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# STUDY OF DIFFERENT PHYSIOLOGICAL AND BIOCHEMICAL MECHANISM WHICH IMPART TOLERANCE AGAINST HEAT STRESS IN CHICKPEA GENOTYPES

**MANISH KUMAR SINGH, SUDHIR KUMAR* and P.S. DESHMUKH****
Arid Forest Research Institute, New Pali Road, Jodhpur-342 005, Rajasthan, India.
*Kisan (P.G.) College, Simbhaoli, Panchsheel Nagar, Meerut, India.
**Division of Plant Physiology, I.A.R.I., New Delhi-110 012, India.
E mail: mksingh@icfre.org

Plants experience high temperature in many different ways and adaptation or acclimation to high temperature occurs over different levels of plant organization. A Phytotron experiment was therefore conducted with four selected chickpea genotypes differing in their sensitivity to temperature stress, so as to know the inbuilt mechanism present in them which impart tolerance against heat stress. 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 372 and KWR 108 exhibited higher RWC (in KWR 108 average decline was 14.21% and in Pusa 372 it was 6.0% while CSJD 884 showed highest decline 18.99% in RWC as compared to control) higher chlorophyll and carotenoid contents, increase activity of SOD, APX, GR and less decrease in MII as compared to susceptible genotypes Phule-G 96006 and CSJD 884. Under HT condition also malic acid content increased by 110% in KWR 108, Pusa 372 (93%) showed highest malic acid content compared to susceptible genotypes under HT condition. Besides this antioxidant enzymes showed positive correlation *(r)* with chlorophyll content, RWC and negative with MII under high temperature stress. This shows that the tolerant genotypes combated with the generation of ROS due to better metabolic activity and due to efficient antioxidant mechanism.

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

## Introduction

In northern India 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[1]. 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[2].

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 cytoxic effects of the reactive oxygen species using antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reudcatase, catalase, ascorbic acid and carotenoides[3]. 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[4,5]. The present work was conducted to study the effect of high temperature stress on membrane injury index, relative water contents and antioxidant enzymes in chickpea genotypes.

## Material and Methods

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 372 and KWR 108 (HT tolerant), Phule-G 96006 and CSJD 884 (HT susceptible). Seeds of these four selected genotypes were collected from Genetics Division, I.A.R.I., New Delhi. They were treated

with *Mesorhizhobium ciceri* SPG strain and were sown in earthen pots (20x30 $cm^2$) 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.

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 $^0$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.

Relative water content in leaves was estimated according to the method described by Barrs and Weatherley[6]. Membrane injury index was estimated from all genotypes in three random replicates as suggested by Deshmukh *et al.*[4]. Chlorophyll content was determined according to Hiscox and lsraelstam[7] and carotenoid content was estimated according to Lichtenthaler and Wellburn[8].

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$^0$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[9]. Ascorbate peroxidase activity was estimated by observing the decrease in absorbance due to ascorbic acid at 290 nm[10] 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]. 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 Gomez and Gomez[12].

**Results and Discussion**

*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 KWR 108 and Pusa 372 and less in Phule-G 96006 and CSJD 884. However, under high temperature (HT) treatment Pusa 372 and KWR 108 showed significantly higher RWC. Average decline in RWC under high temperature stress was 14.21 %, Pusa 372 showed lowest per cent decline (6.0 %), while CSJD 884 showed highest decline (18.99%) in RWC. Significant differences were also obtained between treatment and genotypic interaction.

*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 372 (31.61 %) closely followed by KWR 108, and highest in Pusa-256. Under HT stress, the Pusa 372 showed lowest MII and CSJD 884 exhibited highest MII (46.47 %). The average increase in MII under high temperature stress was 19.33 %, Pusa 372 showed lowest increase (9.80 %), while CSJD 884 showed highest increase (31.74%) in MII. The interaction between treatment and genotypes was also significant.

*Photosynthetic pigments-* Data on total chlorophyll, total carotenoid, and chlorophyll a/b ratio are reported in (Fig.1). Total chlorophyll, total carotenoid and chlorophyll a/b 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 372 followed by KWR 108 exhibited significantly higher total chlorophyll, and chlorophyll a/b ratio compared to Phule-G 96006 and CSJD 884 under both the conditions. Total carotenoid content was significantly higher in Phule-G 96006 under AT condition along with Pusa 372 and KWR 108. There was a general decline of 15.87 % in carotenoid content in all genotypes under HT condition. Under HT condition CSJD 884 showed higher reduction, while Pusa 372 maintained comparatively higher carotenoid content. Higher carotenoid content in tolerant genotypes Pusa 372 and KWR 108 signifies their tolerance capacity.

*Antioxidant enzyme activities-* Superoxide dismutase (SOD) activity showed significant increase under HT compared to AT condition (Table 3). Among the genotypes, KWR 108 showed significantly higher SOD

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

| Genotypes | Control | Treatment | Genotypic mean | Percent decrease |
|---|---|---|---|---|
| Pusa 372 | 80.21 | 75.49 | 80.35 | 6.00 |
| KWR 108 | 83.67 | 71.40 | 77.53 | 14.79 |
| Phule-G96006 | 78.53 | 65.24 | 71.88 | 17.06 |
| CSJD 884 | 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 372 | 31.61 | 34.67 | 33.14 | 9.80 |
| KWR 108 | 34.21 | 39.87 | 37.04 | 16.68 |
| Phule-G 96006 | 36.28 | 43.16 | 39.72 | 19.10 |
| CSJD 884 | 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 | | | |

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

| Genotypes | Control | Treatment | Genotypic mean | Percent decrease |
|---|---|---|---|---|
| Pusa 372 | 3.80 | 5.92 | 4.86 | 55.9 |
| KWR 108 | 3.82 | 6.20 | 5.01 | 62.4 |
| Phule-G 96006 | 2.72 | 4.75 | 3.74 | 74.7 |
| CSJD 884 | 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 x G | 0.25 | | | |

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

| Genotypes | Control | Treatment | Genotypic mean | Percent decrease |
|---|---|---|---|---|
| Pusa 372 | 6.33 | 11.46 | 8.89 | 81.0 |
| KWR 108 | 6.16 | 9.22 | 7.69 | 49.8 |
| Phule-G 96006 | 6.02 | 7.20 | 6.61 | 19.7 |
| CSJD 884 | 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 x G | 0.50 | | | |

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

| Genotypes | Control | Treatment | Genotypic mean | Percent increase |
|---|---|---|---|---|
| Pusa 372 | 5.43 | 4.63 | 5.03 | 14.8 |
| KWR 108 | 5.29 | 4.06 | 4.67 | 23.3 |
| Phule-G 96006 | 4.76 | 3.44 | 4.10 | 27.6 |
| CSJD 884 | 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 %

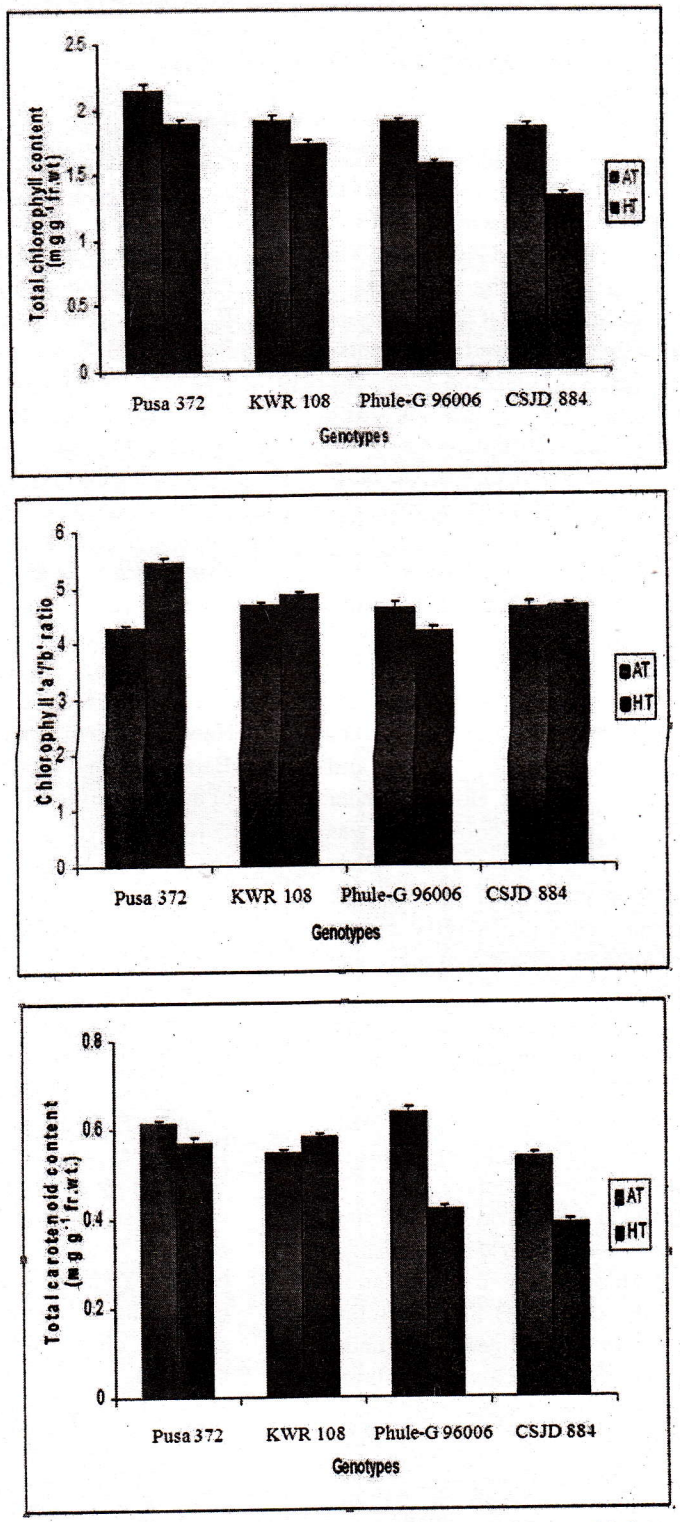


**Fig.1.** Effect of high temperature stress on chlorophyll and carotenoid contents in chickpea genotypes. Vertical bars show ± S.E of mean. Data for treatments (T) and genotypes (G) and TxG interactions were significant (P = 0.05).

activity followed by Pusa 372 and Pusa-256. CSJD 884 exhibited the lowest activity under HT condition. SOD activity increased by two folds in KWR 108 and by 5 % in CSJD 884. Significant interaction was observed between treatment and genotypes.

APX increased significantly under HT condition compared to AT (Table 4). Under both the conditions Pusa 372 possessed higher APX activity, while CSJD 884 showed the lowest activity. KWR 108 and Phule-G 96006 exhibited moderate activity under both the conditions.

Data on glutathione reductase (GR) activity is reported in (Table 5). GR activity decreased significantly under HT condition. Under AT condition Pusa 372 showed higher activity followed by KWR 108. Under HT condition also Pusa 372 and BGD-72 maintained a higher GR activity, while CSJD 884 exhibited a greater decline. Significant interaction was observed between treatment and genotypes for GR activity.

Results on Pearson-product- moment correlation coefficient *(r)* between various antioxidant enzymes, and total chlorophyll content, relative water content and membrane injury index are shown in Table 6. The results revealed that under high temperature condition there exists 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.

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 372 and KWR 108 exhibited a comparatively higher superoxide dismutase, ascorbate peroxidase and slight decline in glutathione reductase compared to susceptible genotypes Phule-G 96006 and CSJD 884. 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 Phule-G 96006 and CSJD 884. Maintenance of lower membrane injury index and high RWC in tolerant genotypes enable them to for better metabolic activities as compared to susceptible genotypes. Hence selection of genotype based on these criteria may help in evolving chickpea genotypes tolerant to high temperature stress with better yield.

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