text{NaNO}_3$ , 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  $30^{\circ}\text{C}$  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 are 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  $\times$  genotype interaction treatments for the RY were not significant (Table 3). The highest RY ( $52.02$  and  $49.95 \text{ t ha}^{-1}$ ) was observed with  $I_1$  and  $I_2$  treatments, respectively (Table 4). The lowest RY ( $28.16 \text{ t ha}^{-1}$ ) was related to  $I_7$  treatment (Table 4). Therefore, drought stress significantly reduced the RY of all the sugar beet genotypes. The decrease in RY in different sugar beet genotypes due to drought stress has been reported by Mahmoodi *et al.* [26], Richter *et al.* [27] and Winter [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  $\times$  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].

Table 3: Analysis of variance for root yield and antioxidant enzymes of sugar beet

Source of variation	Df	Mean square			
		RY	SOD enzyme	CAT enzyme	GPX enzyme
Year	1	211.37 <sup>NS</sup>	138782.52**	6533.33**	27 <sup>NS</sup>
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 <sup>NS</sup>	151224.02 <sup>NS</sup>	1611.33 <sup>NS</sup>	3833.57 <sup>NS</sup>
Error	42	77.85	201148.97	1511.23	4042.3
Genotype	2	129.3 <sup>NS</sup>	9098469.00**	42704.75**	344745.94**
Year × Genotype	2	41.47 <sup>NS</sup>	66116.02**	419.08**	1730.67 <sup>NS</sup>
Irrigation × Genotype	14	52.22 <sup>NS</sup>	23225.31 <sup>NS</sup>	858.33**	3394.96**
Year × Irrigation × Genotype	14	73.05 <sup>NS</sup>	10926.52 <sup>NS</sup>	189.33**	886.48 <sup>NS</sup>
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 (mean of 2008 and 2009)

Irrigation treatment	RY (t ha <sup>-1</sup> )	SOD enzyme (μ mol min <sup>-1</sup> /mg pr)	CAT enzyme (μ mol min <sup>-1</sup> /mg pr)	GPX enzyme (μ mol min <sup>-1</sup> /mg pr)
I <sub>1</sub>	52.02 a	1335.2 d	121.97 cd	309.88 d
I <sub>2</sub>	49.95 ab	1525.1 cd	143.49 bc	334.67 cd
I <sub>3</sub>	41.71 cd	1881.6 ab	179.44 a	373.71 abc
I <sub>4</sub>	43.29 bc	993.80 e	97.320 d	256.17 e
I <sub>5</sub>	41.76 cd	1756.5 bc	168.58 ab	361.29 bc
I <sub>6</sub>	35.51 d	2131.4 a	187.76 a	410.83 a
I <sub>7</sub>	28.16 e	1992.3 ab	186.78 a	391.29 ab
I <sub>8</sub>	36.26 cd	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 2008 and 2009)

Sugar beet genotype	RY (t ha <sup>-1</sup> )	SOD enzyme (μ mol min <sup>-1</sup> /mg pr)	CAT enzyme (μ mol min <sup>-1</sup> /mg pr)	GPX enzyme (μ mol min <sup>-1</sup> /mg pr)
G <sub>1</sub>	39.53 a	1348.16 c	127.21 b	381.48 a
G <sub>2</sub>	41.91 a	2085.41 a	174.18 a	265.17 b
G <sub>3</sub>	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)

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<sub>2</sub> and then G<sub>3</sub> genotypes and about GPX activity related to G<sub>3</sub> genotype (Table 5). The highest CAT activity in interaction treatments related to G<sub>2</sub> and G<sub>3</sub> genotypes in drought stress treatments.

The highest GPX activity in interaction treatments related to G<sub>3</sub> 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].

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 2008 and 2009)

Irrigation × Genotypes		RY (t ha <sup>-1</sup> )	SOD enzyme (μ mol min <sup>-1</sup> /mg pr)	CAT enzyme (μ mol min <sup>-1</sup> /mg pr)	GPX enzyme (μ mol min <sup>-1</sup> /mg pr)
I <sub>1</sub>	G <sub>1</sub>	46.53 abcdef	1121.13 n	111.85 ij	315.25 h
	G <sub>2</sub>	55.27 a	1699.00 hi	133.04 gh	254.25 l
	G <sub>3</sub>	54.24 a	1185.50 n	121.03 hi	360.13 g
I <sub>2</sub>	G <sub>1</sub>	49.84 abc	1201.75 n	116.38 ij	362.75 fg
	G <sub>2</sub>	49.15 abcd	1952.88 ef	159.65 e	255.50 l
	G <sub>3</sub>	50.85 ab	1420.64 m	154.44 ef	385.75 fg
I <sub>3</sub>	G <sub>1</sub>	40.32 defghi	1537.88 kl	142.10 fg	417.75 cde
	G <sub>2</sub>	40.66 defghi	2265.75 c	201.86 abc	276.63 jkl
	G <sub>3</sub>	44.15 bcdefg	1841.13 fg	194.34 bc	426.75 cde
I <sub>4</sub>	G <sub>1</sub>	41.55 cdefg	673.750 p	83.510 k	255.75 l
	G <sub>2</sub>	43.10 cdefg	1445.63 lm	104.86 j	203.25 m
	G <sub>3</sub>	45.23 bcdefg	826.130 o	103.59 jk	309.50 hi
I <sub>5</sub>	G <sub>1</sub>	39.56 efghi	1443.50 lm	133.80 gh	399.63 ef
	G <sub>2</sub>	44.26 abcde	2140.50 d	188.20 cd	279.88 ijkl
	G <sub>3</sub>	41.46 cdefgh	1685.38 ij	183.65 d	404.38 def
I <sub>6</sub>	G <sub>1</sub>	38.52 fghij	1809.63 gh	145.63 efg	462.00 ab
	G <sub>2</sub>	32.66 hijk	2576.75 a	210.13 a	301.25 hij
	G <sub>3</sub>	35.36 ghijk	2007.75 e	207.54 ab	469.25 a
I <sub>7</sub>	G <sub>1</sub>	27.50 k	1585.75 jk	152.14 ef	434.25 bcd
	G <sub>2</sub>	29.79 jk	2450.88 b	206.79 ab	291.25 hijk
	G <sub>3</sub>	27.20 k	1940.25 ef	201.40 abc	448.38 abc
I <sub>8</sub>	G <sub>1</sub>	32.32 ijk	1411.88 m	132.16 gh	404.50 def
	G <sub>2</sub>	36.65 ghij	2151.88 d	188.93 cd	259.38 kl
	G <sub>3</sub>	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

Traits	RY	SOD enzyme	CAT enzyme	GPX enzyme
RY	1	-0.293***	-0.29***	-0.241***
SOD enzyme	-0.293***	1	0.884***	0.116 <sup>NS</sup>
CAT enzyme	-0.29***	0.884***	1	0.33***
GPX enzyme	-0.241***	0.116 <sup>NS</sup>	0.33***	1

\*\*\* = Significant at 0.001 probability level

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

Under drought, CAT activities can increase, decrease or remain unchanged [21]. Manivannan *et al.* [19] reported that CAT and SOD activities were 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 of canola leaves 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]. 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 under different drought stress treatments related to G<sub>2</sub> and G<sub>3</sub> 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, Zhang and Kirkham [21] and Hong *et al.* [36] have suggested that drought tolerance is often correlated with a more efficient antioxidative system. 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<sub>2</sub> and G<sub>3</sub> genotypes with irrigation amount of 80 mm evaporation of pan by surface method were better treatments. The most RY related to G<sub>2</sub> and G<sub>3</sub> genotypes when there was water stress.

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