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RESPONSE OF ANTIOXIDANT ENZYMES ACTIVITIES OF SUGAR BEET TO DROUGHT STRESS

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ABSTRACT

In order to evaluate the response of antioxidant defense system of three sugar beet genotypes to drought stress, a two-year field experiment was conducted at the Research Site of Sugar Beet Seed Institute in Karaj, Iran during 2008 and 2009. 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. Results of the study 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 significant difference on CAT and GPX activities. Results of the study also indicated that drought stress causes production of reactive oxygen species (ROSs), which results in greater membrane permeability, i.e. malondialdehyde (MDA) content and oxidative stress in the plants. Moreover, genotypes having greater levels of antioxidants showed better resistance to drought stress.

Keywords: sugar beet, drought stress, enzyme activities, reactive oxygen species, Iran.

INTRODUCTION

Environmental stresses, such as drought stress and high temperature, influence almost all aspects of plants physiology and biochemistry, and considerably reduce yield (Pitman and Lauchli, 2002). Water is very important for growth and development of plants (Shao *et al*., 2008). Drought stress significantly restricts plants growth and development, and consequently crop productivity. However, in tolerant and/or adaptable plants morphological and metabolic changes occur in response to drought stress, which contribute toward adaptation to these unavoidance ecological limitations (Blum, 1996). Drought stresses are experienced by plants either due to insufficient water supply or due to very high transpiration rate (Manivannan *et al*., 2007b). Improving crop yield under drought stress is one of the most important goals of plant breeding (Cattivelli *et al*., 2008). When plants are subjected to different biotic stresses, some reactive oxygen species (ROS_s) such as super oxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical (OH) and singlet oxygen ($^1O_2$) are produced (Li and Staden, 1998). These ROS_s may start destructive oxidative processes (Scandalios, 1993).

Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic defense system (Meloni *et al*., 2003). Moreover, activities of antioxidant enzymes and the amount of elevated antioxidants under drought stress are very changeable among plant species (Zaman and Das, 1991) and even between the two cultivars of identical plant species (Blum, 1996). A large amount of the damage to plants exposed to drought stress is owing to oxidative damage at the cellular level (Hernandez *et al*., 1993; Farooq *et al*., 2009). If there is a severe difference between the production of ROS_s and antioxidant defense in any cell, oxidative stress and damage occurs (Ouchi *et al*., 1990). Foyer *et al*., (1994) 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 to do so. To overcome oxidative damage under drought stress conditions, plants must have efficient antioxidant system (Stepien and Klobus, 2005). Gunes *et al*., (2008) and Manivannan *et al*., (2008) reported that drought stress increased CAT and SOD activities of the sunflower. Also, increase of SOD, CAT and GPX activities under drought stress in canola was reported by Tohidi-Moghaddam *et al*., (2009). However, depending on crop plant, duration of drought stress and type of antioxidants, antioxidants may increase, decrease or remain unchanged (Zhang and Kirkham, 1996).

Sugar beet is one of the most important crops (Abdel-Motagally and Attia, 2009). Moreover, sugar beet yield are determined by genotype and environment (Hoffman *et al*., 2009). 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 (Ober *et al*., 2003). Therefore, this research was carried to study the effect of drought stress on enzymatic defense systems (SOD, CAT and GPX) in three 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 2008-2009. This site is located at latitude of 35º 59' N, longitude of 51 º6' E and altitude of 1300 m above mean sea level in 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 (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.

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

Year	Depth (cm)	pH	EC (dS m^{-1})	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	0.0
2009	**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

Field method

Eight treatments of irrigation were applied on the three genotypes using a split plot experiment laid out in a 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 genotypes were planted on April 22, 2008 and May 20, 2009. 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 included the amounts of SOD, CAT and GPX (antioxidant enzymes).

Sample preparation for biochemical assay

In 25-30 leaves stage, two leaves of each plant from each experimental unit were removed. Leaves sample were prepared as described by Lowry *et al.* (1951) method. Leaves sample were washed with distilled water and homogenized in 0.16 mol Tries buffer (pH = 7.5) at 4 ºC. Then, 0.5mL 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.

Super oxide dismutase (SOD) activity

SOD activity was determined as described by Misra and Fridovich (1972) 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.

Catalase (CAT) activity

CAT activity was estimated by the method of Cakmak and Horst (1991). 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 (1967) method 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 hydro peroxide. 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 ºC was measured with a spectrophotometer.

Statistical analysis

All data were subjected to Analysis of Variance (ANOVA) using SAS statistical software. Also, means were separated by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

RESULTS AND DISCUSSIONS

Results of ANOVA and comparison of the means for irrigation, genotype and their interactions on different traits during both years of study are presented in Table-3, Table-4, Table-5 and Table-6, respectively. Results showed significant differences ($P \leq 0.01$) for CAT, GPX and SOD activities in irrigation and genotype treatments (Table-3). Also, significant differences ($P \leq 0.01$) were observed for activities of CAT and GPX in irrigation × genotype interactions except SOD 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.* (2004) and Tohidi-Moghaddam *et al*., (2009). 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 (Manivannan *et al*., 2007a).

Table-3. Analysis of variance for antioxidant enzymes of sugar beet.

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

NS = Non-significant
** = Significant at 0.01 probability level
(SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

The highest CAT and SOD activities were found in G_2 and the highest GPX activity was found in G_3 genotype (Table-5). 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 (Stajner *et al*., 1995). Activities of various antioxidant enzymes are known to increase in response to drought (Sairam and Srivastava, 2001; Guo *et al*., 2006;

Manivannan *et al*., 2007b). However, CAT activities may increase, decrease or remain unchanged under drought stress (Zhang and Kirkham, 1996). Manivannan *et al*., (2008) reported that CAT and SOD activities increased under drought stress in Helianthus annuus. Tohidi-Moghaddam *et al*., (2009) reported that plants under drought stress showed a significant increase in SOD, CAT and GPX activities in leaves of canola. 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 (Sgherri *et al*., 2000). The increasing in resistance to drought stress in canola (*Brassica napus* L.) is associated with the antioxidant enzymes activities (Tohidi-Moghaddam *et al*., 2009).

Simple correlation coefficients of examined traits presented in Table-7. Correlation coefficients between studied traits indicated that antioxidant enzymes activities had positive correlation with each other in different genotypes and irrigations treatments. 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 (Smirnoff, 1993). Many researchers have also suggested that drought tolerance is frequently associated with a more efficient antioxidative system (Zhang and Kirk ham, 1996; Hong *et al.*, 2005; Farooq *et al*., 2009). Moreover, Jag tap and Bhargava (1995) stated that activity of SOD increased in drought-tolerant cultivars of maize. Besides, Fu and Huang (2001) 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.

Table-4. Means comparison for antioxidant enzymes between different irrigation treatments using DMRT at 5% (mean of 2008 and 2009).

Irrigation treatment	**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	1335.2 d	121.97 cd	309.88 d
I_2	1525.1 cd	143.49 bc	334.67 cd
I_3	1881.6 ab	179.44 a	373.71 abc
I_4	993.80 e	97.320 d	256.17 e
I_5	1756.5 bc	168.58 ab	361.29 bc
I_6	2131.4 a	187.76 a	410.83 a
I_7	1992.3 ab	186.78 a	391.29 ab
I_8	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.
(SOD: super oxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table-5. Means comparison for antioxidant enzymes between different sugar beet genotypes (mean of 2008 and 2009).

Sugar beet genotype	**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	1348.16 c	127.21 b	381.48 a
G_2	2085.41 a	174.18 a	265.17 b
G_3	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.
(SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table-6. Means comparison for different irrigation treatments and sugar beet genotypes combination on antioxidant enzymes using DMRT at 5% probability (mean of 2008 and 2009).

Irrigation × genotypes		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	1121.13 n	111.85 ij	315.25 h
	G_2	1699.00 hi	133.04 gh	254.25 l
	G_3	1185.50 n	121.03 hi	360.13 g
I_2	G_1	1201.75 n	116.38 ij	362.75 fg
	G_2	1952.88 ef	159.65 e	255.50 l
	G_3	1420.64 m	154.44 ef	385.75 fg
I_3	G_1	1537.88 kl	142.10 fg	417.75 cde
	G_2	2265.75 c	201.86 abc	276.63 jkl
	G_3	1841.13 fg	194.34 bc	426.75 cde
I_4	G_1	673.750 p	83.510 k	255.75 l
	G_2	1445.63 lm	104.86 j	203.25 m
	G_3	826.130 o	103.59 jk	309.50 hi
I_5	G_1	1443.50 lm	133.80 gh	399.63 ef
	G_2	2140.50 d	188.20 cd	279.88 ijkl
	G_3	1685.38 ij	183.65 d	404.38 def
I_6	G_1	1809.63 gh	145.63 efg	462.00 ab
	G_2	2576.75 a	210.13 a	301.25 hij
	G_3	2007.75 e	207.54 ab	469.25 a
I_7	G_1	1585.75 jk	152.14 ef	434.25 bcd
	G_2	2450.88 b	206.79 ab	291.25 hijk
	G_3	1940.25 ef	201.40 abc	448.38 abc
I_8	G_1	1411.88 m	132.16 gh	404.50 def
	G_2	2151.88 d	188.93 cd	259.38 kl
	G_3	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.
(SOD: superoxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

Table-7. Pearson correlation coefficient between antioxidant enzymes of sugar beet.

Traits	SOD enzyme	CAT enzyme	GPX enzyme
SOD enzyme	1	0.884***	0.116^{NS}
CAT enzyme	0.884***	1	0.33***
GPX enzyme	0.116^{NS}	0.33***	1

*** = Significant at 0.001 probability level
(SOD: super oxide dismutase; CAT: catalase; GPX: glutathione peroxidase)

CONCLUSIONS

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 per oxidation by increasing SOD, CAT and GPX activities in leaves.

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