

Protective role of antioxidant enzymes in chickpea (*Cicer arietinum* L.) genotypes under high temperature stress

Manish Kumar Singh

Rain Forest Research Institute, Jorhat 785001, Assam, India; E-mail: kumar.manish21@yahoo.co.in

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ABSTRACT

Four chickpea genotypes differing in their sensitivity to high temperature stress were taken and grown in growth chambers in the phytotron facility of IARI, New Delhi. The plants were maintained at 18/23°C (control) and 25/35°C (temperature stress) night/day temperature after maximum tillering. In all chickpea genotypes high temperature stress increased membrane injury index (MII), activity of superoxide dismutase (SOD) and ascorbate peroxidase (APX), malic acid and slightly decreased the activity of glutathione reductase (GR), relative water content (RWC), chlorophyll and carotenoid contents. Under the HT stress, the tolerant genotypes Pusa-1103 and BGD-72 exhibited higher RWC, chlorophyll and carotenoid, activity of SOD, APX, GR and, and less decrease in MII as compared to susceptible genotypes Pusa-256 and RSG-991. Antioxidant enzymes showed positive correlation (*r*) with chlorophyll content, RWC and negative with MII under high temperature stress. From the results it is apparent that the antioxidant defence mechanism plays an important role in heat stress tolerance of chickpea genotypes.

Keywords: High temperature stress; Phytotron; Membrane injury index; Antioxidant enzymes; Chlorophyll contents; Relative water content.

1. INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important cool-season legumes grown extensively throughout the world, particularly in West and South Asia and North African countries. It has been grown in semiarid regions of the world for hundreds of years. The major chickpea producers India, Pakistan and Turkey contribute 67.0, 9.5 and 6.7%, respectively to the world harvest [1]. India predominates in chickpea supply and it has the distinction of being the largest producer and consumer in the world. The important chickpea growing states of India are Madhya Pradesh, Punjab, Haryana, Rajasthan, Uttar Pradesh, Bihar, Gujarat, Maharashtra, Karnataka and Andhra Pradesh. Latest estimate for 2007-08 indicate that the production of pulses in the country is 16.64 million tonnes from an area of 26.15 million hectares. Chickpea is the most important pulse recorded production of 6.43 million tonnes during 2007-08. In spite of having largest area under chickpea in the world India's position in average productivity is yet to see major breakthrough to meet the per capita availability of 50 g pulses/day to alleviate protein energy malnutrition.

In northern India, however, late planting of chickpea is done after harvest of rice, early potato or cotton. Such late sown chickpea crop experiences high temperature at the end of the cropping season. This high temperature at the end of cropping season

leads to problem of poor biomass and forced maturity [2]. The Inter-Governmental Panel on Climate Change (IPCC) of the United Nations in its recent report has confirmed the global warming trends, and projected that the globally averaged temperature of the air above the earth's surface would rise by 1.4-5.8 °C over the next 100 years [3].

This high temperature stress leads to the production of reactive oxygen species (ROS) which damages the plant cellular and subcellular system. However, plants protect its systems from cytotoxic effects of the reactive oxygen species using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, ascorbic acid and carotenoides [4]. Use of ion leakage and relative water content as simple indices for screening genotypes against heat and drought stress in chickpea and wheat has been suggested by many workers [5, 6].

The present work was conducted to study the effect of high temperature stress on antioxidant enzymes in chickpea genotypes.

2. MATERIALS AND METHODS

2.1. Selection and propagation of genotypes

The experiment was conducted at phytotron facility of I.A.R.I., New Delhi, with four chickpea (*Cicer arietinum* L) genotypes differing in sensitivity to high temperature (HT) stress *i.e.*, Pusa-1103 and BGD-72 (HT tolerant), Pusa-256 and RSG-991 (HT susceptible). Seeds of these four selected genotypes were collected from Genetics Division, I.A.R.I., New Delhi. They were treated with *Mesorhizobium ciceri* SPG strain and were sown in earthen pots (20 × 30 cm²) containing mixture of soil, sand and farmyard manures (FYM) in ratio of 3:1:1. Recommended dose of nitrogen, phosphorus and potassium fertilizers were applied. Seeds were sown in each pot by dibbling method at 2 cm depth. Thinning was done at 10 days after sowing, and five plants were retained in each pot.

2.2. High temperature treatment

Chickpea plants were exposed to temperature stress by covering them with polyvinyl chloride sheets (Capri Hans, sunflex 0.15 mm thickness and

transmittance 85%) mounted on wooden structures of size 3 x 2 x 2 m. The wooden polythene chambers were kept 10 cm above the ground for circulation of air and to control the humidity inside the chamber. Thermometer was placed inside the poly cover and the level of temperature was recorded regularly. The temperature inside the poly cover was 6.1 °C higher than the ambient temperature. Twenty pots from each of the four genotypes were shifted inside the polycovers at the 78 DAS to expose plants to temperature stress. Thirty seven days after temperature stress, all the pots were taken out and kept under the normal environment (ambient temperature), and the physiobiochemical observations were recorded.

2.3. Physiobiochemical estimation

Relative water content in leaves was estimated according to the method described by [7]. Membrane injury index was estimated from all genotypes in three random replicates as suggested by [6]. Chlorophyll content was determined according to [8] and carotenoid content was estimated according to [9].

Enzyme extract for superoxide dismutase, ascorbate peroxidase, glutathione reductase and catalase was prepared by first freezing the weighed amount of leaf samples (1 g) in liquid nitrogen to prevent proteolytic activity followed by grinding with 10 ml extraction buffer (0.1 M phosphate buffer, pH 7.5, containing 0.5 mM EDTA and 1 mM ascorbic acid). Brie was passed through 4 layers of cheese cloth and filtrate was centrifuged for 20 min at 15000 rpm at 4 °C and the supernatant was used as enzyme.

Superoxide dismutase activity was estimated by recording the enzyme induced decrease in absorbance of formazone made by nitro-blue tetrazolium with superoxide radicals [10].

Ascorbate peroxidase activity was estimated by observing the decrease in absorbance due to ascorbic acid at 290 nm and the Glutathione reductase activity was assayed by recording the increase in absorbance in the presence of oxidized glutathione and DTNB (5, 5-dithiobis-2-nitrobenzoic acid [11].

2.4. Statistical analysis

Pearson-product-moment correlation coefficient (r) between various antioxidant enzymes and total chlorophyll content, relative water content and membrane injury index were computed according to [12].

3. RESULTS AND DISCUSSION

3.1. Relative water content

The analysis of data (Table 1) showed that with the increase in temperature stress there was progressive decrease in the RWC of flag leaves. Under ambient temperature, the RWC was higher in BGD-72 and Pusa-1103 and less in Pusa-256 and RSG-991. However, under high temperature (HT) treatment Pusa-1103 and BGD-72 showed significantly higher RWC. Average decline in RWC

under high temperature stress was 14.21%, Pusa-1103 showed lowest per cent decline (6.0%), while RSG-991 showed highest decline (18.99%) in RWC. Significant differences were also obtained between treatment and genotypic interaction.

3.2. Membrane injury index

The membrane injury Index (MII) decreased under heat stress in all the genotypes (Table 2.). However, under ambient temperature (AT) the MII was lowest in Pusa-1103 (31.61%) closely followed by BGD-72, and highest in Pusa-256. Under HT stress, the Pusa-1103 showed lowest MII and RSG-991 exhibited highest MII (46.47%). The average increase in MII under high temperature stress was 19.33%, Pusa-1103 showed lowest increase (9.80%), while RSG-991 showed highest increase (31.74%) in MII. The interaction between treatment and genotypes was also significant.

Table 1. Effect of high temperature stress on relative water content in (%) chickpea genotypes.

Genotypes	Control	Treatment	Genotypic mean	Percent decrease
Pusa 1103	80.21	75.49	80.35	6.00
BGD 72	83.67	71.40	77.53	14.79
Pusa 256	78.53	65.24	71.88	17.06
RSG 991	78.34	63.57	70.95	18.99
Mean	80.19	68.93		14.21
CD at 5%				
Treatment(T)		1.92		
Genotypes(G)		2.71		
T x G		3.83		

Table 2. Effect of high temperature stress on membrane injury index (%) in chickpea genotypes.

Genotypes	Control	Treatment	Genotypic mean	Percent decrease
Pusa 1103	31.61	34.67	33.14	9.80
BGD 72	34.21	39.87	37.04	16.68
Pusa 256	36.28	43.16	39.72	19.10
RSG 991	35.32	46.47	40.90	31.74
Mean	34.36	41.04		19.33
CD at 5%				
Treatment (T)		1.38		
Genotypes(G)		0.97		
T x G		1.95		

3.3. Photosynthetic pigments

Data on chlorophyll 'a', chlorophyll 'b', total chlorophyll, total carotenoid, chlorophyll a/b ratio are reported in (Figs. 1-5). Chlorophyll 'a', chlorophyll 'b', total chlorophyll, total carotenoid and chlorophyll/carotenoid ratio in leaves of chickpea genotypes were higher under AT condition, and a significant decline was observed for all the above parameters under HT condition. Pusa-1103 followed by BGD-72 exhibited significantly higher chlorophyll 'a', chlorophyll 'b', total chlorophyll, and chlorophyll a/b ratio compared to Pusa-256 and RSG-991 under both the conditions.

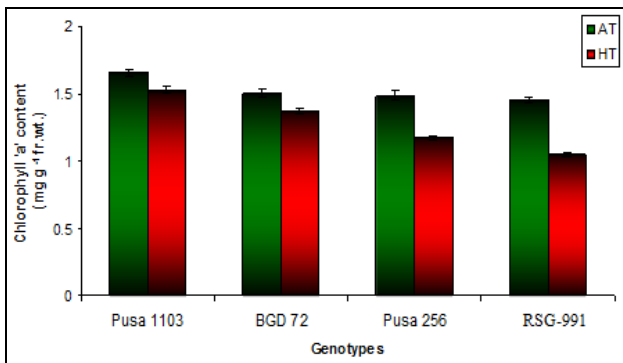


Figure 1. Effect of high temperature stress on chlorophyll 'a' content on chickpea genotypes. Vertical bars show \pm S.E of mean. Data for treatments (T) and genotypes (G) and T x G interactions were significant ($P = 0.05$).

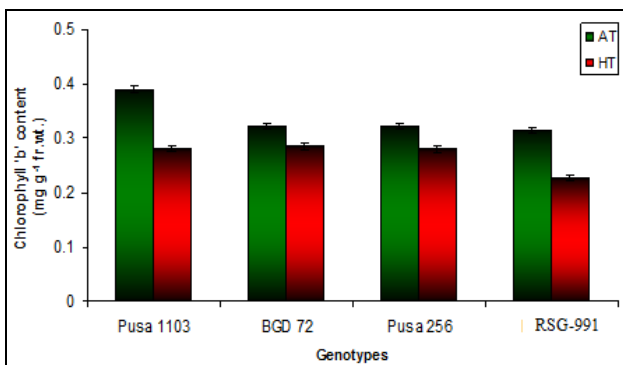


Figure 2. Effect of high temperature stress on chlorophyll 'b' content on chickpea genotypes. Vertical bars show \pm S.E of mean. Data for treatments (T) and genotypes (G) and T x G interactions were significant ($P = 0.05$).

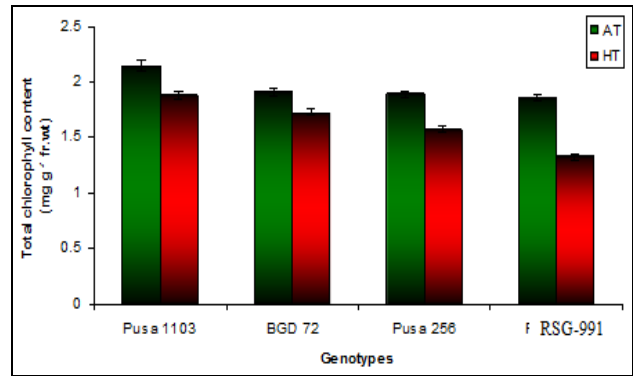


Figure 3. Effect of high temperature stress on total chlorophyll content on chickpea genotypes. Vertical bars show \pm S.E of mean. Data for treatments (T) and genotypes (G) and T x G interactions were significant ($P = 0.05$).

Total carotenoid content was significantly higher in Pusa-256 under AT condition along with Pusa-1103 and BGD-72. There was a general decline of 15.87% in carotenoid content in all genotypes under HT condition. Under HT condition RSG-991 showed higher reduction, while Pusa-1103 maintained comparatively higher carotenoid content. Under AT condition the chlorophyll/carotenoid ratio was higher in BGD-72 and Pusa-1103 closely followed by RSG-991 and lowest in Pusa-256. Under HT treatment, there was a non significant reduction in chlorophyll/carotenoid ratio, and among genotypes Pusa-1103 possessed highest chlorophyll/ carotenoid ratio.

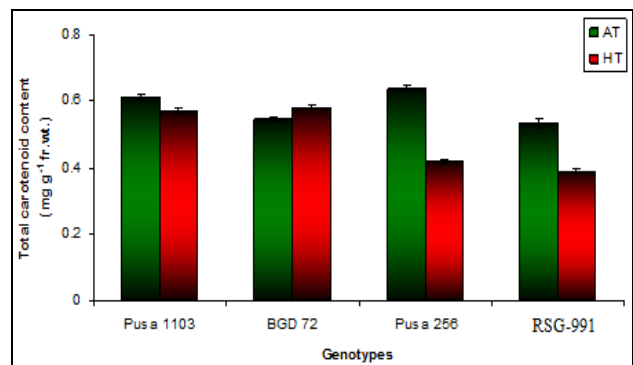


Figure 4. Effect of high temperature stress on total carotenoid content on chickpea genotypes. Vertical bars show \pm S.E of mean. Data for treatments (T) and genotypes (G) and T x G interactions were significant ($P = 0.05$).

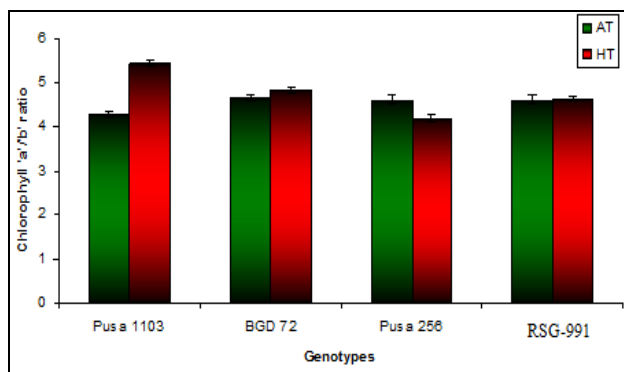


Figure 5. Effect of high temperature stress on chlorophyll 'a'/'b' content on chickpea genotypes. Vertical bars show \pm S.E of mean. Data for treatments (T) and genotypes (G) and T \times G interactions were significant ($P = 0.05$).

3.4. Antioxidant enzyme activities

Superoxide dismutase (SOD) activity showed significant increase under HT compared to AT condition (Table 3). Among the genotypes, BGD-72 showed significantly higher SOD activity followed by Pusa-1103 and Pusa-256. RSG-991 exhibited the lowest activity under HT condition. SOD activity increased by two folds in BGD-72 and by 5% in RSG-991. Significant interaction was observed between treatment and genotypes.

APX increased significantly under HT condition compared to AT (Table 4). Under both the conditions Pusa-1103 possessed higher APX activity, while RSG-991 showed the lowest activity. BGD-72 and Pusa-256 exhibited moderate activity under both the conditions.

Table 3. Effect of high temperature stress on superoxide dismutase activity (units $\text{min}^{-1}\text{mg}^{-1}$ protein) in chickpea genotypes.

Genotypes	Control	Treatment	Genotypic mean	Percent increase
Pusa 1103	3.80	5.92	4.86	55.9
BGD 72	3.82	6.20	5.01	62.4
Pusa 256	2.72	4.75	3.74	74.7
RSG 991	2.92	3.07	2.99	5.1
Mean	3.31	4.98		50.4
CD at 5%				
Treatment (T)		0.12		
Genotype (G)		0.17		
T \times G		0.25		

Table 4. Effect of high temperature stress on ascorbate peroxidase activity (μmol ascorbate oxidized $\text{min}^{-1}\text{mg}^{-1}$ protein) in chickpea genotypes.

Genotypes	Control	Treatment	Genotypic mean	Percent increase
Pusa 1103	6.33	11.46	8.89	81.0
BGD 72	6.16	9.22	7.69	49.8
Pusa 256	6.02	7.20	6.61	19.7
RSG 991	5.97	6.59	6.28	10.4
Mean	6.12	8.62		40.8
CD at 5%				
Treatment (T)		0.25		
Genotype (G)		0.36		
T \times G		0.50		

Table 5. Effect of high temperature stress on glutathione reductase activity ($\Delta A_{412} \text{ min}^{-1} \text{ mg}^{-1} \text{ protein}$) in chickpea genotypes.

Genotypes	Control	Treatment	Genotypic mean	Percent increase
Pusa 1103	5.43	4.63	5.03	14.8
BGD 72	5.29	4.06	4.67	23.3
Pusa 256	4.76	3.44	4.10	27.6
RSG 991	4.12	1.91	3.02	53.8
Mean	4.90	3.51		28.4
CD at 5%				
Treatment (T)		0.19		
Genotype (G)		0.27		
T x G		0.37		

Table 6. Correlation coefficient (r) between various enzymes and chlorophyll content, relative water content and membrane injury index in chickpea genotypes.

Antioxidant enzymes	Chlorophyll content		Membrane injury index		Relative water content	
	AT	HT	AT	HT	AT	HT
SOD	0.559*	0.949**	-0.714**	-0.886**	0.614*	0.855**
APX	0.404	0.884**	0.342	-0.946**	0.052	0.940**
GR	0.092	0.956**	0.473	-0.899**	-0.433	0.836**

* Significant at 5%; ** Significant at 1%.

Data on glutathione reductase (GR) activity is reported in (Table 5). GR activity decreased significantly under HT condition. Under AT condition Pusa-1103 showed higher activity followed by BGD-72. Under HT condition also Pusa-1103 and BGD-72 maintained a higher GR activity, while RSG-991 exhibited a greater decline. Significant interaction was observed between treatment and genotypes for GR activity.

3.5. Correlation studies

Results on Pearson-product-moment correlation coefficient (r) between various antioxidant enzymes, and total chlorophyll content, relative water content and membrane injury index are reported in (Table 6). The results revealed that under high temperature condition there exist a significant positive correlation between antioxidant enzymes and chlorophyll content and RWC, and significant negative correlation with MII. However under ambient temperature non significant correlation was observed between antioxidant enzymes and other parameters. Among the three

antioxidant enzymes, the glutathione reductase showed significantly higher correlation (r) with other three physiological traits under high temperature condition.

4. DISCUSSION

Plants experience high temperature in many different ways and adaptation or acclimation to high temperature occurs over different levels of plant organization [13]. In this study the BGD-72 and Pusa-1103 maintained higher RWC, chlorophyll, carotenoid contents and lower MII under high temperature condition than Pusa-256 and RSG-991. This shows that the tolerant genotype Pusa-1103 had a greater water retention capacity under HT stress. Due to high temperature induced higher transpiration situation similar to water stress is created and RWC becomes important under heat stress. Heat stress injury involves water deficit and cell turgor loss [14]. Maintenance of favorable water status is essential for plant's tolerance to heat stress [15, 16].

Heat stress induced decrease membrane stability has been reported in faba bean leaf discs [17, 18] also reported that the tolerant genotypes possess lower membrane injury index and high RWC, which enable them to maintain better metabolic activities.

Leaf photosynthetic pigment content (chlorophylls and carotenoids) and pigment ratios, such as Chl a/b is good indicators for stress detection and tolerance [18]. Chlorophyll 'a', chlorophyll 'b', total chlorophyll and total carotenoid contents in leaves of chickpea genotypes decreased, while an increase was observed in Chl a/b ratio under HT condition compared to AT condition. Camejo et al. [19] also showed increase in the chlorophyll a/b ratio in stressed Nagcarlang tomato plants, suggesting that these relationships could be used as an indicator of tolerance and physiological status of the plants under stress condition.

Carotenoid protects the photosynthetic systems against singlet oxygen and also plays a pivotal role in thermal dispersion of excess excitation energy [20-23]. Higher carotenoid content in tolerant genotypes Pusa-1103 and BGD-72 signifies their tolerance capacity.

Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzymes activity [24-27] reported significant increase in SOD activity under temperature stress in wheat genotypes, and a greater increase in tolerant genotype C 306, while the susceptible genotypes showed lower activity. In this study the chickpea tolerant genotypes BGD-72 and Pusa-1103 exhibited higher activity of SOD, APX and GR compared to susceptible genotypes. This shows that the tolerant genotypes combated the ROS by maintaining efficient antioxidant mechanism. Sairam et al. [4] and Almeselmani et al. [28] also reported similar results in late sown heat tolerant wheat varieties. Higher activity of various antioxidative enzymes in temperature tolerant genotypes of various crop species has also been reported by various workers [4, 28-30].

Study of correlation coefficient between various antioxidant enzymes with chlorophyll content, relative water content and membrane injury index at high temperature revealed a positive correlation with chlorophyll content, relative water content and negative correlation with membrane

injury index. Almeselmani et al. [28] also reported a significant positive correlation between chlorophyll content and antioxidant enzymes and negative correlation between membrane injury index and antioxidant at high temperature stress condition.

5. CONCLUSION

From the foregoing discussion it is clear that exposure of chickpea genotypes to high temperature stress for a medium duration of thirty seven days i.e., 78 to 115 DAS resulted in increase in the activity of superoxide dismutase, ascorbate peroxidase and glutathione reductase. The temperature tolerant genotypes Pusa-1103 and BGD-72 exhibited a comparatively higher superoxide dismutase, ascorbate peroxidase and slight decline in glutathione reductase compared to susceptible genotypes Pusa-256 and RSG-991. Efficient antioxidant enzymes status in tolerant genotypes under high temperature condition reflected in lower membrane injury index, higher relative water content, chlorophyll and carotenoid content compared to susceptible genotypes Pusa-256 and RSG-991. Hence selection of genotype based on these criteria may help in evolving chickpea genotypes tolerant to high temperature stress with better yield.

TRANSPARENCY DECLARATION

The author declares that there is no conflict of interests.

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