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Effect of heat stress on physio-biochemical characteristics of chickpea (*Cicer arietinum*) genotypes

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ABSTRACT

A study was conducted with three chickpea genotypes Pusa 256, RSG 888 and JG 11 to study the effect of high temperature stress on various physiological and biochemical parameters. In all the chickpea genotypes high temperature stress decreased RWC, MSI, Chl content, dry matter, leaf area and increased activity of antioxidant enzymes such as POX, GR, and SOD. RSG 888 possessed better seedling growth parameters under high temperature as compared to Pusa 256 and JG 11.

Key words: Antioxidant enzymes, Chlorophyll content, Dry matter, Leaf area, Membrane stability index, Protein profile, Relative water content

Chickpea (*Cicer arietinum* L) is the world's most widely cultivated pulse crop with an area of 10 million hectares. India is the largest producer of chickpea contributing to around 70% of the world's total production.

Chickpea is mostly grown in arid and semi arid regions. The average chickpea yield is about 842 kg/ha in 2010 (FAOSTAT 2010). Despite the high yield potential of chickpea of over 4 000 kg/ha, actual yields are quite low. These low yields are considered to be due to a combination of biotic and abiotic stresses.

Chickpea production is significantly affected by abiotic factors like temperature and drought. Changes in seasonal temperature affect the grain yield of chickpea, mainly through physiological and developmental processes.These processes in turn combine to cause altered phenology, reproductive failure and accelerated senescence.At the physiological level, this damage translates into reduced efficiency of photosynthesis, impaired translocation of assimilates and loss of carbon gain. Heat stress causes reduction in Chl content and Chl/carotenoid ratio. Chl content has been related with photosynthesis (Nagarajan and Nagarajan 2010, Priyanka *et al.* 2010).

The extreme temperature stress causes a rapid and excessive accumulation of reactive oxygen species. These ROS under stress conditions react directly with lipids, proteins and nucleic acids and cause lipid peroxidation mediated membrane injury, protein degradation, enzyme inactivation, pigment bleaching and disruption of DNA strands (Foyer *et al*. 1993, Noctor *et al*. 2007). The equilibrium between the production and scavenging of ROS may be perturbed under adverse abiotic stresses thereby reducing crop yield.

Heat stress injury involves water deficit and cell turgor loss, so plants have developed several strategies, which allow them to survive the adverse conditions to avoid stress (Wang *et al*. 2006).The main objective of this study is to understand the physio-biochemical basis of high temperature stress tolerance in chickpea and to identify a suitable genotype for high temperature stress on the basis of these parameters.

MATERIALS AND METHODS

The experiment was conducted with three chickpea genotypes Pusa 256 (National Check-North Zone, recommended for late planting), RSG 888 (West Zone, drought tolerant), JG 11(South Zone, recommended for early planting and rainfed cultivation).Sowing was done in the 6 inch pots under glass house condition in the National Phytotron Facility for 15 days at normal temperature of 25°C (NT). After 15 days chickpea genotypes were transferred from glasshouse to phytotron chamber and exposed to normal (25°C) and high temperature 35° (HT) stress. The temperature treatment was given up to 30 days. Collection of shoot and root samples was done at various stages of growth, i e 5, 10 and 15 days after treatment for various biochemical and physiological observations.

Leaf relative water content was estimated according to the method described by Barrs and Weatherley (1962).

Membrane stability index (MSI) was determined

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according to the method of Premchandra *et al*. (1990) as modified by Sairam and Dube (1997).

Chlorophyll content was determined according to Hiscox and lsraelstam (1979).

Dry weight was estimated after drying to constant weight in a hot air oven at 70°C for 48 hours and expressed as dry weight (g)/plant.

Leaf area/plant was recorded by automatic leaf area meter (LICOR-3000).

Enzyme extract for peroxidase, glutathione reductase and superoxide dismutase 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). After grinding solution was passed through 4 layers of cheese cloth and filtrate was centrifuged for 20 min at 15 000 g and the supernatant was used as enzyme (Dhindsa *et al*. 1981).

Peroxidase [EC 1.11.1.7] activity was assayed by recording the increase in absorbance due to oxidation of guaiacol to tetraguaiacol (Castillo *et al*. 1984)

Glutathione reductase [EC 1.11.1.7] activity was assayed by recording the increase in absorbance in the presence of oxidized glutathione and DTNB (5, 5-dithiobis-2-nitrobenzoic acid (Smith *et al*. 1988).

Superoxide dismutase [EC 1.15.1.1] activity was estimated by recording the enzyme induced decrease in absorbance of formazone made by nitro-blue tetrazolium with superoxide radicals (Dhindsa *et al*. 1981).

RESULTS AND DISCUSSION

Plants experience high temperature in different ways and adaptation or acclimation to high temperature occurs over different levels of plant organization.

Relative water content

Data on relative water content is reported in Fig 1. RWC in leaves of chickpea genotypes showed a significant decrease under high temperature stress as compared to control and showed a continuous decline with the duration of heat stress.In this study the RSG 888 maintained higher RWC, under both normal and high temperature at different stages of treatment followed by Pusa 256 and JG 11. Percent reduction in RWC was also less in RSG 888 followed by JG 11 and Pusa 256.

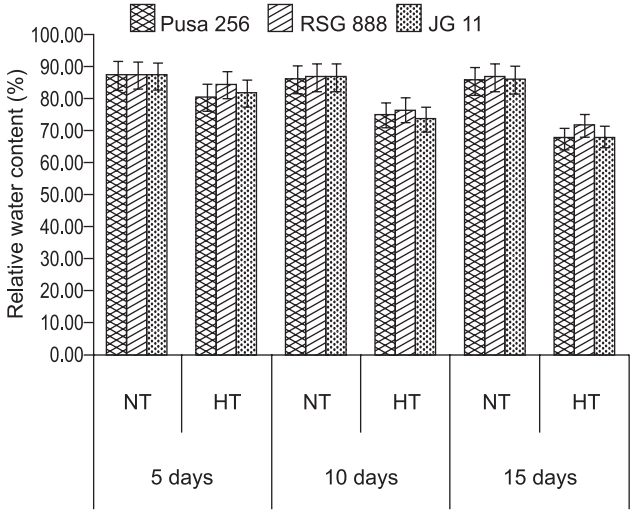

Fig 1 Effect of high temperature stress on relative water content

Relative water content (RWC) represents a useful indicator of the state of water balance of a plant, essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation (Gonzalez and Gonzalez 2001). RWC has been considered as a better indicator of water status as it reflects the balance between water supplied to leaves and transpiration rate through its relation to cell volume. Due to high temperature induced higher transpiration, situation similar to water stress is created and RWC becomes important under heat stress. Various studies have reported that maintenance of favorable water status is essential for plant tolerance to heat stress as it involves water deficit and cell turgor loss (Uprety *et al*. 2007). Heat tolerance may be related to the maintenance of plant water relations by reducing water loss and or increasing water uptake capacity. Decrease in relative water content was a main factor resulting in reduced growth in response to osmotic stress in chickpea. Under drought stress, sensitive chickpea genotypes were more affected by the decline in RWC than tolerant ones (Turner *et al*. 2007).

Membrane stability index

Membrane stability is one of the important parameters to evaluate genetic variability for heat stress (Singh *et al.* 2005). Adverse effect of temperature stress on the membrane leads to disruption of cellular activity or death. Injury to membranes from a sudden heat stress event results from either denaturation of the membrane proteins or from melting of membrane lipids, which leads to membrane rupture and loss of cellular contents (Domonguez-Solis *et al*. 2008).

It was observed that under high temperature condition MSI significantly decreased. There was a continuous decline in MSI as duration of stress increased. Under normal temperature as well as stress the MSI was higher in RSG 888 followed by JG 11and Pusa 256. Under high temperature stress the RSG 888 showed highest membrane stability index at all the three stages, i e 5, 10, and 15 days after treatment.

The heat susceptibility of plasma membrane has been shown by ion leakage studies in many crop plants and the increased leakage of solutes is an indication of damage to membrane (Mittler 2006). However, stable cell membrane that remains functional during stress appears to control adaptation to high temperature and found related to heat and drought tolerance (Sullivan and Ross 1979).

Long term acquisition of heat tolerance in several plants is accompanied by a decrease in fatty acid unsaturation resulting in a decrease in the lipid fluidity and an increase in the phase transition temperature. This leads to the stability of membrane during heat stress. Heat stress induced decrease in membrane stability has been reported in faba bean leaf

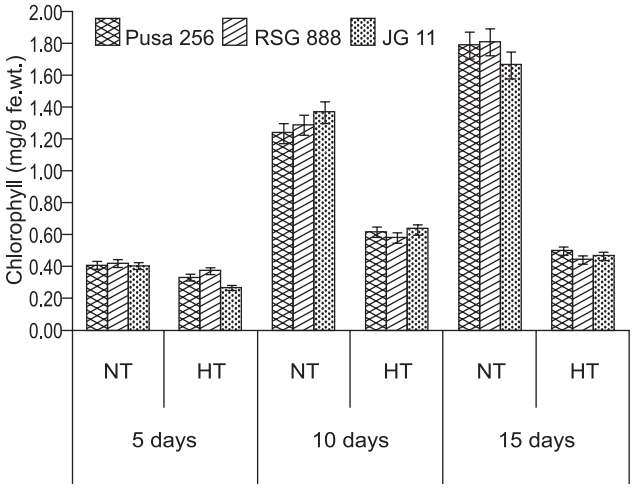

Fig 2 Effect of high temperature stress on chlorophyll content

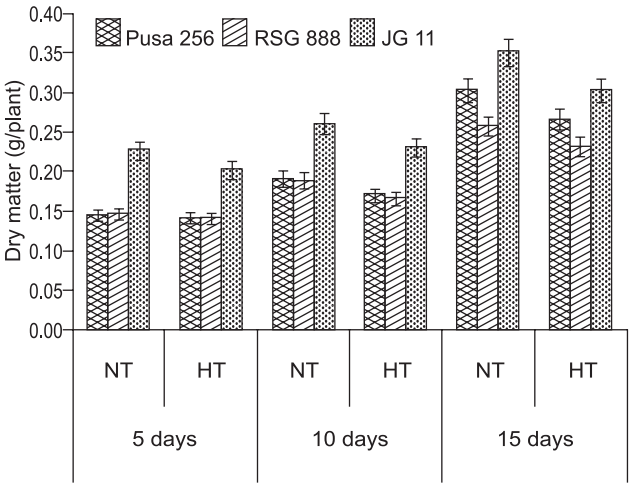

Fig 3 Effect of high temperature stress on dry matter

discs (Raison *et al.* 1980). Deshmukh *et al*. (2006) also reported that the tolerant genotypes possess lower membrane injury index and high RWC, which enable them to maintain better metabolic activities.

Chl content

In the present study among three genotypes there was no significant difference in chlorophyll content under normal as well as high temperature at different stages of treatment though significant decline was observed for all the genotypes under high temperature as compared to control (Fig 2).Under normal temperature as well under stress chlorophyll content was slightly higher in RSG 888 followed by Pusa 256 and JG 11.

Leaf photosynthetic pigment content (chlorophylls and carotenoids) and pigment ratios, such as Chl a/b and chlorophylls/carotenoids are good indicators for stress detection and tolerance (Babani *et al*. 2003).Camejo *et al*. (2005) showed increase in the chlorophyll a/b ratio in stressed Nagcarlang tomato plants, suggesting that this could be used as an indicator of tolerance and physiological status of the plants under stress condition. High Chl a/b ratio was found to be associated with adaptability with low and high temperature. The role of high chlorophyll level under stress situation is a special feature which protects decline in assimilatory system.

Dry matter

Dry matter accumulation is one of the major physiological parameters controlling crop productivity and represents net photosynthesis of the crop. High temperature results in significant decline in dry weight and has adverse effect on all the vigour parameters.

Data on dry matter per plant is reported in Fig 3. Dry matter content per plant increased with the growth of the seedling under normal temperature as well high temperature conditions.However a slight decline as compared to control was observed at different stages of heat stress treatments, decline showed positive correlation with duration of stress. Percentage decrease in dry matter content was minimum in RSG 888 (9.31) followed by Pusa 256 (9.70) and JG 11(12.31).

It is well known that high temperature induces increase in rate of respiration, resulting in loss of stored food material, which results in decline in shoot length and dry weight (Tripathi *et al*. 2009). A given level of stress at vegetative stage can cause a moderate reduction in yield but can eliminate yield entirely if it coincides with reproductive stage. Since the genotypes experienced high temperature stress during vegetative stage for a short period, no significant reduction was observed in leaf area and biomass.

Leaf area

Leaf area increased under normal as well high temperature with the growth of seedlings. However all the genotypes showed a slight decrease as compared to control at all the three stages of treatment.Among the genotypes there was no significant difference under normal and high temperature. However, under both the conditions RSG 888 showed slightly higher leaf area per plant followed by Pusa 256 and JG 11.

Reduction in dry matter of cowpea and greengram due to moisture stress is caused due to reduction in leaf area, absorption of radiation and rate of photosynthesis. Growth and yield are functions of a large number of metabolic processes, which are affected by environmental and genetic factors. Chickpea genotypes under drought condition had lesser leaf area and lesser interception of light. Also leaf area index (LAI) in chickpea genotypes was less than 1 under late sown condition (Collins *et al*. 2008).

Antioxidant enzymes

Glutathione reductase (GR) activity (Table 1) increased under normal as well as under high temperature and showed positive correlation with the growth of the seedlings. GR activity increased significantly under high temperature condition. A similar trend was observed in the root though GR activity par se in root was lower as compared to shoot. GR activity was maximum in RSG 888 genotype under both normal as well high temperature at all the stages, i e 5,

Table 1 Effect of high temperature stress on glutathione reductase activity (nM/min/mg of protein) in shoot of chickpea genotypes

Genotype	5 days		10 days		15 days		Genotypic mean		% Increase
	NT	HT	NT	HT	NT	HT	NT	HT	
Pusa 256	0.55	0.56	8.75	11.43	17.27	29.07	8.86	16.02	44.70
RSG 888	0.32	1.40	9.30	14.13	18.33	29.68	9.32	15.07	38.18
JG 11	1.03	1.31	5.57	7.74	16.38	26.13	7.66	11.73	34.67
CD (P = 0.05)									
Treatment (T) × Genotype (G) = 6.20									
Genotype (G) × Days (D) = 9.30									
Treatment (T) × Genotype (G) × Days (D) = NS									

10, and 15 days after treatment followed by Pusa 256 and JG 11.

Superoxide dismutase activity (SOD) (Table 2) increased under normal as well under high temperature and showed positive correlation with the growth of the seedlings.SOD activity showed significant increase under high temperature compared to normal temperature. A similar trend was observed in the root though SOD activity par se in root was lower as compared to shoot. Among the genotypes, RSG 888 showed significantly higher SOD activity under both normal as well as high temperature conditions followed by JG 11 and Pusa 256. It was also observed that SOD activity is maximum in RSG 888 genotype during high temperature at all the stages, i e 5, 10, and 15 days after treatment.

Data on peroxidase (POX) activity is reported in Table 3. POX activity increased under normal as well under high temperature and showed positive correlation with the growth of the seedlings.Similar trend in POX activity was also observed in root tissue however, POX activity at all the stages of treatment in all the genotypes was higher in root tissue as compared to shoot. Under both high as well as normal temperature, RSG 888 maintained a higher POX activity followed by Pusa 256 and JG 11 in both shoot and root tissue.

Tolerance to high temperature stress in crop plants has been reported to be associated with an increase in antioxidant enzymes activity. In the present study all the antioxidant enzymes, i e POX, GR and SOD showed increased activity under high temperature as compared to control at all the three stages, i e 5, 10 and 15 days after treatment in shoot and root in all the three genotypes. Increase in enzyme activity may be induced by the present reactive oxygen species, thus providing indirect evidence for the extent of generation of reactive species. Sairam*et al* (2006) 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

Table 2 Effect of high temperature stress on superoxide dismutase activity (nM/min/mg of protein) in shoot of chickpea genotypes

Genotype	5 days		10 days		15 days		Genotypic mean		% Increase
	NT	HT	NT	HT	NT	HT	NT	HT	
Pusa 256	274.36	328.76	440.29	676.46	544.77	722.32	419.80	575.84	27.10
RSG 888	407.52	614.55	643.55	842.66	715.35	911.09	588.80	789.43	25.41
JG 11	338.22	383.12	692.15	763.12	736.10	829.65	588.82	658.63	10.60
CD (P=0.05)									
Treatment (T) × Genotype (G) = 58.12									
Genotype (G) × Days (D) =85.34									
Treatment (T) × Genotype (G) × Days(D)=65.92									

Table 3 Effect of high temperature stress on peroxidase activity (nM/min/mg of protein) in shoot of chickpea genotypes

Genotype	5 days		10 days		15 days		Genotypic mean		% Increase
	NT	HT	NT	HT	NT	HT	NT	HT	
Pusa 256	279.21	448.97	325.29	771.43	594.63	1080.48	399.71	766.96	47.88
RSG 888	301.68	510.74	454.87	929.59	504.63	1521.20	420.39	987.18	57.41
JG 11	186.74	422.93	392.33	522.00	615.52	1208.97	398.20	717.97	44.54
CD (P=0.05)									
Treatment (T) × Genotype (G) = 45.46									
Genotype (G) × Days (D) = 55.68									
Treatment (T) × Genotype (G) × Days (D) =78.74									

lower activity. This shows that the tolerant genotypes combated the ROS by maintaining efficient antioxidant mechanism.

Almeselmani *et al.* (2006) also reported similar results in late sown heat tolerant wheat varieties.Higher activity of various antioxidant enzymes in temperature tolerant genotypes of various crop species has also been reported by various workers (Larkhindale *et al*. 2005, Almeselmani *et al*. 2006, Moller *et al*. 2007). Increase in CAT and POX activity under heat stress was also observed by Kaur *et al*. (2009) in *B.juncea* species. The basal activity of APX was also found higher in thermo tolerant genotypes, i e BPR-542-6 when compared to thermo susceptible genotypes, i e NPJ-119.Gulen *et al*. 2008 reported effects of high temperature on the activity of peroxidase (POX) isozymes and leaf proteins. Conversely, total protein content was decreased by heat stress. Although antioxidant enzymes generally do play a role in the antioxidant capabilities of the plant, there is obviously some variability in enzymes among species and genotypes.

Results on Pearson-product-moment correlation coefficient (r) revealed that under high temperature condition there is a significant correlation between antioxidant enzymes and various physiological parameters. POX, GR, SOD showed significant negative correlation with RWC and MSI in shoot and root tissue. Significant positive correlation was also observed with dry matter and leaf area. GR and SOD also showed significant correlation with chl content under high temp.

Almeselmani *et al.* (2006) also reported a significant negative correlation between relative water content and antioxidant enzymes and negative correlation between membrane stability index and antioxidant enzymes. Coefficient of determination was also found lower for RWC and MSI, suggesting that this is very important parameter for maintaining the plant under high temperature stress condition.

CONCLUSIONS

From the foregoing discussion it is clear that exposure of chickpea genotypes to high temperature stress at seedling stage for three different durations resulted in decrease in RWC, MSI, Chl , dry weight, leaf area and also an increase in POX, GR, and SOD. On the basis of present investigation, it is emphasized that tolerant chickpea genotypes to moisture and high temperature stress should possess high relative water content, less injury to leaf tissue (high MSI),high chlorophyll content, high biomass, leaf area and high level of antioxidant enzymes. Such a model genotype possessing these traits will be superior in productivity. The RSG 888 possessed better seedling growth parameters compared to Pusa 256 and JG 11 and can be useful for combating short period of heat stress during initial stage of plant growth.

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