Plant Archives Vol. 25, No. 2, 2025 pp. 1521-1524 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org

DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.no.2.220

MITOCHONDRIAL ANTIOXIDATIVE RESPONSE TO OSMOTIC STRESS IN CHICKPEA

D.A. Sapakale, P.K. Lokhande, S.B. Narale*, S.G. Mohite, U.S. Dalvi, A.A. Kale and R.M. Naik

Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri - 413 722, Maharashtra (India).

*Corresponding author E-mail: sbnarale00@gmail.com

(Date of Receiving-30-06-2025; Date of Acceptance-03-09-2025)

ABSTRACT

Chickpea (*Cicer arietinum* L.) is a one of the most important pulse crops in global agriculture, primarily cultivated in arid and semi-arid regions. The present study investigates the antioxidative mechanisms in chickpea mitochondria under osmotic stress. Three genotypes Vijay, JG-11, and JG-24 were subjected to osmotic stress using PEG-6000 were analysed for proline, glycine betaine, ascorbic acid, lipid peroxidation, and the activity of key antioxidative enzymes, including superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT). The results revealed significant genotypic variations in osmolyte accumulation and antioxidant enzyme activities. Higher proline and glycine betaine levels were observed in tolerant genotypes, along with increased activities of SOD, APX, and CAT. These findings indicate that mitochondrial antioxidative responses play a crucial role in mitigating oxidative damage induced by osmotic stress in chickpea.

***Key words*:** Chickpea, Osmotic Stress, Antioxidative Enzymes, Mitochondria, Proline, Glycine Betaine

Introduction

Chickpea (*Cicer arietinum* L.) ranks as the third most important legume worldwide, with India contributing 85% of the total cultivated area (FAOSTAT, 2015). Drought stress is a significant constraint in chickpea production, reducing yield by 40–50% globally (Ahmed *et al.*, 2005). Mitochondria are essential organelles that play a critical role in stress adaptation through reactive oxygen species (ROS) detoxification and energy metabolism (Millar *et al.*, 2003). The present study aims to evaluate the antioxidative response of mitochondrial enzymes in chickpea genotypes under osmotic stress.

Materials and Methods

Plant material and stress imposition

Three chickpea genotypes (Vijay, JG-11, and JG-24) were obtained from the All India Coordinated Pulses Improvement Project, MPKV, Rahuri (Anonymous, 2013). The plants were grown in pots, and osmotic stress (-0.8 MPa) was induced using PEG-6000 (Turner *et al.*, 2007).

Estimation of Proline and Glycine Betaine

Proline content was estimated using acid ninhydrin (Bates *et al.*, 1973), while glycine betaine was determined using Dragendorff reagent (Stumpf, 1984).

Antioxidant enzyme assays

Superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT) activities were assessed in mitochondrial fractions using spectrophotometric methods (Nakano and Asada, 1981; Aebi, 1984).

Lipid peroxidation assay

Lipid peroxidation was measured in terms of malondialdehyde (MDA) content using thiobarbituric acid reactive substances (TBARS) assay (Heath and Packer, 1968).

Results and Discussion

Osmolyte accumulation

Proline, glycine betaine, and ascorbic acid accumulation increased significantly in stressed plants, with Vijay exhibiting the highest levels. Proline ranged

Table 1: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on free proline content of chickpea varieties.

Varieties	Proline (µmoles/g FW)		
	Control	Stress	Mean
Vijay	6.69	18.66 (178.75)	12.68
JG-11	6.14	16.68 (171.7)	11.41
JG-24	4.81	11.30 (135.1)	8.06
Mean	5.88	15.55 (164.4)	10.72
	Condition	Variety	C × V
SE(±)	0.143	0.175	0.248
CD at 5%	0.425	0.521	0.736

Table 2: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on glycine betaine content of chickpea cultivars.

Varieties	Glycine betaine (µmoles/g FW)		
	Control	Stress	Mean
Vijay	8.61	21.14 (145.62)	14.88
JG-11	8.45	20.26 (139.71)	14.36
JG-24	9.43	18.62 (97.57)	14.02
Mean	8.83	20.01 (126.64)	14.42
	Condition	Variety	C × V
SE(±)	0.109	0.133	0.188
CD at 5%	0.323	0.395	0.559

Table 3: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on Ascorbic Acid content of chickpea varieties.

Varieties	Ascorbic acid (µmoles/g FW)		
	Control	Stress	Mean
Vijay	26.64	21.84 (21.99)	24.24
JG-11	24.63	20.60 (19.55)	22.62
JG-24	23.47	20.53 (14.34)	22.00
Mean	24.92	20.99 (18.7)	22.95
	Condition	Variety	C × V
SE(±)	0.154	0.188	0.266
CD at 5%	0.457	0.560	0.791

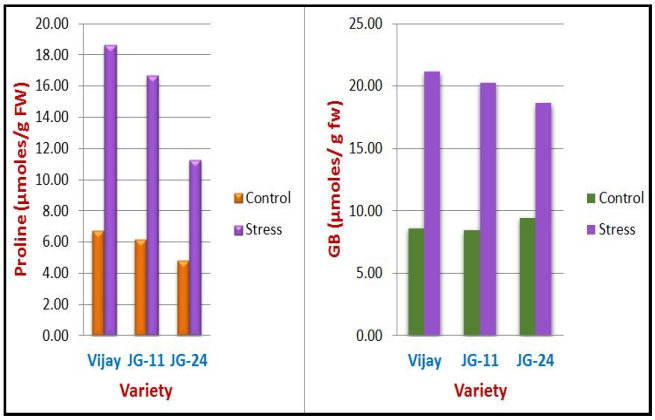

Fig. 1: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on free proline and glycine betaine content of chickpea varieties.

Table 4: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on total superoxide dismutase activity in mitochondrial fraction of chickpea cultivars.

Varieties	Total superoxide dismutage (Units/mg protein)		
	Control	Stress	Mean
Vijay	3.84	5.37 (39.84)	4.61
JG-11	3.44	4.60 (33.72)	4.02
JG-24	3.03	3.51 (15.8)	3.27
Mean	3.44	4.49 (30.73)	3.97
	Condition	Variety	C × V
SE(±)	0.021	0.025	0.036
CD at 5%	0.061	0.075	0.106

Table 5: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on ascorbate peroxidase activity in mitochondrial fraction of chickpea cultivars.

Varieties	Ascorbate peroxidase (nmol ascorbate oxidized mg^{-1} protein min^{-1})		
	Control	Stress	Mean
Vijay	106.04	141.05 (33.01)	123.54
JG-11	113.64	156.37 (37.59)	135.00
JG-24	106.39	113.71 (6.88)	110.05
Mean	108.69	137.04 (26.08)	122.87
	Condition	Variety	C × V
SE(±)	1.461	1.789	2.530
CD at 5%	4.338	5.313	7.514

Table 6: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on catalase activity in mitochondrial fraction of chickpea cultivars.

Varieties	Catalase (μmoles H_2O_2 decompose mg^{-1} protein min^{-1})		
	Control	Stress	Mean
Vijay	5.31	9.23 (73.85)	7.27
JG-11	5.39	9.15 (69.9)	7.27
JG-24	2.92	4.02 (37.58)	3.47
Mean	4.54	7.47 (64.5)	6.00
	Condition	Variety	C × V
SE(±)	0.079	0.097	0.138
CD at 5%	0.236	0.289	0.409

Table 7: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on lipid peroxidation rate of chickpea cultivars.

Varieties	Lipid peroxidation rate (µmoles MDA/g FW)		
	Control	Stress	Mean
Vijay	32.68	40.85 (25)	36.77
JG-11	31.39	41.62 (32.6)	36.51
JG-24	38.61	59.43 (59.9)	49.02
Mean	34.23	47.30 (38.19)	40.76
	Condition	Variety	C × V
SE(±)	0.298	0.365	0.516
CD at 5%	0.885	1.084	1.532

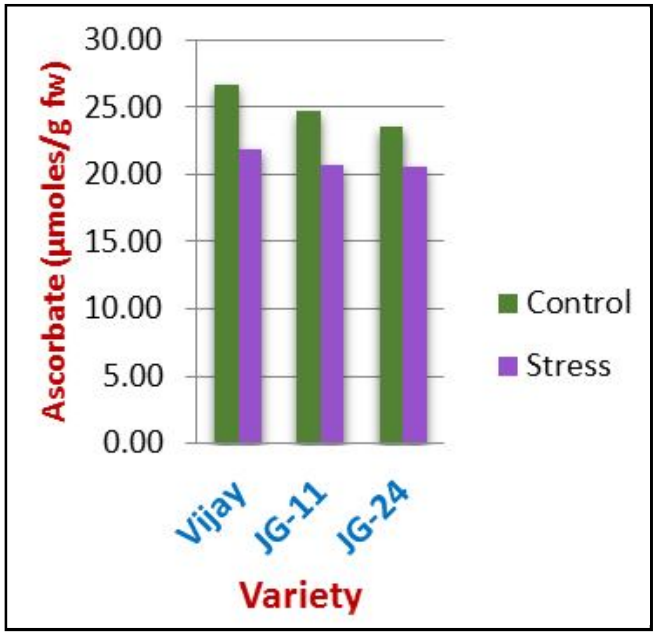

Fig. 2: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on Ascorbate content of chickpea varieties.

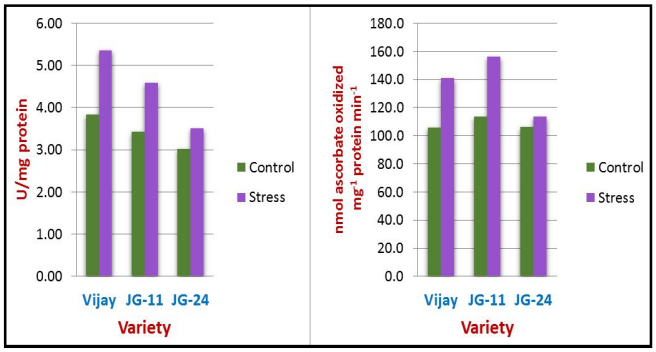

Fig. 3: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on SOD and APX content of chickpea varieties.

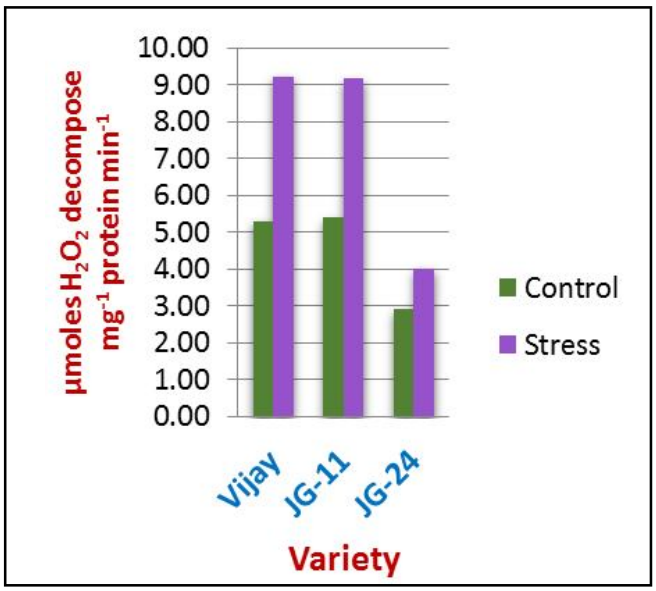

Fig. 4: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on catalase activity in mitochondrial fraction of chickpea cultivars.

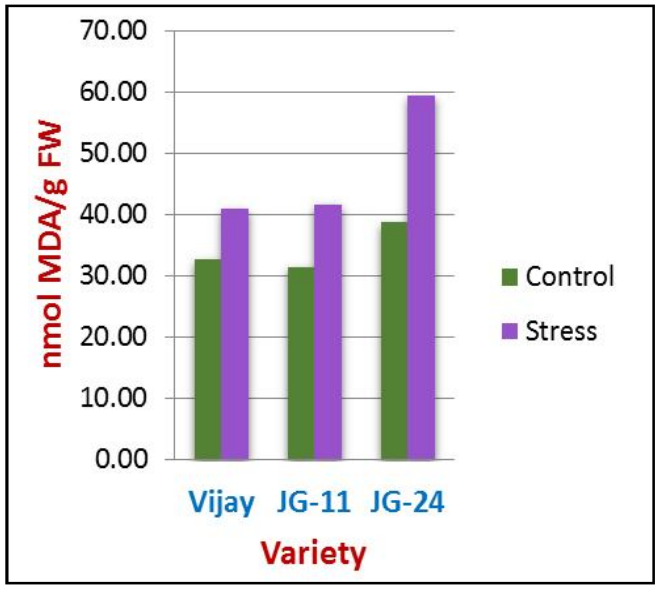

Fig. 5: Effect of PEG-6000 induced osmotic stress (-0.8 Mpa) on lipid peroxidation rate of chickpea cultivars.

from 3.2 to 5.6 µmol/g FW, glycine betaine from 1.8 to 3.2 µmol/g FW, and ascorbic acid from 2.4 to 5.1 mg/g FW. Similar results were reported by Rahbarian *et al.* (2012), who found higher osmolyte accumulation in drought-tolerant chickpea genotypes.

Antioxidant enzyme activities

SOD, APX, and CAT activities were significantly higher in stressed plants. SOD activity ranged from 42 to 68 U/mg protein. APX from 2.1 to 4.3 µmol ascorbate oxidized/min/mg protein and CAT from 5.6 to 9.2 µmol H_2O_2 decomposed/min/mg protein (Table 5 and Fig. 3). Vijay recorded the highest enzyme activity, followed by JG-11 and JG-24, suggesting a robust antioxidative defense mechanism. Alscher *et al.*, (1997) and Pradeep and Hemantaranjan (2012) also reported increased antioxidative enzyme activity in drought-tolerant plants under stress conditions.

MDA content increased under stress, with values ranging from 3.5 to 7.8 nmol/g FW. JG-24 exhibited the highest lipid peroxidation, indicating greater oxidative damage compared to Vijay and JG-11. This is in agreement with the findings of Eyidogan and Oz (2007) and Patel (2011), who reported that higher MDA content is associated with increased oxidative stress in chickpea under salinity and drought conditions.

Conclusion

The study highlights the differential mitochondrial antioxidative responses among chickpea genotypes under osmotic stress. The higher accumulation of osmolytes and enhanced antioxidant enzyme activities in Vijay suggest its superior tolerance. These findings provide

insights into the selection of stress-resilient chickpea genotypes for sustainable agriculture.

References

Aebi, H. (1984). Catalase *in vitro*. *Methods in enzymology*, **105**, 121-126.

Ahmed, S., Nawata E. and Sakuratani T. (2005). Changes in photosynthetic activity, proline and glycine betaine contents in two wheat cultivars under osmotic stress. *Plant Physiology*, **45**, 107-113.

Alscher, R.G., Erturk N. and Heath L.S. (1997). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of Experimental Botany*, 48, 1305-1312.

Bates, L.S., Waldren R.P. and Teare I.D. (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil*, **39(1)**, 205-207.

Eyidogan, F. and Oz M.T. (2007). Effect of salinity on antioxidant responses in chickpea seedlings. *Acta Physiologiae Plantarum*, **29**, 485-492.

FAOSTAT (2015). Food and Agriculture Organization Statistical Database. Rome, Italy.

Heath, R.L. and Packer L. (1968). Photoperoxidation in isolated chloroplasts. *Archives of Biochemistry and Biophysics*, **125**, 189-198.

Millar, A.H., Whelan J., Soole K.L. and Day D.A. (2003). Organization and regulation of mitochondrial respiration in plants. *Annual Review of Plant Biology*, **54**, 455-484.

Nakano, Y. and Asada K. (1981). Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant and Cell Physiology*, **22(5)**, 867-880.

Patel, M.K. (2011). Oxidative stress responses in chickpea under water deficit conditions. *Plant Biochemistry and Biotechnology*, **20(3)**, 302-309.

Pradeep, S. and Hemantaranjan A. (2012). Antioxidative enzyme activities in chickpea (*Cicer arietinum* L.) under drought stress. *Journal of Plant Biochemistry and Biotechnology*, **21**, 68-75.

Rahbarian, R., Khavari-Nejad R.A. and Ganjeali A. (2012). Drought stress induced changes in osmolytes accumulation and antioxidant enzyme activities in chickpea (*Cicer arietinum* L.). *Acta Physiologiae Plantarum*, **34(3)**, 1087-1093.

Stumpf, W. (1984). Estimation of glycine betaine content in plants. *Journal of Plant Research*, **21**, 67-75.

Turner, N.C., Wright G.C. and Siddique K.H.M. (2007). Adaptation of grain legumes to water-limited environments. *Advances in Agronomy*, **63**, 193-231.