NBU Journal of
Plant Sciences

Vol. 3, March 2009
ISSN No 0974 6927

Heat acclimation and chemical pre-treatments induced thermotolerance in chickpea

C Tongden and U Chakraborty*
Plant Biochemistry Laboratory, Department of Botany, University of North Bengal, Siliguri 734013, India

Abstract
Induced heat tolerance triggered by heat acclimation treatment and foliar application of salicylic acid and abscisic acid were evaluated in three different genotypes of chickpea (*Cicer arietinum* L.) distinctly differing in their sensitivity to heat stress. Seedlings pre-treated with 100µM salicylic acid (SA) and 50µM abscisic acid (ABA) showed improved heat tolerance to a lethal temperature of 46°C than the untreated control seedlings. Heat stress increased lipid peroxidation of membranes and reduced plant survival. Protein and proline contents increased significantly in pre-treated seedlings. Cell membrane stability also increased remarkably in pre-treated seedlings of all three genotypes. Changes in activities of antioxidative enzymes like peroxidase, ascorbate peroxidase in pre-treated seedlings revealed increase in enzymatic activities which declined sharply at lethal temperature. Quantum of increase in enzymatic activity was however higher in thermotolerant genotype in comparison to heat susceptible genotype. Thermotolerant genotype also exhibited constitutively higher antioxidative activities. Catalase activity, in contrast, showed a significant decrease in its activity in pre-treated seedlings following exposure to lethal temperature.These results indicate that heat acclimation treatment and application of SA and ABA show great potential in inducing heat tolerance in chickpea seedlings and these can be further analyzed to understand their role in thermoprotection.

Keywords: *Cicer arietinum* L., abscisic acid, antioxidative enzymes, heat acclimation, salicylic acid, thermotolerance

Temperature stress is often one of the most important abiotic stresses which the plant is exposed to. Plants can be damaged in different ways by either high day or high night temperature or by either high air or high soil temperatures. In nature, however, plants often experience mild stresses before they face severe intensity of stresses and plants may be exposed to multiple environmental stresses either sequentially or simultaneously (Srivalli *et al.*, 2003). Exposure to sub-lethal abiotic stresses renders plants more tolerant to a subsequent normally lethal dose of the same stress, a phenomenon referred to as acclimation. Acquired thermotolerance can be induced in plants by a short acclimation period at moderately high or sub-lethal temperatures or by treatment with other non-lethal stress prior to heat stress (Kapoor *et al.*,1990; Burke *et al.*,2000; Massie *et al.*, 2003 and Larkindale *et al.*, 2005).

Cool season legumes like *Cicer arietinum* L. suffers from high temperature stress often coupled with the drought stress when grown during summer months and is considered unfit for cultivation in warmer seasons and regions. Heat stress in this legume causes significant reduction in growth of seedlings, cell membrane stability, photosynthetic rate, photochemical efficiency and gross yield. Therefore, improvement for the high temperature tolerance in chickpea is vital to stabilize the yield in warmer regions. The present study was designed with the objective to induce thermotolerance in chikpea seedlings by treatment with salicylic acid, abscisic acid and heat acclimation and to determine their role in thermoprotection.

*Corresponding author:
E-mail: chakrabortyusha@hotmail.com

Materials and Methods

Plant materials: Seeds of three different genotypes of chickpea (ICC 4918, ICC 5319 and ICC 5003) selected for this study were obtained from the seed germplasm bank of ICRISAT, Patancheru, Andhra Pradesh, India. These seeds were surface sterilized with 0.1 % $HgCl_2$ and grown in 10-inch size clay pots containing steam sterilized soils at the experimental plot, Department of Botany, University of North Bengal. The seedlings required for the present study were then raised from this seed stock.

Determination of lethal Temperature

For the determination of lethal temperature, the seedlings were exposed to different temperatures ranging from 35-46°C. The treatment of seedlings at elevated temperatures (35, 40, 42, 44 and 46°C) for 2 hrs each revealed that all three genotypes could not tolerate a high temperature of 46°C in which only 10-15% recovery was recorded compared to 100% recovery in 35°C and 80-90% recovery in 40°C treatments. Seedlings treated to 46°C for 2 hrs showed no recovery during the recovery period of 96 hrs when returned to normal growing conditions. Hence this temperature of 46°C was considered as the lethal temperature for the seedling growth in all future experiments.

Table 1 : Influence of temperature stress on biochemical components of chickpea

Varieties & Treatments	Protein content (mg/g tissue)	Proline content (mg/g tissue)	MDA content μMg^{-1} fresh wt.	Relative injury RI (%)
ICC 4918 C	44.05±1.76	0.43±0.02	2.23±0.07	29.83±1.98
SA	43.13±1.15	0.56±0.03	2.10±0.09	30.63±1.54
ABA	41.81±0.58	0.51±0.02	2.21±0.07	34.92±2.10
HA	37.26±1.13	0.48±0.02	2.39±0.08	36.32±2.21
L	31.03±0.04	0.48±0.03	2.60±0.07	40.59±2.35
ICC 5339 C	28.23±1.14	0.29±0.02	1.99±0.06	52.97±1.51
SA	32.71±1.13	0.39±0.02	2.08±0.05	75.59±2.08
ABA	32.02±0.69	0.36±0.02	2.23±0.06	78.07±2.40
HA	31.18±0.60	0.33±0.02	2.80±0.05	82.71±1.53
L	18.09±0.49	0.31±0.01	3.99±0.15	84.02±2.51
ICC 8003 C	30.15±1.14	0.28±0.03	1.83±0.09	39.06±1.98
SA	37.14±1.15	0.39±0.01	1.96±0.06	43.95±2.18
ABA	38.62±1.16	0.41±0.02	1.98±0.06	44.07±2.47
HA	35.68±1.18	0.36±0.02	1.92±0.05	48.96±2.51
L	24.02±2.31	0.31±0.02	2.61±0.06	52.06±2.20

Values are mean of three replicates. C: Control; HA: heat acclimation (42°C-2 hrs.); L: Lethal

Lethal temperature treatments were carried out by directly exposing the seedlings to lethal temperatures of 46°C for 2 hrs duration without any pre-treatments.

Heat acclimation treatment

For heat-acclimation (HA) treatments of seedlings, the seedlings were pre-exposed to elevated but sub-lethal temperatures of 35, 40, 42 and 44°C for 2 hours duration prior to lethal temperature treatment. Best heat acclimation was achieved with the exposure of seedlings to 42°C for 2 hrs. Hence this treatment of 42°C for 2 hours was considered as heat acclimation treatment in all experiments.

Foliar spray treatment: Twenty day old seedlings were sprayed twice a day with 100 µM/L of SA and 50 µM/L of abscisic acid (ABA) solution. The same volume (50 mL) of distilled water was sprayed on control seedlings. The spray treatment was carried out for three (3) consecutive days and finally just prior to exposure to heat stress. The SA and ABA pre- treated seedlings were then subjected to heat stress (46°C for 2 hours) and sampled for experimental purposes.

Biochemical assays

Proteins: Soluble protein was extracted in 0.05 M sodium phosphate buffer (pH 7.2) and protein content was estimated following the method of Lowry (1951).SDS- PAGE analysis of total soluble protein was performed on 10% gel.

Proline: This was extracted from the plant tissue in 3% sulfosalicylic acid and estimated following the method of Bates *et al.*(1973).

Membrane stability: The Cell Membrane Stability (CMS) was tested in terms of relative injury (RI) of the membrane following the method of Martineau *et al.* (1979).

Lipid peroxidation: Malondialdehyde (MDA) content was determined and was used as an index of lipid peroxidation as described by Dhindsa *et al.*(1981). The concentration of MDA was calculated by means of an extinction coefficient of 155 $mM^{-1}cm^{-1}$.

Enzymes : For the extraction of enzymes the plant tissues were macerated in 0.05 M Na_2HPO_4/ NaH_2PO_4 buffer (PH 6.9) and the extract was used for the assay of Peroxidase (POX, EC.1.11.1.7), Ascorbate Peroxidase (APOX, EC 1.11.1.11) and Catalase (CAT, EC 1.11.1.6). POX , APOX and CAT activities were assayed spectrophotometrically following the methods of Chakraborty *et al.*(1993), Asada *et al.* (1994) and Chance and Machly (1955) respectively.

Results and Discussion

Enhanced tolerance of chickpea seedlings to lethal temperature was obtained both with SA and ABA foliar spray treatment and by prior heat acclimation. However, higher concentrations of SA and ABA spray did not improve survival of heat shock and instead led to scorching of leaves. Report by Larkindale and Knight (2002), Dat *et al.*(1998a, 1998b) also implicates the possible role of SA and ABA in induction of thermotolerance in *Arabidopsis* plants and mustard seedlings respectively.

The increased heat protection led to significant increase in protein and proline contents in SA and ABA treated and heat acclimatized seedlings over its corresponding untreated controls (Table 1). One of the most important responses of plants to environmental stresses is in their protein metabolism. They respond to environmental stresses either by dis-assembly of pre-formed polysomes resulting in decrease in translation of mRNAs present at the time of induction and their preferential synthesis of stress proteins from newly transcribed stress mRNAs. Since chickpea is cultivated mostly for its proteins it was expected that temperature stress would affect different genotypes to some degree. Protein contents of seedlings increased following moderate heat treatments but showed a rapid decline at lethal temperature in all genotypes. Protein degradation following prolonged heat treatment was maximum in susceptible genotypes like

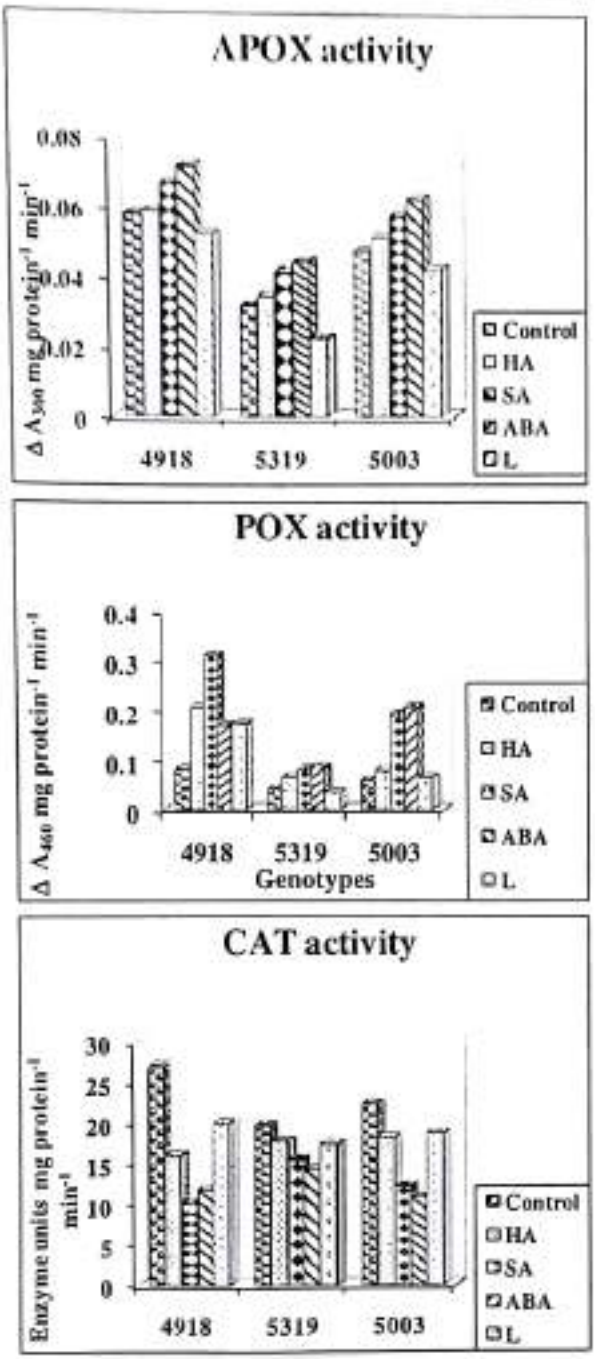

Fig. 1: Changes in activities of antioxidative enzymes in response to lethal temperature treatment in chickpea seedlings

ICC 5319 while it was least in the tolerant genotype ICC 4918.

The most remarkable increase was recorded in the genotype ICC 4918. (Table 1). SDS-PAGE analysis of proteins revealed increase in synthesis and expression of some new proteins in SA and ABA pre- treated plants. Increase in protein content and accumulation of proline in response to various stresses is a well documented phenomenon (Chandra *et al.* 2001) and this has been further confirmed by the results of the present findings.

The cell membrane stability on the other hand decreased with the increasing heat stress and was found to be markedly improved in pre-treated seedlings. The damage of cell membrane was manifested by a dramatic increase in malondialdehyde (MDA) content. However, considerably much lesser MDA accumulation was observed in SA and ABA treated seedlings (Table 1).

Activities of antioxidative enzymes like peroxidase, ascorbate peroxidase, and catalase were analyzed both during temperature stress and induction of thermotolerance by various treatments. It was observed that APOX activities of seedlings decreased on exposure to high lethal temperature but mild temperature treatment prior to lethal temperature resulted in increased activity (Fig. 1). More significant increases were obtained by SA and ABA pre-treatments. APOX gene expression and activity has been reported to be rapidly induced by various stress conditions including chilling (Prasad *et al.*,1994), drought (Mittler and Zilinskas, 1994) and salt stress (Lopez *et al.*, 1996). Larkindale and Huang (2004) reported that in bentgrass APOX activity increased over the first 2 days and 5 days of heating for ACC and $CaCl_2$ respectively but only 12 hrs for H_2O_2 pre-treatment. SA and ABA after pre-treatments had no effect on APOX activity earlier but maintained activity at a significantly higher than in controls after 24 hrs of heating. Jiang and Zhang (2001) also obtained increased activities of APOX in leaves of maize seedlings following ABA treatment. Panchuk *et al.* (2002) reported that heat stress triggers the expression of APX2 gene at the mRNA level and this correlated with the appearance of a new APOX isozyme in *Arabidopsis*.

A pronounced increase in peroxidase activity was observed in all genotypes following heat stress, which was more significant in pre-treated seedlings (Fig. 1). Genotype tolerant to temperature stress was found to have higher constitutive activity than the susceptible one. Exposure to high temperature increased activities in the tolerant genotype while it decreased in the susceptible one. Maximum activity was obtained in SA pre-treatments. In a study with wheat genotypes Gupta and Gupta (2005) reported that exposure to high temperature increased POX activity, which was higher in the tolerant genotype C-306. Peroxidases are often the first enzymes to alter their activities under stress. Enhanced activities have been observed in rice seedlings under anoxia (Lee and Lin, 1995) and low temperature stress (Oidaira *et al.*, 2000). Chakraborty *et al.* (2002) also obtained increased POX activity following water stress in tea plants. Thus, POX would seem to be generally involved with the plants' response to various types of environmental stresses.

Catalases are tetrameric heme containing enzymes that catalyze the breakdown of H_2O_2 to H_2O and O_2. Catalase is indispensable for ROS detoxification during stress (Willekens, 1995). This is also due to the fact that there is proliferation of peroxisomes during stress which might help in scavenging of H_2O_2 diffusing form the cytosol (Lopez Hupertez, 2000). However, reports on effects of stresses on CAT activities vary. Jiang and Huang (2001) showed that CAT activities declined under drought, heat and a combination of the two

stresses. Results of the present study also revealed that CAT activity decreased during high temperature stress in all genotypes. On the other hand, CAT activity also decreased during induction of thermotolerance by pre-treatments. Dat *et al.* (1998b) working with induction of SA or heat acclimation (HA) in mustard seedlings reported a parallel decrease of both H_2O_2 and CAT during the initial period of thermoprotection. The decline in H_2O_2 content may be indicative of the enhanced antioxidant potential in the tissue, which could contribute to enhanced thermotolerance. CAT activity reached a minimum during the thermoprotection period but the reason for this still remains unknown. H_2O_2 production being an ongoing process in plants, inhibition of CAT activity-one of the main routes of H_2O_2 degradation could result in H_2O_2 accumulation which would then activate defense related genes by acting as a second messenger (Keshamma *et al.*, 2004). The observed decrease in CAT activity during induction of thermotolerance in the present study (Fig. 1) may also be due to the above mechanism of accumulation of H_2O_2 during initial stages. Larkindale and Huang (2004) also obtained lower CAT activities in *Agrostis stolonifera* plants treatd with SA, $CaCl_2$, H_2O_2 and HA over the control plants prior to heating and within 48 hrs of heat stress.

In conclusion, the present study shows that SA and ABA pre- treatments induce thermoprotection in chickpea seedlings. The thermoprotection obtained with these treatments though similar to that obtained by heat acclimation is more efficient in reducing physiological wilting and conferring longer period of protection. The thermotolerance induced by such pre-treatments may be associated at least in part, with the control and / or prevention of oxidative damage.

Acknowledgements

Ms. Cyuria Tongden gratefully acknowledges the financial assistance received from Council of Scientific and Industrial Research (CSIR), New Delhi, India, in the form of CSIR Ad- hoc fellowship. Authors also wish to thank Dr. H.D. Upadhayaya of ICRISAT Patancheru, Andhra Pradesh for providing the seeds for carrying out this work.

References

Asada K. 1984. Chloroplasts: Formation of active oxygen and its scavenging. *Methods Enzymol.* 105: 422-429.

Bates HS., Waldren RP., Treane, D. 1973. Rapid estimation of free proline for water stress determination. *Plant Soil.* 39: 205-207.

Burke JJ., O'Mahoney PJ, Oliver, MJ. 2000. Isolation of *Arabidopsis* mutants lacking components of acquired thermotolerance. *Plant Physiol.* 123:575-588.

Chakraborty U., Dutta S, Chakraborty BN. 2002. Responses of tea plants to water stress. *Biol. Planta.* 45(4):557-562.

Chakraborty U, Chakraborty BN, Kapoor, M. 1993. Changes in the levels of peroxidase and phenyl alanine ammonia lyase in *Brassica napus* cultivars showing variable resistance to *Leptosphaeria maculans*. *Folia Microbiol.* 38: 491-496.

Chance B, Machly, AC. 1955. Assay of catalases and peroxidases. *Methods Enzymol.* 2:764-775.

Chandra A., Anand A., Mandal PK, Saxena, P. 2001. Influence of Salicylic Acid on protein content and catalase activity in relation to systemic acquired resistance in cowpea against root rot. *Indian Phytopath.* 54: 284-287.

Dat JF, Foyer CH, Scott, IM. 1998 a.Changes in salicylic acid and antioxidants during induced thermotolerance in mustard seedlings. *Plant Physiol.* 118:1455-1461.

Dat JF, Lopez- Delgado H, Foyer CH, Scott, IM.1998 b. Parallel changes in H_2O_2 and catalase during thermotolerance induced by salicylic acid or heat acclimation in mustard seedlings. *Plant Physiol.* 116: 1351-1357.

Dhindsa RS, Plumb-Dhindsa P, Thorpe, TA.1981. Leaf senescence: correlated with increased leaves of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 32: 93-101.

Gupta S, Gupta, NK. 2005. High temperature induced antioxidative defense mechanism in seedlings of contrasting wheat genotypes. *Indian J. Plant Physiol.* 10 (1):73-75.

Jiang Y, Huang, B. 2001. Drought and Heat Stress Injury to Two Cool- Season Turfgrasses in Relation to Antioxidant Metabolism and Lipid Peroxidation. *Crop Sci.* 41: 436-442.

Jiang M, Zhang, J. 2001. Effect of abscisic acid on active oxygen species, antioxidative defence system and oxidative damage in leaves of maize seedlings. *Plant Cell Physiol.* 42(11):1265-1273.

Kapoor M, Sreenivasan GM, Goel N, Lewis, J.1990. Development of thermotolerance in *Neurospora crassa* by heat shock and other stresses eliciting peroxidase induction. *J Bacteriol.* 172:2798-2801.

Keshamma E, Jayalakshmi SK, Sreerumulu, K. 2004. Effect of salicylic acid, spermine and cinnamic acid on some defense enzymes of chickpea (*Cicer arietinum* L.) CV JG 62. *J. Plant Biol.* 31(1):13-20.

Larkindale J, Hall JD, Knight MR, Vierling, E. 2005. Heat stress phenotypes of *Arabidopsis* mutants implicate multiple signaling pathways in the acquisition of thermotolerance. *Plant Physiol.* 138:882-897.

Larkindale J, Huang, B. 2004. Thermotolerance and antioxidant systems in *Agrostis stolonifera*: involvement of salicylic acid, abscisic acid, calcium, hydrogen peroxide, and ethylene. *J. Plant Physiol.* 161(4):405-13.

Larkindale J, Knight, MR. 2002.Protection against heat stress-induced oxidative damage in *Arabidopsis* involves calcium, abscisic acid, ethylene, and salicylic acid. *Plant Physiol.* 128: 682-695.

Lee TM, Lin, YN. 1995. Changes in soluble and cell wall-bound peroxidase activities with growth in anoxia-treated rice (*Oryza sativa* L.) coleoptiles and roots. *Plant Sci.* 106:1-7.

Lopez-Huperter E, CharltonWL, Johnson B, Graham IA, Baker, A. 2000. Stress induces peroxisome biogenesis genes. *Embo J.* 19:6770-6777.

Lopez F, Vansuyt G, Casse-Delbart F, Fourcroy, P. 1996. Ascorbate peroxidase activity not the mRNA level, is enhanced in salt-stressed *Raphanus sativus* plants. *Physiol.Plant.* 97:13-20.

Lowry OH, Rosebrough NJ, Farr AL, Randall, RJ. 1951. Protein measurement with folin phenol reagent. *J. Biol.*

Chem. 193:265-275.

Martineau JR, Specht JE, Williams JH, Sullivan CY 1979. Temperature tolerance in soybeans. I. Evaluation of a technique for assessing cellular membrane thermostability. *Crop Sci.*19:75-78.

Matsse MR, Lapoczka EM, Boggs KD, Stine KE, White, GE. 2003. Exposure to the metabolic inhibitor sodium azide induces stress protein expression and thermotolerance in the nematode *Caenorhabditis elegans*. *Cell Stress Chaperones* 8:1-7.

Mittler R, Zalinskas, BA. 1994. Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *Plant J.* 5:397–405.

Oidaira H, Sano S, Koshiba T, Ushimaru, T 2000. Enhancement of antioxidative enzyme activies in chilled rice seedlings. *J. Plant Physiol.* 156:811-813.

Panchuk IP, Volkov RA, Schoffl, F. 2002. Heat stress- and heat-shock transcription factor- dependent expression and activity of ascorbate peroxidase in *Arabidopsis*. *Plant Physiol.* 129:838-853.

Prasad TK, Anderson MD, Stewart, CR. 1994. Acclimation, hydrogen peroxide, and abscisic acid protect mitochondria against irreversible chilling injury in maize seedlings. *Plant Physiol.* 105:619-627.

Sevalli B, Sharma G, Khanna-Chopra, R 2003. Antioxidative defense system in an upland rice cultivar subjected to increasing intensity of water stress followed by recovery. *Physiol. Plantz.* 119(4):503.

Willekens H, Inze D, Van Montagu M, Van Camp, W 1995. Catalases in plants. *Mol. Breeding* 1:207-228.