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Physiological and Biochemical Responses to Drought Stress of Chickpea Genotypes

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***Abstract*—**The experimental design was 4 x 5 factorial with three replications in fully controlled research greenhouse in Department of Soil Sciences and Plant Nutrition, Faculty of Agriculture, University of Selcuk in the year of 2009. Determination of tolerant chickpea genotypes to drought was made in the research. Additionally, sophisticated effects of drought on plant growth and development, biochemical and physical properties or physical defense mechanisms were presented. According to the results, the primary genotypes were Ilgın YP (0.0063 g/gh) for leaf water capacity, 22235 70.44(%) for relative water content, 22159 (82.47%) for real water content, 22159 (5.03 mg/l) for chlorophyll a+b, Ilgın YP (125.89 nmol H_2O_2.dak^{-1}/ mg protein^{-1}) for peroxidase, Yunak YP (769.67 unit/ mg protein^{-1}) for superoxide dismutase, Seydişehir YP (16.74 µg.TA^{-1}) for proline, Gökçe (80.01 nmol H_2O_2.dak^{-1}/ mg protein^{-1}) for catalase. Consequently, all the genotypes increased their enzyme activity depending on the increasing of drought stress consider with the effects of drought stress on leaf enzyme activity. Chickpea genotypes are increasing enzyme activity against to drought stress.

***Keywords*—**Chickpea, drought, enzyme, tolerance to drought

I. Introduction

WATER plays a vital role in all living organisms [1]. Human is still seeking to find solutions for the water scarcity problem. The solutions should be environmentally friendly for human health and sustainable agriculture [2]. Losing of crops yield which induced by drought is quite important and may also exceeds losses from all other stress factors. Drought stress cause to many changes on plants in terms of biologically, physiologically and biochemically. Data collecting about the effects of ecological conditions on plants would allow to improvement of plant quality and productivity parameters [3].

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The objective of this study was to investigate and identify the drought tolerant chickpea genotypes in terms of the plant response to different levels of water shortage.

II. Material and Method

A total of 10 genotypes were used in the research which were consisted from four local population (Bozkır, Seydişehir, Yunak and Ilgın) collected from city of Konya, 1 standard cultivar (Gökçe) and 5 genotypes (22245, 22159, 22146 and 2235, 22243) from ICARDA (drought tolerant). The pots which had a volume of 1 liter (14 x 13 cm) were washed and sterilized for planting in greenhouse. The seeds of genotypes were exposed to 5% sodium hypochlorite for 10 minute and then washed for 3 times with de-ionized (dl-H_2O) for sterilization. Subsequently, sowing was made to the pots.

The experiment was conducted in "Randomized Plots Factorial Design with Two Factors" with three replications in fully controlled research greenhouse in Department of Soil Sciences and Plant Nutrition, Faculty of Agriculture, University of Selcuk in the year of 2009. Sowing was made by hand on 20th of September 2009.

The top sides of pots were closed for one week following to sowing where 25^{0}C temperature and 40-50% relative humidity conditions of greenhouse. The top sides of each genotype were opened after seeds were germinated. The seedlings were grown under 25^{0}C temperature and 40-50% relative humidity conditions for 40 days after emergence. Then, the plants were classified as control (0. Day), and three stress groups (3rd day, 5th day and 7th day) groups [4]. The three stress groups were treated to stress by non-irrigation for 3, 5 and 7 days. The harvest was made in the same order with stress groups which was started with 0 day (40 days after emergence), and following 3rd, 5th and 7th days [4].

Some physiological and biochemical analysis were made on the leaf tissue of harvested stress and control groups in both of trials.

A. Leaf Water Capacity

Leaves were weighed after samples were taken to investigation of wet weight (W_0) and then, the leaves were held under 25^{o}C and 50 % humidity by weighing on 2nd, 4th and 6st hours (W_2, W_4 and W_6) and finally, they were hold under 50^{o}C for 24 hours and leaf samples were also weighed (Wd). After that, leaf water capacity was calculated by using the following formula [5]:

$$YSTK = (W_0 - W_2) + (W_2 - W_4) + (W_4 - W_6)/3 * W_d (T_2 - T_1)$$

B. Relative and Real Water Content

The leaf segments of control and stress groups were weighed (fresh weight) and hold in glass tube (containing 5 ml of distilled water) under light, room temperature for 24 hours to determination of relative and real water content. At the end

of this time, the hydrated leaf segments were weighed again and the weight in turgor was investigated. Eventually, the leaf segments were dried in incubator under 80°C for 48 hours and also weighed for investigation of dry weight. Thus, relative and real water content were determined according to the fallowing formula [6]:

Relative water content (%) = (TA-KA) / (HA-KA)* 100
GSI (%)= (TA – KA) / TA* 100
TA: fresh weight
HA: weight in turgor condition
KA: dry weight

C. Chlorophyll Content

The amount (mg/l) of chlorophyll a, chlorophyll b and total chlorophyll (a+b) were determined according to Lichtenthaler [7]. To determine pigment content in plant tissue, a total of 6 plants were used which were taken from control and stress groups with 2 plants of each replication with 3 replications. The acetone was added on sliced leaf samples in 1 ml eppendorf tubes. The samples were hold in freezer (+4°C) for 1 week under dark to passing of pigment to solution. At the end of this period, the data was collected by spectrophotometer (Shimadzu Mini-1240 UV-Vis) under 470 and 644.8 nm of wave length.

D. Preparation of Enzyme Extracts

An amount of 5 x 0.5 g leaf samples (separately) were frozen in liquid nitrogen and hold in deep freezer (80°C). Likewise, 0.1 g leaf samples (3 pieces) were also frozen in liquid nitrogen and hold in deep freezer (80°C). a total of 0.5 g leaf samples were homogenized in liquid nitrogen with % 2 w/v polyvinylpolyprrolidone (PVPP) and 1 mM EDTA, pH 7,8 and 50 mM Na-phosphate buffer medium, after filtration, centrifuge was made on +4°C, 14 000 rpm for 30 minutes. These processes were made separately for each of the analysis.

E. Peroxidase (POD; EC 1.11.1.7) Content

Peroxidase content was determined according to the method which was reported by Kumar and Kahn [8]. The mixture which was prepared to POD was as fallowing; 2 ml of 0.1 M buffer phosphate (pH=6.8), 1 ml of 0.01 M pyrogallol, 1 ml of 0.005 M H_2O_2 and 0.5 ml of enzyme extract. An amount of 1 ml from 2.5 M H_2SO_4 was added to the previously prepared solution and it was hold 25 °C for 5 minutes.

The value of purpurogallin was investigated in 420 nm wave length. Enzyme activity was described as U/(mg protein).

F. Superoxide Dismutase (SOD; EC 1.15.1.1) Content

The report of Beauchamp and Fridovich [9] was used as method. An amount of 1.17 M riboflavin, 0.1 M methionin, 2 x 10^{-5} M KCN and 5.6 x 10^{-5} M NBT reaction mixture was dissolved in 3 ml of 0.05 M sodium phosphate (pH=7.8) buffer solution. 1 ml of enzyme extract was added to the medium. The mixture was illuminated inside of monolayer Philips 40 W fluorescence tube. Illumination was continued for 1 hour. Evaluation was made in spectrophotometer under 560 nm. The activity of SOD was determined as U/(mg protein).

G. Proline Analysis

Determination of free proline content was made according to Bates, Waldren and Teare et al. [10]. Toluene fraction of liquid phase was evaluated by spectrophotometer under 520 nm wave lengths. Concentration of proline was calculated by using of calibration curve and µmol proline g^{-1} fresh weight.

H. Catalase (CAT; EC 1.11.1.6) Content

The method of Bergmeyer [11] was used to investigate the CAT enzyme activity. Analyze was made by determination of H_2O_2 reduction rate towards blind in 240 nm UV light zone. Reduction of absorbance during reaction was fallowed throughout 180 seconds. The activity of catalase was described as the amount of µmol H_2O_2 consumption per minute. Analysis of variance was performed using according to "Randomized Plots Factorial Design with Two Factors" and, analysis of LSD and grouping were made on significance level of 1% and 5% [12]. Analysis was performed using "JUMP 5.0.1a" computerized statistical program.

III. Results and Discussions

A. Leaf Water Capacity

Analysis of variance showed that, stress applications and genotype x stress applications had effect on leaf water capacity (Table I). As the means of stress groups, Ilgın YP genotype had the highest value (0.0063 g/gh) while 22243 exhibited the lowest value (0.0036 g/gh). As the means of genotypes, the highest value (0.0111 g/gh) was in control group, while 7th day stress application showed the lowest value (0.0016 g/gh). A reduction for leaf water capacity was occurred in all the used genotypes by drought stress (Table II).

B. Relative Water Content

Analysis of variance showed that, stress applications and genotype x stress applications had effect on relative water content (Table I). As the means of stress groups, 22235genotype had the highest value (70.44%) while 22243 exhibited the lowest value (60.33%). As the means of genotypes, the highest value (69.38%) was in control group, while 7th day stress application showed the lowest value (62.97%). A reduction for relative water content was occurred in all the used genotypes by 7th day stress (Table II).

Many researchers revealed that drought had negative effects on relative water content of plants [13, 14, 15, 16, 17]. A previous study was reported that, reduction of relative water content was continued after closing of stoma in bean [18]. Kalefetoğlu [4] was also reported that relative water content was decreased with the same stress application on chickpea.

C. Real Water Content

Analysis of variance showed that, stress applications and genotype x stress applications had effect on real water content (Table I). As the means of stress groups, 22159 had the highest value (82.47%) while 22243exhibited the lowest value (75.13%). As the means of genotypes, the highest value (83.30%) was in control group, while 7th day stress application showed the lowest value (73.54 %). A reduction for real water content was occurred by increasing of drought

stress (Table II). A previous study was also reported the similar results [4].

D.Chlorophyll A Content

Analysis of variance showed that, stress applications and genotype x stress applications had effect on chlorophyll A content (Table I). As the means of stress groups, 22159 had the highest value (3.51 mg/l) while Yunak YP exhibited the lowest value (1.57 mg/l). As the means of genotypes, the highest value (2.62 mg/l) was in the 3rd day group, while 7th day stress application showed the lowest value (2.15 mg/l). A reduction for real water content was occurred by increasing of drought stress (Table II).

Many of previous researches were also reported results that the amount of chlorophyll A decreases under drought stress [4, 13, 18, 19, 20, 21, 22, 23, 24].

E. Chorophyll B Content

Analysis of variance showed that, stress applications and genotype x stress applications had effect on chlorophyll B content (Table I). As the means of stress groups, Yunak YP had the highest value (2.55 mg/l) while Ilgın YP exhibited the lowest value (0.96 mg/l). As the means of genotypes, the highest value (1.86 mg/l) was taken from control group, while 5th day stress application showed the lowest value (1.31 mg/l).

Many researches were revealed similar results and also reported that the content of chlorophyll B decreases by drought stress [4, 13, 18, 21, 23].

F. Total Chlorophyll (A+B) Content

Analysis of variance showed that, stress applications and genotype x stress applications had effect on total chlorophyll (A+B) content (Table I). As the means of stress groups, 22159 had the highest value (5.03 mg/l) while Seydişehir YP exhibited the lowest value (3.15 mg/l). As the means of genotypes, the highest value (4.19 mg/l) was taken from control group, while 5th day stress application showed the lowest value (3.67 mg/l).

A great number of previously made study are also in accordance with these results [4,13, 18, 21, 23]. Exceptionally, total chlorophyll (A+B) content of Gökçe, Yunak YP, 22159 and 22243 were found lower than some of the stress groups. The reason of the difference could be the genetically structure of genotypes.

G.Prexidase Content

Analysis of variance showed that, genotypes, stress applications and genotype x stress applications had effect on peroxidase content (Table I). As the means of genotypes, Ilgın YP had the highest value (125.89 nmol H_2O_2.dak^{-1} / mg protein^{-1}) while Seydişehir YP exhibited the lowest value (88.44 nmol H_2O_2.dak^{-1} / mg protein^{-1}). As the means of stress groups, the highest value (134.99 nmol H_2O_2.dak^{-1} / mg protein^{-1}) was taken from 7th day stress group, while control group showed the lowest value (85.98 nmol H_2O_2.dak^{-1} / mg protein^{-1}). The amount of peroxidase content was increased by increasing of drought stress. The highest value was taken from 7th day stress group in all the genotypes (Table III).

Plants are developing antioxidant defense system against to drought effect on the leaves and they are using some enzymatic antioxidants such as peroxidase [4, 25]. Additionally, the POD enzyme which produced by the stressed plants and catalyzing of SOD enzyme by dismutation of O_2 and eliminating of H_2O_2 are the most important enzymes [26]. A lot of researches was also revealed that POD was affected by drought stress [4, 23, 27, 28, 29, 30].

H.Superoxide Dismutase Content

Analysis of variance showed that, stress applications and genotype x stress interactions had effect on superoxide dismutase content (Table I). As the means of genotypes, Yunak YP had the highest value (769.67 unit/mg protein^{-1}) while 22146 exhibited the lowest value (645.56 unit/mg protein^{-1}). As the means of stress groups, the highest value (1070.79 unit/mg protein^{-1}) was taken from 7th day stress group, while control group showed the lowest value (278.02 unit/mg protein^{-1}). The amount of superoxide dismutase content was increased by increasing of drought stress (Table III). The results were in accordance with previous researches [4, 31].

I. Proline Content

Analysis of variance showed that, stress applications and genotype x stress interactions had effect on proline content (Table I). As the means of genotypes, Seydişehir YP had the highest value (16.74 μg.TA^{-1}) while 22243 exhibited the lowest value (12.12 μg.TA^{-1}). As the means of stress groups, the highest value (23.35 μg.TA^{-1}) was taken from 7th day stress group, while control group showed the lowest value (2.04 μg.TA^{-1}). The highest proline content was taken from the 7th day stress application (Table III).

Collecting of osmotic protective is one of the important responses of plants to drought stress. Proline is also one of these osmolites and it is common on plants [4, 32, 33, 34, 35]. Kalefetoğlu (2006) was revealed that, proline provides to protecting of cell water by controlling turgor and also supplies to make a cover on membrane and macromolecules which provides structure protecting and take a role on removing of free radicals.

J. Catalase Content

Analysis of variance showed that, stress applications and genotype x stress interactions had effect on catalase content (Table I). As the means of genotypes, Gökçe had the highest value (80.01 nmol H_2O_2.dak^{-1} / mg protein^{-1}) while 22243 exhibited the lowest value (64.70 nmol H_2O_2.dak^{-1} / mg protein^{-1}). As the means of stress groups, the highest value (77.01 nmol H_2O_2.dak^{-1} / mg protein^{-1}) was taken from 7th day stress group, while control group showed the lowest value (60.71 nmol H_2O_2.dak^{-1} / mg protein^{-1}). The amount of catalase content was increased significantly by increasing of drought stress (Table III).

It was reported [29] that, the content of catalase is quite important in the beans (*Phaseolus acutifolius* L.) which are known as tolerant to drought. Kalefetoğlu (2006) was also reported that the content of catalase was increased as depending on drought. These findings are in accordance with the present results.

TABLE I
MEANS SQUARES OF INVESTIGATED CHARACTERISTICS IN CHICKPEA GENOTYPES UNDER DIFFERENT LEVELS OF DROUGHT

Sources	DF	Leaf water capacity	Relative water content	Real water content	Chlorophyll A content	Chlorophyll B content
Total	119					
Genotype (G)	9	0.000**	99.032**	49.482**	5.307**	0.557**
Stress Groups (SG)	3	0.000**	262.692**	495.105**	1.144**	7.061**
G x S.G. Int.	27	0.000**	179.242**	37.127**	0.347**	0.785**
Error	80	0.000	33.124	8.108	0.009	0.016

Sources	DF	Chlorophyll A+B content	Peroxidase content	Superperoxide Dismutase content	Proline content	Katalase content
Total	119					
Genotype (G)	9	3.893**	14118.598**	24453.3333**	24.822**	583.896**
Stress Groups (SG)	3	9.400**	1621.508**	4364043.67**	2592.517**	1506.207**
G x S.G. Int.	27	1.045**	279.942**	27872.1481**	8.778**	167.356**
Error	80	0.019	14.773	176	0.366	8.259

*** P< 0.01*

TABLE II
LEAF WATER CAPACITY, RELATIVE WATER CONTENT, REAL WATER CONTENT, CHLOROPHYLL A CONTENT, CHLOROPHYLL B CONTENT, CHLOROPHYLL A + B CONTENT OF THE CHICKPEA GENOTYPES

Genotypes	Leaf water capacity (g/gh)					Relative water content (%)				
	Control	3rd day	5th day	7th day	Mean	Control	3rd day	5th day	7th day	Mean
Bozkır	0.0090	0.0032	0.0020	0.0015	**0.0039**	69.55	60.46	69.40	63.85	**65.82**
22245	0.0080	0.0046	0.0015	0.0024	**0.0041**	70.92	58.34	66.84	61.02	**64.28**
Seydişehir	0.0065	0.0016	0.0015	0.0018	**0.0029**	71.60	73.38	71.36	59.19	**68.88**
Gökçe	0.0067	0.0021	0.0016	0.0016	**0.0030**	66.19	59.78	82.16	64.90	**68.26**
Yunak	0.0062	0.0015	0.0024	0.0018	**0.0030**	70.30	73.56	65.81	61.15	**67.71**
22159	0.0114	0.0016	0.0018	0.0013	**0.0040**	63.35	72.77	72.47	69.21	**69.45**
22146	0.0149	0.0019	0.0018	0.0014	**0.0050**	75.63	76.87	48.23	71.99	**68.18**
Ilgın	0.0174	0.0020	0.0025	0.0014	**0.0058**	69.27	71.39	67.81	59.07	**66.88**
22235	0.0149	0.0018	0.0019	0.0019	**0.0051**	73.47	70.33	77.17	60.80	**70.44**
22243	0.0158	0.0027	0.0018	0.0014	**0.0054**	63.55	73.89	46.54	58.52	**60.63**
Mean	**0.0111**	**0.0023**	**0.0019**	**0.0016**	**0.0042**	**69.38**	**69.08**	**66.78**	**62.97**	**67.05**
	LSD_G: 0.001017; **LSD_{SG}:** 0.0006813; **LSD_{SG}:** 0.002154					**LSD_G:** 6.200; **LSD_{SG}:** 3.921; **LSD_{SG}:** 12.40				

Genotypes	Real water content (%)					Chlorophyll A content (mg/l)				
	Control	3rd day	5th day	7th day	Mean	Control	3rd day	5th day	7th day	Mean
Bozkır	84.22	78.45	78.16	76.73	**79.39**	2.03	1.70	1.40	1.33	**1.62**
22245	82.99	77.37	78.01	70.24	**77.15**	1.87	1.93	1.67	1.57	**1.76**
Seydişehir	83.17	78.54	82.60	72.32	**79.16**	2.37	2.50	2.43	1.27	**2.14**
Gökçe	83.46	73.99	79.98	72.97	**77.60**	2.50	3.27	3.03	3.00	**2.95**
Yunak	82.13	81.49	76.81	72.98	**78.35**	1.80	1.87	1.40	1.20	**1.57**
22159	83.27	82.47	85.13	79.00	**82.47**	2.60	4.03	3.67	3.73	**3.51**
22146	84.26	81.31	67.14	79.21	**77.98**	2.50	3.20	2.83	2.87	**2.85**
Ilgın	82.70	79.24	78.37	71.40	**77.93**	2.50	2.80	2.47	2.27	**2.51**
22235	85.30	83.90	82.56	71.71	**80.87**	2.47	1.97	1.73	1.43	**1.90**
22243	81.50	80.40	69.79	68.81	**75.13**	2.63	2.97	3.00	2.83	**2.86**
Mean	**83.30**	**79.72**	**77.86**	**73.54**	**78.61**	**2.33**	**2.62**	**2.36**	**2.15**	**2.37**
	LSD_G: 3.067; **LSD_{SG}:** 1.940; **LSD_{SG}:** 6.135					**LSD_G:** 0.1022; **LSD_{SG}:** 0.06463; **LSD_{SG}:** 0.2044				

Genotypes	Chlorophyll B content (mg/l)					Chlorophyll A+B content (mg/l)				
	Control	3rd day	5th day	7th day	Mean	Control	3rd day	5th day	7th day	Mean
Bozkır	2.17	2.34	1.70	1.26	**1.87**	4.21	4.06	3.09	2.61	**3.49**
22245	2.24	2.02	1.92	1.52	**1.93**	4.09	3.96	3.58	3.11	**3.69**
Seydişehir	1.52	0.91	0.86	0.73	**1.01**	3.88	3.42	3.30	1.99	**3.15**
Gökçe	1.87	1.50	1.11	0.75	**1.31**	4.36	4.79	4.13	3.73	**4.25**
Yunak	2.30	2.13	2.39	3.37	**2.55**	4.10	3.99	3.76	4.55	**4.10**
22159	1.99	1.40	1.57	1.13	**1.52**	4.59	5.41	5.25	4.87	**5.03**
22146	1.93	1.12	0.90	1.32	**1.32**	4.47	4.29	3.73	4.21	**4.18**
Ilgın	1.53	0.86	0.68	0.75	**0.96**	4.07	3.66	3.14	3.00	**3.47**
22235	1.67	1.45	1.37	1.50	**1.50**	4.13	3.44	3.09	2.94	**3.40**
22243	1.42	0.72	0.58	3.18	**1.48**	4.04	3.67	3.58	6.03	**4.33**
Mean	**1.86**	**1.45**	**1.31**	**1.55**	**1.54**	**4.19**	**4.07**	**3.67**	**3.70**	**3.91**
	LSD_G: 0.1363; **LSD_{SG}:** 0.08618; **LSD_{SG}:** 0.2725					**LSD_G:** 0.1485; **LSD_{SG}:** 0.09391; **LSD_{SG}:** 0.2970				

TABLE III
PEROXYDASE CONTENT, SUPERPEROXIDE DISMUTASE CONTENT, PROLINE CONTENT AND CATALASE CONTENT OF THE CHICKPEA GENOTYPES

Genotypes	Peroxides Content (nmol H_2O_2.dak^{-1} / mg protein^{-1})					Superperoxide Dismutase Content (unit/mg protein^{-1})				
	Control	3rd day	5th day	7th day	Mean	Control	3rd day	5th day	7th day	Mean
Bozkır	86.63	115.57	122.63	151.53	**119.09**	260.20	427.93	871.87	1097.60	**664.40**
22245	94.07	126.90	146.73	127.17	**123.72**	277.83	401.07	823.97	1158.30	**665.29**
Seydişehir	55.27	86.63	103.87	108.00	**88.44**	272.17	356.53	1060.27	922.83	**652.95**
Gökçe	95.13	117.50	138.63	141.60	**123.22**	284.23	521.63	892.03	977.17	**668.77**
Yunak	89.07	140.63	134.30	135.20	**124.80**	276.17	618.23	1086.47	1097.80	**769.67**

22159	89.67	115.67	119.23	136.33	**115.23**	263.67	305.67	968.30	1264.53	**700.54**
22146	87.93	124.33	138.77	132.33	**120.84**	286.47	575.03	853.87	866.87	**645.56**
Ilgın	88.77	144.10	134.27	136.43	**125.89**	291.17	474.53	1067.57	1221.33	**763.65**
22235	86.60	93.20	105.43	141.57	**106.70**	283.50	491.20	931.10	957.60	**665.85**
22243	86.70	134.93	134.60	139.73	**123.99**	284.83	455.67	991.13	1143.90	**718.88**
Mean	**85.98**	**119.95**	**127.85**	**134.99**	**117.19**	**278.02**	**462.75**	**954.66**	**1070.79**	**691.56**
	LSD_G: 4.140; **LSD_{SG}:** 2.619; **LSD_{SG}:** 8.281					**LSD_G:** 14.29; **LSD_{SG}:** 9.039; **LSD_{SG}:** 28.58				
Genotypes	**Proline Content (µg.TA^{-1})**					**Catalase Content (nmol H_2O_2.dak^{-1} / mg protein^{-1})**				
	Control	**3rd day**	**5th day**	**7th day**	**Mean**	**Control**	**3rd day**	**5th day**	**7th day**	**Mean**
Bozkır	1.72	16.72	19.42	25.43	**15.82**	65.67	74.20	70.53	65.70	**69.03**
22245	2.25	18.90	20.07	23.83	**16.27**	58.23	64.50	73.83	72.20	**67.19**
Seydişehir	3.29	14.88	21.85	26.95	**16.74**	75.73	65.43	67.67	72.47	**70.33**
Gökçe	1.62	16.62	20.38	24.08	**15.68**	73.53	76.30	82.87	87.33	**80.01**
Yunak	1.67	16.25	18.48	21.81	**14.55**	56.23	68.27	76.50	65.87	**66.72**
22159	1.75	12.04	23.28	26.42	**15.87**	60.07	76.13	86.33	96.23	**79.69**
22146	1.94	16.33	18.96	22.52	**14.94**	60.03	65.57	77.33	72.17	**68.78**
Ilgın	1.98	14.46	17.29	19.30	**13.26**	64.60	69.53	74.33	70.33	**69.70**
22235	2.33	16.23	20.56	24.10	**15.81**	56.83	70.87	80.87	85.70	**73.57**
22243	1.86	11.56	15.98	19.08	**12.12**	36.13	65.07	75.47	82.13	**64.70**
Mean	**2.04**	**15.40**	**19.63**	**23.35**	**15.11**	**60.71**	**69.59**	**76.57**	**77.01**	**70.97**
	LSD_G: 0.6517; **LSD_{SG}:** 0.4122; **LSD_{SG}:** 1.303					**LSD_G:** 3.096; **LSD_{SG}:** 1.958; **LSD_{SG}:** 6.192				

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REFERENCES

[1] O. Önder, M., Ceyhan, E. and Kahraman, A., 2011. Effects of Agricultural Practices on Environment. Biology, Environment and Chemistry (ICBEC 2011), Volume 24, Page 28-32, December 28-30, Dubai, UAE.

[2] Kahraman, A., Önder, M. and Ceyhan, E., 2011. Biodiversity and biosecurity in Turkey. Biology, Environment and Chemistry (ICBEC 2011), Volume 24, Page 33-37, December 28-30, Dubai, UAE.

[3] Ceyhan, E., Kahraman, A. and Önder, M., 2012. The effects of environment on plant products. International Journal of Bioscience, Biochemistry and Bioinformatics, Volume 2, no 1, Page 48-51.

[4] Kalefetoğlu, T., 2006, Nohut (Cicer arietinum L.) çeşit ve hatlarının kuraklık stresine karşı dayanıklılığının karakterizasyonu. Haccetepe Universiy, Institute of Life Sciences, Master Thesis. pp: 142.

[5] Clarke, J.M. and McGaig, T.N.1982, Excised-leaf water retention capability as an indicator of drought. J. Plant Sci., 62: 571-578.

[6] Farrant, J.M., 2000, A comparison of mechanisms of desiccation tolerance among three angiosperm resurrection plant species. Plant Eco., 151, 29-39.

[7] Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth Enzymol, 148: 350–382.

[8] Kumar, K.B. and P.A. Khan, 1982. Peroxidase and polyphenol oxidase in excised ragi (Eleusine coracana cv. PR 202) leaves during senescence. Indian J. Experimental Botany, 20: 412-416.

[9] Beauchamp, C. ve Fridovich, I., 1971, Superoxide Dismutase: Improved Assays and Applicable to Acrylamide Gels. Analytical Biochemistry. 44: 276-287 p.

[10] Bates, L.S., Waldren, R.P., Teare, I.D., 1973, Rapid determination of free proline for water–stress studies. Plant Soil, 39, 205– 207.

[11] Bergmeyer, H.U., 1970. Methods of Enzymatic Analysis. Verlag Chemie, Weinheim/Bergstr, German.

[12] Düzgüneş O., Kesici T., Kavuncu, O. ve Gürbüz, F. 1987, Araştırma ve Deneme Metodları (İstatiksel Metodlar-II). Ankara Univ.Agricultural Fac. Press No:1021, Class Book Serial No:295. Ankara.

[13] Fu, J. and Huang, B., 2001, Involvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. Environ. Exp. Bot., 45, 105–114.

[14] Egert, M. and Tevini, M. 2002, Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (Allium schoenoprasum). Environ. Exp. Bot., 48. 43–49.

[15] Liu, F. and Stützel, H. 2002, Leaf water relations of vegetable amaranth (Amaranthus spp.) in response to soil drying. Eur. J. Agron., 16, 137–150.

[16] Tambussi, E. A., Casadesus, J., Munné-Bosch, S., Araus, J. L., 2002, Photoprotection in water-stressed plants of durum wheat (Triticum turgidum var. durum): changes in chlorophyll fluorescence spectral signature and photosynthetic pigments. Funct. Plant Biol., 29, 35-44.

[17] Anyia, A.O. and Herzog, H., 2004. Genotypic Variability in Drought Performance and Recovery in Cowpea under Controlled Environment. J. Agronomy & Crop Science, 190, 151—159.

[18] Lucero, M. E., Mueller, W., Hubstenberger, J., Phillips, G. C., O'Connell, M. A., 1999. Tolerance to nitrogenous explosives and metabolism of TNT by cell suspensions of Datura innoxia. In Vitro Cell Div. Biol. Plant, 35: 480-486.

[19] Costa França, M.G., Pham-Thi, C.A.T., Pimentel, R.O.P., Rossiello, Y., Fodil, Z., Laffray, D., 2000, Differences in growth and water relations among Phaseolus vulgaris cultivars in response to induced drought stress. Environ. Exp. Bot., 43, 227–237.

[20] Soltani, A., Khooie, F. R., Ghassemi-Golezani K. and Moghaddam, M. 2000. Thresholds for chickpea leaf expansion and transpiration response to soil water deficit. Field Crops Res., 68 (3), 205-210.

[21] Munné-Bosch, S., Jubany-Mari, T., Alegre, L., 2001, Drought-induced senescence is characterised by a loss of antioxidant defences in chloroplasts. Plant. Cell Environ., 24, 1319-1327.

[22] Srivalli, B., Sharma, G. and Khanna-Chopra, R., 2003. Antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. Physiologia Plantarum, 119: 503–512.

[23] Jung, S., 2004, Variation in antioxidant metabolism of young and mature leaves of Arabidopsis thaliana subjected to drought. Plant Sci., 166, 459-466.

[24] Lazaridou, M. and Koutroubas, S.D., 2004. Drought effect on water use efficiency of berseem clover at various growth stages . Proceeding for the 4th International Crop Science Congress, Brisbane, Australia, 26 September-1 October 2004.

[25] Jiang, H.F. and Ren, X.P., 2004, The effect on SOD activity and protein content in groundnut leaves by drought stress. AAS, 30, 169- 174.

[26] Asada, K. and Takahashi, M., 1987, Production and scavenging of active oxygen radicals in photosynthesis. Photoinhibition. Kyle. D.J. (ed.). Elsevier. pp. 227-297.

[27] Ramachandra Reddy, A., Viswanatha Chaitanya, K., Vivekanandan, M., 2004, Drought-induced responses of photosynthesis and antioxidant metabolism in higher plants. J. Plant Physiol.,161, 1189–1202.

[28] HongBo, S., ZongSuo, L., MingAn, S., 2005, Changes of anti-oxidative enzymes and MDA content under soil water deficits among 10 wheat (Triticum aestivum L.) genotypes at maturation stage. Colloids and Surfaces B: Biointerfaces, 45, 7-13.

[29] Türkan, İ., Bor. M., Özdemir, F., Koca, H. 2005, Differential responses of lipid peroxidation and antioxidants in the leaves of drought-tolerant P. acutifolius Gray and drought-sensitive P. vulgaris L. subjected to polyethylene glycol mediated water stress. Plant Sci., 168, 223-231.

[30] Ge, T., Sui, F., Bai, L., Lu, Y., Zhou, G., 2006, Effects of water stress on the protective enzyme activities and lipid peroxidation in roots and leaves of summer maize. ASC, 5(4), 291-298.

[31] Shao L, Young LT, Wang JF. Chronic treatment with mood stabilizers lithium and valproate prevents excitotoxicity by inhibiting oxidative stress in rat cerebral cortical cells. Biol Psychiatry 2005;58:879–84.

[32] Tıpırdamaz, R. ve Çakırlar. H. 1990, Buğday (Triticum aestivum L.) bitkisinin Türkiye'de yetiştirilen iki çeşidinde tuz ve su stresinin oransal su kapsamı prolin ve betain değişimine etkisi. DOĞA-Tr. J. of Biology. 14 (2), 125-148.
[33] Hsu, S.Y., Hsu, Y.T., Kao, C.H., 2003, The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant., 46, 73–78.
[34] Kavi Kishore, P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R., Rao, K.R.S.S., Rao, S., Reddy, K.J., Theriappan, P., Sreenivasulu, N., 2005, Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr. Sci., 88, 424–438.
[35] Tan, Y., Liang, Z., Shao, H., Du, F., 2006, Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix astragali at seeding stage. Colloid. Surface. B., 49, 59–64.