УДК 57.2788

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EFFECT OF DROUGHT STRESS ON CONTENT OF SOME ENZYMATIC ANTIOXIDANTS FOR VARIOUS CHICKPEA GENOTYPES

Annotation: *The aim of this study was to evaluate the effect of drought stress on antioxidant enzymes activity ascorbate peroxidase (APX) , superoxide dismutase (SOD) and catalase (CAT). Also some growth parameters as number of leaves plants, length of shoot, root length were measured for three genotypes chickpea ILC3279, GAB_3, GAB_4. The results show that there was a significant increases in the shoot length, number of leaves and root length measured in GAB_4. Inaddition There was significant differences between studied genotypes about SOD, APX and CAT activities. The highest CAT and SOD enzyme activities under the drought-stressed condition were observed in GAB_3, GAB_4. The results indicated that there was relevant correspondence between the activity of APX and the least content of H_2O_2% in ILC3279. Consequently GAB_4 genotype is the most tolerant to drought stress among the studied genotypes.*

Keywords: *Drought stress, Chickpea, Enzymatic antioxidants, Ascorbate peroxidase, Superoxide dismutase, Catalase, Growth parameters.*

ВЛИЯНИЕ ЗАСУШЛИВОГО СТРЕССА НА СОДЕРЖАНИЕ НЕКОТОРЫХ ФЕРМЕНТАТИВНЫХ АНТИОКСИДАНТОВ ДЛЯ РАЗЛИЧНЫХ ГЕНОТИПОВ НУТА

Аннотация: *Целью настоящего исследования явилась оценка влияния засушливого стресса на активность антиоксидантных ферментов аскорбат-пероксидазы (АПХ), супероксиддисмутазы (СОД) и каталазы (кат). Также для трех генотипов нута ILC3279, GAB3, GAB4 были измерены такие параметры роста, как количество листьев растения, длина побега, длина корня. Результаты показывают, что наблюдалось значительное увеличение длины побега, количества листьев и длины корней, измеренных в GAB4. Кроме того, между изученными генотипами наблюдались значительные различия в отношении активности СОД, АПХ и кат. Наиболее высокая активность ферментов CAT и SOD в условиях засушливого стресса наблюдалась в GAB3, GAB4. Результаты показали, что существует релевантное соответствие между активностью APX и наименьшим содержанием H2O2% в ILC3279. Следовательно, генотип GAB4 является наиболее толерантным к засуховому стрессу среди изученных генотипов.*

Ключевые слова: *Засушливый стресс, нут, ферментативные антиоксиданты, аскорбат-пероксидаза, супероксиддисмутаза, каталаза, параметры роста.*

1- Introduction:

Chickpea (*Cicer arietinum* L.) is a member of the family of fabaceae, among the nine species that are annuals and the world's leading legume crop in cooler regions after dry beans and peas [7,c.300]. Chickpea is a valuable crop for improving soil fertility, particularly in rained or dry regions, due to nitrogen-fixing root nodulation capacity [6,c.83]. For human food and animal feed, chickpea seed has primary significance because it contains 17-31% protein and 52 to 78 % protein biological activity. The readily

digestible seed protein contains essential amino acids such as leucine, isoleucine, lysine, valine and phenylalanine in significant amounts [5,c.39]. It is grown either in semi-arid zones as a dry weather plant or in cool climates as a rainfed crop [16,c.175]. In practice, about 90 % of chickpea crops are grown without reliance on irrigation under rained conditions [7,c.300]. The second significant constraint on the productivity of chickpeas after diseases is drought. Drought-related global economic losses for chickpeas are about 40-50 % [8,c.81]. Different defense systems have been used by plants that allow them to adapt to stress conditions such as drought stress for survival. An increase in reactive oxygen species (ROS) is one of the main plant defensive mechanisms at the cell level during abiotic stresses [12,c.1063]. The common sources of ROS generation are distinct subcellular organelles such as chloroplasts, mitochondria and peroxisomes. In response to stress, the accumulated ROS, including singlet oxygen, hydroxyl radical, superoxide and hydrogen peroxide (H_2O_2), act as signaling molecules [7,c.300]. Excessive ROS production can lead to cellular damage, inhibition of enzymes, degradation of proteins, damage to DNA and RNA, and ultimately cell death [14,c.995]. In order to defend the plant against the toxic impact of ROS [15,c.13561], the ROS detoxification system in cells is therefore necessary. The ROS detoxification strategy contains The enzymatic and non-enzymatic antioxidant elements. superoxide dismutase (SOD), Catalase (CAT), ascorbate peroxidase (APX) are a part of enzymatic antioxidants [12,c.1063]. In all subcellular organelles, these enzymes are actually present. In general, there is more than one antioxidant enzyme in an organelle capable of eliminating a single type of ROS [4,c.548]. Germination, seedling stabilization and plant growth are adversely affected by low moisture availability at the sowing stage. Genotypical (dormancy and integument thickness) or environmental effects could be responsible for differences between genotypes for germination responses. Ultimately, poor seedling stands result in decreased seed yield [17,c.2382]. Germination rate, spread of germination, germination potential and development of seedlings are more imperatively affected by chickpea drought stress. In drier conditions, seedling establishment is a critical growth phase, as seedling mortality

is caused by insufficient availability of water. High returns would likely be provided by understanding the process of drought tolerance in plants and considering biochemical traits in breeding programs [7,c.300]. In this study, the key enzymatic pathway involved in ROS detoxification is highlighted and the most drought-tolerant chickpea cultivar is determined.

2. Experimental

2.1.Plant materials:

Seeds of three genotypes chickpea ILC3279, GAB_3, GAB_4 were germinated under two levels of normal (70% FC) and drought stress (40% FC) and were grown in the village of Jandar, south of Homs, during the growing season 2019. The seeds were planted in polyethylene bags (20 cm diameter, one seed per pot) and placed under a transparent polyethylene cover in order to avoid exposing the plants to rain. The experimental design was a spilt-split plot in the form of a randomized complete block design with three replications. To perform the enzyme assay, The young leaves and the roots samples were randomly collected in the vegetative growth stage. Some parameters as length of shoot, root length and number of leaves plants were measured. Analysis were performed in the Biotechnology Center, Albaath University, Homs, Syria.

2.2. Methods and Statistical analysis:

2.2.1. Activity definition of antioxidant enzymes:

0.5 g of leaf samples were crushed in 10 ml extraction buffer (0.1) M phosphate buffer, pH 7.5 then centrifuged at 4 °C for 10 min at 5000 rpm. The supernatant was collected and used for all enzymatic activities to be evaluated [11,c.445]. Total SOD (EC 1.15.1.1) activity was estimated by its ability to inhibit the photochemical reduction of nitro-blue-tetrazolium (NBT) according to [3,c.559]. At 560 nm, the absorbance was documented. The Ascorbate peroxidase APX (EC 1.11.1.1) activity was measured as the decrease in optical density due to ascorbic acid at 290 nm by the method of [10,c.867]. The assay mixture contained 200 μl ethylene diaminetetra acetic acid (EDTA 0.2 mM), 200 μl ascorbate (0.5 mM), 200 μl H_2O_2 (2.5 mM) and 1.2 ml phosphate buffer (pH

7). Catalase (EC 1.11.1.6) was assayed by measuring the disappearance of H_2O_2. 1 mL of 30 mM H_2O_2 was added in 1.9 mL of 50 mM phosphate buffer (pH 7). The decrease in absorbance at 240 nm was observed during 3 min and enzyme activity was computed by calculating the amount of H_2O_2 decomposed through this time[1,c.121].

The method of [2,c.1337] was used to determine hydrogen peroxide. 5 ml of 0.1% trichloroacetic acid (TCA), 500 µl of 0.01 mM potassium phosphate buffer, and 500 µl of 1 mM KI. The absorbance was possessed at 390 nm. Data were done as µmoles per gram fresh weight (µmol g^{-1} fw).

2.2.2. Data analysis:

All statistical analyses were performed by using GENSTAT11 program to determine the coefficient of variation and determine the significance of the studied values. The Least Significant Difference (LSD) test was used to compare the averages and determine the significant differences between them at the 1% level.

3. Results and Discussion:

3.1. Growth parameters:

Drought resulted in decreases in shoot length and number of leaves compared with normal irrigation (Table. 1,2). There was a significant increases in shoot length and number of leaves in GAB_4 compared with GAB_3, ILC3279. There was not any significant difference in shoot length and number of leaves in both genotypes GAB_3 and ILC3279.

Table 1. Effect of Drought Stress on length of shoot

Average A	Decreases %	Stress B		Genotypes A
		Non Stress	Stress	
21.67	16.90	23.67	19.67	GAB_3
23.17	4.22	23.67	22.67	GAB_4
21.17	13.23	22.67	19.67	LIC3279
	11.43	23.34	20.67	Average B
A*B		B	A	LSD 0.01
1.440	0.962	0.831	1.018	

The results also show that there was a significant increase in root length in GAB_4 compared with ILC3279, GAB_3 (Table. 3). Also there was a significant increase in root length in ILC3279 compared with GAB_3. Drought stress caused significant increase in root length compared with non stress condition. Consequently the effect of stress was most clearly observed in GAB_4 which the shoot length, number of leaves and root length measured increases in it. So these parameters play a major role in selecting genotypes for drought tolerance.

Table 2. Effect of Drought Stress on number of leaves plants

Average A	Decreases%	Stress B		Genotypes A
		Non Stress	Stress	
14.17	62.89	20.67	7.67	GAB_3
19.17	38.02	23.67	14.67	GAB_4
15.17	70.00	23.33	7.00	LIC3279
	56.64	22.56	9.78	Average B
A*B		B	A	LSD 0.01
2.200	14.02	1.270	1.555	

Table 3. Effect of Drought Stress on root length

Average A	Increases %	Stress B		Genotypes A
		Non Stress	Stress	
17.83	5.46	17.33	18.33	GAB_3
26.17	21.58	23.00	29.33	GAB_4
20.00	9.52	19.00	21.00	LIC3279
	13.59	19.78	22.89	Average B
A*B		B	A	LSD 0.01
2.036	6.484	1.176	1.440	

3.2. H_2O_2 content

stresses caused significant increases in H_2O_2% content in accumulated in genotypes due to drought compared with non stress condition. There were increments of H_2O_2%

content in the GAB_4, ILC3279 and GAB_3 respectively under drought condition compared to normal treatment (Table. 4).

Table 4. Analysis of Hydrogen peroxide μmol g^{-1} fw

Average A	Increases %	Stress B		Genotypes A
		Non Stress	Stress	
0.39	19.75	0.35	0.43	GAB_3
0.43	38.74	0.33	0.53	GAB_4
0.50	34.66	0.40	0.61	LIC3279
	31.94	0.36	0.53	Average B
A*B		B	A	LSD 0.01
0.027	9.51	0.015	0.019	

3.3. Antioxidant enzymes activity:

The increments of SOD and CAT activities in drought stress were higher compared to non stress (Table. 5,6). CAT activities of GAB_3 were increased by 50.80% compared with GAB_4 47.50%, LIC3279 18.97% under drought condition (Table. 5). whereas SOD activities of GAB_4 were increased by 95.77% compared with GAB_3 53.97%, LIC3279 (-21.84)% under drought condition(Table. 6). There were significant differences between studied genotypes about SOD and CAT activities.

Table 5. Analysis of CAT (μmol g^{-1} fw)

Average A	Increases %	Stress B		Genotypes A
		Non Stress	Stress	
1.49	50.80	0.98	2.00	GAB_3
0.96	47.50	0.66	1.25	GAB_4
1.40	18.97	1.25	1.54	LIC3279
	39.70	0.96	1.60	Average B
A*B		B	A	LSD 0.01
0.044	3.607	0.026	0.031	

Table 6. Analysis of SOD (unit g^{-1} fw)

Average A	Increases %	Stress B		Genotypes A
		Non Stress	Stress	
0.35	53.97	0.22	0.48	GAB_3
0.24	95.77	0.02	0.46	GAB_4
0.44	-21.84	0.48	0.40	LIC3279
	45.94	0.24	0.45	Average B
A*B		B	A	LSD 0.01
0.031	8.761	0.018	0.022	

Whereas the minimum activity of APX was found in genotypes GAB_3, GAB_4 and LIC3279 respectively (Table. 7).

Table 7. Analysis of APX (μmol g^{-1} fw)

Average A	Decreases%	Stress B		Genotypes A
		Non Stress	Stress	
1.02	62.84	1.48	0.55	GAB_3
4.18	45.76	5.42	2.94	GAB_4
2.76	2.5	2.80	2.73	LIC3279
	35.6	3.23	2.08	Average B
A*B		B	A	LSD 0.01
0.116	2.296	0.067	0.082	

There were significant differences for the enzymes activity and between the genotypes in terms of activities of all three enzymes at the level of 1% in vegetative stage. Results of this study showed that under drought stress condition, activity of antioxidant enzymes (superoxide dismutase, catalase and ascorbate peroxidase) increased this is agreement with [9,c.782]. Superoxide from the photosynthetic and respiratory electron leakage in chloroplast was produced by drought stress. The superoxide dismutase (SOD) and ascorbate peroxidase (APX) enzymes have dismutated H_2O_2 into superoxide and water. In contrast, in the peroxisome, catalase (CAT) mostly decomposed photorespiration mediated H_2O_2 [13,c.1]. In this research ILC3279 genotype had the maximum activity of APX and the least content of H_2O_2%. Whereas activities enzymes of SOD and CAT

played the active role in tolerant of drought in GAB_3, GAB_4 genotypes. Finally, GAB_4 genotype is the most tolerant one then GAB_3 genotype. while ILC3279 genotype was sensitive to drought stress.

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