Bangladesh J. Bot. **44**(2): 177-183, 2015 (June)

EFFECTS OF DROUGHT STRESS ON GROWTH PARAMETERS, ENZYME ACTIVITIES AND PROLINE CONTENT IN CHICKPEA GENOTYPES

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Key words: Chickpea, Drought, Growth, Enzyme, Proline

Abstract

Drought stress decreased leaf water absorption capacity and real water content and increase relative water content in genotypes of chickpea. It decreased chlorophyll a and b content. Drought increased peroxidase superoxide dismutase, glutathione reductase, ascorbate peroxidase and catalase in stress groups. Proline content increased drastically index stress condition.

Introduction

Chickpea is the third pulse crop followed by bean and pea in world production. It is clear that the sowing area, production and yield of chickpea decreased during the last ten years (Ceyhan *et al.* 2007, Ceyhan *et al.* 2012a). The main cause of reducing of yield is biotic (*Ascochyta rabiei*) and abiotic (high temperature and drought) factors. A closed basin Central Anatolian region is one of the most affected regions by drought (Ceyhan *et al.* 2012a). This region has almost 50% of the total chickpea production in Turkey. Drought considerably affect chickpea cultivation, drought tolerant line of chickpea is none (Singh 1997). Drought is known as the most important abiotic stress factor over the world (Ceyhan *et al.* 2012a) and it causes loss in yield of the cold climate pulses (Singh 1997). Drought stress in plants induces physiological, biochemical and molecular changes for adaptation (Arora *et al.* 2002, Kalefetoğlu 2006).

Number of work related to drought stress in chickpea is very few. Effect of drought on physiological and biochemical changes chickpea genotypes is reported.

Materials and Methods

A total of ten genotypes were collected from six local population (Kadinhani LP, Altinekin LP, Hadim LP, Cumra LP, KarapinarLP and Beysehir LP) from city of Konya, two standard cultivar (Canitez and Kusmen-99) and two genotypes (22117 and 22223) from ICARDA (drought tolerant). The pots which had a volume of 1 liter (14 × 13 cm) were washed and sterilized for planting in greenhouse. The seeds of genotypes were exposed to 5% sodium hypochlorite for 3 min and then washed for 3 times with de-ionized water for sterilization. Subsequently, seeds were sown in uncontaminated pots.

The experiment was conducted in "Randomized Plots Factorial Design with Two Factors" with three replications in a fully controlled research greenhouse in Department of Soil Sciences and Plant Nutrition, Faculty of Agriculture, University of Selcuk in 2009. Sowing was made by hand on 15th of February 2009.

The top sides of pots were closed for one week after sowing and placed in the greenhouse at 25^0C temperature and 40 - 50% relative humidity. The top sides of pots were opened after seeds were germinated. The seedlings were grown in the greenhouse under 25^0C and 40 - 50% relative

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humidity conditions for 40 days after emergence. Then, the plants were classified as control (0 Day), and three stress groups (3rd, 5th and 7th day) (Ceyhan *et al.* 2012 b). Drought stress was applied 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 of stress (Ceyhan *et al.* 2012 b).

Some physiological and biochemical analysis were made on the leaf tissue of harvested stress and control groups in trial. Leaf water absorption capacity (LWAC), relative water content (RWC) and real water content (REWC) were determined according to the method of Clarke and McGaig (1982) and Farrant (2000). The amount (mg/l) of chlorophyll a, chlorophyll b and total chlorophyll (a+b) were determined according to Lichtenthaler (1987).

Leaf samples (0.5 g) were frozen (separately) in liquid nitrogen and stored in deep freezer (80^{o}C). Likewise, 0.1 g leaf samples were also frozen in liquid nitrogen and stored in deep freezer (80^{o}C). 0.5 g leaf samples were homogenized in liquid nitrogen with %2 w/v polyvinyl-polyprrolidone (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 min. These processes were made separately for each of the peroxidase (POD), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and proline analysis (Kumar and Kahn 1982, Beauchamp and Fridovich 1971, Bates *et al.*1973, Foyer and Halliwell 1976, Nakano and Asada 1981, Bergmeyer 1970).

Results and Discussion

LWAC of stress groups showed that the genotypes Karapinar LP (0.0063 g/gh), Kusmen 99 (0.0060 g/gh) and 22223 (0.0054 g/gh) had more LWAC. Means of genotypes showed that the highest LWAC belongs to control group (0.0132 g/gh). As increasing in drought stress; LWAC decreased (Table 1). An important level of reduction in the LWAC of the genotypes occurred by application of drought stress compared with their own controls. The same result was also reported by Ceyhan *et al.* (2012b).

RWC of stress groups showed that the highest value was observed in the genotype Canitez (81.89%) while the lowest value was observed in genotype Beysehir LP (66.40%) (Table 2). The RWC values decreased compared to their control genotypes Kadinhani LP, Altinekin LP, Hadim LP, Karapinar LP and Beysehir LP but that decreased in the other genotypes (Table 2). Many researchers implicated that drought had negative effect on RWC in the plants (Anyia and Herzog, 2004, Ceyhan *et al.* 2012b). RWC decreased in bean even after closing of stoma (Costa Franca *et al.* 2000) and in chickpea (Kalefetoğlu 2006) following stress application. On the other hand, Turkan *et al.* (2005) described the high RWC in the drought-resistant *Phaseolus acutifolius* was due to high content of proline.

REWC of stress groups showed that the highest value was observed on the genotype Kusmen 99 (83.41%). Means of the genotypes showed that the highest REWC value (84.47%) was found on control group (Table 1). The REWC values decreased comparing with their control of the genotypes Kadinhani LP, Altinekin LP, Hadim LP, Karapinar LP and Beysehir LP while an increasing was observed in the other genotypes (Table 2). Kalefetoğlu (2006) working with chickpea observed reducing in REWC compared to controls by increasing the drought.

Effect of drought on chlorophyll content: Chlorophyll a of the stress groups ranged from 1.62 (Kadinhani LP) to 3.78 mg/l (Kusmen 99). Means of the genotypes showed the highest value (2.640 mg/l) in control group while the lowest value (2.08 mg/l) was observed on 7 days stress group (Table 1). All the genotypes showed an important decrease for chlorophyll a content as from 3 days compared with their own control group (Table 1).

Chlorophyll b of the stress groups produced the highest value Canitez and it was followed by Kadinhani and Altinekin (2.518, 1.960 and 1.935 mg/l, respectively). The lowest value occurred on Karapinar LP with 0.842 mg/l of chlorophyll b content (Table1).

Table 1. Effects of drought on LWAC, RWC, REWC and chlorophyll a, b, a+b inchickpea genotypes.

	LWAC	RWC	REWC	Chlorophyll a	Chlorophyll b	Chlorophyll a+b
	g/gh	%	%	mg/l	mg/l	mg/l
Stress groups						
Control	0.0132 a*	73.92	84.47 a	2.640 a	1.613 a	4.243 a
3rd day	0.0021 b	76.31	80.69 b	2.520 b	1.464 b	3.975 b
5th "	0.0018 b	78.18	80.69 b	2.380 c	1.301 d	3.681 c
7th "	0.0018 b	76.31	76.30 c	2.076 d	1.386 c	3.463 d
Genotypes						
Kadinhani	0.0040 efg	68.25 cd	79.75 cd	1.617 h	1.960 b	3.577 f
Altinekin	0.0046 de	72.69 bcd	79.34 d	1.775 g	1.935 b	3.714 e
Hadim	0.0045 def	76.50 ab	80.33 cd	2.100 f	0.925 de	3.023 i
Cumra	0.0048 cd	81.85 a	80.30 cd	3.258 b	1.009 d	4.259 b
Canitez	0.0038 fg	81.89 a	80.84 bcd	1.483 i	2.518 a	3.989 d
Kusmen	0.0060 ab	80.43 ab	83.41 a	3.783 a	1.376 c	5.159 a
22117	0.0042 d-g	81.30 a	83.07 ab	2.975 c	1.039 d	4.013 cd
Karapinar	0.0063 a	75.55 abc	79.56 cd	2.508 e	0.842 e	3.346 g
22223	0.0054 bc	76.95 ab	81.78 abc	1.733 g	1.448 c	3.183 h
Beysehir	0.0036 g	66.40 d	77.02 e	2.808 d	1.356 c	4.143 bc

Chlorophyll (a+b) content of the stress groups showed that the highest values were as followed: 5.159, 4.259 and 4.143 mg/l on the genotypes Kusmen 99, Cumra LP and Beysehir LP respectively. The lowest value was 3.023 mg/l which occurred on the genotype Hadim LP (Table 1). Many researchers found that the plants under drought stress decreased content of chlorophyll a, b, (a+b) content (Costa Franca *et al.* 2000, Fu and Huang 2001, Kalefetoğlu 2006, Ceyhan *et al.* 2012b).

Effect of drought on peroxidase, superoxide dismutase and glutathione reductase content: POD content of the genotypes of stress groups ranged from 98.88 (control) to 228.38 nmol H_2O_2 min/mg/protein (7 day stress). The highest value of the POD was 194.16 nmol H_2O_2. min/mg/protein on the genotype Kusmen 99. POD values of the genotypes increased by increasing of stress (Table 2).

Many of the previous researchers reported POD is affected by drought stress (Turkan *et al.* 2005, Kalefetoğlu 2006, Ceyhan *et al.* 2012b). Kalefetoğlu (2006) implicated that POD activity increases depending on increasing of drought stress in chickpea and, the cell membranes might be protected by high level of POD activity. Similarly, Turkan *et al.* (2005) found that POD content was higher in drought resistant bean genotype.

SOD content of the genotypes showed the highest value on 7 days of stress group (1351.00 unit mg/protein) while the lowest value was taken from the control group (373.53 unit mg/protein). The highest value was 1155.72 unit mg/protein on the genotype Kadinhani LP (Table 2). Kalefetoğlu (2006) stated that total SOD activity varied between the genotypes and lines under

various intensity of drought stress. Shao *et al.* (2005) reported that wheat genotypes under water scarcity in the soil should had different levels of SOD content. The reduction in SOD content of the genotypes Kadinhani, 22117 and Canitez implicated that drought was the limiting factor for SOD activity (Fu and Huang 2001, Jiang and Ren 2004, Kalefetoğlu 2006, Ceyhan *et al.* 2012b).

GR stress groups showed the highest GR contents ranged from 67.26 (Kadinhani LP) to 100.45 n mol NADPH min/mg/protein (Cumra LP). According to the means of the genotypes, the highest GR value was 101.90 n mol NADPH min/mg/protein in 7 days of stress group while the lowest GR value was 64.50 n mol NADPH. min/mg/protein in the control group. The stress groups of 3 days and 5 days were in between the values of 83.51 and 96.09 n mol NADPHmin^{-1} mg/protein, respectively (Table 2).

Table 2. Effects of drought on POD, SOD, GR, APX, CAT and proline in chickpea genotypes.

	POD	SOD	GR	APX	CAT	Proline
	nmol H_2O_2/min/ mg/protein	unit/mg/ protein	nmol NADPH.min/mg/ protein	nmol ascorbate.min^{-1}mg protein^{-1}	nmol H_2O_2.min/ mg/protein	µg/TA
Stress groups						
Control	98.88 d	373.53 b	64.50 d	78.02 d	60.71 c	1.68 d
3rd day	137.94 c	1223.63 a	83.51 c	111.08 c	69.59 b	10.82 c
5th day	176.69 b	1317.14 a	96.09 b	145.99 b	76.57 a	12.25 b
7th day	228.38 a	1351.00 a	101.90 a	182.46 a	77.01 a	15.26 a
Genotypes						
Kadinhani	128.39 f	1155.72	67.26 f	101.90 d	69.02 cd	8.80 ef
Altinekin	192.27 a	1154.88	85.11 cd	132.79 b	67.19 d	9.66 de
Hadim	149.05 d	822.51	94.42 b	130.45 b	70.32 c	9.80 cd
Cumra	132.01 f	1053.07	100.45 a	133.77 b	80.01 a	8.44 f
Canitez	167.73 b	849.80	81.89 e	132.17 b	66.72 de	12.27 a
Kusmen	194.16 a	956.89	79.32 e	125.54 c	79.69 a	10.85 b
22117	159.80 c	1014.21	82.13 de	126.39 c	68.77 cd	12.59 a
Karapinar	140.84 e	965.11	87.36 c	126.15 c	69.70 c	8.60 f
22223	190.95 a	1053.38	94.70 b	153.35 a	73.57 b	10.70 bc
Beysehir	149.53 d	989.08	92.36 b	131.38 b	64.70 e	8.30 f

*Figures in the same line column a common letter are not significantly different.

The chickpea genotypes Canitez and AkN 209 showed an increasing in the GR content on the stress level of seven days (Kalefetoğlu 2006). The leaves of chickpea (Kalefetoğlu 2006, Ceyhan *et al.* 2012b) and bean plants (Turkan *et al.* 2005) exposed drought increased the content of GR. Moran *et al.* (1994) reported that content of GR decreased in pea following drought stress while Keles and Öncel (2002) observed an increase in wheat.

Ascorbate peroxidase content: Drought stress groups showed the lowest APX activity in the genotype Kadinhani LP (101.90 nmol ascorbate/min mg/protein) while the highest value was in the genotype 22223 (153.35 nmol ascorbate/min mg/protein) (Table 2). Means of the genotypes showed the highest APX content as a value of 182.46 nmol ascorbate/min mg/protein from 7 days stress application while the lowest value was 78.02 nmol ascorbate/min mg/protein in the control group. The stress groups of 3 and 5 days ranged from 111.08 to 145.99 nmol ascorbate/min

mg/protein, respectively (Table 2). Turkan *et al.* (2005) implicated that, in the drought resistant bean (*Phaseolus acutifolius* L.), content of APX plays an important role in combating drought stress. Kalefetoğlu (2006) and Ceyhan *et al.* (2012b) revealed that content of APX increased depending on drought in chickpea.

Catalase content: Means of the stress groups showed the highest value as 80.01 nmol H_2O_2/min mg/protein in the genotype of Cumra LP while the lowest value obtained as 64.70 nmol H_2O_2/min mg/proteinin the genotye Beysehir LP and, the rest of the genotypes were ranged between these values. Activity of CAT in all the genotypes increase following 3 days of drought stress (Table 2). Turkan *et al.* (2005) reported that content of CAT acts significantly in the drought resistant bean (*Phaseolus acutifolius* L.). Research results of Kalefetoğlu (2006) and Ceyhan *et al.* (2012b) showed that content of CAT increased depending on drought.

Proline content: Means of the genotypes showed that proline contents (Table 1) of the stress groups ranged from 1.68 (control) to 15.26 µg/FW (7 days). The highest proline content occurred in the genotypes 22117 (12.59 µg/FW) and Canitez (12.27 µg/FW). The lowest proline content was 8.30 µg/FW in Beysehir LP genotype (Table 2).

Proline was found to be occumulated in a large scale under drought stress (Kalefetoğlu 2006, Tan *et al.* 2006, Ceyhan *et al.* 2012b). The present result also showed increasing of proline content in chickpea genotypes under drought stress. Kalefetoğlu (2006) suggested that proline controls turgor and protects cell water. Similarly, Tan *et al.* (2006) detected an increasing proline content depended on the level of water scarcity and time.

Table 3. Means squares of investigated characteristics in chickpea genotypes under different levels of drought.

Variation sources	LWAC	RWC	REWC	Chlorophyll a	Chlorophyll b	Chlorophyll a+b
Genotypes (G)	**	**	**	**	**	**
Stress groups (SG)	**	ns	**	**	**	**
G × SG İnt.	**	**	*	**	**	**
Variation sources	POD	SOD	Proline	GR	AP	CAT
Genotypes (G)	**	ns	**	**	**	**
Stress groups (SG)	**	**	**	**	**	**
G × SG İnt.	**	ns	**	**	**	**

*: $p < 0.05$; **: $p < 0.01$; ns: non significant.

In conclusion, statistical analysis for LWAC, RWC, REWC, chlorophyll a, b, (a+b), POD, SOD, GR, APX, CAT and proline content in genotypes, stress and genotypes × stress were significant (Table 3). The most drought resistant genotypes were Cumra LP, Canitez, Kusmen-99, 22117 and 22223 may be due to enzyme activity in the leaves. It is clear that drought stress changed the activity of antioxidant enzymes and proline content in all the used genotypes significantly and response of genotypes varied in a large scale. Future researcher supposed to analyze of all the drought stress tolerant chickpea genotypes throughout the widely used lines and breeding programs should be supported as well as using of the promising genotypes.

Acknowledgment

The authors are thankful for the support extended by TUBITAK (Project number: 108 O 742) and Selcuk University Scientific Research Projects (BAP). This paper is a part of this project and was summarized from M.Sc. thesis of the first author (EG).

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(Manuscript received on 7 April, 2014; revised on 7 April, 2015)