Chemical and biochemical responses of soybean (*Glycine max* L.) cultivars to water deficit stress

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Abstract

Plant responses to oxidative injuries and content of antioxidants were comparatively studied in five soybean cultivars (L_{17}, Clean, T.M.S, Williams×Chippewa and M_9) at the three levels of irrigation. In order to measure the water deficit stress, the irrigation was applied based on three levels of evaporation from the 'class A' pan (50 mm; optimum condition of irrigation, 100 mm; moderate water deficit and 150 mm; extreme water deficit). A two-year field experiment was conducted in 2008 and 2009. The experimental design was a split plot in a randomized complete block design with four replications. The output results showed that, water deficit stress significantly increased activities of some antioxidant enzymes (catalase and glutathione redoctase) as well as ABA and Proline content. Lipid and protein oxidation (malondialdehyde and dityrosine contents) also increased significantly at the moderate and extreme water deficit stress. The relative water content, seed and oil yield were affected by water deficit and were reduced by both levels of water deficit. Among cultivars, L_{17} and Williams×Chippewa produced the highest seed and oil yield at the optimum condition of irrigation and both levels of water deficit stress, respectively. Assessment of correlation results indicated that, there was a positive and significant correlation among activity of antioxidant enzymes, ABA and proline content with seed and oil yield in both levels of water deficit stress, as well.

Keywords: ABA, Antioxidant enzymes, Oxidative injury, Proline.
Abbreviations: ABA- Abscisic acid; CAT- Catalase; GRD- Glutathione redoctase; RCBD- Randomized Complete Block Design; ROS- Reactive Oxygen Species; RWC- Relative Water Content; S_1- Optimum condition of irrigation; S_2- Moderate water deficit; S_3- Extreme water deficit; MDA- Malondialdehyde.

Introduction

Plants are immobile and therefore unable to escape stressful environments (Chai et al., 2005). Drought stress is one of the several environmental factors greatly limiting crop production and plant distribution worldwide (Zhang et al., 2010). A common consequence of drought stress is an increased production of reactive oxygen species (ROS) such as superoxide radical ($O2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$). These ROS are all toxic (Movahhedy-Dehnavy et al., 2009) and very reactive and cause severe damage to DNA, proteins and lipids (Clement et al., 2008). To eliminate or reduce toxicity of ROS, plants have evolved various protective mechanisms (includes enzymatic and non enzymatic antioxidant defense systems), which are effective at different levels of stress-induced deterioration (Creissen and Mullineaux, 2002). Some antioxidant enzymes include SOD (Nayyar and Gupta, 2006), CAT and GRD (Demiral and Turkan, 2005) play key roles in the formation and degradation of H_2O_2, too. The level of damages may be limited by enzymatic and nonenzymatic scavenjers of free radicals (Aroca et al., 2003). The degree to which the activities of antioxidants enzymes and the amount of antioxidants are elevated under drought stress varies among several plant species (Johnson et al., 2003) and even between two cultivars of the same species (Selote and

Khanna-Chopra, 2004). Catalases (CAT) are heme-containing antioxidant enzymes that catalyzes the dismutation of H_2O_2 into H_2O and O_2, using either an iron or manganese cofactor. This enzyme protects cells from damage caused by ROS (Zhenmei et al., 2009). Glutathione has antioxidant properties since the thiol group in its cydtein moiety is a reducing agent and can be reversibly oxidized and reduced (Zhen et al., 2009). Under water stress conditions, the lowering of the cellular water potential and the ABA accumulation give rise to a reorganization of the cellular metabolism, accumulation of osmolytes, such as proline, glycine, betaine can be one of the most molecular responses to water stress (Hernandez et al., 2001). The principal role of proline probably is not to reduce the osmotic potential, but to protect enzymes against dehydration and salt accumulation (Heidari and Moaveni, 2009). Abscisic acid is a lipid hormone that inhibits cell growth in plants and is produced in large amounts when the plant lacks sufficient water, promoting closure of stomata and hence reducing further water losses (Efetova et al., 2007). Levels of ABA increase suddenly in response to various forms of stress, including heating, chilling and drought stress (Lee et al., 2009). Ohashi et al., (2009) reported that seed yield increased as the amount and number of irrigation increased. They also showed limited

irrigation can significantly decrease seed yields especially during two growth periods: flowering and filling stage. Crop yield depends on environmental and genetic factors (Chapman, 2008) and genetic characteristics can interact with environmental stress factors to affect crops growth and yield (Faisal Anwar Malik et al., 2009). The identification of attributes useful for the process of screening genotypes for drought tolerance is a major challenge to plant breeder. Thus, study of the agronomic and physiological characteristics associated to high yield potential under sub-optimal environmental conditions could be used as stress tolerance indexes in future elite germplasm. The aim of the study was to investigate the effect of different levels of irrigation on seed and oil yield, RWC, ABA and proline content, activities of some antioxidants enzymes (CAT and GRD), lipid and protein oxidation (MDA and dityrosine content) for five cultivars of soybean.

Materials and methods

Geographical location, climate and soil conditions of the experimental field

This study carried out during the 2007-2008 and 2008-2009 growing seasons at the educational farm of Karaj Islamic Azad University in Iran. The site had semi-arid climate characterized by warm and dry summers, long-term (30 years) mean annual rainfall and temperature of were 246 mm and 23.36 $^{\circ}$C, respectively. The meteorological data were taken from the Karaj meteorological station during the trial period in each growing season. The meteorological data are shown in Fig.1 and Fig. 2. Prior to the experiment, two composite soil samples were taken at depths of 0-30 and 30-60 cm. The samples were sent to laboratory and analysed for pH, electrical conductivity (EC), organic carbon, total N, available P and available K. Details of soil properties are shown in Table 1.

Statistical design

The experimental design was a split plot in a randomized complete block design with four replications. The irrigation treatments (three levels) were randomized to the main plots and soybean cultivars (five cultivars) were randomly distributed within the subplots of the water deficit treatments (main plots).

Irrigation treatments

The water deficit treatments were applied by changing in irrigation intervals. Irrigations were carried out when an amount of evaporated water from the class "A pan" evaporation reached 50 (S_1; optimum conditions of irrigation), 100 (S_2; moderate water deficit) and 150 (S_3; extreme water deficit) mm, respectively. Amount of irrigation was identical for all water deficit treatments from the beginning of planting time till complete establishment of plants. In order to make sure the identical amount of water discharge to every plot, the water contour instruments were used. Total irrigation water applied in S_1, S_2 and 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

Soybean cultivars were: V_1; L_{17}, V_2; Clean, V_3; T.M.S, V_4; Williams×Chippewa and V_5; M_9.

- L_{17}, Clean (group III maturity) and M_9 (group II maturity) are commercial cultivars in Iran and are being

cultivated in many arid and semi-arid regions of the country.

- T.M.S and Williams×Chippewa (group II maturity) are the two of the promising lines which have been selected for assessment of their tolerances to water deficit stress in Iran.

Agricultural practices

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). Triple super phosphate fertilizer was applied before sowing at a rate of 150 kg ha^{-1}. Also, 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 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.

Measurement of the seed and oil yield

After the soybean cultivars reached physiological maturity, seed yield was determined by harvesting two central rows (to avoid border effects) in the first week of October in both years. The oil percentage was estimated by Nuclear Magnetic Resonance (model SLK NMR-100) and the oil yield was calculated via multiplying seed yield to oil percentage.

Measurement of relative water content (RWC)

In order to measure the RWC, fresh leaves were weighed (FW) and immersed on distilled water for 4 h to regain turgidity, then blotted dry gently on filter paper and re-weighed (turgidity weight TW). The samples were dried at 80 $^{\circ}$C for 24 h to determine the dry weight (DW). Relative water content was calculated as in following equation (Ghoulam et al., 2002): RWC (%) = [(FW - DW) / (TW - DW)] *100

### Measurement of chemical and biochemical properties

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. In order to prepare samples for enzyme assays and protein measurement, leaves from each plant 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.

Catalase (CAT) activity

Catalase (CAT, EC 1.11.1.6) activity was measured by method of Paglia and Valentine (1987), hydrogen peroxide is

Table 1. Physico-chemical properties of the soil in the experimental field.

Soil properties	Values			
	2007-2008		2008-2009	
	0-30 cm	30-60	0-30 cm	30-60
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 (mg kg^{-1})	6.1	5.7	7.6	7.2
Available K (mg kg^{-1})	182	186	195	188
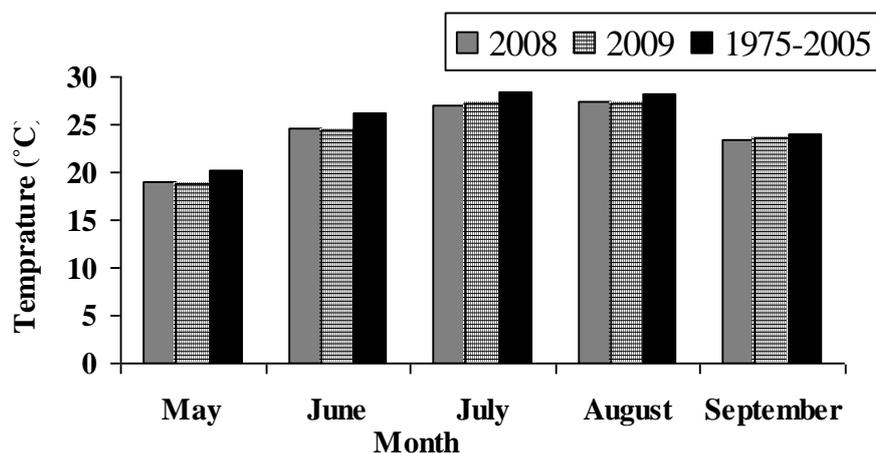
Fig 1. Mean monthly temperature during the soybean growing season (2007-2008 and 2008-2009) and the long term period (1975–2005).

used as substrate and one unit of catalase is defined as the constant rate of the first order reaction (k).

Glutathione redoctase (GRD) activity

Activity of Glutathione reductase (GR) (EC 1.6.4.2) was determined by the method of Foyer and Halliwell (1976) and modified by Rao (1992). The supernatant was immediately used to assay GR activity through glutathione-dependent oxidation of NADPH at 340 nm. About 1 ml reaction mixture, containing 0.2 mM NADPH, 0.5 mM GSSG and 50 μl of enzyme extract, was run for 5 min at 25°C by using UV–vis spectrophotometer. The activity was calculated by using extinction coefficient 6.2 mM^{-1} cm^{-1} and expressed as U mg^{-1} protein. One enzyme unit (U) determines the amount of enzyme necessary to decompose 1 μmol of NADPH per min at 25°C.

Lipid peroxidation (malondialdehyde Content)

The level of lipid peroxidation was measured in terms of MDA content using thiobarbituric acid (TBA)-reactive substances following the protocol of Sairam et al., (1998). Leaf samples of 0.5 g were homogenized in 10mL of 0.1% trichloroacetic acid (TCA). The homogenate was centrifuged at 15,000g for 5 min. Four milliliter of 0.5% TBA in 20% TCA was added to 2mL of aliquot of the supernatant. The mixture was heated at 100 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000g for 10min, the absorbance of supernatant was recorded at 532 nm. The value for non-specific absorption at 600 nm was subtracted.

Protein Damage (dityrosine content)

Fresh tissue material (1.2 g) were homogenized with 5 ml of ice-cold 50mM HEPES-KOH, pH 7.2, containing 10 mM EDTA, 2 mM PMSF, 0.1 mM p-chloromercuribenzoic acid, 0.1 mM DL-norleucine and 100 mg polyclar AT. The plan tissue homogenate was centrifuged at 5000 g for 60 min to remove debris. o,o-dityrosine was recovered by gradient elution from the C-18 column (Econosil C18, 250mm × 10 mm) and was analyzed by reversed-phase HPLC with simultaneous UV-detection (280 nm). A gradient was formed from 10 mM ammonium acetate, adjusted to pH 4.5 with acetic acid, and methanol, starting with 1% methanol and increasing to 10% over 30 min. A standard dityrosine sample was prepared according to Amado et al., (1984). Dityrosine was quantified by assuming that it's generation from the reaction of tyrosine with horseradish peroxidase in the presence of H_2O_2 was quantitative (using the extinction coefficient $e_{315} = 4.5$ mM^{-1} cm^{-1} at pH 7.5).

Abscisic acid (ABA)

Abscisic acid was extracted, purified and assayed following the procedure described by Li et al. (1992) with some modifications using GC–MS technique as reported earlier (Nayyar et al., 2005).

Proline

The proline content was examined according to the methods of Bates et al., (1973) and Lowry et al., (1951), elaborated elsewhere (Nayyar and Gupta, 2006).
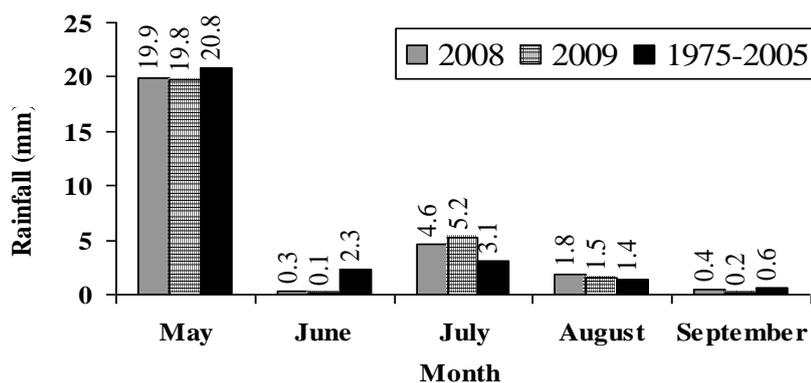
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Fig 2. Mean monthly rainfall during the soybean growing season (2007-2008 and 2008-2009) and the long term period (1975–2005).

Statistical analysis

Main and interaction effects of experimental factors were determined from analysis of variance (ANOVA) in SAS (SAS Institute Inc., 2002). The assumptions of variance analysis were 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 analyses using PROC CORP in SAS were conducted to determine the relationship between measured parameters and seed yield.

Results

The mean monthly temperature and precipitation often had the same trend in both years during the growth season (Fig 1 and Fig 2). The negligible variation between the two years could explain the non significant interaction of the years and treatments in most traits.

Abscisic Acid (ABA)

Significant change occurred in ABA content of leaves at different levels of irrigation (Table 2). Abscisic acid content increased with intense of water deficit stress in all of cultivars. The results showed, at the optimum condition of irrigation (S_1), cultivars of L_{17} and T.M.S had the highest and lowest ABA content in leaves, respectively. Moreover, in both levels of water deficit stress cultivars of Williams× Chippewa and T.M.S indicated the highest and lowest ABA content. Of course, at the level of S_3, cultivars of M_9 and Clean were not significant in ABA content (Table 3). Assessment of correlation tables at all of the irrigation levels indicated that, there was a positive and significant correlation between ABA content with Proline, GRD, CAT, RWC (with the exception at S2), seed and oil yield as well as negative and significant correlation with MDA and dityrosine content (Tables 4 ,5 ,6).

Proline

Significant differences in proline accumulation were revealed under water deficit stress. In water deficit treated leaves, the proline content was significantly higher (Table 2). In optimum condition of irrigation, the differences in proline content among all of cultivars were significant. In this condition (S_1), the highest level of proline, obtained from cultivar of L_{17}. In moderate water deficit stress level (S_2), the

proline content in cultivars had a decline order of Williams×Chippewa>L_{17}>M9>Clean>T.M.S. In extreme water deficit, the highest and lowest proline content were obtained from Williams×Chippewa and T.M.S. moreover in this condition, the difference among cultivars of L_{17} and Clean was not significant (Table 3). There was a positive and significant relationship between proline content and ABA, antioxidant enzymes, RWC, seed and oil yield (with the exception of S_1) in all of irrigation levels (S_1, S_2, S_3). Furthermore, proline content and biochemical biomarkers (MDA and dityrosine) had negative and significant correlation under fully irrigated condition (S_1) and water deficit stress levels (S_2, S_3) (Tables 4,5,6).

Glutathione redoctase (GRD)

The main effect of irrigation levels, cultivars and the interaction of irrigation levels×cultivars were significant (Table 2). The water deficit stress increased the GRD level compare to optimum condition of irrigation. Similar to SOD, the GRD level was more in S_2 than S_3. In the first level of irrigation (S_1), the highest of GRD content obtained from cultivar of L_{17}. Furthermore, the differences in GRD level among Williams×Chippwa and Clean were not significant. The highest GRD content was observed in Williams×Chippwa under the moderate and extreme water deficit. In these conditions, the lowest GRD content was observed in T.M.S. Also, there was no significant difference in GRD content between M_9 and Clean under the moderate water deficit condition as well as M_9, Clean and L_{17} under extreme water deficit level (Table 3). There was a positive and significant correlation between GRD level with CAT, seed and oil yield in all of irrigation levels. Although, the relationship between GRD level and biomarkers content (MDA and dityrosine) were negative and significant under three levels of irrigation (Tables 4, 5, 6).

Catalase (CAT)

Analysis of variance for CAT content showed that, there were significant differences ($P<0.01$) among irrigation levels, cultivars and the interaction of irrigation levels×cultivars (Table 2). The CAT content increased in moderate and extreme water deficit stress (S_2, S_3) compared to normal condition of irrigation (S_1). But, the CAT content was more in mild than high level of water deficit stress. At the optimum condition of irrigation, the highest and lowest CAT content were observed in cultivars of L_{17} and T.M.S. At the moderate

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Table 2. The mean squares of ANOVA for effect of irrigation levels on abscisic acid (ABA), proline, glutathione redoctase (GRD), catalase (CAT), malondialdehyde (MDA) content, dityrosine content, relative water content (RWC), seed yield and oil yield in soybean cultivars

Features		Mean Square								
	df	ABA	Proline	GRD	CAT	MDA	Dityrosine	RWC	Seed yield	Oil yield
Year	1	ns	ns	ns	ns	ns	ns	ns	ns	ns
Irrigation levels	2	**	**	**	**	**	**	**	**	**
Year* Irrigation levels	2	ns	ns	ns	ns	ns	ns	ns	ns	ns
Cultivars	4	**	**	**	**	**	**	**	**	**
Cultivars * Irrigation levels	8	**	**	**	**	**	**	**	**	**
Year * Cultivars	4	ns	ns	ns	ns	ns	ns	ns	ns	ns
Year * Cultivars * Irrigation levels	8	ns	ns	ns	ns	ns	ns	ns	ns	ns

Table 3. Effects of irrigation levels on abscisic acid (ABA), proline, glutathione redoctase (GRD), catalase (CAT), malondialdehyde (MDA) content, dityrosine content, relative water content (RWC), seed yield and oil yield in soybean cultivars

Levels of irigation	Cultivar	ABA (mg kg^{-1})	Proline (µmol g^{-1} fw)	GRD (mg protein)	CAT (u mg^{-1} protein)	MDA (nmol mg protein)	Dityrosine (nmol mg protein)	RWC (%)	Seed yield (kg ha^{-1})	Oil yield (kg ha^{-1})
S_1	L_{17}	8.33[a]	9.49[a]	3.2548[a]	119.15[a]	1.87[d]	9.66[d]	72.58[a]	2869.17[a]	636.29[a]
S_1	Clean	7.72[b]	8.99[b]	2.75[b]	114.09[ab]	2.19[c]	11.81[c]	69.63[b]	2458.74[b]	499.89[b]
S_1	T.M.S	5.15[e]	5.03[e]	1.98[d]	83.86[d]	3.240[a]	21.46[a]	64.98[c]	2002.83[e]	402.43[d]
S_1	Williams*Chippewa	7.27[c]	7.29[c]	2.65[b]	106.96[b]	2.249[c]	12.48[c]	69.23[b]	2245.03[c]	472.98[c]
S_1	M_9	5.98[d]	6.34[d]	2.30[c]	98.72[c]	2.53[b]	18.32[b]	66.37[c]	2120.80[d]	465.20[c]
S_2	L_{17}	9.18[b]	10.79[b]	5.73[ab]	134.29[b]	2.59[c]	18.14[c]	60.66[c]	1184.72[b]	282.70[b]
S_2	Clean	8.04[d]	9.10[c]	5.37[b]	127.77[c]	2.96[b]	23.88[b]	62.31[bc]	1161.83[c]	251.69[c]
S_2	T.M.S	5.44[e]	6.40[d]	4.38[c]	119.06[d]	3.89[a]	27.39[a]	58.94[d]	356.47[d]	76.94[d]
S_2	Williams*Chippewa	10.17[a]	11.92[a]	6.03[a]	140.39[a]	2.54[c]	15.25[d]	66.64[a]	1246.63[a]	*294.19[a]*
S_2	M_9	8.53[c]	9.29[c]	5.62[b]	130.01[bc]	2.62[c]	19.14[c]	63.47[b]	1147.92[c]	250.58[c]
S_3	L_{17}	10.67[c]	11.25[c]	3.60[c]	123.68[b]	3.68[b]	25.94[b]	54.74[b]	418.22[d]	107.01[c]
S_3	Clean	11.78[b]	11.70[c]	3.77[bc]	122.63[b]	3.265[c]	25.43[b]	55.11[b]	456.52[c]	102.45[c]
S_3	T.M.S	7.96[d]	7.75[d]	2.90[d]	96.51[c]	4.58[a]	28.71[a]	48.83[c]	252.62[e]	55.38[d]
S_3	Williams*Chippewa	12.95[a]	13.62[a]	4.38[a]	135.76[a]	2.87[d]	18.76[d]	60.12[a]	634.97[a]	169.20[a]
S_3	M_9	12.06[b]	12.58[b]	3.96[b]	129.87[a]	3.04[d]	20.47[c]	55.11[b]	581.75[b]	144.98[b]

Levels of irrigation: S_1; optimum condition of irrigation, S_2; moderate water deficit stress level, S_3; extreme 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

and extreme 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 (Table 3). At the extreme water deficit stress, the differences in CAT content among Williams×Chippewa and M_9 were not significant. In the meanwhile, a positive and significant correlation between CAT content and seed yield at the levels of S_1 and S_3 was observed (Tables 4, 5, 6).

Lipid peroxidation (MDA content)

Water deficit stress significantly increased MDA content in all of cultivars (Table 3). Assessment of interaction between irrigation levels×cultivars indicated that, leaves of all cultivars suffered more oxidative damage at the moderate and extreme levels of water deficit stress. According to consequences, cultivars of T.M.S and L_{17} had the highest and lowest MDA content at the optimum condition of irrigation, respectively. Furthermore, mean investigations indicated that, membranes in cultivars of M_9, L_{17} and Williams×Chippewa had higher endurance to moderate water deficit stress and obtained lowest MDA content in this condition. The same result (except L_{17}) was observed in extreme water deficit. In the present study and at the optimum condition of irrigation (S_1), output results between MDA content with antioxidant enzymes activity (CAT and GRD content) and stress hormone content (ABA) were significant and negatively correlated. The same trend was observed in both of water deficit stress (S_2, S_3). By the way, a negative and significant correlation among lipid peroxidation (MDA content) with seed and oil yield in these conditions was observed (Tables 4, 5, 6).

Protein damage (dityrosine content)

The dityrosine levels were found to be significantly higher in the water deficit stress levels (S_2, S_3) than optimum condition of irrigation (S_1) (Table 2). Among cultivars and at the optimum conditions of irrigation, the highest and lowest dityrosine level was observed in T.M.S and L_{17}, respectively. Although, there were no significant differences in dityrosine level between cultivars of Williams×Chippewa and Clean at the optimum condition of irrigation. At both moderate and extreme water deficit stress, cultivars of Williams×Chippewa and T.M.S had the lowest and highest dityrosine level. Also, differences between cultivars of M_9 and L_{17} at the S_2 level as well as cultivars of L_{17} and Clean at the S_3 level were not significant (Table 3). Assessment of correlation tables indicated that, there was a negative and significant correlation between protein damage (dityrosine level) and ABA, proline, GRD, CAT (exception in level of S_3) at all three levels of irrigation. Although, a negative and significant correlation between dityrosine level with seed and oil yield was observed only at the extreme water deficit stress (S_3) (Tables 4, 5, 6).

Relative Water Content (RWC)

Relative water content (RWC) altered by intensification in water deficit stress and decreased significantly in all of cultivars. Among cultivars and at the optimum conditions of irrigation, the highest RWC observed in L_{17}. Although, the differences between cultivars of Clean and Williams× Chippewa and also T.M.S and M_9 were not significant. At both moderate and extreme water deficit stress (S_2, S_3), Williams×Chippewa had more total RWC and least percent

of reduction in RWC compared with optimum condition of irrigation. Also, differences between cultivars of M_9 and Clean as well as Clean and L_{17} at level of S_2 and cultivars of Clean, L_{17} and M_9 at level of S_3 were not significant (Table 3). Assessment of correlation tables indicated that, there is a positive and significant correlation between RWC and ABA, proline, antioxidant enzymes, seed and oil at the extreme water deficit stress (S_3) (Tables 4, 5, 6).

Seed yield

Analysis of variance for Seed yield indicated significant differences ($P<0.01$) among irrigation levels, soybean cultivars and their interactions (Table 2). Seed yield decreased from first level of irrigation (S_1) to S_2 and S_3 in all of cultivars, significantly. At the optimum conditions of irrigation, the highest and lowest seed were produced by cultivars of L_{17} and T.M.S whereas, at the moderate and extreme water deficit stress conditions, the highest and lowest seed yield were observed in cultivars of Williams×Chippewa and T.M.S (Table 3). 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%).

Oil yield

The soybean oil yield was calculated via multiplying seed yield by oil percentage. The Seed yield and oil percentage were affected by irrigation levels and cultivars as well as the interaction of irrigation levels×cultivars treatments. The water deficit reduced seed yield, increased oil percentage (data were not shown) and consequently decreased oil yield in soybean cultivars. The L_{17} and Williams×Chippewa had the highest oil yield in full and limited irrigation conditions, respectively. Among the cultivars, the T.M.S had less oil yield under the well-irrigated and both water deficit levels (Table 3).

Discussion

The data obtained from this study indicated that, the responses of some physiological parameters symptomatic for oxidative stress and the related enzymes strongly depend on the severity of water deficit stress. Also, our results clearly demonstrated a wide variation in water deficit tolerance in soybean cultivars. They did differ significantly for water deficit stress injury in their seed and oil yield, RWC, lipid and protein oxidation (MDA and dityrosine content), antioxidant enzymes (CAT, GRD), ABA and proline contents at moderate and extreme levels of water deficit stress (S_2, S_3). Under water deficit conditions (S_2, S_3), seed and oil yield decreased in all of the assessed cultivars. The decrease in seed yield under water deficit conditions is largely due to the reduction in the number of pods per plant (Ohashi et al., 2009). However, when soil moisture reaches the lower values of available soil water, the number of seeds per pod and the weight of individual seeds may play an important role in diminishing the harvest index and final yield (Kirnak et al., 2008). Reductions in oil yield of soybean cultivars were also reported to take place under drought stress (Lee et al., 2008). The differences between oil content of cultivars were mainly due to the genetic differences (Sari and Ceylan, 2002) and irrigation levels (Movahhedy-Dehnavy et al., 2009). However, environmental conditions and management practices may also affect the oil content of the cultivars

Table 4. Correlation coefficient between contents of abscisic acid (ABA), proline, glutathione redoctase (GRD), catalase (CAT), malondialdehyde (MDA), Dityrosine content, relative water content (RWC), seed yield and oil yield at the optimum condition of irrigation (S_1).

Features	ABA	Proline	GRD	CAT	MDA	Dityrosine	RWC	Seed yield	Oil yield
ABA	1								
Proline	0.94605**	1							
GRD	0.97365**	0.95323*	1						
CAT	0.98488**	0.98046**	0.96008**	1					
MDA	-0.9576*	-0.93101*	-0.94898*	-0.98309**	1				
Dityrosine	-0.99541**	-0.95284*	-0.95915**	-0.97503**	0.95598*	1			
RWC	0.97986**	0.94847*	0.99581**	0.95226*	-0.93484*	-0.97028**	1		
Seed yield	0.90839*	0.92782*	0.96612**	0.89122*	-0.85497	-0.87232	0.95636*	1	
Oil yield	0.86098*	0.87072	0.95146*	0.86239	-0.86233	-0.82617	0.92838*	0.97653**	1

* and ******: significant at the 5% and 1% levels of probability, respectively. Numbers without symbols are non significant.

Table 5. Correlation coefficient between contents of abscisic acid (ABA), proline, glutathione redoctase (GRD), catalase (CAT), malondialdehyde (MDA), Dityrosine content, relative water content (RWC), seed yield and oil yield at the moderate water deficit stress level (S_2)

Features	ABA	Proline	GRD	CAT	MDA	Dityrosine	RWC	Seed yield	Oil yield
ABA	1								
Proline	0.98797**	1							
GRD	0.99432**	0.96662**	1						
CAT	0.98224**	0.99361**	0.9588**	1					
MDA	-0.95889**	-0.91193*	-0.98191**	-0.89521*	1				
Dityrosine	-0.95533*	-0.94803*	-0.94632*	-0.96672**	0.91186*	1			
RWC	0.79975	0.96279*	0.79547	0.80723	-0.71217	-0.77444	1		
Seed yield	0.93085*	0.88134*	0.95497*	0.84835	-0.96669**	-0.81093	0.7108	1	
Oil yield	0.96348**	0.93101*	0.97646**	0.90024*	-0.97618**	-0.86162	0.70739	0.99131**	1

* and ******: significant at the 5% and 1% levels of probability, respectively. Numbers without symbols are non significant.

Table 6. Correlation coefficient between contents of abscisic acid (ABA), proline, glutathione redoctase (GRD), catalase (CAT), malondialdehyde (MDA), Dityrosine content, relative water content (RWC), seed yield and oil yield at the extreme water deficit stress level (S_3)

Features	ABA	Proline	GRD	CAT	MDA	Dityrosine	RWC	Seed yield	Oil yield
ABA	1								
Proline	0.99069**	1							
GRD	0.98128**	0.9879**	1						
CAT	0.9731**	0.993753**	0.96986**	1					
MDA	-0.99594**	-0.98647**	-0.97392**	-0.96747**	1				
Dityrosine	-0.8743*	-0.89794*	-0.92996*	-0.87248	0.89141*	1			
RWC	0.94359*	0.95709*	0.97648**	0.94653*	-0.91758*	-0.86087	1		
Seed yield	0.95947**	0.97235**	0.98041**	0.9538*	-0.96926**	-0.97382**	0.92033*	1	
Oil yield	0.92586*	0.95649*	0.97178**	0.94624*	-0.93063*	-0.97939**	0.93078*	0.98843**	1

* and ******: significant at the 5% and 1% levels of probability, respectively. Numbers without symbols are non significant.

(Sabzalian et al., 2008). In this study, cultivar of Williams×Chippewa showed the highest seed and oil yield as well as smaller reduction in these parameters during the drought stress period compared to other cultivars. In this study, the ABA content increased significantly in the leaves of tested cultivars with an increase in intensity of water deficit stress. Among cultivars, the highest ABA content and also the highest percentage of the increase in ABA content at extreme water deficit stress (S_3), were observed in cultivars of Williams×Chippewa and M_9 (12.95 mg kg^{-1} and 101.67%), respectively. Heidari and Moaveni, (2009) reported that, the increase in ABA content is suggested to be associated with maintenance of growth of roots and shoots under water stress due to suppression of ethylene in case of maize. Here, higher ABA content in cultivars of Williams×Chippewa and M_9 perhaps may also impose greater stomatal restrictions in these cultivars to reduce water loss more effectively in contrast with other cultivars having lower ABA content. Similar to ABA, the proline level was significantly increased in all of cultivars. This accumulation may be a response characteristic of cultivars under water deficit (Urano et al., 2009), which it works as osmotic adjustor. At the moderate and extreme levels of water deficit stress (S_2, S_3), the highest content of proline was observed in cultivar of Williams×Chippewa (S_2, 11.92 and S_3, 12.95 μmol g^{-1} fw). Sircelj et al., (2005) in their study reported that, the proline accumulation in water-stress leaves of sorghum is associated positively with "recovery resistance", possibly by serving as a source of respiratory energy to the recovering plant. In present study, greater amount of ABA and proline in cultivar of Williams×Chippewa at extreme water deficit (S_3) might raise their ability to counter the oxidative injury relative to other cultivars. Water deficit treatments induced a reduction in the RWC of leaves. This reduction was more pronounced in the less tolerant cultivar T.M.S than in the more tolerant Williams×Chippewa. Reduction of RWC indicates a loss of turgor that results in limited water availability for cell expansion. Thus, the growth inhibition in T.M.S could be related to reduction of RWC provoked by the salt treatment. Shaoyun et al., (2009) indicated that, RWC reflects water status of plants, while ion leakage implicates the injury of plasmalemma. Higher RWC and lower ion leakage in cultivars under drought stress indicating that, those cultivars had an increased drought resistance. Output of results indicated that, activities of the antioxidants (CAT, GRD) were increased in all of the cultivars and both levels of water deficit stress (S_2, S_3). Induction of oxidative stress in drought-stressed plants reported in the previous studies (Borrmann et al., 2009; Manavalan et al., 2009). They showed that enzymatic antioxidants content played an important role in scavenging harmful oxygen species and the activities of antioxidant enzymes were altered when plants were subjected to stress. Results of our research also showed that, the content of antioxidants were higher at level of moderate than extreme water deficit stress ($S_2>S_3>S_1$). This subject would be explained such away, 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 two 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 the excessive physiological damages resulted of water deficit stress, the antioxidant activities are less than mild water deficit level ($S_2>S_3>S_1$). These findings can be related as the ability of the crops against different intensities of water deficit stress. Previously, an increase in the level of antioxidants was reported with an 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. Moreover, Lee et al., (2009) reported a positive and significant correlation between CAT, SOD and ascorbate peroxidase (APX) under both conditions of well irrigated and water deficit stress conditions. Furthermore, 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 measured antioxidants, GRD content relatively showed larger increase than CAT content, suggesting its vital involvement in deciding the oxidative response. Among cultivars, antioxidant contents were more in Williams×Chippewa at both water deficit stress levels (S_2, S_3). Considering that, the cultivar of Williams×Chippewa had the highest seed yield in both water deficit stress levels, it seems that this cultivar has more effective alternative mechanisms for defense against free radicals and oxidative stress.The MDA and dityrosine contents in leaves increased markedly to a higher extent in all of the cultivars at both moderate and extreme water deficit stress. Among cultivars and in the extreme water deficit (S_3), the lowest MDA and dityrosine contents were observed in Williams×Chippewa (2.87 and 18.76 nm/mg protein, respectively). This might explain lower lipid and Protein Oxidation of this cultivar relative to other cultivars. In other words, this cultivar appeared to has experienced less oxidative damage as compared to other cultivars, which is perhaps due to it superior capacity to counter the oxidative stress as well as higher water content. The same results reported by Dolatabadian et al., (2008), who showed that salt stress increased lipid peroxidation (MDA content) in canola cultivars.

Conclusion

Our results clearly demonstrate that all tested soybean cultivars responded positively with respect to antioxidant enzymes to water stress conditions. In all of soybean cultivars, enzymatic antioxidant defense systems were activated in response to the increase in intensity of water deficit stress by a significant increase in antioxidants content. The elevation of MDA and dityrosine in our experiment could be a direct reflection of an oxidative injury of the cells after water deficit stress. Furthermore, it was observed that, cultivars with higher antioxidant levels had lower lipid and protein oxidation as well as more seed and oil yield. This may be due to the protective effect of antioxidant enzymes on the membranous structure in cells. The findings of this research also showed that water deficit decreased the cell turgor or water potential gradients (RWC) as well as increased proline and ABA content. Finally, the present findings revealed that cultivars of L_{17} and Williams×Chippewa are more suitable than other cultivars for sowing at the optimum condition of irrigation (S_1) and water deficit conditions (S_2, S_3), respectively.

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