

# Effects of Different Levels of Water Deficit Stresses on the Morphologic Properties of Root, Antioxidants Activity and the Seed Yield in Five Cultivars of Soybean (*Glycine max* L)

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**Abstract** – A two-year field experiment was studied for assessment responses of five soybean cultivars (L<sub>17</sub>, Clean, T.M.S, Williams\*Chippewa, M<sub>9</sub>) to oxidative injuries and some antioxidants content in 2011 and 2012. The experimental design was split plot in a randomized complete block design with four replications. In this study, three levels of irrigation were applied based on 50 mm (S<sub>1</sub>; optimum condition of irrigation), 100 mm (S<sub>2</sub>; moderate water deficit) and 150 mm (S<sub>3</sub>; extreme water deficit) evaporation from class A pan. Results showed that, water deficit stresses was significantly increased contents antioxidants (superoxide dismutase and glutathione peroxidase) and root-shoot volume ratio, and also, was significantly decreased length and volume of roots. Among cultivars, L<sub>17</sub> and Williams\*Chippewa were produced the highest seed yield at the optimum condition of irrigation and both of water deficit stress levels. Assessment of correlation results indicated, there was positive and significant correlation between seed yield with antioxidants activity (superoxide dismutase and glutathione peroxidase) and there also was a negative and significant correlation between seed yield with root-shoot volume ratio.

**Keywords** – Drought, Oxidative Stress, Root-Shoot Ratio.

## I. INTRODUCTION

Soybean is one of the most important oil producing crops in Iran which due to the semiarid climatic conditions of the country, always finds itself exposing to the stress of water deficit and decrease of seed yield, setting in the agricultural pattern as the second cultivation (substituting crop) and the presence of competing produces during some of its growth phases. The water deficit stresses cause the change in biologic process in plants and their change to the autonomous and incontrollable processes, similar to all oxidative stresses, with the production of free oxygen radicals such as super oxide radical (O<sub>2</sub><sup>-</sup>), singlet Oxygen (O<sub>2</sub>), hydroxyl radical (OH<sup>-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>). These processes provide the arrangements to decrease the photosynthesis rate and even the death of the plant by producing the new specialization cells and damage to different parts of cell (DNA, Proteins and lipids) (2). On the contrary, the plants also step forward to

activate different defensive mechanisms such as the increase of anti oxidant compounds rate to protect different parts of its cell from undesired effects of free radicals (7). In the mechanism of antioxidant related defense, the enzymes by their direct and indirect interfere in the processes involved in the oxidative stress cause the speed decrease of oxidation and slowing of their accomplishment course (16). These compounds, in fact, by donating a hydrogen atom to the free radicals, prevent the expansion of chain reaction of oxidation (19). Dismutase super oxide, a type of storable protein is very powerful/strong with a catalytic role that catalyzes the superoxide dismutase to the hydrogen peroxide and oxygen. As this anti oxidant exists in all shoots and most of cell components (with the ability to produce the active species of oxygen), it is assumed that they play very fundamental role in defending against the oxidative stresses (10). Glutathione peroxidase is also a tri-peptide that breakouts its antioxidant properties by removing the peroxides formed in the lipid per-oxidation reactions (20). As mentioned above, the accessible water is the main factor of limiting the growth in the dry environments. Hence, the maximum yield from the viewpoint of growth and production is achieved when the maximum absorption takes place from the limited water existing in the soil (3). These properties would be achieved only through the adaptation mechanism related to the root system. As the higher ratio of root to the shoot (the water absorbing organs in relation to the water consuming organs) improves the plant ability to increase the tolerance to soil, therefore, most of the physiology specialists introduce this ratio/proportion as a standard to select the genotypes resistant to the drought (22). The results of studies carried out by Gislum and Boelt shown that the more growth of roots and their embranchment in the plantlets of pulses grains has relation with their resistance to drought (5). It shows too, that the plants which have longer main roots, the number of sub roots, the accumulation of root length and the root ratio to the shoots, indicate more resistance and tolerance to water shortage and drought stress as compared with the plants which have less such properties.

Considering the defensive roles of anti oxidants against the decrease of negative effects of active oxygen as well as the activity role and the distribution of roots in the effective water absorption from the soil in the environments experiencing the drought stress, it seems that the study of chemical, biochemical, morphologic and physiologic changes of the species and different cultivars out of the desired conditions, would play a considerable role in the better understanding the resistance mechanisms of drought and access to the required genetic resources in correcting the cultivars tolerable to the oxidative stresses.

## II. MATERIALS AND METHODS

### *Geographical coordinate and environmental conditions (climate, soil and water) of the experimental field*

This study carried out during the 2010-2011 and 2011-2012 growing seasons at an educational farm in zone of Varamin (Iran). Based on long term statistics (30 years) of Pishva climatology station, mean temperature of the experiment site in years 2011 and 2012 were 26.31 and 26.67 °C, respectively. Mean annual precipitation during the growing season were 31.63 and 33.32 mm, too (Table 1). 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 2. Also, field water quality results are presented in Table 3.

### *Statistical design and irrigation treatments*

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). 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 (S1; optimum conditions of irrigation), 100 (S2; moderate water deficit) and 150 (S3; 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 S1, S2 and S3 were 465, 234.5 and 146.56 m<sup>3</sup>, respectively. After this stage, the plots were irrigated according to their prescribed treatment.

### *Soybean cultivars*

Soybean cultivars were: V1; L<sub>17</sub>, V2; Clean, V3; T.M.S, V4; Williams×Chippewa and V5; M<sub>9</sub>.

- L<sub>17</sub>, Clean (group III maturity) and M<sub>9</sub> (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

Seeds were obtained from the Iran oilseeds corporation.

### *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<sup>-1</sup>. Also, the nitrogen fertilizer (15 kg ha<sup>-1</sup>) 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 handplanted on 24<sup>th</sup> May 2011 and 26<sup>th</sup> May 2012 at the rate of 20 seeds per m<sup>2</sup> of row and then were thinned to achieve a density of approximately 333,333 ha<sup>-1</sup>. During the whole growth season, weeds and insects were effectively controlled.

### *Measurement of the seed 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. Then, the seed were separated from pods and were weighted with a precision balance. Finally, seed were calculated based on kg ha<sup>-1</sup>, too.

### *Measurement of the length of roots and root-shoot ratio*

In order to measurement of the morphological characteristics, ten plants were selected randomly from every treatment after the last irrigation. Roots were carefully removed from the soil and were washed rapidly. Roots length was measured by a ruler and immediately after washing. Root volumes were calculated from the difference between the root volumes in the normal condition with roots volumes after being in a certain volume of water. Root-shoot ratio was determined based on dividing the root dry weight to shoot dry weight.

### *Measurement of chemical properties*

#### *Sampling and preparation of samples for measurement of protein*

In order to measurement of the anti oxidants activity (superoxide dismutase and glutathione peroxidase, fifteen leaves were taken from each plot randomly and were placed in liquid N<sub>2</sub> 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 Lowery et al., method (13). 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)*

Superoxide dismutase activity was determined by a modification of the protocol of Misra and Fridovich at alkaline pH,  $O_2^-$  serves as chain propagation species for the autoxidation of epinephrine to adrenochrome (15). Superoxide dismutase competes with this reaction, thus decelerating the adrenochrome 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  $\mu$ L of buffer I (62.5 mM  $Na_2CO_3/NaHCO_3$  [pH 10.2], 125  $\mu$ M EDTA), 10  $\mu$ L catalase and 10  $\mu$ L epinephrine (10.5-11.0 mg epinephrine in 2 mL 0.1 N HCl). 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

#### *Glutathione peroxidase (GPX)*

The activity was measured by the Paglia and Valentine 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 (18).

#### *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.

### III. RESULTS

As shown in the tables 1 to 3, the monthly mean temperature and precipitation, soil and water quality during growth season during every two years in the farm limits of test implementation has been relatively enjoying a similar trend. Such a situation may be a reason on a non eruption of significant dispute among treatments in addition to their mutual impacts during the years of test implementation.

#### *Chemical Properties*

##### *Superoxide dismutase*

Based on the table results of variance analysis, the treatment of water deficit stress had a significant effect on the activity rate of antioxidant of superoxide dismutase (Table. 4), such that the amount of this enzyme was increased by increasing the intensity of water deficit stress (increase of irrigation course). Among the cultivars and in the desired conditions of irrigation ( $S_1$ ), the maximum and the minimum amount of antioxidant superoxide dismutase was observed in the cultivars of  $L_{17}$  and T.M.S. In both conditions of moderate and extreme of water deficit ( $S_2$ ,  $S_3$ ), the highest and lowest amount of this antioxidant was respectively assigned to the cultivars of Williams\*Chippewa and T.M.S (Table 5). In this research, a positive and significant correlation was observed between the amount of superoxide dismutase with glutathione peroxidase and seed yield in both conditions of moderate and extreme of water deficit stress. Furthermore, there was a negative and significant correlation between superoxide dismutase and the root-shoot ratio (Tables 6, 7 and 8).

##### *Glutathione peroxidase*

The mean comparison of interaction effects between irrigation levels and cultivars indicated that by increasing the intense of water deficit stress, the amount of antioxidant Glutathione peroxidase was increased significantly in all cultivars (Table 4), however, like the

Table 1: Average monthly of temperature and precipitation on during the growing season of 2010-2011 and 2011-2012 in experimental location

Month	Precipitation (mm)		Average of temperature ( $^{\circ}$ c)					
			Minimum		Mean		Maximum	
	2010-2011	2011-2012	2010-2011	2011-2012	2010-2011	2011-2012	2010-2011	2011-2012
May	16.49	17.66	12.36	13.16	22.22	20.78	28.09	26.79
June	0.10	0.30	20.12	19.29	29.94	27.38	37.19	39.05
July	4.43	3.83	20.72	23.45	29.89	34.03	44.68	37.87
August	1.32	1.67	22.23	22.01	28.23	31.98	39.31	39.80
October	0.18	0.37	18.43	18.00	26.37	25.70	35.44	36.40
November	9.11	9.49	13.85	13.26	21.21	20.14	27.73	29.32
Average	31.63	33.32	17.95	18.20	26.31	26.67	35.41	34.87

Statistics were gathered from Pishva Climatology station.

**Table 2: Physical and chemical properties of experimental soil**

Soil properties	Content in different depths			
	2010-2011		2011-2012	
	0-30 (cm)	30-60 (cm)	0-30 (cm)	30-60 (cm)
Electrical Conductivity (ds/m)	1.0	1.2	1.2	1.25
pH	7.2	7.5	7.00	7.00
Organic carbon (%)	0.6	0.55	0.55	0.61
Total nitrogen (%)	0.05	0.04	0.05	0.05
Available phosphor (mg/kg)	7	5.5	7.4	6.7
Available potassium (mg/kg)	155	159	160	163

**Table 3: Results of water quality water in years of 2011 and 2012**

Parameters	Content	
	2010-2011	2011-2012
Electrical Conductivity (Ec) (ds.m <sup>-1</sup> )	1.3	1.11
pH	7.52	7.29
TDS (Mg.l <sup>-1</sup> )	545.4	548.08
Sodium Absorption Rate (SAR)	3.87	3.89
Na <sup>+</sup> (eq.l <sup>-1</sup> )	5.11	5.07
Ca <sup>++</sup> (eq.l <sup>-1</sup> )	130	133
Mg <sup>++</sup> (eq.l <sup>-1</sup> )	48	48
So <sub>4</sub> <sup>-</sup> (eq.l <sup>-1</sup> )	72	74
Cl <sup>-</sup> (eq.l <sup>-1</sup> )	3.9	3.7
Hco <sub>3</sub> (eq.l <sup>-1</sup> )	135	131

superoxide dismutase, the amount of this antioxidant was higher in moderate water deficit than the extreme water deficit stress and optimum condition of irrigation (S<sub>2</sub>>S<sub>3</sub>>S<sub>1</sub>). The study of interaction effects between irrigation levels and cultivars indicated that during the optimum conditions of irrigation, the cultivars of L<sub>17</sub> and T.M.S and in both conditions of moderate and extreme of water deficit, the cultivars of Williams\*Chippewa and T.M.S were respectively the highest and the lowest content of antioxidant Glutathione peroxide. The study of correlation coefficient tables across the studied properties indicated that, there was a positive and significant correlation between the rate of this antioxidant with the seed yield during the optimum condition of irrigation (S<sub>1</sub>) and extreme of water deficit (S<sub>3</sub>). In addition, a negative correlation was observed between the content of Glutathione peroxide with the root-shoot ratio at all irrigation levels but, this correlation was only significant during the optimum condition of irrigation (Tables 6, 7 and 8).

#### Agricultural properties

##### Root-Shoot ratio

The water deficit stresses in this study affected the root-shoot ratio such that by increasing in intense of water deficit stress, this ratio increased significantly in all cultivars. In all the cultivars, the highest root-shoot ratio was observed at extreme water deficit stress (Table 5). Based on the obtained results, during the optimum condition of irrigation (S<sub>1</sub>), the cultivars of T.M.S and L<sub>17</sub> indicated the highest and lowest root-shoot ratio from them. While during the moderate and extreme water

deficit stresses, the cultivars of Williams\*Chippewa and M<sub>9</sub> had the lowest content of this ratio. Assessment of the correlation tables also indicated that, there was a negative and significant correlation between the root-shoot ratio with the seed yield during the moderate and extreme levels of water deficit. In addition, a negative correlation was observed between this parameter and the root volume (Tables 6, 7 and 8).

##### Length of main root

The results of variance analysis table indicated that, the irrigation levels, the cultivars and the interaction effects of irrigation levels in cultivars had significant effect on the length of the main root (Table 4). Such that, by increasing in irrigation intervals and exercise moderate and extreme water deficit stresses, the amount of this property was decreased significantly in all cultivars. Based on the obtained results, at all levels of irrigation, the maximum length of main root was observed in Williams\*Chippewa cultivar. In moderate and extreme water deficit stresses levels; the shortest length of main root was assigned to the cultivar of T.M.S (Table 5). The study of correlation tables among properties also indicated that only during the optimum condition of irrigation, there was a positive and significant correlation between the length of main root with the seed yield and the root volume (Table 6).

##### Root Volume

The main effects of irrigation levels, cultivars as well as the interaction cultivars and irrigation levels on the root volume have been presented in Table 5. Under the conditions of water deficit stress, the roots volume was decreased significantly in all cultivars such that the least rate of root volume in the cultivars was observed at the extreme water deficit stress (S<sub>3</sub>). Under the optimum conditions of irrigation (S<sub>1</sub>), the maximum root volume of 12/35 cm<sup>3</sup> was observed in the cultivar of L<sub>17</sub>. At the level of moderate water deficit stress, the cultivars were arranged in descending order in this form: Williams\*Chippewa > Clean > T.M.S > L<sub>17</sub> > M<sub>9</sub>. At the level of extreme water deficit stress, the highest and the lowest rate of root volume were assigned to the cultivars of Williams\*Chippewa and T.M.S with 2/4 and 7/01cm<sup>3</sup>. The calculation results in the correlation part among the under test properties also indicated that, there was a significant and positive correlation between the root volume with the length of main root and the seed yield during optimum condition of irrigation in addition, there was only a positive correlation in the conditions of moderate and extreme water deficit stresses (Tables 6, 7 and 8).

### Seed yield

The results of variance analysis table for seed yield in this study indicated the significant difference between the main levels of irrigation, the cultivars and interaction effects of irrigation levels in cultivars (Table 4). The results indicated that, the seed yield from the optimum condition of irrigation to the levels of moderate and extreme water deficit stresses was significantly decreased in all cultivars. Under the optimum condition of irrigation ( $S_1$ ), the least and highest seed yield was produced by the

cultivars of  $L_{17}$  and T.M.S. While, at moderate and extreme levels of water deficit stresses, the highest and lowest seed yield was observed respectively in the cultivars of Williams\*Chippewa and T.M.S (Table 5). In addition, the lowest and highest rate of reduce in seed yield was observed from the optimum condition of irrigation ( $S_1$ ) to the extreme water deficit stress ( $S_3$ ) respectively equal to 87/39 and 71/72 % in the cultivars of T.M.S. and Williams\*Chippewa.

Table 4: The mean square of ANOVA for effect of irrigation levels on superoxide dismutase (SOD), glutathione peroxidase (GPX), length of main root, roots volume, root-shoot ratio and seed yield in soybean cultivars

Sources of Variation	Degree of freedom (df)	SOD	GPX	Length of main root	Root volume	Root-shoot ratio	Seed yield
Year	1	ns	ns	ns	ns	ns	Ns
Irrigation levels	2	**	**	**	**	**	**
Year*Replication	2	ns	ns	ns	ns	ns	Ns
Cultivars	4	**	**	**	**	**	**
Cultivars*Irrigation levels	8	**	**	**	**	**	**
Cultivars *Year	4	ns	ns	ns	ns	ns	Ns
Year*Cultivars*Irrigation levels	8	ns	ns	ns	ns	ns	Ns

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

## IV. DISCUSSION

The results of present study has clearly indicated that there was a significant difference between the under test cultivars from the optimum condition of irrigation to the moderate and extreme levels of water deficit from the viewpoint of antioxidant superoxide dismutase and glutathione peroxide, root-shoot ratio, length of main root, root volume and the seed yield. This difference may be the result of resistance degree difference for different cultivars of one species on different levels of water deficit stress. In this study, under the conditions of moderate and extreme levels of water deficit ( $S_2$  and  $S_3$ ), despite the increase of root-shoot ratio, the root volume, the length of main root and the seed yield have been decreased in all cultivars. The past researches have also indicated that, decrease in seed yield of cowpea under the conditions of irrigation constraint has been associated with the density and the volume decrease of root (17). The other research has also shown that the water deficit stress had finally as well, decreased the seed yield of sunflower significantly, in addition to the significant decrease of root volume at the phase of flowering (9). It seems that the water deficit stresses by decreasing the leaf surface, closing of stoma, decrease of absorbing and water transfer and the food elements in the root area and as a whole the use of resistance and tolerance mechanisms to drought by the plant, on one hand has led to the decrease of production and transfer of assimilate to the roots and on the other hand to the demand increase to receive the photosynthesis substance by the grains under growth. The outcome of these factors has probably caused that the attributes such as volume and the root length and more possibly the dry

weight of the root are gravely influenced and decrease quantitatively. In the field of root-shoot ratio, several studies have indicated that the drought stress increases it in most of the plants. This increase, almost relates to the more decrease of shoots growth in comparison with the root (8). Of course, there are many evidences that approve the increase of roots growth independently from the shoots under the conditions of drought stress (11).

In a study has been reported that the effect of water deficit stress on the increase of root-shoots ratio in different genotypes of chickpea are majorly related to the blooming stages and establishing of sheath and its main cause is also the single strategy that is more or less followed by the genotypes of chickpea. Based on this strategy, the allocation of more photosynthesis substance to the under growth grains and less allocation to the growing organs and probably the repeated transfer from the growing organs such as the leaves, petioles, stems and roots to the grain under growth would cause the increase of root-shoot ratio (1). Of course, Sagzena in his research announced that this process continues till the plant reaches the final stage of growth and physiological maturity and under such conditions, the root-shoot ratio and the root density is continuously decreased by completing the physiologic stages of plant and reaches the minimum amount of it at the time of maturation and harvest (21). In addition, it was determined during the study of all investigations that the root-shoot ratio was rather under the control of internal factors (genetic capacity) and the environmental factors (specially the drought stress) play role as a stimulant to express these capacities in relation with the allocation rate to the roots and shoots. Of course, the role of these factors in the allocating rate of shoot-root

Table 5: Effects of irrigation levels on content of superoxide dismutase (SOD) ( $\mu\text{mg}^{-1}$  protein), glutathione peroxidase (GPX) ( $\mu\text{mg}^{-1}$  protein), length of main root (cm), roots volume ( $\text{cm}^3$ ), root-shoot ratio and seed yield ( $\text{kg}\cdot\text{ha}^{-1}$ ) in soybean cultivars

Irrigation levels	Cultivars	SOD	GPX	Length of main root	Roots volume	Root-shoot ratio	Seed yield
S <sub>1</sub>		1591.86 <sup>c</sup>	18.14 <sup>c</sup>	104.21 <sup>a</sup>	10.22 <sup>a</sup>	0.7 <sup>c</sup>	2339.31 <sup>a</sup>
S <sub>2</sub>		2442.48 <sup>a</sup>	34.75 <sup>a</sup>	56.62 <sup>b</sup>	7.27 <sup>b</sup>	0.89 <sup>b</sup>	1019.52 <sup>b</sup>
S <sub>3</sub>		2152.15 <sup>b</sup>	28.14 <sup>b</sup>	45.83 <sup>c</sup>	4.57 <sup>c</sup>	1.11 <sup>a</sup>	468.82 <sup>c</sup>
	L <sub>17</sub>	2216.43 <sup>b</sup>	29.00 <sup>b</sup>	68.74 <sup>b</sup>	8.03 <sup>b</sup>	0.84 <sup>b</sup>	1490.70 <sup>a</sup>
	Clean	2089.41 <sup>d</sup>	25.64 <sup>d</sup>	79.42 <sup>a</sup>	7.59 <sup>b</sup>	0.86 <sup>b</sup>	1359.03 <sup>b</sup>
	T.M.S	1556.41 <sup>e</sup>	20.45 <sup>e</sup>	44.61 <sup>d</sup>	6.4 <sup>c</sup>	1.19 <sup>a</sup>	870.64 <sup>d</sup>
	Williams*Chippewa	2304.09 <sup>a</sup>	32.95 <sup>a</sup>	85.68 <sup>a</sup>	8.30 <sup>a</sup>	0.78 <sup>c</sup>	1375.54 <sup>b</sup>
	M <sub>9</sub>	2144.5 <sup>c</sup>	26.92 <sup>c</sup>	65.96 <sup>c</sup>	6.44 <sup>c</sup>	0.83 <sup>b</sup>	1283.49 <sup>c</sup>
S <sub>1</sub>	L <sub>17</sub>	1856.15 <sup>a</sup>	22.68 <sup>a</sup>	92.23 <sup>c</sup>	12.35 <sup>a</sup>	0.54 <sup>d</sup>	2869.17 <sup>a</sup>
S <sub>1</sub>	Clean	1772.70 <sup>b</sup>	19.87 <sup>b</sup>	118.04 <sup>b</sup>	10.93 <sup>b</sup>	0.63 <sup>c</sup>	2458.74 <sup>b</sup>
S <sub>1</sub>	T.M.S	1307.56 <sup>d</sup>	14.42 <sup>e</sup>	87.8 <sup>d</sup>	9.54 <sup>c</sup>	0.94 <sup>a</sup>	2002.83 <sup>e</sup>
S <sub>1</sub>	Williams*Chippewa	1524.68 <sup>c</sup>	17.59 <sup>c</sup>	128.94 <sup>a</sup>	9.38 <sup>c</sup>	0.65 <sup>c</sup>	2245.03 <sup>c</sup>
S <sub>1</sub>	M <sub>9</sub>	1498.20 <sup>c</sup>	16.14 <sup>d</sup>	94.03 <sup>c</sup>	8.89 <sup>d</sup>	0.73 <sup>b</sup>	2120.80 <sup>d</sup>
S <sub>2</sub>	L <sub>17</sub>	2711.18 <sup>b</sup>	40.14 <sup>b</sup>	58.98 <sup>b</sup>	6.83 <sup>d</sup>	0.8 <sup>c</sup>	1184.72 <sup>b</sup>
S <sub>2</sub>	Clean	2303.22 <sup>d</sup>	30.56 <sup>d</sup>	73.13 <sup>a</sup>	7.54 <sup>b</sup>	0.9 <sup>b</sup>	1161.83 <sup>c</sup>
S <sub>2</sub>	T.M.S	1858.18 <sup>e</sup>	25.91 <sup>e</sup>	52.08 <sup>c</sup>	7.22 <sup>c</sup>	1.19 <sup>a</sup>	356.47 <sup>d</sup>
S <sub>2</sub>	Williams*Chippewa	2803.12 <sup>a</sup>	43.87 <sup>a</sup>	74.10 <sup>a</sup>	8.5 <sup>a</sup>	0.77 <sup>c</sup>	1246.63 <sup>a</sup>
S <sub>2</sub>	M <sub>9</sub>	2536.71 <sup>c</sup>	33.04 <sup>c</sup>	57.59 <sup>b</sup>	6.22 <sup>e</sup>	0.8 <sup>c</sup>	1147.92 <sup>c</sup>
S <sub>3</sub>	L <sub>17</sub>	2081.14 <sup>d</sup>	24.19 <sup>d</sup>	53.98 <sup>b</sup>	4.91 <sup>b</sup>	1.17 <sup>b</sup>	418.22 <sup>d</sup>
S <sub>3</sub>	Clean	2192.30 <sup>c</sup>	26.50 <sup>c</sup>	47.1 <sup>c</sup>	4.29 <sup>c</sup>	1.04 <sup>c</sup>	456.52 <sup>c</sup>
S <sub>3</sub>	T.M.S	1503.48 <sup>e</sup>	21.02 <sup>e</sup>	26.71 <sup>e</sup>	2.4 <sup>d</sup>	1.46 <sup>a</sup>	252.62 <sup>e</sup>
S <sub>3</sub>	Williams*Chippewa	2584.45 <sup>a</sup>	37.41 <sup>a</sup>	55.03 <sup>a</sup>	7.01 <sup>a</sup>	0.91 <sup>d</sup>	634.97 <sup>a</sup>
S <sub>3</sub>	M <sub>9</sub>	2398.58 <sup>b</sup>	31.59 <sup>b</sup>	46.27 <sup>d</sup>	4.21 <sup>c</sup>	0.97 <sup>d</sup>	581.75 <sup>b</sup>

- Levels of irrigation: S<sub>1</sub>; optimum condition of irrigation, S<sub>2</sub>; moderate water deficit stress level, S<sub>3</sub>; 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).
- $\mu\text{mg}^{-1}$  protein : International Units of activity per milligram protein

Table 6: Correlation coefficient between contents of superoxide dismutase (SOD), glutathione peroxidase (GPX), length of main root, roots volume, root-shoot ratio and seed yield at the optimum condition of irrigation (S<sub>1</sub>).

Features	Superoxide Dismutase	Glutathione Peroxidase	Length pf Main Root	Roots Volume	Root-Shoot Ratio	Seed Yield
superoxide dismutase	1					
glutathione peroxidase	0.97 <sup>**</sup>	1				
length of main root	0.85	0.91 <sup>*</sup>	1			
roots volume	0.96 <sup>**</sup>	0.96 <sup>*</sup>	0.95 <sup>*</sup>	1		
root-shoot ratio	-0.91 <sup>*</sup>	-0.90 <sup>*</sup>	-0.65	-0.78	1	
seed yield	0.94 <sup>*</sup>	0.99 <sup>**</sup>	0.94 <sup>*</sup>	0.954 <sup>*</sup>	-0.85	1

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

Table 7: Correlation coefficient between contents of superoxide dismutase (SOD), glutathione peroxidase (GPX), length of main root, roots volume, root-shoot ratio and seed yield at the moderate water deficit stress level (S<sub>2</sub>)

Features	Superoxide Dismutase	Glutathione Peroxidase	Length Of Main Root	Roots Volume	Root-Shoot Ratio	Seed Yield
superoxide dismutase	1					
glutathione peroxidase	0.94 <sup>*</sup>	1				
length of main root	0.06	0.16	1			
roots volume	0.75	0.58	0.35	1		
root-shoot ratio	-0.96 <sup>**</sup>	-0.82	-0.61	-0.84	1	
seed yield	0.89 <sup>*</sup>	0.74	0.04	0.92	-0.97 <sup>**</sup>	1

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

Table 8: Correlation coefficient between contents of superoxide dismutase (SOD), glutathione peroxidase (GPX), length of main root, roots volume, root-shoot ratio and seed yield at the extreme water deficit stress level ( $S_3$ )

Features	Superoxide Dismutase	Glutathione Peroxidase	Length Of Main Root	Roots Volume	Root-Shoot Ratio	Seed Yield
superoxide dismutase	1					
glutathione peroxidase	0.91*	1				
length of main root	0.85	0.64	1			
roots volume	0.58	0.33	0.64	1		
root-shoot ratio	-0.99**	-0.88	-0.82	-0.65	1	
seed yield	0.99**	0.95*	0.77	0.46	-0.97**	1

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

during the final physiologic stages of plant (grain maturation) is gradually decreased and probably the physiologic conditions dominant on plant, will be independently, from the environmental factors, be determinant of transfer root of photosynthesis substance to the organs including to the grains under maturation (6, 23). The chemical and biochemical effects of water shortage stresses in different plants species as well as in different cultivars and genotypes have been presented in some research reports (4, 14). In the present study the results indicated that the amount of antioxidants activity measured (superoxide dismutase and glutathione peroxidase) in both levels of moderate and extreme water deficit stresses were increased as compared with optimum condition of irrigation. Of course, the amount of these antioxidants at the level of medium water deficit stress was far higher than the extreme water shortage stress level antioxidants ( $S_2 > S_3 > S_1$ ). This theme may be in relation with the abilities rate of different cultivars as compared with the levels of different oxidative stresses (including water deficit stress). On the whole, when the crops are exposed to oxidative stresses, the production rate of antioxidants is increased in them to combat with free radicals and increasing their destructive effects. In fact, the defensive mechanism of antioxidants is activated in them (16). The results obtained from this research also approve this topic. Since, by increasing the irrigation cycle (implementing the moderate water deficit stress), the values of antioxidants measured at the level of medium stress was increased significantly compared with the optimum condition of irrigation ( $S_2 > S_1$ ). But it seems when the intensity of water deficit stress is increased more than a limit, the physiologic damages inflicted to plant are so grave that not only the defense mechanism of antioxidants does not improve but even there may be disturbance in the activity of this system. Therefore, the amount of antioxidants may reach a lower limit than their amount at previous levels in this level of stress. This result ( $S_2 > S_3 > S_1$ ) is also registered in this test. In the previous studies, the increase of antioxidants values in the conditions of water deficit stress have been reported as compared with complete irrigation conditions in the corn and soybean plants (24). They had pointed out that the resistant species in response to drought stress have higher antioxidant enzymes activity, while the plant sensitive to drought in such conditions lack this mechanism. In addition, Lie et al., in their research observed the positive and significant correlation among the amounts of Catalase

antioxidants, Superoxide dismutase and Ascorbate peroxidase with each other and with the hormones of Abscisic acid and the seed yield under the conditions of drought stress (12). In this research and among the measured antioxidants, glutathione peroxidase enjoyed more increase under the conditions of extreme water deficit stress as compared with superoxide dismutase in comparison with the optimum conditions of irrigation. Among the cultivars, the maximum amount of glutathione peroxidase and superoxide dismutase at both levels of extreme and moderate water deficit stresses ( $S_2, S_3$ ) was observed in the cultivar of Williams\*Chippewa. In addition, the maximum percentage of increase in the rate of antioxidants from the desired irrigation conditions to the extreme stress of water deficit was also observed in the same cultivar. Considering that the Williams\*Chippewa cultivar enjoyed the maximum rate of seed yield and the measured antioxidants, hence it seems that the defensive mechanism of antioxidants of this cultivar is more powerful than those all cultivars against the stresses of water shortage.

## V. CONCLUSION

As a whole the following results are presentable in this research:

- The physiologic responses of soybean cultivars were different at the levels of desired irrigation and the implemented stresses of water deficit.
- The water deficit stresses activated the defensive mechanism of antioxidants in all cultivars and increased the amounts of antioxidants in them.
- Despite the increase in the amount of antioxidants, the stresses of water deficit decreased the volume and the length of main root due to the disorder in the physiologic process (such as unbalancing in allocating the photosynthesis substance across the root and grains as well as disorder in the repeated transfer from growing organs) and increased the root-shoot ratio.
- The cultivars with the higher antioxidants amounts enjoyed the higher volume and length of main root in addition to the higher seed yield.
- Considering the research results, under the optimum conditions of irrigation in  $L_{17}$  cultivar as well as under the moderate and extreme conditions of water deficit stresses, the cultivar of Williams\*Chippewa enjoys the cultivation priority.

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