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Evaluation of Water Deficit Stress on Seedling Growth, Antioxidant Enzyme Activity and Yield of Four Cultivars of Cotton

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Abstract **– Cotton (*GossypiumhirsutumL.*), is an indeterminate perennial, oilseed and fiber crop. Water availability and quality affect the growth and physiological processes of all plants. In order to investigation of drought stress on germination, physiological and agronomical traits of cotton a study was done in laboratory and greenhouse condition. In laboratory study an experiment was planned with two factors (variety and drought stress) as a factorial experiments based on completely randomized design with 4 replications. Osmotic potentials were conducted with D-Mannitol (0, -0.4, -0.8 and -1.6 Mega Pascal) and Germination characteristics were studied. Green house experiment was done at 25×5.5 cm pots. Control, medium stress and high level of stress were known as 75, 15 and 5% relative soil water content. The effect of drought on total of germination was significant. The highest value of this trait was obtained in control and the lowest of that was related to -1.6 Mpa. Total germination of Golestan was the highest. Sahel has the least total germination. The effect of drought stress levels was significant on all agronomy traits except stem fresh weight. Based on LSD test of drought stress levels on agronomy traits, with increasing of stress all traits were decreased. Maximum of root length was observed in 75 % of field capacity (75%FC=control) and minimum of it was obtained in 5%FC (8.55). LSD test of cultivars showed that the Sahel has the best performance of agronomy traits, as the highest of root length and dry weight, leaf fresh weight, height, total dry weight were observed in Sahel. With increasing in drought stress from control to higher level of stress, leaf area index (LAI) was decreased. The results of Duncan test showed that in totally with increasing of stress level LAI, super oxide dismutase, stomata conductance and photosynthesis (Phn) almost were decreased and catalase (CAT), acrobat peroxidase (APX) and fv/fm (quantum yield) were increased. CAT was decreased as stress level was increased. As the same, minimum of APX was obtained in control and maximum of it was related to the high level of stress. Stomata conduction was decreased as stress level was increased. Phn also was decreased as stress level was increased.**

Keywords **– Water Deficit, Seedling Growth, Antioxidant Enzyme Activity, Leaf Area Index, Quantum Yield, Cotton.**

I. INTRODUCTION

Cotton (GossypiumhirsutumL.), an indeterminate perennial, oilseed and fiber crop, is grown in more than seventy countries worldwide and plays an important role in the global economy (Hearn, 1979). However, the productivity of cotton is adversely affected by biotic and abiotic stresses.

Water deficit is the major abiotic factor limiting plant growth and crop productivity around the world (Kramer, 1983). In general, plant water stress is defined as the condition where a plant's water potential and turgor are decreased enough to inhibit normal plant function (Hsiao et al., 1973). Approximately one third of the cultivated area of the world suffers from chronically inadequate supplies of water (Massacci et al., 2008).Water availability and quality affect the growth and physiological processes of all plants since water is the primary component of actively growing plants ranging from 70-90% of plant fresh mass (Gardner et al., 1983). The effects of water stress depend on the severity and duration of the stress, the growth stage at which stress is imposed, and the genotype of the plant (Kramer, 1983).

As soil dries, changes in root metabolism send biochemical signals to the shoots, which respond with decreased growth, stomatal conductance, photosynthesis and osmotic potential (Kramer and Boyer, 1995). When plants are subjected to water stress there is a decrease in plant height (Gates, 1964; Slatyer, 1969), leaf size, leaf number (Slatyer, 1967; Silva, 1973), flower primordia formation, as well as in root growth. Water-deficit stress reduces cell and leaf expansion, stem elongation, and leaf area index (Jordan et al., 1970; McMichael and Hesketh, 1982; Turner et al., 1986; Ball et al., 1994; Geriket al., 1996). Leaf, stem and root growth rate are very sensitive to water stress because they are dependent on cell expansion (Hsiao, 1976; Hearn, 1994). Krieg and Sung (1986) reported that water stress caused a reduction in the whole plant leaf area by decreasing the initiation of new leaves, with no significant changes in leaf size of leaf abscission. McMichael and Quisenberry (1991) observed decreased shoot-to-root ratios of plants grown under conditions of severe water stress. Photosynthesis plays a major role in determining crop productivity in all species and is directly affected by water stress. Photosynthetic rates of the leaves decrease as the relative water content and leaf water potential decrease (Lawlor and Cornic, 2002). In cotton, several reports have indicated that water

stress causes a reduction in photosynthesis rates due to a combination of stomatal and non-stomatal limitations (Pallas et al., 1967; McMichael and Hesketh, 1982; Turner et al., 1986; Sung and Krieg, 1979; Gentyet al., 1987; Ephrathet al., 1990; Faveret al., 1996, Lacapeet al., 1998; Leidiet al., 1999). Stomatal closure decreases water loss, but also decreases the movement of CO2 into the plant. Significant correlations between leaf water potential and stomatal conductance under conditions of water-deficit stress have been reported (Sociaset al., 1997).

Drought stress has been reported to induce an oxidative stress due to inhibition of photosynthesis (Smirnoff, 1993; Miller et al., 2008; Choudhury et al., 2013) resulting from the production and accumulation of toxic oxygen species such as peroxide radicals, hydrogen peroxide and hydroxyl radicals (Foyer et al., 1997). At cellular level, drought stress often leads to the accumulation of reactive oxygen species (ROS). Excessive ROS production can cause oxidative stress to the photosynthetic apparatus and seriously impair the normal function of cells (Dietz and Pfannschmidt, 2011; Foyer and Noctor, 2009). These reactive oxygen species produced during water-deficit stress can damage many cellular components including lipids, proteins, carbohydrates, DNA and nucleic acids (Monk et al., 1987; Apel and Hirt, 2004). Membrane lipid peroxidation and protein oxidation constitute the simplest criteria of assessing the extent of oxidative damage in the tissue (Noctor and Foyer, 1998; Mittler 2002). Efficient antioxidant systems in the plant can minimize the level of oxidative stress and protect the tissues. Such antioxidant systems can be enzymatic or non-enzymatic. The major antioxidant species in the plants are superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (AP), and glutathione reductase (GR) (Gaspar et al., 2002; Gill and Tuteja, 2010; Suzuki et al., 2012). The levels of these antioxidant systems, however, have shown increases, decreases or no effect, depending on the species, duration of drought stress and the specific antioxidants investigated (Reddy et al., 2004). Mahan and Wanjura (2005) performed field studies to identify changes in antioxidant metabolism in cotton. They observed that ascorbate peroxidase activity was increased in water-stressed plants compared to the well-watered plants. However, Kawakami et al. (2010) also reported that superoxide dismutase of water-stressed plants was significantly decreased compared to the control.

II. Materials and Methods

Laboratory study was done at germination and seedling stages in control and stress conditions. An experiment was planned with two factors (variety and drought stress) as a factorial experiments based on completely randomized design with 4 replications. Osmotic potentials were conducted with D-Mannitol (0, -0.4, -0.8 and -1.6 Mega Pascal). In order to reach this potentials, 0, 14.6, 29.2, 58.3 gr D-Mannitol were dissolved in 500 mL Distilled water, respectively (Springer, 2005). The experiment was done in germinator at 25±˚C (ISTA, 2001). After Disinfection with alcohol and fungicide, 400 seeds of each variety were sown in papers (100 seed in each replication). Germinated seeds were counted at 4th and 7th days after experiment.

Green house experiment was done at 25×5.5 cm pots. Dried soil weight was calculated at 80˚C after 24 h. fertilization was done with 50 ml N-P-K (20-20-20) based on 0.8 % weight to volume ratio (W/V) (Hugh, 2003). The start of fertilization was after thinning as twice in week. Temperature of green house was constant in 27±3. With daily pot weighting and hold its water content between 55-85% field capacity prevent of any stress condition. Based on variety deference in growth, stress was started at 35-45 days after sowing date. Drought stress was conducted as relative soil water content (RSWC):

RSWC= (pot weight-pot dry weight-pot wet weight) / (pot weight after extra water draining- pot dry weight)

Control (without stress), medium stress and sever (high) stress were known as 75, 15 and 5% RSWC (Hugh, 2003). After the onset of the first wilting signs, constant value of water (based on stress treatment) was added to pots. After second round of stress and onset of first wilting sign irrigation was done at field capacity after 12 hour. After 48 hour morphologic and physiologic assessments and hormonal measurements were done. Chlorophyll content of leaves was measured base on Jensen et al (1999) method. In order to measuring of root wet weight seedling were placed in water container. After that seedling were kept at room temperature in laboratory for 1 hour. Seedling length and weight were measured. Samples were kept at 80 ˚C for 48 h and dry weight of seedlings was measured. Root length, root dry weight, root fresh weight, stem dry weight, stem fresh weight, seedling length, leaf fresh weight, seedling total dry weight, seedling total fresh weight were measured. Measurement of chlorophyll (Lichtenthaler, 1987), Measurement of antioxidant enzyme activities (Cakmak and Horst, 1991), Measurement of protein content (Bradford, 1976), Measurement of Proline content (Bates, 1973), Measurement of lipid peroxidation (Vos et al., 1991) were done. Analysis of data, means compression based on multiple Duncan test, was done with SAS (1992).

III. Results and Discussions

The effect of drought, cultivar and its interactions on germination traits are presented in table 1. The effect of drought on germination after 4 and 7 days and total of germination was significant. The effect of cultivar, also, was significant on all of these traits. There was no significant effect of drought × cultivar on any traits.

Based on LSD test of drought stress on gerafter4 showed in fig.1 with increasing in drought level, gerafter4 was decreased significantly. The highest value of this trait was obtained in control (drought level equal to zero) and the lowest of that was related to -1.6 Mpa. Significant difference was observed between drought levels. Total germination has the same trend, with increasing of drought stress the value of total germination has decreased. Maximum of total germination has occurred in control level and the minimum of that was obtained in the -1.6 Mpa. Germination after 7 days has the different pattern,

more gerafter7 was observed in high level of drought stress. It seems that in these levels, germination rate was decreased and the number of germination seeds was increased in 7th day. Between -0.8 and -1.6 Mpa there was no significant difference. The lowest gerafter7 was obtained in control that there was no significance difference with -0.4 Mpa.

Table 1: Anova analysis of drought, cultivar and its interaction on germination traits: germination after 4 days (gerafter4), germination after 7 days (gerafter7) and total of germination (totalger).

Source	DF	gerafter4	gerafter7	totalger
drought	3	24863.85**	133.43**	22254.6**
cultivar	3	87.182**	13.307*	167.896**
drought*cultivar	9	9.321	6.085	6.215
Error	48	17.984	4.338	11.656
Total	63			
C.V		7.325	38.640	5.395

** and * were significant in 0.01 and 0.05 %, respectively.
C.V: coefficient of variance

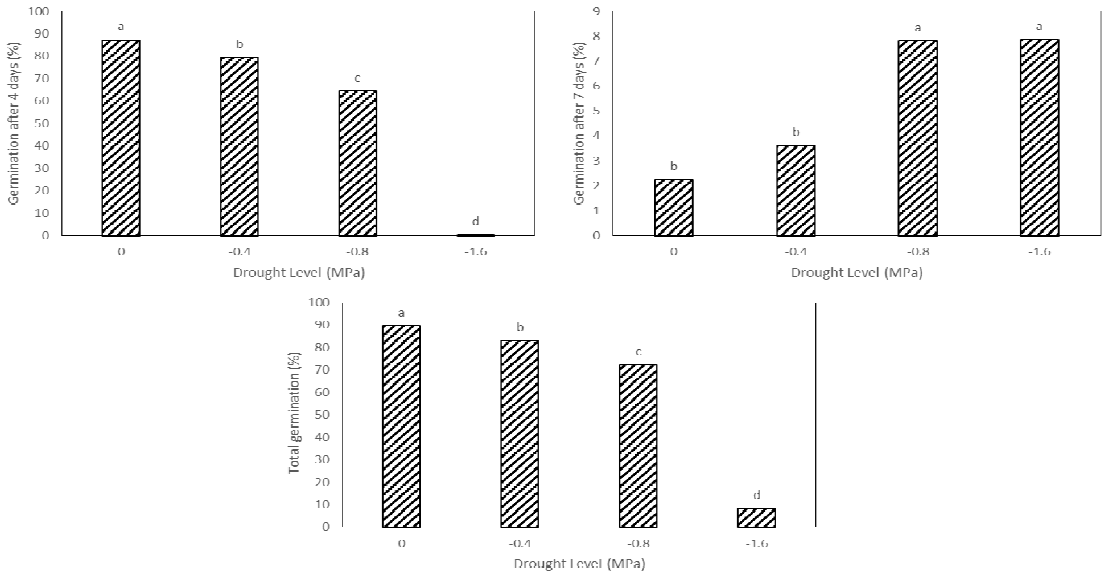

Fig.1. Lsd test of drought stress levels on germination (gerafter4, gerafter7 and totalger are germination after 4 days, germination after 7 days and total germination). Similar letter means that there is no statically difference.

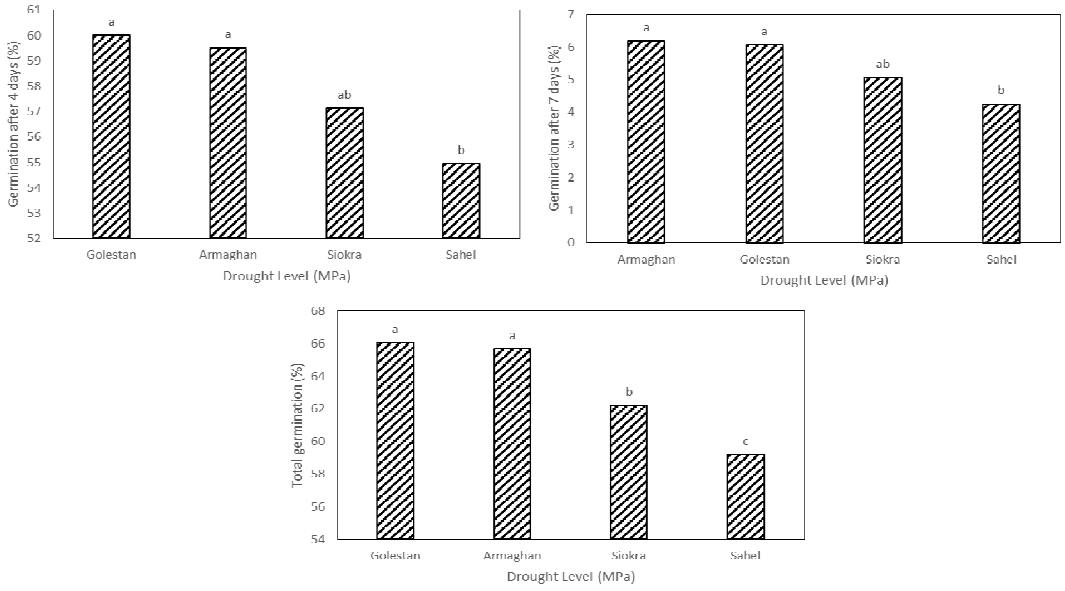

Fig.2. Lsd test of variety effect on germination (gerafter4, gerafter7 and totalger are germination after 4 days, germination after 7 days and total germination). Similar letter means that there is no statically difference.

Anova analysis of treatments on Agronomy traits are shown in table 2. The effect of drought stress levels was significant on all agronomy traits except stem fresh weight. The effect of cultivar was not significant on root fresh weight, stem fresh weight and stem dry weight but its effect was significant on others. The effect of interaction of drought × cultivars was significant on root dry weight, leaf dry weight, leaf fresh weight and total dry weight.

Table 2: Anova of droughts, cultivars and their interactions on agronomy traits (rootdw=root dry weight, rootfw=root fresh weight, stemdw= stem dry weight, stemfw= stem fresh weight, leafdw=leafdryweight, leaffw= leaf fresh weight, height= seedling height, totaldw= seedling total dry weight, totalfw=total fresh weight, chlorophyll= chlorophyll content).

Source	DF	RL	rootdw	rootfw	Stemdw	stemfw	leafdw	leaffw	height	Totaldw	Totalfw	chlorophyll
Drought	2	141.537**	0.003**	0.012**	0.009**	0.056	0.0153**	0.777**	214.684**	0.076**	1.487**	0.185*
Cultivar	3	20.320**	0.0003**	0.0006	0.0004	0.003	0.002**	0.046**	18.717*	0.006**	0.088*	0.234**
Drought* Cultivar	6	1.524	0.00003*	0.0001	0.00007	0.002	0.0005**	0.024*	2.488	0.001**	0.039	0.0005
Error	36	1.039	0.00001	0.0002	0.0001	0.026	0.00007	0.010	4.854	0.0003	0.026	0.038
Total	47											
cv		9.032	15.712	45.143	15.588	72.923	7.327	10.966	15.303	7.917	14.073	13.885

* and ** are significant at ($p<0.05$ and $p<0.01$), respectively.
C.V = coefficient of variance.

Based on LSD test of drought stress levels on agronomy traits presented in table 3, with increasing of stress all traits were decreased. Maximum of root length was observed in 75 % of field capacity (75%FC=control) and minimum of it was obtained in 5%FC (8.55). Also root dry weight and fresh weight have the same pattern. Stem dry weight was decreased with increasing of drought stress levels. Dry and fresh weight of leaf were decreased as drought stress were increased. The highest value of total dry weight was observed in 75%FC (0.284). The least value of chlorophyll content was obtained in 5%FC.

Table 3: LSD test drought stress levels (75%FC= 75% field capacity, control; 15%FC= medium stress; 5%FC= High stress level) on agronomy traits (rootdw=root dry weight, rootfw=root fresh weight, stemdw= stem dry weight, stemfw= stem fresh weight, leafdw=leafdryweight, leaffw= leaf fresh weight, height= seedling height, totaldw= seedling total dry weight, totalfw=total fresh weight, chlorophyll= chlorophyll content)

	Root length	rootdw	rootfw	stemdw	leafdw	leaffw	totaldw	chlorophyll
75%FC	14.45^{a}	0.037^{a}	0.066^{a}	0.103^{a}	0.144^{a}	1.116^{a}	0.284^{a}	1.532^{a}
15%FC	10.844^{b}	0.017^{b}	0.025^{b}	0.074^{b}	0.105^{b}	0.881^{b}	0.196^{b}	1.357^{b}
5%FC	8.55^{c}	0.010^{c}	0.015^{b}	0.055^{c}	0.083^{c}	0.676^{c}	0.148^{c}	1.336^{b}

Similar letter in each column means that there was no statically significant difference.

LSD test of cultivars showed that the Sahel has the best performance of agronomy traits, as the highest of root length and dry weight, leaf fresh weight, height, total dry weight were observed in Sahel (Table 4). Armaghan after former has the almost acceptable function. The least values of agronomy traits were occurred in Golestan. In chlorophyll these trend was inverted. The highest value of chlorophyll was obtained in Golestan and the least of it was observed in Sahel. That is namely that the Sahel cultivar were less delicate to stress than the others.

Table 4: LSD test of cultivars on agronomy traits (rootdw=root dry weight, rootfw=root fresh weight, stemdw= stem dry weight, stemfw= stem fresh weight, leafdw=leafdryweight, leaffw= leaf fresh weight, height= seedling height, totaldw= seedling total dry weight, totalfw=total fresh weight, chlorophyll= chlorophyll content).

Cultivar	root length	rootdw	leafdw	leaffw	height	totaldw	fwtotal	chlorophyll
Sahel	13.1^{a}	0.027833^{a}	0.126083^{a}	0.96746^{a}	16.25^{a}	0.23925^{a}	1.25533^{a}	1.23452^{c}
Armaghan	11.3^{b}	0.022333^{b}	0.112183^{b}	0.91596^{ab}	13.6917^{b}	0.210683^{b}	1.17396^{ab}	1.41045^{b}
Saiokra	10.5667^{bc}	0.019158^{c}	0.102617^{c}	0.84322^{b}	14.0417^{b}	0.197025^{bc}	1.08563^{b}	1.41171^{b}
Golestan	10.1583^{c}	0.017133^{c}	0.10085^{c}	0.83805^{b}	13.6083^{b}	0.190067^{c}	1.07101^{b}	1.57631^{a}

Similar letters in each column means that there is no statically significant difference.

Dancan test of cultivar×stress interaction showed that the highest root dry weight is related to Sahel×75%FC. Other 75%FC also has no significant difference with Sahel×75%FC (Table 5). With increase of stress levels to 15 and 5%FC root dry weight was decreased, the least root dry weight obtained from Golestan×5%FC. Leaf dry

weight has the same pattern, maximum of leaf dry weight was related to the Sahel×75%FC and minimum of that was related to the Golestan×5%FC. Leaf fresh weight was decreased from 75%FC to 5%FC stress level. Sahel×75%FC has the highest value of leaf fresh weight. Total dry weight was decreased as stress level was increased. In totally Sahel and 75%FC have the the best performance in traits.

Duncan test of interaction agronomy traits

Table 5: Duncan test of cultivar× drought stress (75%FC= 75% field capacity, control; 15%FC= medium stress; 5%FC= High stress level) interaction on agronomy traits (rootdw, leaf dw, leaffw and total dw are roor dry weight, leaf dry weight, leaf fresh weight and total dry weight, respectively).

	RootDW	LeafDW	LeafFW	TotalDW
Sahel×75%FC	0.0465a	0.1758a	1.3033a	0.3365a
Armaghan×75%FC	0.03875b	0.1475b	1.1635a	0.2838b
Saiokra×75%FC	0.03325c	0.1271c	1.01b	0.2613bc
Golestan×75%FC	0.0295c	0.125cd	0.9885b	0.2532c
Sahel×15%FC	0.02425d	0.1142de	0.8785b	0.2228d
Armaghan×15%FC	0.0175e	0.1082ef	0.9258b	0.2015de
Saiokra×15%FC	0.01425ef	0.099fg	0.8521bc	0.1837ef
Golestan×15%FC	0.0135efg	0.097fg	0.8687b	0.1755fg
Sahel 5%FC	0.01275efg	0.0882gh	0.7206cd	0.1585gh
Armaghan×5%FC	0.01075fg	0.0808h	0.6585d	0.1468g
Saiokra×5%FC	0.00997fg	0.0817h	0.6675d	0.146g
Golestan×5%FC	0.0084g	0.0805h	0.657d	0.1414g

Similar letters in each column means that there is no statically significant difference.

Anova analysis of drought, cultivar and their interactions demonstrated that the effect of all treatments and their interaction have the significant effect on all traits ($p<0.01$) (Table 6).

Table 6: Drought stress, cultivar and their interactions on physiology traits (LAI= leaf area index, CAT= catalase (Units / mg protein), SOD=super oxide dismutase (Units / mg protein), APX= acrobat peroxidase (Units / mg protein), fv/fm= quantum yield, stomatcon= stomata conduction and Phn=photosynthesis)

Source	DF	LAI	CAT	SOD	APX	fvfm	stomatcon	phn
drought	2	0.020**	69789.77**	6.114**	0.452**	0.000001**	9544.771**	348.837**
cultivar	3	0.001**	827.333**	3.020**	0.152**	0.00001**	506.139**	7.365**
drought*cultivar	6	0.00007**	98.688**	0.662**	0.003**	0.0000004**	19.826**	0.290**
Error	11	0.000008	0.222	0.38	0.003025	0.000009	161	0.084
Total	47							
C.V		2.260	0.369	1.765	1.268	0.060	2.570	0.469

* and ** are significant at ($p<0.05$ and $p<0.01$), respectively.
C.V= coefficient of variance

With increasing in drought stress from control to higher level of stress, leaf area index (LAI) was decreased (Table 7). Maximum of LAI was obtaine in control (0.164) and least of LAI was related to the high level of stress (0.093). CAT was decreased as stress level was increased. As the same, minimum of APX was obtained in control and maximum of it was related to the high level of stress. Stomata conduction was decreased as stress level was increased. Phn also was decreased as stress level was increased.

Table 7: LSD test of stress (control=75%FC, medium=15%FC and high=5%FC) on physiological traits (LAI= leaf area index, CAT= catalase (Units / mg protein), SOD=super oxide dismutase (Units / mg protein), APX= acrobat peroxidase (Units / mg protein), fv/fm= quantum yield, stomatcon= stomata conduction and Phn=photosynthesis)

drought	LAI	CAT	SOD	APX	fvfm	stomatcon	phn
control	0.164^{a}	64.437^{c}	6.469^{a}	0.555^{c}	0.838^{a}	107.562^{a}	15.414^{a}
medium	0.130^{b}	122.125^{b}	5.756^{b}	0.723^{b}	0.839^{ab}	80.5^{b}	9.095^{b}
high	0.093^{c}	196.188^{a}	5.238^{c}	0.891^{a}	0.839^{a}	58.812^{c}	6.299^{c}

Similar letters in each column means that there is no statically significant difference.

The results of Duncan test showed that in totally with increasing of stress level LAI, SOD, stomatcon and pnn almost were decreased and cat, APX and fv/fm were increased (Table 8). Sahel×control has the highest LAI

(0.175) and Armaghan×high has the least LAI (0.083). Maximum of SOD was related to Golestan×control (7.300) and minimum of that is belong to Sahel×high (5.100). Syokra×control has the maximum of stomatcon(112.75) and Armaghan×high has the minimum of stomatcon. Golestan×Control has the highest pnn (16.16) and least of pnn occurred in Sahel×medium (7.83). Maximum of cat is related to Golestan×high and minimum of it was related to Sahel×control. Golestan×high has the highest APX and Sahel×control has the least APX.

Table 8: Duncan multiple means compression test of cultivar× drought stress levels (control=75%FC, medium=15%FC and high=5%FC) interaction on physiological traits (LAI= leaf area index, CAT= catalase (Units / mg protein), SOD=super oxide dismutase (Units / mg protein), APX= acrobat peroxidase (Units / mg protein), fv/fm= quantum yield, stomatcon= stomata conduction and Phn=photosynthesis)

	LAI	CAT	SOD	APX	fvfm	stomatcon	phn
Armaghan×high	0.083^{i}	181.2^{c}	5.200^{e}	0.858^{b}	0.840^{a}	50.25^{k}	6.00^{k}
Golestan×high	0.084^{i}	214.8^{a}	5.525^{d}	0.985^{a}	0.838^{bc}	62.75^{i}	7.12^{i}
Sahel×high	0.099^{h}	194.2^{b}	5.100^{e}	0.748^{e}	0.840^{a}	54.50^{j}	5.38^{l}
Sayokra×high	0.105^{g}	194.5^{b}	5.125^{e}	0.975^{a}	0.838^{bc}	67.75^{h}	6.70^{j}
Golestan×medium	0.125^{f}	131.2^{d}	6.825^{b}	0.822^{c}	0.838^{c}	83.50^{f}	10.12^{e}
Armaghan×medium	0.125^{f}	116.5^{g}	5.550^{d}	0.732^{f}	0.840^{a}	70.50^{h}	8.70^{g}
Sayokra×medium	0.131^{e}	120.0^{f}	5.250^{e}	0.802^{d}	0.838^{bc}	87.75^{e}	9.74^{f}
sahel×medium	0.139^{d}	120.8^{e}	5.400^{d}	0.535^{j}	0.840^{a}	80.25^{g}	7.83^{h}
Golestan×control	0.154^{c}	71.8^{h}	7.300^{a}	0.628^{g}	0.838^{bc}	109.25^{b}	16.16^{a}
Armaghan×control	0.156^{c}	61.0^{j}	5.900^{c}	0.580^{i}	0.840^{a}	102.25^{d}	15.15^{c}
Sayokra×control	0.170^{b}	62.8^{i}	6.850^{b}	0.602^{h}	0.838^{bc}	112.75^{a}	15.51^{b}
Sahel×control	0.175^{a}	62.2^{i}	5.825^{c}	0.410^{k}	0.838^{bc}	106.00^{c}	14.84^{d}

Similar letters in each column means that there is no statically significant difference.

Under conditions of drought, fv/fm was negatively affected (Kitao, and Lei, 2007). The inhibitory effect of drought on photosynthetic activity has been widely described and is mainly associated with stomatal and metabolic limitations (Aranjuelo et al., 2010; Chaves et al., 2009; Lawlor and Tezara, 2009). The decrease in leaf relative water content and the increase in leaf osmotic potential minimize evapotranspiration.

It was demonstrated in cotton that the drought stress resulted in more than four folds increase in the anthocyanin content Similar results have been reported in cowpea seedlings (Deeba et al., 2012; Balakumar, 1993) resurrection plants (Sherwin and Farrant, 1998) and Arabidopsis (Giraud et al., 2008). It has been suggested that increased anthocyanin content helps reduce leaf water potential (Chalker-Scott, 1999). In addition, anthocyanins also have anti-oxidative capabilities. Tsuda et al. (1994) in their work with liposomes, microsomes and membrane systems noted that introduced anthocyanins scavenged oxygen radicals and inhibited lipid peroxidation.

MDA is one of the end products of lipid peroxidation. MDA levels reflect the degree of peroxidation of membrane lipids. H_2o_2 as an ROS can damage membrane lipids, proteins and DNA (Foyer and Noctor, G. 2009) as responses to drought stress, significant increases in MDA and H_2o_2 levels were detected in cotton leaves. Plants respond to increases in contents of MDA and H_2o_2 with the whole regulatory network composed of a series of events, including increases in activities of antioxidant enzymes. Antioxidant enzymes enable detoxification of excess ROS, and maintain balance in the formation and removal of ROS (Alvarez et al., 2009). In the present study, significant increase in activity of antioxidant enzyme, SOD was detected. SODs are metalloenzymes located in various cell compartments that catalyze the disproportionation of o2- to o2 (Fridovich, 1995).

Various researchers have reported that drought stress induces the up-regulation of this enzyme (Faize et al., 2011; França et al., 2007; Pandey et al., 2010). Catalase activity, however, was found to be consistently reduced in the present study. As already mentioned, H_2O_2 levels were increased as the drought intensity increased. It is proposed that a controlled down-regulation of catalase activity occurs during stress. This down-regulation apparently is necessary to maintain a certain level of H_2O_2 required to activate $H_2o_2^-$ dependent signaling pathways (Shao et al., 2008).

Various environmental stresses including drought and heat cause oxidative damage by the formation of ROS, which leads to loss of cellular homeostasis (Sgherri et al., 1996; Navari-Izzo and Rascio,1999) and inhibition of growth. Moreover, combination of drought and heat stresses causes a greater detrimental effect on the growth of plants (Savin and Nicolas, 1996; Jiang and Huang, 2001).

In two of previous studies, Pettigrew (2004) and Massacci et al. (2008) showed an increase in the fv/fm of field grown cotton plants under drought as compared to well irrigated conditions.

Also In addition to these, similar to Jiang and Huang (2000) findings in Kentukey bluegrass, Sekmen et al., (2014) reported that, the Fv/fm cotton had the most severe decrease by combination of hot and drought stresses as compared to individual stress treatments alone.

The SOD constitutes the first line of the enzymatic defence system via conversion of the toxic O2- to the more stable H2O2. Sekmen et al (2014) showed that in Cotton, as compared with 84-S, M-503 demonstrated

slightly higher constitutive and induced levels of SOD under control and stress conditions, which indicate that this cultivar might have a better O2- scavenging capacity. Similar to our findings, Turkan et al. (2005), and Almeselmani et al. (2006) observed a significant increase in SOD levels in drought tolerant Phaseolusacutifolius, and heat tolerant wheat cv. HD2815 under drought and heat stress, respectively. On the other hand, Jiang and Huang (2001)reported that increased SOD activity was related to enhancement of O2- production under stress.

CAT and POX are the major scavengers of H2O2, which is produced through dismutation of O_2^-in peroxisomes, chloroplasts and cytosols (Asada and Takashi, 1987). Reports related to the effects of stresses on CAT activity vary (Jiang and Huang, 2001; Turkan et al., 2005; Almeselmani et al., 2006; Bian and Jiang,2009).

In the Sekmen et al., (2014) study, CAT activity of drought-sensitive 84-S (cotton) was increased under drought stress.

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