LR-5204
[1-7]

RESEARCH ARTICLE Legume Research- An International Journal

Effect of Different Biochemical Parameters and Antioxidant Enzymes Activities on Drought Indices in Chickpea (*Cicer arietinum* L.)

Rajitha Sistu[1], Sushma Tiwari[1,5], M.K. Tripathi[1,5], Sangeeta Singh[2], Neha Gupta[3], Niraj Tripathi[4], Ruchi Asati[5], Rakesh Kumar Yadav[5]

10.18805/LR-5204

ABSTRACT

Background: Drought is the foremost environmental restraint that effects the growth and yield of chickpea. The mechanism of response to drought differs with genotype and growth stages of individual crop species. The activation of antioxidant enzymes is an alternate defensive system against oxidative stress that ultimately decide development of putative drought tolerant mechanism.

Methods: Eighty-three chickpea genotypes were grown under normal and drought stress conditions and leaf samples were used to estimate different biochemical parameters including total sugar, lipid peroxidation (MDA), chlorophyll, proline and protein along with estimation of activities of different antioxidant enzymes *viz*., catalase (CAT) (EC 1.11.1.6), ascorbate peroxidase (APX) (EC 1.11.1.11), superoxide dismutase (SOD) and peroxidase (POX).

Result: Positively significant correlation was found among proline under control with proline underwent stress (r=0.441), MDA under stress with proline under control (r=0.365) and MDA under control (r=0.336) at 1% level of significance. Positively significant correlation was also investigated between SOD under stressed condition with SOD under control (r=0.665), POX (0.449) and APX under stress (0.423), CAT under control (0.471) and CAT under stress condition (0.374) at 1% probability level. Heatmaps along with dendrograms represented expression levels of different antioxidant enzymes activities that showed variations among different genotypes. In conclusion. total sugar, proline and Malondialdehyde, have been increased under drought stressed condition whilst total chlorophyll and protein were decreased. While antioxidant enzymes *viz*., POX, APX, CAT and SOD levels increased under drought stressed conditions.

Key words: Antioxidant enzyme activities, Biochemical parameters, Chickpea, Drought stress.

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the oldest cultivated food legumes with high nutritional, agronomical and economically important crop in the world. It is an important cool season legume crop with a genome size of 738 Mb (Varshney *et al*., 2013). India is the largest producer of chickpea having annual production of 10.13 million tonnes from a land area of 9.44 million hectares and a productivity of 1073 kgha^{-1} (Asati *et al.,* 2023). Among different chickpea-growing countries including Africa, south America and Indian Subcontinents, India alone contributes more than 70% of the world's total production. Moreover, it is the most important legume crop and a source of nutrition to millions of people globally owing to its richness in protein, fibre and minerals (Asati *et al*., 2023). Thus, characterization of chickpea genotypes to select superior line (s) resistant /tolerant against different biotic and abiotic stresses is one of the most important requirements being applied widely at present (Sahu *et al.,* 2020a).

Abiotic stresses tolerance is a complex trait, owing to the interactions between diverse stress factors and various molecular Tripathi *et al*. (2023), biochemical Sahu *et al*. (2020b); Tomar *et al*. (2022); Tiwari *et al*. (2023a) and physiological mechanisms (Aarif *et al.,* 2021). Affecting plant growth at different developmental stages. Among diverse abiotic stresses, drought is one of the major abiotic constraints that alters the plant growth and development. Even though chickpea is good in storing water in the soil within the root zone, but it suffers from terminal drought which

[1]Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior-474 002, Madhya Pradesh, India.
[2]National Institute of Plant Genome Research, Delhi-110 067, New Delhi, India.
[3]School of Studies in Biotechnology, Jiwaji University, Gwalior-474 002, Madhya Pradesh, India.
[4]Directorate of Research Services, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur-482 004, Madhya Pradesh, India.
[5]Department of Genetics and Plant Breeding, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior-474 002, Madhya Pradesh, India.

Corresponding Author: Sushma Tiwari, Department of Plant Molecular Biology and Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior-474 002, Madhya Pradesh, India. Email: sushma2540@gmail.com

How to cite this article: Sistu, R., Tiwari, S., Tripathi, M.K., Singh, S., Gupta, N., Tripathi, N., Asati, R. and Yadav, R.K. (2023). Effect of Different Biochemical Parameters and Antioxidant Enzymes Activities on Drought Indices in Chickpea (*Cicer arietinum* L.). Legume Research. DOI: 10.18805/LR-5204

Submitted: 04-07-2023 **Accepted:** 11-10-2023 **Online**: 19-10-2023

delays flowering. Drought stress inhibits the uptake of nitrogen and subsequent fixation in the roots of chickpea as well. Drought generally affects chickpea production in areas lacking satisfactory and constant rainfall, it affects overall crop performance starting from germination, with losses of up to 40-50% in crop productivity (Sachdeva *et al*., 2022).

Drought stress is known to alter the chlorophylls and carotenoids concentrations in plant tissues (Tiwari *et al*., 2023b). It is generally associated with the accumulation of osmoprotectants such as proline. Likewise, the role of reactive oxygen species (ROS) in stress signalling have also been extensively investigated in recent years. The reactive oxygen species in plants are removed by a variety of antioxidant enzymes and/or lipid soluble and water-soluble scavenging molecules (Mishra *et al*., 2023). The antioxidant enzymes being the most efficient mechanisms against oxidative stress (Sharma *et al.,* 2021). Estimation of different biochemical parameters and antioxidant enzymes activities may be effectively employed for selecting putative drought tolerant chickpea genotype(s). The present study was accomplished to screen drought tolerant genotype (s) based on manifestation of diverse biochemical parameters and antioxidant enzymes activities.

MATERIALS AND METHODS

A total 83 chickpea genotypes including 63 desi and 20 kabuli were obtained from All India Coordinated Research Project on chickpea, College of Agriculture, Sehore, Madhya Pradesh, India. The genotypes grown in randomized block design with spacing of 30 cm × 15 cm (row to row × plant to plant), in two replications during *Rabi* 2021-22 at the experimental field of Rajmata Vijayraje Scindia Krishi Vishwa Vidyalaya, Gwalior, India. The soil type of experimental field was sandy loam having pH 7.1 and containing available N, P and K 202.5 kg/ha, 12.4 kg/ha and 249.7 kg/ha respectively with an average organic matter. First irrigation was given to plants at 30 days after sowing then field was kept unirrigated, for imposing drought condition, 40-day old plants were left unirrigated until podding, which occurred when soil moisture content (SMC) dropped to 20-40%. For the well-watered control, plants received water at regular intervals especially after 60 days to keep the SMC at 50 to 55 per cent. Plants were sampled at 75 days after sowing for measurement of different biochemical parameters from control as well as stressed conditions.

Estimation of different biochemical parameters

Total chlorophyll

Total chlorophyll was calculated as per method given by Arnon *et al.* (1949). It was estimated 2 times first on 30 days and second on 75 days after sowing, Leaf samples was crushed in 10 ml (80% acetone) finely and was transferred into a falcon tube. Centrifuged for 15 minutes at 10000 rpm and the green supernatant was transferred into a fresh 15 ml falcon tube. Then readings were taken in a spectrophotometer at 643 nm, 663 nm and 470 nm wave lengths.

Lipid peroxidation

MDA (malondialdehyde) was measured by following the procedure as described by Heath and Packer (1968). Twenty-five mg of leaf sample was taken and crushed in liquid nitrogen. Then 500 microliters of 0.1% trichloro-acetic acid were added and vortexed and centrifuged at 10000 rpm for 10 min. Hundred microliters of supernatant in an Eppendorf tube was taken and 200 microliters of 0.5% TBA was added subsequently. The reaction mixture was heated at 95°C for 30 minutes and quickly kept at -80°C for 2 minutes to stop the reaction. After 2 minutes it was centrifuged at 10000 rpm for 10 min in room temperature and supernatant was taken for reading at 532 nm.

Total sugar

Determination of total sugar was done by anthrone reagent method as described by Dubois *et al.* (1956). Hundred mg fresh random leaf samples) was taken and crushed in a mortar pestle until the leaf completely disappears and fine liquid solution was made with addition of 5 ml of 80% ethanol. The solution was poured in the 15 ml falcon tube and was centrifuged at 1000 rpm for 10 minutes. Then the supernatant was transferred in a fresh 15 ml falcon tube and more 5 ml (80% ethanol) was added into the old tube which has leaf extract and again centrifuged at 10000 rpm for 10 minutes. Total 10 ml (5 ml+5 ml), dry 10 ml supernatant in glass bottle was incubated at 65°C until it gets dried. After drying 1ml distilled water was added and it was left until it has gotten dissolved. Then 100 microlitre was taken from a glass bottle with the help of a pipette then anthrone reagent was added in a falcon tube and it was heated at 100°C for 30 minutes and cooled until it comes at room temperature and then reading was recorded at 630 nm in the spectrophotometer.

Proline

Free proline content in leaves was determined according to the method proposed by Bates *et al.* (1973) based on the formation of red coloured formazine by proline with ninhydrin in acidic medium, which is soluble in organic solvents like toluene.

Protein

Protein content was estimated by the method given by Lowry *et al.* (1951) Five hundred mg of plant materials were weighed and macerated in a pestle and mortar with 10 ml of 20 per cent trichloroacetic acid. The homogenate was centrifuged for 15 min at 6000 rpm. The supernatant was discarded. To the pellet, 5 ml of 0.1 N NaOH was added and centrifuged for 5 min. The supernatant was saved and made up to 10 ml with 0.1 N NaOH. This extract was used for the estimation of the protein.

Estimation of antioxidant enzymes

Leaf samples (250 mg) were ground to a fine powder using liquid nitrogen. Ground powder was homogenized in 1.5 ml

of ice-cold extraction buffer containing phosphate buffer (100 mM, pH 7.0), 1% PVP and 1mM EDTA before centrifugation at 1000 rpm and 40°C for 15 min. The supernatant was separated and stored at 40°C till the assay of enzymatic activities using spectrophotometer.

Catalase (CAT) activity (EC 1.11.1.6)

CAT activity was determined by the method of Luck (1965) with some modifications. For assay 100 µl of diluted enzyme extracted from leaves was taken and added with 800 ìl of 50 mM potassium phosphate buffer (pH 7.0). Reaction was initiated by adding 100 µl of 100 mM hydrogen peroxide. The change in absorbance was recorded at 240 nm at an interval of 15 sec for 2 min. The enzyme activity was expressed as unit mg^{-1} protein min^{-1}. One unit can be defined as 0.1 decrease in absorbance.

Ascorbate peroxidase (APX) activity (EC 1.11.1.11)

APX activity was measured by the method of Nakano and Asada (1981). To prepare reaction mixture for determining APX activity, diluted enzyme extract (20 µl) was added in 50 mM potassium phosphate buffer (880 µl) containing 0.5 mM ascorbate. The reaction was started by the addition of 1 mM H_2O_2 (100 µl). Diminishing absorbance was recorded at 290 nm at 15 sec intervals for 2 min.

Superoxide dismutase (SOD) activity

Twenty-five mg leaf sample was taken and crushed in liquid nitrogen. Two hundred and fifty microliters of 0.1% trichloro acetic acid was added in an Eppendorf tube. Then it was vortexed for 10 min and centrifuged for 20 minutes at 10000 rpm followed by taking 160 microliters supernatant in an Eppendorf tube and 160 microliters of phosphate buffer was added. Then 680 microliters of 1 M potassium iodide were added. The reaction mixture was kept in dark for 1 hour and then absorption was taken at 390 nm.

Peroxidase (POX)

POX activity was measured by estimating the oxidation of guaiacol as proposed by Putter *et al*. (1974). To prepare reaction mixture for determining POX activity diluted enzyme extract (100 µl) were added in 50 mM potassium phosphate buffer (700 µl). Reaction was initiated by adding 100 µl of 100 mM H_2O_2. The change in absorbance was recorded at 15s interval for 2 min at 470 nm.

Statistical analysis, hierarchical cluster and expression analysis

Statistical analysis of antioxidant enzymes was performed and the coefficient of correlation among the traits was calculated using software SPSS ver 19.0. Heat map and dendrogram analysis was prepared using “heatmap” function of ‘R’ package (Zhao *et al.*, 2014).

RESULTS AND DISCUSSION

The chickpea has narrow genetic variation which extremely affects chickpea enhancement (Sachdeva *et al*., 2022). Low moisture stress influences the early vegetative growth stages of chickpea plant which ultimately affects yield. Therefore, biochemical and anti-enzymatic activities performances of selected chickpea genotypes were considered for identification of putative drought linked-selection indices with improved drought tolerance under drought stress.

Analysis of biochemical parameters for drought in chickpea

Total sugar content ranged between19.6 mg/g (RVSSG) to 32.5 mg/g (RVSSG36) under control condition and 30.3 mg/g (RVSSK86) to 38.4 mg/g (JG16) under stressed condition. While proline content arrayed between 1.3 µg/g (RVSSG44) to 2.2 µg/g (JG16) under control and 2.6 µg/g (SAGL162380) to 6.4 µg/g (SAGL190008) under stressed circumstances. Present findings agree with earlier findings that drought stress causes a substantial increase in accumulation of osmolytes *i.e*., sugar and proline activities (Tiwari *et al*., 2023a).

MDA was evidenced 1.6 nmol/g (for 6 genotypes including JAKI 9218) to 1.9 nmol/g (for 23 genotypes including JG74) under control and 2.2 nmol/g (RVSSG 44) to 3.9 nmol/g (SAGL171017) under stressed conditions. Crops have become more prone to oxidative damage due to unpredicted climate changes by excessive production of toxic ROS such as H_2O_2, superoxide and hydroxyl radicals. The previous investigations on chickpea emphasized that under low moisture stress condition, oxidant status of sensitive chickpea genotypes increased owing to increased oxidative stress (Kaloki *et al*., 2019; Jameel *et al*., 2021). In order to assess the severity of plasma membrane damage and the capacity of plants to withstand drought stress, malondialdehyde (MDA), a chemical formed by membrane lipids in response to reactive oxygen species (ROS), may be utilized as a drought indicator (Zhang *et al*., 2021). There was a positive association between MDA content and lipid peroxidation and it can deteriorate the integrity of cell wall (Rani *et al*., 2020). The outcomes of present investigation are in accordance with an earlier report, in which tolerant chickpea genotypes accumulated low MDA concentration compared to sensitive genotypes (Chaudhary *et al.,* 2020).

Total protein content varied between 20.4 mg/g (SAGL19005) to 24.4 mg/g (RVSSG78) under control and 17.2 mg/g (SAGL152348) to 20.4 mg/g (SAGL152218) under stressed situations. Remarkable reduction was evident in protein content under water stress conditions in the current study. It was stated earlier that low water status in plant caused a noteworthy reduction in protein production, which might be due to involvement of diverse factors. Protein molecules play a crucial role in appropriate functions of cell. Since proteins directly influence the development of novel phenotypes by altering physiological features in response to environmental changes, their role is essential in the stress responses of plants. This reduction in protein content ultimately leads to reduce in plant growth and crop yield in sensitive chickpea genotypes.

Chlorophyll ranged between 1.84 µg/ml (BGD112) to 2.11 µg/ml (SAGL162380) under controlled condition whilst 1.03 µg/ml (BGD112) to 2.04 µg/ml (SAGL162380) under

stressed conditions. Photosynthesis plays a vital role in deciding growth and development in plants. Pioneer investigations exhibited that abiotic stress caused a decline in photosynthesis rate which can be computed by assessing photosynthetic pigments. Under stress circumstances, chlorophyll a and chlorophyll b were lesser reduced in tolerant genotypes. Identical findings were also addressed in an earlier investigation in which chickpea heat tolerant genotypes depicted high chlorophyll than sensitive genotypes (Kaloki *et al*., 2019). Our findings are also in agreement with the results of Keerthi *et al*. (2023) where chlorophyll content decreased in the chickpea cultivars under water stress conditions. The contents of photosynthetic pigments are directly related to water stress tolerance. Reduction in chlorophyll contents may be because of disturbance in biosynthesis or their breakdown under water stress. Correlation coefficient among different biochemical parameters exhibited significant corelation at 0.01 and 0.05 probability level. Total sugar under control condition was positively correlated with total sugar under stress circumstances (r=0.446) at 1% significant level. Similarly, positively significant correlation was also evident among proline content under control with proline under stressed conditions (r=0.441), MDA under stress condition with proline control (r=0.365) and MDA control (r=0.336) at 1% level of significance (Table 1).

Analysis of antioxidant enzymes activities

POX ranged from 7.8 U/mg (RVSSG36) to 12.8 U/mg (SAGL152405) under controlled condition and 10.2 U/mg to 25.1 U/mg (RVSSG78) under stress condition. Whereas APX arrayed between 1.4 U/mg (SAGL152348) to 3.8 U/mg (SAGL154805) under control and 2.9 U/mg (SAGL162380) to 6.9 U/mg (SAGL 152216) under stress circumstances. CAT varied between 12.2 U/mg (SAGL162380) to 42 U/mg (JG14) under control and 22.3 U/mg (SAGL162380) to 69.2 U/mg (RVG201) under stress situations. Whilst SOD ranged between 31.6 U/mg (RVSSG68) to 100 U/mg (JG14) under controlled and 87.6 U/mg (RVSSG44) 194.4 U/mg (SAGL190028) under stressed conditions respectively. In present investigation, the influence of drought stress was exhibited least in drought

Table 1: Correlation coefficient analysis among different biochemical parameters of chickpea genotypes.

	Correlations									
	TS_C	TS_S	P_C	P_S	MDA_C	MDA_S	Prt_C	Prt_S	C_C	C_S
TS_C	1	0.446**	0.005	0.013	-0.087	0.121	0.256*	-0.081	-0.040	0.028
TS_S		1	0.213	0.020	-0.126	-0.163	-0.052	-0.099	-0.051	0.068
P_C			1	0.441**	0.005	0.365**	0.054	-0.088	-0.110	-0.224*
P_S				1	0.036	0.336**	0.063	-0.042	-0.036	-0.142
MDA_C					1	0.222*	-0.012	-0.193	-0.027	-0.029
MDA_S						1	0.261*	0.013	-0.066	-0.163
Prt_C							1	0.189	0.020	-0.097
Prt_S								1	-0.038	0.247*
C_C									1	0.181
C_S										1

**Correlation is significant at the 0.01 level (2-tailed)*. Correlation is significant at the 0.05 level (2-tailed).

TS_C=Total sugar in control; TS_S=Total sugar in stress; P_C= Proline control; P_S=Proline stress; MDA_C= Malondialdehyde Control; MDA_S= Malondialdehyde stress; Prt_C= Protein control; Prt_S= Protein stress; C_C= Chlorophyll control; C_S= Chlorophyll stress conditions.

Table 2: Correlation coefficient analysis among antioxidant enzymes activities of chickpea genotypes.

	Correlations							
	POX_C	POX_S	APX_C	APX_S	CAT_C	CAT_S	SOD_C	SOD_S
POX_C	1	0.428**	0.047	0.023	0.222*	0.167	-0.008	0.150
POX_S		1	0.032	0.373**	0.651**	0.511**	0.323**	0.449**
APX_C			1	0.445**	0.147	0.302**	0.211	0.126
APX_S				1	0.332**	0.344**	0.248*	0.423**
CAT_C					1	0.687**	0.500**	0.471**
CAT_S						1	0.356**	0.374**
SOD_C							1	0.665**
SOD_S								1

**Correlation is significant at the 0.01 level (2-tailed)*. Correlation is significant at the 0.05 level (2-tailed).

Where, POX-C and POX S= Peroxidase in control and stress; APX-C and APX S= ascorbate peroxidase in control and stress; CAT-C and S= Catalase in control and stress and SOD-C and S= superoxide dismutase in control and stress conditions.

tolerant genotypes compared to rest of the genotypes which might be because of accumulation of osmolytes and increased activities of antioxidant enzymes. Chickpea stress tolerant genotypes accumulate more osmolytes for instance, proline, glycine betaine and reduced level of glutathione. To detoxify the effects of ROS generated under abiotic stresses, specialized enzymatic antioxidants, *i.e.*, SOD, CAT, POD and APX get triggered and act as first line of defence (Tang *et al*., 2019). Increased activity of antioxidants enzymes was evident in tolerant genotypes under drought stressed circumstances compared to normal conditions. It has been earlier investigated that drought tolerance in chickpea is strongly correlated with increased antioxidant enzyme activities (Tiwari *et al.,* 2023a). A remarkable

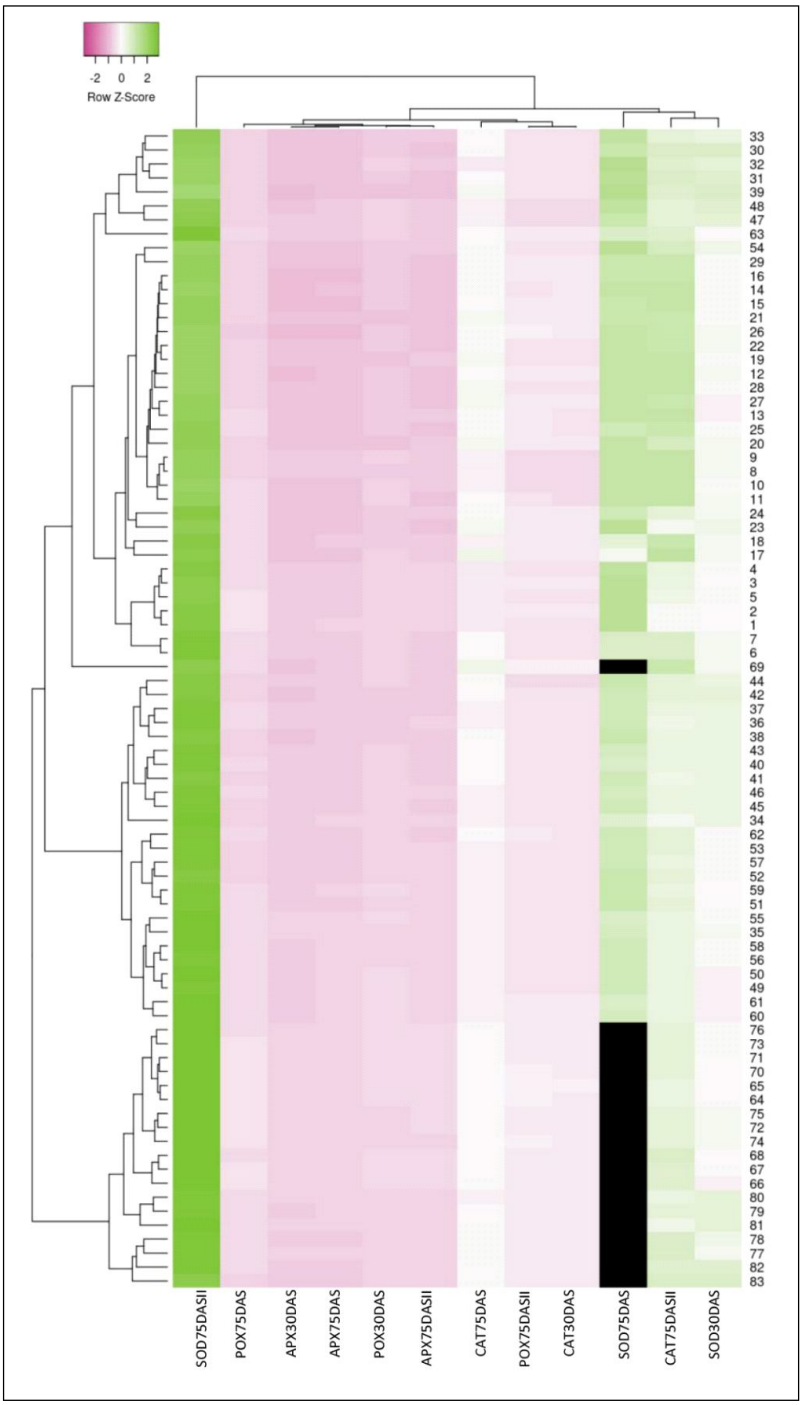

Fig 1: Heat map of chickpea genotypes based on antioxidant enzymes activities in different chickpea genotypes.

enhancement in POD was investigated under combined (drought plus heat) stress in D-09027 and CH24/07 in comparison to control by (Jameel *et al*., 2021).

Correlation coefficient analysis among different antioxidant enzymes of chickpea genotypes under controlled and drought stresses conditions was also analysed. POX under control was significantly correlated with POX under drought stress (r=0.428) at 1% level of significance (Table 2). Likewise, significant positive correlation was also evident among APX under stress with POX under stress (r=0.373) and APX under control (r=0.445), CAT under stress with POX under stress (r=0.511), APX under control (r=0.302), APX under stress (r=0.344) and CAT under control (0.687) at 1% probability level. Positively significant correlation was also found between SOD under stress with SOD under control (r=0.665), POX under stress (0.449), APX under stress (0.423), CAT under control (0.471) and CAT under stress conditions (0.374) at 1% significant level (Table 2).

Phylogenetic cluster analysis and expression profiling

Based on different antioxidant enzymes activities, two major groups were formed where the first group is further divided into one cluster and one subgroup. Genotype JG315 alone represented the cluster-1 and the subgroup included 40 genotypes which further divided into two clusters where cluster-2 includes 25 genotypes and cluster-3, 15 genotypes. Another major group consists of 42 genotypes which further divide into two subgroups. Genotypes *viz*., RVG 205, RVSSG30, SAGL15221 and JSC37 are considered into subgroup forming cluster- 4 and other 38 genotypes were comprinted into a subgroup further grouped into two clusters *namely* cluster-5 including 17 genotypes and cluster-6 having 21 genotypes (Fig 1). Diversity assessment based on heatmaps for representing expression levels of different antioxidant enzymes showing variations among studied genotypes (-2 to 2). The increasing intensity of color from pink to black and then to green represents increasing values accordingly (Fig 1). Similar kind of studies were also performed by Sharma *et al*. (2021) and Tomar *et al.* (2022) as they have also estimated different biochemical parameters and represented heat map for showing the level of expression. Current research work found significant difference in different biochemical parameters and anti-enzymatic activities under imposed drought conditions in chickpea.

CONCLUSION

Chickpea is one of the most important legume crops economically and a prime source of proteins. Among several abiotic stresses drought is most devasting problem due to changing scenario of environment. Estimation of biochemical parameters and antioxidant enzyme activity is one of the important aspects to study drought effect. Total sugar, proline and malondialdehyde, has been increased under drought conditions whilst total chlorophyll and protein decreases under drought stress conditions. Likewise, antioxidant enzymes *viz*., POX, APX, CAT and SOD level increased under drought stress conditions. Highest sugar was found in chickpea genotype JG16, proline in SAGL190008, MDA in SAGL 171017 and highest protein was recorded in genotype RVSSG 78. Whereas, the highest POX enzyme activity was investigated in genotype RVSSG78, APX activity in genotype SAGL152216, CAT in RVG201 and highest SOD was recorded in genotype SAGL190028. Biochemical parameters and antioxidant enzymes play very crucial role to observe effect of drought stress in chickpea genotypes. Such kind of studies are important to select superior chickpea genotype(s) for further breeding programme.

Conflict of interest: None.

REFERENCES

Asati, R., Tripathi, M.K., Tiwari, S., Yadav, R.K., Tripathi, N. (2023). Molecular breeding and drought tolerance in chickpea. Life. 12: 1846. https://doi.org/10.3390/life12111846.

Arif, A., Parveen, N., Waheed, M.Q., Atif, R.M., Waqar, I., Shah, T.M. (2021). A comparative study for assessing the drought-tolerance of chickpea under varying natural growth environments. Front. Plant Sci. 11: 607869.

Arnon, D.I. (1949). Copper enzymes in isolated chloroplasts. Polyphenol oxidase in Beta vulgaris. Plant Physiology. 24: 1-15.

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

Chaudhary, S., Devi, P., Bhardwaj, A., Jha, U.C., Sharma, K. and Prasad, D. *et al* (2020). Identification and characterization of contrasting genotypes/cultivars for developing heat tolerance in agricultural crops: Current status and prospects. Front Plant Sci. 11:1505. doi: 10.3389/fpls.2020.587264.

Dubois, M., Gillea, K.A., Hamilton, J.K., Rebers, P.A. and Smith, F. (1956). Calorimetric method for determination of sugars and related substances. Analytical Chemistry. 28(3): 350-356.

Heath, R.L. and Packer, L. (1968). Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Archives in Biochemistry and Biophysics. 125:189-198.

Jameel, S., Hameed, A. and Shah, T.M. (2021). Investigation of distinctive morpho-physio and biochemical alterations in desi chickpea at seedling stage under irrigation, heat and combined stress. Front. Plant Sci. 12: 692745.

Keerthi Sree, Y., Lakra, N., Manorama, K., Ahlawat, Y., Zaid, A., Elansary, H.O, Sayed, S.R.M., Rashwan, M.A. and Mahmoud E.A. (2023). Drought-induced morpho-physiological, biochemical, metabolite responses and protein profiling of chickpea (*Cicer arietinum* L.). Agronomy. 13: 1814. DOI:10.3390/13071814.

Kaloki, P., Devasirvatham, V. and Tan, D. (2019). Chickpea abiotic stresses: Combating drought, heat and cold. Abiotic. Biotic. Stress. Plants. doi: 10.5772/intechopen.83404.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193(1): 265-75. PMID: 14907713.

Luck, H. (1965). Catalase. In: Method of Enzymatic Analysis, Academic Press, [Bergmeyer, H.U., (ed)]., New York and London. 885-894. http://dx.doi.org/10.1016/B978-0-12-395630-9.50158-4.

Mishra, N., Jiang, C., Chen, L., Paul, A., Chatterjee, A. and Shen, G. (2023). Achieving abiotic stress tolerance in plants through antioxidative defense mechanisms. Front. Plant Sci. 14: 1110622. doi: 10.3389/fpls.2023.1110622.

Nakano, Y., Asada, K. (1981). Hydrogen peroxide is scavenged by Ascorbate-specific peroxidase in spinach chloroplasts. Plant Cell Physiol. 22(5): 867-880.

Pütter J. (1974). Peroxidase. In: Methods of Enzymatic Analysis. [Bergmeyer, H.U., (eds)]. New York: Academic Press. Pp: 685-690.

Rani, A., Devi, P., Jha, U.C., Sharma, K.D., Siddique, K.H. and Nayyar, H. (2020). Developing climate-resilient chickpea involving physiological and molecular approaches with a focus on temperature and drought stresses. Fron Plant Sci. 10: 1759. https://doi.org/10.3389/fpls.2019.01759.

Sahu, V.K., Tiwari, S., Tripathi, M.K., Gupta, N., Tomar, R.S., Yasin, M. (2020a). Morpho-physiological and biochemical traits analysis for Fusarium wilt disease using gene-based markers in desi and Kabuli genotypes of chickpea (*Cicer arietinum* L.). Indian J. Genet. 80(2): 163-172.

Sahu, V.K., Tiwari, S., Gupta, N., Tripathi M.K., Yasin, M. (2020b). Evaluation of physiological and biochemical contents in desi and kabuli chickpea. Legume Research: Doi 10.188 05/LR-4265.

Sharma, A., Tripathi, M.K., Tiwari, S., Gupta, N., Tripathi, N. and Mishra, N. (2021). Evaluation of soybean (*Glycine max* L.) genotypes on the basis of biochemical contents and anti-oxidant enzyme activities. Legume Research. 10.188 05/LR-4678.

Sachdeva, S., Bharadwaj, C., Patil, B.S., Pal, M., Roorkiwal, M. and Varshney, R.K. (2022). Agronomic performance of chickpea affected by drought stress at different growth stages. Agronomy. 12: 995. doi: 10.3390/plants12 183175.

Tang, J., Wang, S.Q., Hu, K.D., Huang, Z. Q., Li, Y.H. and Han, Z. *et al.* (2019). Antioxidative capacity is highly associated with the storage property of tuberous roots in different sweet potato cultivars. Sci. Rep. 9: 1-10. doi: 10.1038/s41598-019-47604-8.

Tiwari, P.N., Tiwari, S., Sapre, S., Babbar, A., Tripathi, N., Tiwari, S. and Tripathi, M.K. (2023a). Screening and selection of drought tolerant high yielding chickpea genotypes based on physio-biochemical selection indices and yield trials. Life. 13: 1405, DOI: 10.3390/life13061405.

Tiwari, P.N., Tiwari, S., Sapre, S., Tripathi, N., Payasi, D.K., Singh, M., Thakur, S., Sharma, M., Tiwari, S. and Tripathi, M.K. (2023). Prioritization of physio-biochemical selection indices and yield-attributing traits towards the acquisition of drought tolerance in chickpea (*Cicer arietinum* L.). Plants. 12: 3175. https://doi.org/ 10.3390/plants121 83175.

Tomar, Y.S., Tiwari, S., Tripathi, M.K., Singh, S. and Gupta, N. (2022). Genetic diversity, population structure and biochemical parameters estimations driving variations in groundnut germplasm. Legume Research - An International Journal. 10.18805/LR-4965.

Tripathi, M.K., Tripathi, N., Tiwari, S. Mishra, N., Sharma, A., Tiwari, S. and Singh, S. (2023). Identification of Indian soybean (*Glycine max* [L.] Merr.) genotypes for drought tolerance and genetic diversity analysis using SSR markers SCI. 3(3): 31-46.

Varshney, R.K., Song, C., Saxena, R.K., Azam, S., Yu, S. and Sharpe, A.G. *et al*. (2013). Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. Nat. Biotechnol. 31: 240-246.

Zhao, S., Guo, Y., Sheng, Q. and Shyr, Y. (2014). Advanced heat map and clustering analysis using heatmap 3. Biomed Res Int. 986048. doi: 10.1155/2014/986048.

Zhang, Y., Luan, Q., Jiang, J. and Li, Y. (2021). Prediction and utilization of malondialdehyde in exotic pine under drought stress using near-infrared spectroscopy. Front. Plant. Sci. 12: 735275. doi: 10.3389/fpls.2021.735275.