Available online at www.notulaebotanicae.ro

Print ISSN 0255-965X; Electronic 1842-4309

Not. Bot. Hort. Agrobot. Cluj 38 (3) 2010, 86-94

Notulae Botanicae Horti Agrobotanici Cluj-Napoca

Change in several Antioxidant Enzymes Activity and Seed Yield by Water Deficit Stress in Soybean (*Glycine max* L.) Cultivars

Hassan MASOUMI[1)], Mozhgan MASOUMI[2)], Farrokh DARVISH[1)], Jahanfar DANESHIAN[3)], Ghorban NOURMOHAMMADI[1)], Davood HABIBI[4)]

[1)] *Islamic Azad University, Department of Agronomy and Plant Breeding, Science and Research Branch, Post code 14778, Tehran, Iran; Masoumi_hassan118@yahoo.com*

[2)] *Islamic Azad University, Department of Microbiology, Tehran Medical Branch, Tehran, Iran*

[3)] *Seed and Plant Improvement Institute, Department of Oil Seed Crops, Karaj, Iran*

[4)] *Islamic Azad University, Faculty of Agriculture and Natural Sciences, Karaj Branch, Karaj, Iran*

Abstract

Drought stress is one of several environmental factors greatly limiting crop production. In order to study the effect of water deficit on antioxidant enzymes activity and seed yield of five soybean cultivars, an experiment was conducted in two growing seasons in 2008 and 2009. The experimental design was randomized complete block in a split plot arrangement with four replications. Irrigation treatments were (S_1, 50; S_2, 100 and S_3, 150 mm evaporation from the Class "A pan" evaporation) and cultivars were ('L_{17}', 'Clean', 'T.M.S.', 'Williams*Chippewa' and 'M_9'). The results showed that, water deficit stress increased antioxidants content [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)] significantly, but content of them were more at mild than high water deficit stress (S_2>S_3>S_1). Furthermore, water deficit stress, decreased total chlorophyll content, number of pods per plant, thousand seed weight, seed yield and harvest index in all of cultivars. Among cultivars, 'L_{17}' and 'Williams*Chippewa' produced the highest seed yield at the optimum condition of irrigation and both water deficit stress levels, respectively. Assessment of correlation results indicated that, there was a positive and significant correlation among SOD and seed yield in both water deficit stress levels, too.

Keywords: drought, oxidative stress, soybean, yield components

Abbreviation: ROS: Reactive oxygen species; SOD: Superoxide dismutase; CAT: Catalase; GPX: Glutathione peroxidase; MDA: Malondialdehyde; ABA: Abscisic acid; APX: Ascorbate peroxidase; HI: Harvest Index; a.s.l: above sea level; RCBD: Randomized Complete Block Design

Introduction

Glycine max is one of the most important oil seed crops in Iran that usually is confronted with water deficit stress and reduction of yield. The reasons are concerned to summer cultivation of soybean (substituting crop) and presence of competitor crops which have partially the same growth season. Drought stress significantly limits plant growth and crop productivity. A common consequence of drought stress is an increased production of ROS such as superoxide radicals ($^{\cdot}O_2^{-}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Agarwal *et al.*, 2005). One of the primary effects of ROS and their Products in cells is the peroxidation of membranes which leads to leakage of low-molecular-weight solutes, particularly K^+ (Hajiboland and Joudmand, 2009). The reaction of plants to water stress differs significantly at various organizational levels depending upon intensity and duration of stress as well as plant species and its stage of development (Demiral and Turkan, 2005). Mechanisms of active oxygen species detoxification exist in all the plants and include activation of enzymatic [superoxide dismutase (SOD, EC 1.1.5.1.1), catalase (CAT, EC 1.11.1.6), glutathione peroxidase (GPX)] (Johnson *et al.*, 2003) as well as non-enzymatic (flavones, anthocyanins, carotenoids and ascorbic acid) antioxidants (Nayyar and Gupta, 2006). Superoxide dismutase, is a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, it is an important antioxidant defense in nearly all cells exposed to oxygen (Corpas *et al.*, 2006) and plays a key role in the reduction of oxidative damages. Catalase is a common enzyme found in nearly all living organisms that are exposed to oxygen, where it functions to catalyze the decomposition of hydrogen peroxide to water and oxygen (Vertuani *et al.*, 2004). Catalase has one of the highest turnover number of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen per second (Chelikani *et al.*, 2004). Also, glutathione peroxidase is the general name of an enzyme family with peroxidase activity whose main biological role is reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water (Ran *et al.*, 2007).

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 (Zhang and Kirkham, 1995) and even between two cultivars of the same species (Bartoli *et al.*, 1999). However, under conditions of environmental stress, production of ROS can increase and endogenous protective activity may then become inadequate. Various associations between water deficit stress and endogenous levels of water-soluble antioxidants have been described (Zaman and Das, 1991; Borrmann *et al.*, 2009; Manavalan *et al.*, 2009). Environmental stresses including drought and temperature affect nearly every aspect of the physiology and biochemistry of plants and significantly diminish yield (Vranov *et al.*, 2002). Drought stress induces cellular accumulation of ROS which can damage membrane lipids, proteins and nucleic acids (Munns, 2002; Lovelli *et al.*, 2007). Several studies have pointed out 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 (Foyer *et al.*, 1994; Selote and Khanna-Chopra, 2004). In addition, plants are subjected to the interaction of two or more environmental stress factors under natural conditions and many studies have been carried out to study the effects of these stress factors on plant metabolism separately. Therefore, the aim of the study was to investigate the effect of water deficit stress levels on number of pods per plant, thousand seed weight, seed yield, total chlorophyll content and activities of three antioxidant enzymes (CAT, SOD, GPX) for five cultivars of soybean.

Materials and methods

Experimental set-up

The experiment was carried out at educational farm of Karaj Islamic Azad University (35° 48' 29" N, 51° 10' 29" E, and 1321 meter a.s.l), Iran during the 2007-2008 and 2008-2009 growing seasons. This location is classified in a semiarid area (according to the Köppen climate classification) characterized by warm and dry summers, long-term (30 years) mean annual rainfall and temperature of 246 mm and 23.36 °C, respectively. The meteorological data recorded during the trial period in each growing season are given in Tab. 1. Before the experimental began, two composite soil samples were taken at depths of 0-30 and 30-60 cm. The samples were sent to laboratory and tested for pH, electrical conductivity (EC), organic carbon, total N, available P and available K. Details of soil properties are shown in Tab. 2.

The experimental design was a RCBD arranged in split plot with four replications. Each replication was divided into three main plots, which differed in severity of imposed water shortage. The water deficit treatments were applied by changing in irrigation intervals. Irrigations were carried out when an amount of evaporated water from class "A pan" evaporation reached 50 (S_1; optimum conditions of irrigation), 100 (S_2; mild water deficit) and 150 (S_3; high water deficit) mm, respectively. Irrigation levels were randomized to the main plots. Amount of irrigation was identical for all water deficit treatments from the beginning of planting time till complete establishment of plants (appearance of fourth and fifth nodes; R_5). Total irrigation water applied in optimum conditions of irrigation (S_1), mild and high water deficit stress levels (S_2, S_3) were 465, 234.5 and 146.56 m^3, respectively. After this stage, the plots were irrigated according to their prescribed treatment. Soybean Cultivars included 'L_{17}' (V_1), 'Clean' (V_2), 'T.M.S.' (V_3), 'Williams*Chippewa' (V_4) and 'M_9' (V_5), that were arranged in sub plots.

Before planting, the soil surface was ploughed during autumn and then disked twice in the spring (at the beginning of April and middle of May). Ttriple super phosphate fertilizer was applied before sowing at a rate of 150 kg ha^{-1}. The nitrogen fertilizer (15 kg ha^{-1}) in the form of urea was applied before planting (one third of the application). The rest of nitrogen fertilizer, distributed before starting the first stress treatment.

Plots were 7-m long and consisted of six rows, 0.6 m apart. Between all main plots, a 3-m wide strip was left bare to eliminate all influences of lateral water movement. Soil surface of cultivated area was thoroughly irrigated 6 days before planting. The soybean seeds were inoculated with *Rhizobium japonicum* before planting and were

Tab. 1. Monthly temperature and precipitation during the growing season in 2007-2008 and 2008-2009*

Month	Average temperature (°C)						Precipitation (mm)	
	Minimum		Maximum		Mean			
	2007-2008	2008-2009	2007-2008	2008-2009	2007-2008	2008-2009	2007-2008	2008-2009
May	12.1	12.0	26.0	25.8	19.1	18.9	19.9	19.8
June	16.9	16.8	32.6	32.5	24.7	24.7	0.3	0.1
July	19.1	19.2	35.1	35.2	27.1	27.2	4.6	5.2
August	19.6	19.6	35.1	35.0	27.4	27.3	1.8	1.5
September	15.9	15.8	31.2	31.3	23.5	23.5	0.4	0.2
October	11.8	12.0	24.9	25.1	18.4	18.6	11.6	10.6

* Data recorded at the Karaj meteorological Station

hand-planted on 24th May 2008 and 26th May 2009 at the rate of 20 seeds per m^2 of row and then were thinned to achieve a density of approximately 333,333 ha^{-1}. During the whole growth season, weeds and insects were effectively controlled.

Tab. 2. Physico-chemical properties of the soil in the experimental field

Soil properties	Values			
	2007-2008		2008-2009	
	0-30 cm	30-60 cm	0-30 cm	30-60 cm
EC (dSm^{-1})	1.4	2.25	1.31	2.1
pH	7.8	7.6	7.6	7.3
Organic carbon (%)	0.72	0.49	0.67	0.51
Total N (%)	0.07	0.05	0.05	0.03
Available P ($mgkg^{-1}$)	6.1	5.7	7.6	7.2
Available K ($mgkg^{-1}$)	182	186	195	188

Measurement of number pods per plants, thousand seed weight seed yield and harvest index

Six representative plants per plot were sampled to calculate the number of pods per plant and harvest index (HI). Number of pods were added for all six plants separately, and then averaged. Harvest index (HI) was computed as the division of seed yield per biological yield. Biological yield was by sampling six plants in each plot. Samples were dried in an oven at 60˚C for 72 hours and then weighted. One thousand seed weight was determined by measuring the weight of 500 seeds from each plot and multiplying by 2 in order to express as 1000 seeds. After the soybean cultivars reached physiological maturity seed yield was determined by harvesting two central rows in first week of October in both years.

Measurement of Antioxidant Enzymes Activities

To quantify antioxidant enzymatic activity, fifteen leaves were taken from each plot randomly and were placed in liquid N_2 and then stored at -80°C pending biochemical analysis.

Samples preparation for enzyme assay and protein measurement

Leaves from each sample were washed with distilled water and homogenized in 0.16M Tris 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.* (1951) method. 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

Superoxide dismutase activity was determined by a modification of the protocol of Misra and Fridovich (1972) at alkaline pH, O_2^- serves as chain propagation species for the autoxidation of epinephrine to adrenochrome. Superoxide dismutase competes with this reaction, thus decelerating the adrenchrome formation. One unit of superoxide dismutase is defined as the amount of extract that inhibits the rate of adrenochrome formation by 50%.

The modified assay contained 800 μL of buffer I (62.5 mM Na_2CO_3/$NaHCO_3$ [pH 10.2], 125 μM EDTA), 10 μl catalase and 10 μl epinephrine (10.5-11.0 mg epinephrine in 2 mL 0.1 N HCI). The final volume of 1 ml was adjusted by addition of buffer II (20 mM KH_2PO_4/K_2HPO_4 [pH 7.8], 0.5% [v/v] Triton X-100). The epinephrine solution was adjusted to cause a change in absorption of 0.021 to 0.024 per minute at 480 nm in the controls. The assay was performed in thermostated cuvettes at 30°C. The changes of absorption in controls and different dilutions of extract were recorded by a spectrophotometer. For the determination of superoxide dismutase activity each assay was repeated for five volumes of extract and five controls three to four times. The superoxide dismutase activity of the extracts were expressed as SOD units per milligram of protein

Catalase (CAT) activity

Catalase activity was estimated at 25°C as previously described by Paglia and Valentine (1987) that used hydrogen peroxide as substrate and one unit of catalase was defined as the rate constant of the first order reaction (k).

Glutathione peroxidase (GPX) activity

The activity was measured by the Paglia and Valentine (1987) method in which 0.56M (pH=7) phosphate buffer, 0.5M EDTA, 1mM NaN_3, 0.2mM NADPH were added to the extracted solution. Glutathione peroxidase (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 was measured with a spectrophotometer.

Measurement of Total Chlorophyll

Fresh leaves (1 g) were extracted with 80% acetone and centrifuged at 5000×*g* for 10 min. The absorbance of the supernatant was read at 645 and 663 nm and calculated for total chlorophyll (Arnon, 1949).

Statistical analyses

All data were analyzed with an analysis of variance (ANOVA) using the GLM procedure in SAS (SAS Institute, 2002). The assumptions of variance analysis was tested by ensuring that the residuals were random and homogenous, with a normal distribution about a mean of zero. The LSMEANS command was used to compare means at a $P<0.05$ probability. Correlation analysis using PROC

CORP in SAS were conducted to determine the relationship between measurement parameters and seed yield.

Results

The mean monthly of temperature and precipitation often had the same trend in both years during the growth season (Tab. 1). The negligible variation between the two years could explain the non significant interaction of the years and treatments in most traits.

Superoxide dismutase (SOD)

Superoxide dismutase content was affected by water deficit and cultivars as well as the interaction of water deficit×cultivars (Tab. 3). Mean comparison indicated that the SOD content in water deficit conditions (S_2, S_3) were higher than the optimum condition of irrigation (S_1). Of course, the SOD content had a decline order of $S_2>S_3>S_1$ (Tab. 4). Among cultivars and at the optimum conditions of irrigation, the highest and lowest SOD content was observed in 'L$_{17}$' and 'T.M.S.', respectively. In both mild and high water deficit stress levels, the highest and lowest SOD content were obtained from 'Williams*Chippewa' and 'T.M.S.' A positive and significant correlation was among SOD content with seed yield, CAT and GPX content in mild and high water deficit stress. Also, there was a positive correlation among SOD content and total chlorophyll in high water deficit stress (Tab. 8, 9).

Tab. 3. The mean squares of ANOVA for effect of irrigation levels on some antioxidants content in soybean cultivars

Features	df	Superoxide dismuatase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPX)
Year	1	ns	ns	ns
Irrigation levels	2	**	**	**
Year* Irrigation levels	2	ns	ns	ns
Cultivars	4	**	**	**
Cultivars * Irrigation levels	8	**	**	**
Year * Cultivars	4	ns	ns	ns
Year * Cultivars * Irrigation levels	8	ns	ns	**

ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively

Catalase (CAT)

Analysis of variance for CAT content showed that, there were significant differences ($P<0.01$) among irri-

Tab. 4. Effects of irrigation levels on some antioxidants content in soybean cultivars

Levels of irrigation	Cultivar	Superoxide dismuatase (u mg^{-1} protein)	Catalase (u mg^{-1} protein)	Glutathione peroxidase (u mg^{-1} protein)
S_1		1591.86^c	104.56^c	18.14^c
S_2		2442.48^a	130.30^a	34.75^a
S_3		2152.15^b	121.69^b	28.14^b
	'L$_{17}$'	2216.43^b	125.71^a	29.00^b
	'Clean'	2089.41^d	121.50^b	25.64^d
	'T.M.S.'	1556.41^e	99.81^c	20.45^e
	'Wiliams*Chippewa'	2304.09^a	127.71^a	32.95^a
	'M$_9$'	2144.5^c	119.53^b	26.92^c
S_1	'L$_{17}$'	1856.15^a	119.15^a	22.68^a
S_1	'Clean'	1772.70^b	114.09^{ab}	19.87^b
S_1	'T.M.S.'	1307.56^d	83.86^d	14.42^e
S_1	'Wiliams*Chippewa'	1524.68^c	106.96^b	17.59^c
S_1	'M$_9$'	1498.20^c	98.72^c	16.14^d
S_2	'L$_{17}$'	2711.18^b	134.29^b	40.14^b
S_2	'Clean'	2303.22^d	127.77^c	30.56^d
S_2	'T.M.S.'	1858.18^e	119.06^d	25.91^e
S_2	'Wiliams*Chippewa'	2803.12^a	140.39^a	43.87^a
S_2	'M$_9$'	2536.71^c	130.01^{bc}	33.04^c
S_3	'L$_{17}$'	2081.14^d	123.68^b	24.19^d
S_3	'Clean'	2192.30^c	122.63^b	26.50^c
S_3	'T.M.S.'	1503.48^e	96.51^c	21.02^e
S_3	'Wiliams*Chippewa'	2584.45^a	135.76^a	37.41^a
S_3	'M$_9$'	2398.58^b	129.87^a	31.59^b

Levels of irrigation: S_1; optimum condition of irrigation, S_2; mild water deficit stress level, S_3; high water deficit stress level; for a given means within each column of each section followed by the same letter are not significantly different ($P<0.05$); u mg^{-1} protein: International Units of activity per milligram protein

Tab. 5. The mean squares of ANOVA for effect of irrigation levels on total chlorophyll content, number of pods per plant, thousand seed weight, seed yield and harvest index in soybean cultivars

Features	df	Total Chlorophyll	Number of pods per plant	Thousand seed weight	Seed yield	Harvest Index
Year	1	ns	ns	ns	ns	ns
Irrigation levels	2	**	**	**	**	**
Year* Irrigation levels	2	ns	ns	ns	ns	ns
Cultivars	4	**	**	**	**	**
Cultivars * Irrigation levels	8	**	**	**	**	**
Year * Cultivars	4	ns	ns	**	ns	ns
Year * Cultivars * Irrigation levels	8	ns	**	ns	ns	*

ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively

gation levels, cultivars and also interaction of irrigation levels×cultivars (Tab. 3). The CAT content increased in mild and high water deficit stress (S_2, S_3) compared to normal condition of irrigation. But CAT content was more in mild than high water deficit stress levels. At the optimum condition of irrigation, the highest and lowest CAT content were observed in cultivars of 'L_{17}' and 'T.M.S.' Furthermore, the differences in CAT content among 'Williams*Chippewa' and 'M_9' were not significant. At the mild and high water deficit stress levels (S_2, S_3), there were significant differences among cultivars. In both conditions, the highest and lowest CAT content were obtained from 'Williams*Chippewa' and 'T.M.S.' (Tab. 4). In the meanwhile, there was observed a positive and significant correlation between CAT content and seed yield at optimum condition of irrigation and high water deficit stress (Tab. 7, 8).

Glutathione peroxide (GPX)

Mean comparison of interactions effects between irrigation levels×cultivars indicated that GPX content increased with intensification in water deficit (Tab. 3). But similar to CAT and SOD, GPX content was more in mild water deficit stress than high water deficit stress and op-

Tab. 6. Effects of irrigation levels on total chlorophyll content, number of pods per plant, thousand seed weight, seed yield and harvest index in soybean cultivars

Levels of irrigation	Cultivar	Seed yield ($kg.ha^{-1}$)	Harvest Index (%)	Thousand seed weight (g)	Number of pods per plant	Total Chlorophyll (mg/g fw)
S_1		2339.31^a	46.43^a	146.76^a	27.65^a	5.33^a
S_2		1019.52^b	38.034^b	116.77^b	19.67^b	4.49^b
S_3		468.82^c	27.92^c	90.41^c	14.45^c	3.65^c
	'L_{17}'	1490.70^a	34.70^d	111.08^d	24.51^a	4.59^b
	'Clean'	1359.03^b	32.46^e	112.51^c	21.61^b	4.48^d
	'T.M.S.'	870.64^d	35.12^c	103.05^e	17.92^e	4.03^e
	'Wiliams*Chippewa'	1375.54^b	43.39^a	135.52^a	20.41^c	4.82^a
	'M_9'	1283.49^c	41.63^b	127.72^b	18.49^d	4.53^c
S_1	'L_{17}'	2869.17^a	45.80^c	148.50^c	33.41^a	5.65^a
S_1	'Clean'	2458.74^b	42.87^d	142.97_d	29.58^b	5.43^{ab}
S_1	'T.M.S.'	2002.83^e	46.96^b	130.15^e	25.82^c	5.12^b
S_1	'Wiliams*Chippewa'	2245.03^c	50.10^a	158.00^a	25.39^c	5.26^b
S_1	'M_9'	2120.80^d	46.43^{bc}	154.18^b	24.04^d	5.21^b
S_2	'L_{17}'	1184.72^b	33.25^d	103.59^d	23.11^a	4.57^b
S_2	'Clean'	1161.83^c	33.05^d	109.09^c	20.39^b	4.39^{bc}
S_2	'T.M.S.'	356.47^d	38.40^c	101.79^d	19.53^c	4.21^c
S_2	'Wiliams*Chippewa'	1246.63^a	43.98^a	140.76^a	18.48^d	4.80^a
S_2	'M_9'	1147.92^c	41.46^b	128.63^b	16.82^e	4.48^b
S_3	'L_{17}'	418.22^d	25.05^c	81.17^d	17.02^a	3.56^c
S_3	'Clean'	456.52^c	21.45^d	85.49^c	14.85^b	3.60^c
S_3	'T.M.S.'	252.62^e	20.01^e	77.21^e	8.42^c	2.75^d
S_3	'Wiliams*Chippewa'	634.97^a	36.08^b	107.81^a	17.35^a	4.40^a
S_3	'M_9'	581.75^b	37.00^a	100.36^b	14.59^b	3.91^b

Levels of irrigation: S_1; optimum condition of irrigation, S_2; mild water deficit stress level, S_3; high water deficit stress level; for a given means within each column of each section followed by the same letter are not significantly different (P<0.05); mg/g fw : Milligram per gram fresh weight

Tab. 7. Correlation coefficient between antioxidant content, total chlorophyll content, number of pods per plant, thousand seed yield, seed yield and harvest index at the optimum condition of irrigation (S_1)

Features	Superoxide dismuatase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPX)	Total chlorophyll	Number pods per plant	Thousand seed weight	Seed yield	Harvest Index
Superoxide dismuatase (SOD)	1							
Catalase (CAT)	0.96**	1						
Glutathione peroxidase (GPX)	0.97	0.95**	1					
Total chlorophyll	0.96**	0.90*	0.99**	1				
number pods per plant	0.85	0.73	0.91*	0.95*	1			
Thousand seed weight	0.31	0.52	0.30	0.2	-0.11	1		
Seed yield	0.93*	0.89*	0.99**	0.99**	0.94*	0.23	1	
Harvest Index	-0.53	-0.31	-0.38	-0.44	-0.50	0.40	-0.36	1

ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively

Tab. 8. Correlation coefficient between antioxidant content, total chlorophyll content, number of pods per plant, thousand seed yield, seed yield and harvest index at the mild water deficit stress (S_2)

Features	Superoxide dismuatase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPX)	Total chlorophyll	Number pods per plant	Thousand seed weight	Seed yield	Harvest Index
Superoxide dismuatase (SOD)	1							
Catalase (CAT)	0.97**	1						
Glutathione peroxidase (GPX)	0.94*	0.97**	1					
Total chlorophyll	0.94	0.99**	0.97**	1				
number pods per plant	0.06	0.03	0.16	0.55	1			
Thousand seed weight	0.62	0.69	0.59	0.75	-0.69	1		
Seed yield	0.9*	0.85	0.74	0.78	0.04	0.53	1	
Harvest Index	0.22	0.31	0.28	0.43	-0.81	0.84	0.006	1

ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively

Tab. 9. Correlation coefficient between antioxidant content, total chlorophyll content, number of pods per plant, thousand seed yield, seed yield and harvest index at the high water deficit stress (S_3)

Features	Superoxide dismuatase (SOD)	Catalase (CAT)	Glutathione peroxidase (GPX)	Total chlorophyll	Number pods per plant	Thousand seed weight	Seed yield	Harvest Index
Superoxide dismuatase (SOD)	1							
Catalase (CAT)	0.98**	1						
Glutathione peroxidase (GPX)	0.91*	0.85	1					
Total chlorophyll	0.99**	0.97**	0.94*	1				
number pods per plant	0.85	0.92*	0.64	0.85	1			
Thousand seed weight	0.88*	0.81	0.99**	0.91*	-0.56	1		
Seed yield	0.99**	0.95*	0.95*	0.98**	0.77	0.94*	1	
Harvest Index	0.82	0.77	0.89*	0.83	0.54	0.93*	0.89*	1

ns, *, and **: non-significant and significant, at the 5% and 1% levels of probability, respectively

timum condition of irrigation (S_2>S_3>S_1). Assessment of interaction between irrigation levels×cultivars showed that at the optimum condition of irrigation, cultivars of 'L_{17}' and 'T.M.S.' had the highest and lowest GPX content, respectively. Whereas, at both mild and high water deficit stress levels, cultivars of 'Williams*Chippewa' and 'T.M.S.' indicated the highest and lowest GPX content (Tab. 4). In this experiment, there was a positive and significant correlation between GPX content and seed yield at the optimum condition of irrigation and high water deficit stress and the same correlation was observed among GPX content and total chlorophyll content in all of the irrigation levels (Tab. 7, 8, 9).

Total chlorophyll

Total chlorophyll content was altered by intensification in water deficit stress and decreased significantly in all of cultivars (Tab. 5). Among cultivars and at the optimum conditions of irrigation, the highest total chlorophyll content was observed in 'L_{17}'. Although, differences among cultivars of 'Clean', 'T.M.S.', 'Williams*Chippewa' and 'M_9' as well as 'L_{17}' and 'Clean' were not significant. At both mild and high water deficit stress, 'Williams*Chippewa' had more total chlorophyll content and least percent of declined compared with optimum condition of irrigation. Also, differences between cultivars of 'Clean' and 'L_{17}' at both conditions were not significant (Tab. 5). Assessment of correlation tables indicated that, there was a positive and significant correlation between total chlorophyll content and seed yield at the optimum condition of irrigation and high water deficit stress. Such a positive and significant correlation was observed among total chlorophyll and measured enzyme activities (SOD, CAT and GPX) in different irrigation levels of this experiment (Tab. 7, 8, 9).

Number of pods per plant

The analysis of variance indicated that irrigation levels, cultivars and interaction of cultivars×irrigation levels had significant effect on number of pods per plant. The number of pods per plant decreased significantly at mild and high water deficit (Tab. 6). At the optimum condition of irrigation and mild water deficit stress, cultivars of 'L_{17}' and 'M_9' had the highest and lowest number of pods per plant, whereas, at the high water deficit stress, the highest and lowest number of pods per plant were obtained from 'L_{17}', 'Williams*Chippewa' and 'T.M.S.' At the optimum condition of irrigation, the differences among cultivars of 'T.M.S.' and 'Williams*Chippewa' and at the high water deficit stress differences among cultivars of 'L_{17}' and 'Williams*Chippewa' as well as 'Clean' and 'M_9' were not significant. A noticeable point was that, a positive and significant correlation between number of pods per plant and seed yield was observed only at the optimum condition of irrigation (Tab. 7).

Thousand seed weight

Assessment of variance analysis and mean comparison tables indicated that, seed thousand weight decreased with intensification in water deficit stress in all of cultivars significantly (Tab. 5). The differences in thousand seed weight among cultivars were significant in all of irrigation levels. At the optimum condition of irrigation and high water deficit stress, the highest and lowest thousand seed weight were from 'Williams*Chippewa' and 'T.M.S.', respectively. Furthermore, the highest and lowest thousand seed weight were in cultivars of 'Williams*Chippewa' and 'L_{17}', 'T.M.S.', respectively (Tab. 6). There was a positive and significant correlation between thousand seed weight and GPX content, total chlorophyll, harvest index and seed yield at the high water deficit stress (Tab. 9).

Seed yield

Analysis of variance for Seed yield indicated significant differences (P<0.01) among irrigation levels, soybean cultivars and their interactions (Tab. 5). Seed yield decreased from optimum condition of irrigation to mild and high water deficit stress levels in all of cultivars, significantly. At the optimum conditions of irrigation, the highest and lowest seed were by cultivars of 'L_{17}' and 'T.M.S.' whereas, at the mild and high water deficit stress conditions, the highest and lowest seed yield were observed in cultivars of 'Williams*Chippewa' and 'T.M.S.' (Tab. 6). Also within this period, the highest and the lowest percent of decrease in seed yield were in cultivars of 'T.M.S.' (87.39%) and 'Williams*Chippewa' (71.72%).

Harvest Index (HI)

There were significant differences (P<0.01) between irrigation levels, cultivars and interaction of cultivar×irrigation levels (Tab. 5). Mean comparison showed that, harvest index decreased with increase in intensity of water deficit stress in all of the cultivars (Tab. 6). At the optimum condition of irrigation, the highest and lowest harvest index were in cultivars of 'Williams*Chippewa' and 'Clean'. In this condition, the differences among cultivars of 'M_9', 'T.M.S.' and 'L_{17}' were not significant. The same trend was observed in mild water deficit stress. Although, the difference in harvest index among 'L_{17}' and 'Clean' was not significant. At the high water deficit stress, cultivars of 'M_9' and 'T.M.S.' had the highest and lowest harvest index, too. From the correlation aspect, a positive and significant correlation was observed between harvest index and GPX content, thousand seed weight and seed yield at the high water deficit stress (Tab. 9).

Discussion

The present findings revealed that all of the cultivars had varying ability to deal with oxidative stress that might govern their differential sensitivity to water deficit stress. They did differ significantly for water deficit stress injury in their seed yield, harvest index, thousand seed weight, number of pods per plants, antioxidant enzymes (SOD, CAT and GPX) and total chlorophyll contents at moderate and high water deficit stress levels (S_2, S_3). However, the differences between them became evident as the degree of the stress increased to moderate and higher levels. The water deficit stress levels (S_2, 100 and S_3, 150 mm evaporation from the Class "A pan" evaporation) decreased total chlorophyll, number of pods per plants, thousand seed weight, seed yield and harvest index in all of the assessed cultivars. The decrease in yield and yield components in different soybean cultivars due to water deficiency has also been reported by other researchers (Dominique *et al.*, 2007; Vicki Hufstetler *et al.*, 2007; Ohashi *et al.*, 2009). The results indicated that, cultivars of 'L_{17}' and 'Williams*Chippewa' had the highest seed yield in optimum condition of irrigation and both water deficit stress levels, respectively (Tab. 6). Also, Larry and Heatherly (2000) in their experiment showed, thousand seed weight is positively associated with seed yield.

Induction of oxidative stress in drought-stressed plants is well known in several cases (Borrmann *et al.*, 2009; Manavalan *et al.*, 2009) and its magnitude indicates the stress sensitivity of the genotype (Nayyar and Kaushal, 2002; Selote and Khanna-Chopra, 2004). Our results indicated that, activities of all measured antioxidants (SOD, CAT, GPX) were increased in all of cultivars and both water deficit stress levels (S_2, S_3) but, antioxidants levels (degree of antioxidant activity) were higher at mild than high water deficit stress ($S_2>S_3>S_1$).

This finding can be related to the ability of the crops against different intensities of water deficit stress. In other words, when crops are exposed in mild water deficit stress conditions, their antioxidant defensive mechanism is activated and the content of antioxidants will raise in them. Results of this research indicate the same trend, too. Thus, the content of all three measured antioxidants increased in all of the cultivars ($S_2>S_1$). Furthermore, it seems, when the intensity of water deficit stresses increase too much in crops, the physiological damages will increase, too. Thus, they can not promote their antioxidant defensive mechanism along with the intense of water deficit in parallel manner. In other words, in extreme water deficit stress condition, the antioxidant defensive mechanism of crops will be activated as well and the antioxidants content will increase as compared to the full-irrigated. But, due to excessive physiological damages resulted of water deficit stress, the antioxidant activities are less than mild water deficit level ($S_2>S_3>S_1$). Previously, an increase in the level of antioxidants was reported with increase in stress intensity in maize and soybean by Vasconcelos *et al.* (2009) and Jiang and Zhang (2002) which might be attributed to inhibitory effects of water stress on protein turnover causing depletion of antioxidants (Bartoli *et al.*, 1999). Also, Lee *et al.* (2009) reported a positive and significant correlation between CAT, SOD and APX (ascorbate peroxidase) in both well irrigated and water deficit stress conditions. Frthermore, Lobato *et al.* (2008) have also been found a positive and significant correlation between content of antioxidants with accumulation of ABA and seed yield in soybean cultivars. Among the various antioxidants examined, GPX content relatively showed larger increase than others suggest its vital involvement in deciding the oxidative response. Among cultivars, antioxidants contents were more in 'Williams*Chippewa' at both water deficit stress levels (mild and high). As well as, the highest percent of increase in antioxidants content (with the exception of CAT) was observed in this cultivar. Considering that, the cultivar of 'Williams*Chippewa' had the highest total chlorophyll content, thousand seed weight and seed yield in both water deficit stress levels, it seems that, this cultivar have more effective alternative mechanisms for defense against free radicals and oxidative stress.

Conclusions

In conclusion this study has shown that, all of the soybean cultivars responded to different water deficit stress levels and activated antioxidant defensive mechanism against free radical by a significant increase in antioxidants content. Also there was observed that, cultivars with higher antioxidants levels had more yield and yield components in water deficit stress. In other words, antioxidants enzymes could increase the survival capacity of soybean cultivars under conditions of water deficit stress.

Finally, the present findings revealed that, cultivars of 'L_{17}' and 'Williams*Chippewa' are more suitable than others for sowing at the optimum condition of irrigation (S_1) and water deficit conditions (S_2, S_3).

Acknowledgment

The authors thanks from Mr. Taghi Mahlooji and Mohammad Ali Dehbari (Department of agronomy and irrigation in the Djame Iran consulting engineering, Tehran, Iran) for their technical and valuable guidance.

References

Agarwal, S., R. K. Sairam, G. C. Srivastava and R. C. Meena (2005). Changes in antioxidant enzymes activity and oxidative stress by abscisic acid and salicylic acid in wheat genotypes. Biol. Plantarum 49(4):541-550.

Arnon, D. (1949). Copper enzymes in isolated chloroplasts: polyphenol oxidases in *Beta vulgaris*. Plant Physiol. 24:1-15.

Bartoli, C. G., M. Simontacchi, E. Tambussi, J. Beltrano, E. Montaldi and S. Puntarulo (1999). Drought and watering-dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. J. Exp. Bot. 50:375–383.

Ben Amor, N., A. Jimenez, W. Megdiche, M. Lundqvist, F. Sevilla and C. Abdelly (2007). Kinetics of the anti-oxidant response to salinity in the halophyte *Cakile maritime*. J. Integr. Plant Biol. 49:982-992.

Borrmann, D., R. D. Junqueira, P. Sinnecker, M. S. D. Gomes, I. A. Castro and U. M. L. Marquez (2009). Chemical and biochemical characterization of soybean produced under drought stress. Ciênc. Tecnol. Aliment. 29(3):676-681.

Chelikani, P., I. Fita and P. C. Loewen (2004). Diversity of structures and properties among catalases. Cell. Mol. Life Sci. 61(2):192-208.

Corpas, F., A. Fernandez, A. Carreas, R. Valderrama, F. Luque and F. J. Esteban (2006). The expression of different superoxide dismutase forms is cell-type dependent in olive leaves. Funct. Plant Biol. Chem. 38:1213-1216.

Demiral, T. and I. Turkan (2005). Comparative lipid peroxidation, antioxidant defense systems and proline content in roots of two rice cultivars differing in salt tolerance. Environ. Exp. Bot. 53:247-257.

Dominique, D., L. Tung and P. Roumet (2007). Identification of Soybean Plant Characteristics That Indicate the Timing of Drought Stress. Crop Sci. 40:716-722.

Foyer, C. H., M. Lelandais and K. J. Kunert (1994). Photo oxidative stress in plants. Plant Physiol. 92:696-717.

Hajiboland, R. and A. Joudmand (2009). The K/Na replacement and function of antioxidant defence system in sugar beet (*Beta vulgaris* L.) cultivars. Acta. Agr. Scand. Section B. Soil and Plant Sci. 59:246-259.

Jiang, M. Y., and J. H. Zhang (2002). Role of abscisic acid in water stress-induced antioxidant defense in leaves of maize seedlings. Free Radic. Res. 36:1001-1015.

Johnson, S. M., S. J. Doherty and P. R. Croy (2003). Biphasic superoxide generation in potato tubers: a response to stress. Plant Physiol. 13:1440-1449.

Larry, G. and F. Heatherly (2000). Drought stress and irrigation effects on germination of harvested soybean seed. Crop sci. 33:777-781.

Lee, B. R., L. S. Li, W. J. Jung, Y. L. Jin, J. C. Avice, A. Ouryy and T. H. Kim (2009). Water deficit-induced oxidative stress and the activation of antioxidant enzymes in white clover. Bio. Plantaru. 53(3):505-510.

Lobato, A. K. S., R. C. L. Costa, C. F. Oliveira Neto, B. G. Santos Filho, F. J. R. Cruz, J. M. N. Freitas and F. C. Cordeiro (2008). Physiological and biochemical behavior in soybean (*Glycine max* L.) plants under water deficit. Aus. J. of Crop Sci. 2(1):25-32.

Lovelli, S., M. Perniola, A. Ferrara and T. Di Tommaso (2007). Yield response factor to water (Ky) and water use efficiency of *Carthamus tinctorius* L. and *Solanum melongena* L. Agric. Water Manage. 92:73-80.

Lowry, O., A. Rosebrough, A. Far and R. Randall (1951). Protein measurement with folin phenol reagent. J. Bio. Chem. 193:680-685.

Manavalan, L. P., S. K. Guttikonda, L. S. P. Tran and H. T. Nguyen (2009). Physiological and Molecular Approaches to Improve Drought Resistance in Soybean. Plant and Cell Physiol. 50(7):1260-1276.

Misra, H. P. and I. Fridovich (1972). The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 247:3170-3175

Mittler, R., S. Vanderauwera, M. Gollery, F. V. Breusegem (2004). Abiotic stress series. Reactive oxygen gene network of plants. Trends Plant Sci. 9(10):490-498.

Munns, R. (2002). Comparative physiology of salt and water stress. Plant Cell Environ. 25:239-250.

Nayyar, H. and S. K. Kaushal (2002). Chilling induced oxidative stress in germinating wheat grains as affected by water stress and calcium. Biol. Plant. 45:601-604.

Nayyar, H. and D. Gupta (2006). Differential sensitivity of C_3 and C_4 plants to water deficit stress: Association with oxidative stress and antioxidants. Environ. Exp. Bot. 58:106-113.

Ohashi, Y., N. Nakayama, H. Saneoka, P. K. Mohapatra and K. Fujita (2009) Differences in the responses of stem diameter and pod thickness to drought stress during the grain filling stage in soybean plants. Acta. Physiol. Plant 31(2):271-277.

Paglia, D. E. and W. N. Valentine (1987). Studies on the quantitative and qualitative characterization of glutathione peroxidase. J. Lab. Med. 70:158-165.

Ran, Q., H. Liang, Y. Ikeno and Y. Jang (2007). Reduction in glutathione peroxidase for increases life span through increased sensitivity to apoptosis. J. Gerontol. A Biol. Sci. Med. Sci. 62:932-42.

SAS Institute. 2002. The SAS system for windows. Released 9.1. SAS Inst., Cary, NC.

Selote, D. S. and R. Khanna-Chopra (2004). Drought-induced spikelet sterility is associated with an inefficient antioxidant defense in rice panicles. Plant Physiol. 121:462-471.

Tuanhui, B., L. Cuiying, M. Fengwang, F. Fengjuan and S. Huairui (2010). Responses of growth and antioxidant system to root-zone hypoxia stress in two Malus species. Plant Soil. 327:95-105.

Vasconcelos, A. C. F., X. Z. Zhang, E. H. Ervin and J. D. Kiehl (2009). Enzymatic antioxidant responses to biostimulants in maize and soybean subjected to drought. Sci. Agricola. 66(3):395-402.

Vertuani, S., A. Angusti and S. Manfredini (2004). The antioxidants and pro-antioxidants network: an overview. Curr. Pharm. Des. 10(14):1677-94.

Vicki Hufstetler, E., H. Roger Boerma, T. E. Carter and J. Hugh (2007). Genotypic variation for three physiological traits affecting drought tolerance in soybean. Crop Sci. 47:25-35.

Vranov, E., D. Inze and F. Breusegem (2002). Signal transduction during oxidative stress. Exp. Botani. 372:1227-1236.

Zaman, A. and P. K. Das (1991). Effect of irrigation and nitrogen on yield and quality of safflower. Indian J. Agron. 36:177-179.

Zhang, J. X. and M. B. Kirkham (1995). Water relations of water-stressed, splitroot C_4 (*Sorghum bicolor; Poaceae*) and C_3 (*Helianthus annuus; Asteraceae*) plants. Am. J. Bot. 82:1220-1229.