American-Eurasian J. Agric. & Environ. Sci., 11 (6): 782-785, 2011
ISSN 1818-6769

Effect of Drought Stress on Antioxidant Enzymes Activity of Some Chickpea Cultivars

[1]Abdollah Mohammadi, [1]Davood Habibi, [1]Mahyar Rohami and [2]Saeed Mafakheri

[1]Department of Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran
[2]Department of Agronomy, Karaj Branch, Islamic Azad University, Karaj, Iran

Abstract: To evaluate the effects of drought stress on antioxidant enzymes activity of three chickpea cultivars, an experiment was conducted at the experimental field of the Islamic Azad University, Karaj branch and laboratories of Tarbiyat Moallem University of Tehran. For field studies, a spilt-split plot design on the basis of RCBD with four replications was used. The main factor was irrigation (at two levels of normal and drought stress), the sub factor was selenium (in two levels of selenium spraying and without selenium) and the sub-sub factor was three chickpea cultivars. Evaluated traits in this study were super oxide dismutase enzymes, catalase, glutation peroxidase and malondialdehyde. The results showed that treatments significantly affected the activity of MDA, CAT, GPX and SOD enzymes. The results of biochemical analysis showed the activity of antioxidant enzymes increases under stress conditions. Application of 20 g/ha selenium increased enzymes activity except the catalase. Finally, this research indicated that the Bivanij cultivar is the most tolerant to drought stress among the studied cultivars.

Key words: Selenium · Environmental stresses · Soil water content · Enzymes · Tolerance

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a member of the fabaceae family, the world's third most important pulse crop and a major source of protein for millions of peoples in developing countries [1, 2]. This plant is cultivated on 700,000 hectares in Iran; the fourth rank in the world after India, Turkey and Pakistan [3, 4]. The origin of chickpea is believed to be in the region near to today's Turkey, Iran, Afghanistan and the former USSR [5]. Generally, this crop is planted in the spring and taking advantages of conserved soil moisture [6]. Legume crops are often grown in drought-prone areas, subjected to water stress [7]. Drought is the most common adverse environmental factor; limiting crop production in different parts of the world especially in Iran which is considered as an arid and semiarid country [4]. Drought stress is the second important yield limiting factor in chickpea, after disease [8]. Late season drought stress, during flowering, podding and grain filling stages, is a major abiotic stress which reduces chickpea yield capacity [9, 4].

Antioxidant enzymes activity increases in plant cells as a response to environmental stresses. Environmental stresses can result in the production of Reactive Oxygen Species (ROS), including O_2^-, H_2O_2 and OH^-; these ROS adversely affect crops yield and quality [10, 11]. ROS are highly reactive and can alert normal cellular metabolism through oxidative damage to membranes, proteins and nucleic acids; they also cause lipid peroxidation, protein denaturation and DNA mutation [10]. To prevent damage to cellular components by ROS, plants have developed a complex antioxidant system. The primary components of this system include carotenoids, ascorbate, glutathione and tocopherols, in addition to enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), peroxidases and the enzymes involved in ascorbate-glutathion cycle such as ascorbate peroxidase (APX) and glutathione reductase (GR) [10]. These enzymes have key role in the defense against oxidative stress [11].

Studies on barley, wheat, soybean and chickpea determined that the Catalase activity is effective in reducing the damages of stress [16]. Researches on sunflower, sorghum and soybean showed that drought stress increased the activity of superoxide dismutase (SOD), glutathione peroxidase (GPX) and catalase (CAT) [13, 14, 15]. Another studies showed that applying 21 g of selenium boosted the catalase activity [15]. Sairam and

Corresponding Author: Abdollah Mohammadi, Department of Plant Breeding, Karaj Branch, Islamic Azad University, Karaj, Iran.

Saxena [16] studies on three wheat cultivars indicated that drought stress increased lipid peroxidation and enzymes ascorbate peroxidase, glutathione reductase and peroxidase, but reduced the membrane resistance, chlorophyll and carotenoids. Zhuang and Chen [17] studied three drought stress tolerant plants, *Tamarix ramosissima*, *Populus euphratica* and *Apocynum venetumas*. They showed at different levels of water content in soil, physiological factors such as MDA, SOD, ABA, POD, IAA and CK had the highest sensitivity to drought stress.

Breeding for resistance or tolerance to drought and high temperature stresses in chickpea is limited by the lack of adequate selection criteria for stress tolerance. Most breeding programs are based on visual scoring in the controlled or field conditions [8]. Understanding the drought tolerance process in plants and considering biochemical and physiological traits in breeding programs would probably provide high returns [7]. So this experiment was conducted to determine the most drought tolerant chickpea cultivar.

MATERIALS AND METHODS

This experiment was conducted at the experimental field of the Islamic Azad University, Karaj branch, Iran (51° 6' E, 35° 45' N and 1313m above the sea level) and laboratories of Tarbiyat Moallem University of Tehran, Iran. The experimental design was a spilt-split plot in the form of a randomized complete block design with four replications.

The main factor was irrigation in two levels of normal and drought stress. To measure soil moisture depletion, gyps blocks were used. The field was irrigated when soil electric conductivity was 60 (which mean 12% of soil moisture according to the calibration conducted before the experiment). The sub factor was selenium in two levels of spraying 20 g/ha selenium and without selenium. For selenium spraying, $Na_2O_3Se.5H_2O$ (M=256) was used. The sub-sub factor was three chickpea cultivars (Jam, ILC 482 and Bivanij).

To measure the traits, leaves were harvested from plants and the method of Lowry *et al*. [18] was used for protein assays. Glutathione peroxidase (GPX) enzyme activity and catalase activity were measured to Paglia [19]. For measurement of SOD enzyme Misra [20] method was used. For measurement of Malondialdehyde (MDA) the chromatography method was used, this method was conducted based on the reaction between Tiobarbetiuric acid and MDA [21].

RESULTS AND DISCUSSION

Superoxide Dismutase (SOD): To select drought tolerant genotypes, there are several advanced molecular techniques; one of them is determination of SOD. According to analysis of variances (Table 1), irrigation levels were significantly different. The maximum activity of SOD (1127 u/g protein) found in drought stress condition and the minimum activity of the enzyme found in normal irrigation (709 u/g protein; Table 2). Application of selenium significantly increased SOD activity ($P \leq 0.01$). The interaction of selenium × irrigation significantly affected this trait ($P \leq 0.05$; Table 1). There were no significant differences between genotypes. These results are in agreement with those of Saei [14] on sorghum, Shafei [15] on soybean and Amman [13] on sunflower.

Glutathione Peroxidase (GPX). Results of analysis of variances (Table 1) showed the significant effect of irrigation on GPX (Table 1). This enzyme increased under drought stress condition, but decreased under normal irrigation (Table 2). These results are similar with the findings of Saei [14] and Amman [13]. Application of selenium and different varieties and the interaction between irrigation × cultivar significantly affected GPX ($P \leq 0.05$). The three-fold interaction between irrigation × selenium × varieties had also significant effect on GPX ($P \leq 0.05$). The maximum enzyme quantity obtained under drought stress condition (10.554 u/g protein; Table 2). Among the cultivars, Bivanij had the highest amount of GPX. Studying the interaction between irrigation × genotypes indicated that Bivanij and Jam had higher GPX under drought stress condition. The interaction between irrigation × selenium × genotypes significantly affected the trait. Bivanij and Jam under drought stress and selenium application had the maximum enzyme activity (Tables 1 and 2).

Catalase (CAT). CAT has role in decomposition of peroxidase under environmental stresses and plant tolerance to the stresses. Drought stress treatment (103.84 μ/g protein) and normal irrigation treatment (59.95 u/g protein) had the highest and the lowest CAT enzyme activity, respectively (Table 2). There were no significant differences between genotypes, but different selenium levels had significant effect on this trait ($P \leq 0.01$). Amman [13], Saei [14] and Shafei [15] obtained the similar results. Kafi and Mahdavi Damghani [12] conducted an experiment on barley, wheat, soybean and chickpea and concluded that CAT activity is effective on reducing the adverse influences of environmental stresses.

Table 1: Analysis of variance for Superoxide Dismutase, Glutathione Peroxidase, Catalase enzymes and Malondialdehyde

		S.O.D		G.P.X		C.A.T		M.D.AS.O.V	
	df	Ms	Prob	Ms	Prob	Ms	Prob	Ms	Prob
Replication	3	27430.167	ns	2.262	ns	1296.152	ns	0.034	ns
Irrigation (A)	1	2096688.0	**	147.48	**	23115.72	**	3.521	**
Error	3	53681.722		1.428		211.986		0.511	
Selenium (B)	1	489244.083	**	37.646	*	2382.703	**	0.625	**
AB	1	201502.083	*	13.353	ns	378.642	ns	0.34	*
Error	6	28681.639		5.026		72.514		0.044	
Cultivars (C)	2	12040.146	ns	1.648	*	9.814	ns	0.016	ns
AC	2	8708.813	ns	2.655	*	5.613	ns	0.012	ns
BC	2	1382.271	ns	0.575	ns	39.836	ns	0.012	ns
ABC	2	5537.521	ns	1.964	*	107.842	ns	0.003	ns
Error	24	5354.431		0.490		79.9		0.009	

Ns, nonsignificant; *, Significant at $p \leq 0.05$; **, Significant at $p \leq 0.01$

Table 2: The mean Comparison of Superoxide Dismutase, Glutathione Peroxidase, Catalase enzymes and Malondialdehyde

Treatments	S.O.D (µ/g protein)	G.P.X (µ/g protein)	C.A.T (µ/g protein)	M.D.A (µm/g protein)
Normal	708.917b	7.048b	59.95b	0.635b
Drought Stress	1126.917a	10.554a	103.84a	1.173a
With Selenium	1018.875a	9.687a	74.847b	1.017a
Without Selenium	816.958b	7.915b	88.938a	0.788b
Jam	948.313a	8.975a	82.556a	0.933a
ILC 482	895a	8.431b	82.093a	0.904a
Bivanij	910.435a	8.997a	81.029a	0.870a

Means in a column followed by the same letter are not significantly different at $p \leq 0.01$

Malondialdehyde (MDA). According to analysis of variances (Table 1), irrigation and selenium significantly affected MDA activity ($P \leq 0.01$). Mean comparisons of the effect of drought stress and irrigation showed that MDA was the lowest under normal irrigation (0.63 µm/g protein) and the highest under drought stress (1.17 µm/g protein; Table 2); showing the decomposition of lipids and production of MDA in plants as the result of drought stress. Results indicated that application of selenium increased the MDA. Application of selenium increased MDA activity (1.02 µm/g protein) compared with the control (0.79 µm/g protein). Zhuang and Chen [17] studied three drought stress tolerant plants and concluded that at different levels of soil water content, physiological factors such as MDA had the highest sensitivity to drought stress.

CONCLUSION

Results of this study showed that under drought stress condition, activity of antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) increased. Application of selenium also increased enzymes activity, except for catalase. In drought tolerant cultivars lipid peroxidation is less which is related to higher activity of antioxidant enzymes. Finally, results of laboratory and field studies showed that Bivanij cultivar is the most tolerant one among the studied cultivar.

REFERENCES

1. Nasre Esfahani, M., A. Mostajeran and G. Emtiazi, 2010. The effect of drought stress on nitrogenase and antioxidant in nodules formed from symbiosis of chickpea with tow strain of *Mesorhizobium ciceri*. World Applied Science Journal, 10: 621-626.
2. Rahman, M.S., N.K. Sana, M.M. Hasan, M.E. Huque and R.K. Shaha, 2008 Enzyme activities and degradation of nutrients in chickpea (*Cicer arietinum* L.) seeds during germination. J. Biosciences, 16: 29-34.
3. Gorbanli, M., M. Nojavan, R. Heidari and T. Farboodnia, 2001. Changes in soluble sugars starch and proteins in two cultivars of Pea under drought stress. J. Teachers Training University, Tehran, 1: 38-53.
4. Sabaghpour, S.H., A.A. Mahmodi, A. Saeed, M. Kamel and R.S. Malhotra, 2006. Study on chickpea drought tolerance lines under dryland condition of Iran. The Indian J. Crop Sci., 1: 70-73.

5. Witcombe, J.R. and W. Erskine, 1984. Genetic resources and their exploitation: chickpeas, faba beans and lentils. Springer, pp: 272.
6. Malhotra, R.S. and M.C. Saxena, 2002. strategies for overcoming drought stress in chickpea. Caravan, 17: 20-22.
7. Bourgault, M. and D.L. Smith, 2010. Comparative study of common bean (*Phaseolus vulgaris* L.) and mungbean (*Vigna radiata* L. Wilczek) response to seven watering regimes in a controlled environment. Crop and Pasture Sci., 61: 918-928.
8. Toker, C., J. Gorham and M.I. Cagirgan, 1998. Assessment of response to drought stress of chickpea (*Cicer arietinum* L.) lines under rainfed conditions. Turkish J. Agriculture and Forestry, 22: 615-621.
9. Molina, C., B. Rotter, R. Horres, S.M. Udupa, B. Besser, L. Bellarmino, M. Baum, H. Matsumura, R. Terauchi, G. Kahl and P. Winter, 2008. SuperSAGE: the drought stress-responsive transcriptome of chickpea roots. BMC Genomics, 9: 553. doi:10.1186/1471-2164-9-553.
10. Baby, J. and D. Jini, 2011. Development of salt stress-tolerant plants by gene manipulation of antioxidant enzymes. Asian J. Agric. Res., 5: 17-27.
11. Rahimizadeh, M., D. Habibi, H. Madani, G.N. Mohammadi, A. Mehraban and A.M. Sabet, 2007. The effect of micronutrients on antioxidant enzymes metabolism in sunflower (*Helianthus annuus* L.) under drought stress. HELIA, 30: 167-174.
12. Kafi, M. and A. Mahdavi damghani, 2000. Mechanism of tolerance to environmental stress in plant. Ferdowsi University, Mashhad, Iran (In Farsi).
13. Amman, A., 2004. Drought effect of drought conditions at yield, yield components and physiological characteristics in different genotypes of sunflowers. M.Sc. Thesis. Faculty of Agriculture and Natural Resources, Karaj-Branch, Islamic Azad University (In Farsi).
14. Saei, M., 2004 Study on relationship morph-physiological traits with drought tolerance in different genotypes of sorghum. M.Sc. Thesis. Faculty of Agriculture and Natural Resources, Karaj-Branch, Islamic Azad University (In Farsi).
15. Shafei, S., 2005 Study on water shortage effect at some of physiological traits, yield and yield components in some genotypes of soybean. M.Sc. Thesis. Faculty of Agriculture and Natural Resources, Karaj-Branch, Islamic Azad University (In Farsi).
16. Sairam, R.K. and D.C. Saxena, 2000. Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. J. Agronomy and Crop Sci., 184: 55-61.
17. Zhuang, L. and Y.N. Chen, 2006. Physiological responses of three contrasting plant species of groundwater level changes in aria environment. J. Integrative Plant Biol., 48: 520-110.
18. Lowry, O., A. Rosebrough, A. Far and R. Randall, 1951. Protein measurement with folin phenol reagent. J. Biological Chemistry, 193: 680-685.
19. Paglia, D.E. and W.N. Valentine, 1987 Studies on the quantitative and qualities characterization of glutathione proxidase. J. Laboratory and Clinical Medicine, 70: 158-165.
20. Misra, H.P. and I. Fridorich, 1972. The generation of super oxide radical during auto oxidation, The J. Biological Chemistry, 247: 6960-6969.
21. Bahl, P.N., K.P. Singh and D. Singh, 1984. Evaluation of tall chickpea genotypes for normal and late sowings. The Indian J. Agric. Sci., 54: 110-113.