DISCUSSION

Plants are exposed to various environmental stresses throughout the course of their life span and hence have an inbuilt ability to adjust to seasonal and other environmental variables. The ability to withstand and to acclimate to supra-optimal temperatures results from both prevention of heat damage and repair of heat sensitive components (Larkindale *et al.*, 2005). Organisms must also maintain metabolic homeostasis during stress or be able to re-establish homeostasis subsequent to the stress period. Apart from the regular circadian and seasonal perturbations, there may be certain other rapid and unpredicted disturbances in the environment resulting in stressful conditions (Grover *et al.*, 2001). Abiotic stress negatively influences survival, biomass production and grain yield of most crops. Different crop eco-systems are affected by different abiotic stress factors and to a differential extent. The degree of susceptibility of different plant species and of different genotypes within the species is often varied. There is also some level of variation associated with specific developmental stages of the plant.

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).

Chickpea (*Cicer arietinum* L.) is one of the important cool season legumes of India and grows best at 18-25°C. With the potentially serious effects of radical global temperature change on agriculture, in the near future it is expected that by the later half of twenty-first century global warming would seriously jeopardize agriculture, forestry and other industries using the natural environment (Iba, 2002). However, although much research is being conducted to evaluate the effects of

global warming on plant growth and productivity, effects to search for specific and practical approaches to improve the adaptability of plants to their temperature environments have only recently begun (Grover *et al.*, 2000, Sharkey, 2000).

In the present study, therefore, attempts have been made to identify genotypes of *Cicer arietinum* L. showing temperature tolerance and to further induce tolerance through various treatments. At the onset, seeds of fifteen genotypes of *Cicer arietinum* were exposed to elevated temperatures upto 55°C and the effect of elevated temperatures on seed germination and seedling growth were evaluated. Germination of seeds was found to be retarded at 50°C and completely inhibited at 55°C which was considered as the lethal temperature for seed germination. Seedlings of most genotypes could not tolerate a maximum temperature of 46°C. Based on tolerance index (TI) of different genotypes, they could be roughly categorized into tolerant (ICC 4918, ICC 1852, ICC 2042, ICC C37, ICC V2 and ICC V10), moderately tolerant (ICC 6119, ICC 14340, ICC 5003 and ICC 4969) and susceptible (ICC 5319, ICC 10035, ICC 16359, ICC 7344 and ICC V1). Tolerance and susceptibility of the different genotypes to temperature stress was further confirmed by cell membrane stability (CMS) test. Similar findings in CMS test to that of tolerance index (TI) confirmed the genotypic variations with respect to temperature tolerance. In a study by Porch (2006), stress tolerance index (STI), stress susceptibility index (SSI) and geometric mean (GM) were used to evaluate the genotypic performance of 14 genotypes of common bean under variable temperature conditions. Their results also indicated that it was possible to identify superior genotypes for heat tolerance based on their indices. In their evaluation of heat tolerance indices, STI and GM although correlated were found to be effective stress indices for the selection of genotypes with good yield potential under stress and low stress conditions. Several previous authors have also confirmed the importance of using thermostability of cell membranes for screening heat tolerant genotypes (Sadalla *et al.*, 1990; Moffet *et al.*,1990 and Agarie *et al.*, 1995). Talwar *et al.* (2002) could group groundnut genotypes into two groups based on the relative injury of cell membranes caused by high temperature.

Chickpea seeds as well as seedlings were treated with known signaling molecules like salicylic acid (SA), calcium chloride ($CaCl_2$), abscisic acid (ABA)

and also to a sub-lethal temperature treatment prior to exposure to lethal temperature in order to induce thermotolerance. It was observed that SA and $CaCl_2$ pre-treatments enhanced the rate of germination while ABA initially inhibited germination to a certain level. Seedlings pre-treated with the chemicals or subjected to heat-acclimation treatment before exposure to lethal temperature showed more tolerance in comparison to the untreated seedlings directly exposed to lethal temperature. High degree of thermotolerance was induced by these treatments in the heat susceptible genotypes – ICC 5319, ICC 10035, ICC 7344 and ICC V1. Similar results have also been reported previously by workers who have shown that pre-treating plants with certain endogenous signaling compounds or pre-exposing plants to mild heat stress can induce thermotolerance (Dat *et al.*,1998a and b; Larkindale and Knight, 2002 and Larkindale and Huang, 2004). SA had been the focus of attention of researchers mainly because of its ability to induce protection against plant pathogens (Raskin, 1992; Katoch *et al.*, 2003). However it has now been shown that SA has an equally important role to play for induction of thermotolerance (Dat *et al.*,1998a and Larkindale and Knight, 2002). Larkindale and Huang (2004) showed that pre-treatment with SA, ABA, ACC, $CaCl_2$, and H_2O_2 improved the tolerance of creeping bentgrass to heat stress. The leaves of treated plants remained greener and shoot density was higher than untreated control plants during heat stress. The observed effects of chemical treatments were similar to the effects of acquired thermotolerance by pre-exposure of plants to mild heat stress. In the present study, also, it was observed that while exposure of seedlings to lethal temperature resulted in wilting and collapse of seedlings, pre-treatment with the chemicals or heat acclimation led to lesser wilting of the seedlings. Besides the other treatments in the present study, seeds or seedlings were also pre-treated separately with a bacterium *Bacillus megaterium* with known plant growth promoting activity. The idea behind the treatment was that since *Bacillus megaterium* increases plant growth and enhances vigour such plants may show more tolerance to temperature. Results of this treatment also showed that it was equally effective as SA or $CaCl_2$ in inducing thermotolerance. Only a few PGPR strains have been studied previously for their capacity to enhance plants' tolerance of environmental stresses. Plants with reduced ethylene level due to application of PGPR showing 1, amino cyclopropane, 1

carboxylic acid (ACC) deaminase activity revealed a substantial tolerance to flooding stress (Grichko and Glick, 2001) and metal contaminants (Nie *et al.*,2002). Hu and Kloepper (2003) reported that PGPR treatments increased tomato seedling survival rate and enhanced the shoot weight even under heat stress conditions.According to them the response of PGPR treated plants subjected to heat stress mimicked the classic heat-shock response.

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. The response of chickpea seeds and seedlings of various genotypes subjected to high temperature as well as various pre-treatments, with respect to the quantitative changes in protein as well as nature of proteins were analyzed in heat stress condition. Protein content of seeds varied with the genotypes. Protein contents of seeds and 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 ICC V1, ICC 7344 and ICC 10035 while it was least in the tolerant genotypes (ICC 4918, ICC 1852 and ICC C-37). Induction of thermotolerance by pre-treatments with chemicals or PGPR treatment was accompanied by an increase in the protein content. New proteins, mostly in the small and intermediate molecular weight range were observed following heat-acclimation, SA, ABA and $CaCl_2$ treatments. The exposure to lethal temperature showed a considerable reduction in the number of protein bands and also revealed genotypic differences in the protein profile. Low molecular weight heat shock proteins have been shown to be exclusively expressed in plants in response to heat stress (Agarwal *et al.*, 2003) Apart from induction by heat stress, there are also reports that show induction of low molecular weight heat shock proteins (HSPs) by other stresses. Almoguera and Jordano (1992) noted expression of Hasp 17.4 transcript in seedlings exposed to ABA and other osmotic shock. Similar results have also been reported by other workers during water and other osmotic stresses (Coca *et*

al., 1996; Pla *et al.*, 1998 and Sun *et al.*, 2001). The positive correlation noted between the synthesis of HSPs and development of thermotolerance in time dependent and temperature dependent manner in several studies as well as in the present study suggest that accumulation of HSPs is an essential component of the protection process. Studies with *Arabidopsis* plants containing an antisense DNA sequence that reduces HSP 70 synthesis showed that the high temperature extreme at which the plants could survive was reduced by 2°C compared with controls although the mutant plants grew normally at optimum temperature (Lee and Schoffel,1996). Presumabably, failure to synthesize the entire range of HSPs that are usually induced in the plants would lead to a much more dramatic loss of thermotolerance. Other studies with both *Arabidopsis* mutants (Hong and Vierling, 2000) and transgenic plants (Queitsch *et al.*,2000) demonstrate that at least HSP 101 is a critical component of both induced and constitutive thermotolerance in plants. The accumulation of 104 kDa protein in rice in response to several abiotic stresses including high temperature was reported by Singla *et al.* (1998). In the present study, however more of low molecular weight proteins were induced during thermotolerance.

Heat stress is known to have a complex impact on cell function indicating that many processes are involved in thermotolerance. Some processes may be specific to basal thermotolerance, others may be induced during acquired thermotolerance and many may be involved in both (Larkindale *et al.*, 2005).Though the best characterized aspect of thermotolerance is the production of heat shock proteins, several lines of evidence indicate that HSP synthesis is only one aspect of protection against heat induced damage.High temperatures are known to affect membrane linked processes due to alteration in membrane fluidity and permeability (Alfonso *et al.*,2001; Sangwan *et al.*,2002). Enzyme function is also sensitive to changes in temperature. Heat induced alterations in enzyme activity can lead to imbalance in metabolic pathways or heat can cause complete enzyme inactivation due to protein denaturation (Vierling, 1991; Kampinga *et al.*,1995). Membrane and protein damage lead to the production of active oxygen species (AOS) that cause heat induced oxidative stress (Dat *et al.*, 1998 a, 1998b; Gong *et al.*, 1998a,b Larkindale and Knight, 2002; Suzuki and Mittler, 2006). These different types of

damage translate into reduced photosynthesis, impaired translocation of assimilates and reduced carbon gain leading to altered growth and production (Hall, 2001).

Heat stress induced oxidative damage and scavenging by antioxidative enzymes and non enzymatic antioxidants were evaluated in chickpea genotypes in the present study. Activities of antioxidative enzymes like ascorbate peroxidase (APOX), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR) and peroxidae (POX) were analyzed both during temperature stress and induction of thermotlerance 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 in most of the genotypes. More significant increases were obtained by SA and $CaCl_2$ pre-treatments. Maximum activity was seen in ICC 4918 following $CaCl_2$ pre-treatment. APOX is one of the most important antioxidative enzymes of plants that detoxify H_2O_2 using ascorbate for reduction. It has a higher affinity to H_2O_2 than CAT and POX and it may have a more crucial role in the management of reactive oxygen species (ROS) during stress or it may be responsible for fine modulation of ROS for signaling (Srivalli *et al.*, 2003). 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*. It was shown previously that mRNA levels of pea and *Arabidopsis* APX 1 gene were induced by heat stress and oxidative stress and there was evidence that in *Arabidopsis* heat induction of APX 1 requires an HSE sequence present in the promoter upstream region of APX 1 (Storozhenko *et al.*, 1998).

Peroxidase activity in chickpea seedlings was found to vary among the different genotypes. Genotypes tolerant to temperature stress were found to have higher constitutive activity than the susceptible ones. Exposure to high temperature increased activities in the tolerant genotypes while it decreased in the susceptible ones. All pre-treatments followed by lethal temperature led to an increase in activity in all genotypes. Maximum activity was obtained in SA pre-treatments. Seed treatment with SA was also found to enhance POX activity in seeds and roots of chickpea (Keshamma *et al.*, 2004). 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. In mulberry, increase in salinity was found to induce higher activity of POX (Harinasut *et al.*, 2003). In the present study it was observed that pre-treatments not only enhanced activities but also induced two new isozymes of the enzyme. Thus, POX would seem to be generally involved with the plants' response to various types of environmental stresses. Though in the present study, increased POX activity was obtained following induction of thermotolerance, this was not in conformity with the results of Larkindale and Huang (2004) in creeping bent grass. They found that SA and ABA pre-treatments had no effects on POX activity, ACC treatment significantly increased activity while $CaCl_2$, H_2O_2 and heat acclimation reduced activity particularly during later phase of heating.

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. Increased, decreased or unchanged CAT activities under drought stress have been observed (Smirnoff, 1993; Zhang and Kirkham,1994; Castillo,1996). 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. Isozyme analysis of catalse also did not reveal any induction of new isozymes. 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. They suggested that the metabolic and molecualar mechanisms associated with the observed decline in H_2O_2 content and CAT activity during this period may be relevant to thermotolerance.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. It would seem possible that the changes in CAT activity may vary according to intensity of stress, time of assay and induction of new isozymes. The dual role of H_2O_2 as a signaling molecule as well as the toxic metabolite could make it a very variable enzyme. 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 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. ABA and ACC pre-treatments maintained higher CAT activity than controls after 48 hrs of heating.Increased activity following ABA treatments have also been reported by Jiang and Zhang (2001) in maize seedlings.

Superoxide dismutases (SOD) are very important reactive oxygen species (ROS) scavenging enzymes and they catalyze the dismutation of O_2^- into H_2O_2. It has been reported that under stress conditions different plants and tissues respond differently with regard to SOD induction suggesting that different mechanisms may be involved in protection against oxidative stress (Blokhina *et al.*, 2003). The reduced activity of SOD leading to accumulation of singlet oxygen has been shown in flooding stress in maize (Yan *et al.*, 1996). While no significant differences in

SOD activity in two cultivars of rice differing in sensitivity to chilling was observed (Saruyama and Tanida, 1995), other studies have reported increase in SOD activity in tolerant cultivars compared to susceptible ones (Hernandez *et al.*, 1993). Similar results were also reported by Pal *et al.* (2004) who reported that though SOD activity increased under salt stress in all three tested genotypes increase in the tolerant genotype was higher. In the present study, while the exposure of chickpea seedlings to lethal temperature decreased SOD activity, induction of thermotolerance by various pre-treatments enhanced activity over control. Maximum enhancement was obtained due to $CaCl_2$ and SA pre-treatments while heat-acclimation did not significantly increase activity. It was also observed that increase was higher in the tolerant cultivars. It would seem that the increased activity of SOD might contribute to the protection of plants from oxidative injury during induction of thermotolerance. SOD was also reported to be enhanced continuously with increase in temperature in two wheat genotypes though the magnitude was comparatively lower in the susceptible genotypes (Gupta and Gupta, 2005). Authors suggested that the comparatively higher increment of SOD activity in tolerant genotypes might have decreased the possible toxic concentration of O_2^- radical more effectively. Mazorra *et al.* (2002) also reported a role of SOD activity in imparting temperature stress tolerance to tomato. Larkindale and Huang (2004) however, reported that SA and ABA pre-treatments for thermotolerance induction had no effects on SOD activity under heat stress while pre-treatment with ACC, $CaCl_2$ or heat acclimation (HA) increased activity to some extent.

Glutathione reductase (GR) activity was found to be enhanced in pre-treated seedlings while a decrease in activity was observed during high temperature exposure. However, the decrease in activity of susceptible genotypes was greater. SA, ABA and $CaCl_2$ pre-treatments were most effective in enhancing activities. Jiang and Zhang (2001) reported increased activities of GR in maize seedlings following ABA treatment. Jiang and Huang (2001) obtained decreased activity of GR under heat stress, which would have resulted in H_2O_2 accumulation. Since GR catalyzes the NADPH dependent reaction of the disulphide bond of GS-SG and is responsible for maintaining the reduced pool of glutathione, increased GR activity would facilitate improved stress tolerance (Tyystjärvi *et al.*, 1999). Increase in GR

activity has been reported during water stress in various plants (Pastori and Trippi, 1993; Jiang and Huang 2001) and has also been correlated with increased resistance to paraquat exposure (Broadbent *et al.*, 1995). Increase in the activity has been linked with the increase in synthesis of proteins (Edwards *et al.*, 1994). Over expression of GR in chloroplast has been reported to confer increased antioxidant protection to cold induced photo inhibition (Foyer *et al.*, 1995).

Besides antioxidative enzymes a few non-enzymatic antioxidants are also known to be involved in scavenging of ROS of which ascorbate and carotenoids are most well known. Ascorbate is one of the most extensively studied antioxidants and has been detected in majority of plant cell type organelles and apoplast. Carotenoids also function as antioxidants in several cases. The present study indicated that both ascorbate and carotenoids were reduced following exposure to high lethal temperature, which was greater in the susceptible genotypes. However, all pre-treatments increased both of the antioxidants to a certain degree. ABA did not induce any significant changes in the content of antioxidants in the leaves of maize seedlings within first 12 hrs of treatment (Jiang and Zhang, 2001). Reduction in ascorbate content in response to drought was reported in sorghum (Zhang and Kirkham, 1996) and wheat leaves (Bartoli *et al.*,1999). Agarwal and Pandey (2003) on the other hand obtained increased ascorbate content in *Cassia* seedlings subjected to water stress which were able to adapt themselves to the stress. Mahan *et al.* (2006) also showed that oxidative damage resulting from temperature extremes in cotton seedlings (*Gossypium hirsutum* L.) cultivar Fibermax 958 could be by mitigated by minor alterations in the antioxidant metabolism.

The results of the present study and those of other studies point to the role of ROS scavenging antioxidant mechanism in inducing tolerance to stresses including temperature stress. It is interesting to note that while ROS have the potential to cause oxidative damage to cells during environmental stress, they may also play a key role in plants as signal transduction molecules involved in mediating responses to pathogen infection, environmental stresses, programmed cell death and developmental stimuli (Mittler *et al.*,2004; Torres and Dangl, 2005; Suzuki and Mittler,2006). The rapid increase in ROS production referred to as 'oxidative' burst was shown to be essential for many of these processes and genetic studies have

shown that respiratory burst oxidases homologue (Rboh) genes encoding NADPH oxidases are the main producers of signal transduction associated ROS in cells during these processes. Thus the two somewhat opposing faces of ROS i.e., the damaging toxic molecule on one hand and beneficial signal transduction molecule on the other might explain the somewhat confusing results obtained in some cases in relation to the detoxifying antioxidant systems.

Oxidative stress which induces the production of free radicals can result in lipid peroxidation causing membrane damage. Larkindale and Knight (2002) found that treatment of *Arabidopsis* at 40°C caused a significant increase in lipid peroxidation after 2 days which increased even further after 3 days. In contrast, seedlings treated in the same way but subjected to a prior treatment at 30°C for 1hr showed no significant increase in lipid peroxidation over three days. Results of the present study indicated that lethal temperature led to an increase in lipid peroxidation as measured by malondialdehyde (MDA) content in all genotypes. This effect was partially overcome by the pre-treatments where lipid peroxidation though higher than the untreated controls were lesser than the lethal temperature treatment. Similar results were also obtained by Larkindale and Huang (2004) in creeping bentgrass where lipid peroxidation increased during heat stress but was lowered by pre-treatments with chemicals and sub-lethal temperature. Drought and heat stress have also been reported to increase MDA content in tall Fescue and Kentucky blue grass similar to what has been found in other species (Rensburg and Kruber 1994; Behl *et al.* 1996; Gong *et al.*, 1997; Liu and Huang, 2000; Jiang and Zhang, 2001).The increase in MDA content according to Jiang and Huang (2004) may be related to reduction in SOD, CAT, APOX and GR activities.

Disruption of membrane stability was found to be a major result of imposition of temperature stress in chickpea seedlings. Relative injury to the membranes was much lesser in the tolerant genotypes than in the susceptible ones. Similarly, induction of thermotolerance by pre-treatments also decreased the injury to membranes. Higher thermostability of cell membranes and photosynthesis has been reported to contribute to adaptation at high temperature in several crops (Shannan *et al.*, 1990; Ibrahim and Quick, 2001; Talwar *et al.*,2002). Photosynthesis is one of the physiological processes that are most sensitive to high temperature

stress (Yang *et al.*, 2005). Inhibition of photosynthesis by high temperature stress is of common occurence for plants in tropical and sub-tropical regions and the temperate zones where the plants are exposed periodically to high temperatures (Larcher, 1995). Hence the effect of temperature stress on chlorophyll contents and Hill activity were also determined in the present study, as these would indicate disruptions in photosynthesis. Both chlorophyll contents and Hill activity decreased due to lethal temperature treatment. Pre-treatments reversed the effects to a certain degree and among all treatments SA and $CaCl_2$ were most effective. Since Hill activity was affected it would seem that PS II was inhibited. Previous studies also report that photosystem II is inhibited by severe heat stress with temperatures higher than 45°C (Havaux, 1993, 1996; Sharkey, 2000). However, Aarti *et al.* (2006) reported that oxidative stress showed greater impact on chlorophyll biosynthesis than on photosystem II in *Cucumis sativus* (cucumber). The authors also suggested that oxidative stress impedes key steps in chlorophyll biosynthesis by either directly or indirectly inhibiting the activity of Mg-chelatase, Fe-chelatase and protoporphyrinogen IX oxidase.

Studies were also conducted on the effect of temperature stress on other important biochemical components i.e. proline, carbohydrates and phenols. Proline showed an increase in accumulation in all treatments including lethal temperature treatment. Proline being a stress metabolite is known to increase under various stresses (Kramarova *et al.*, 1999; Chakraborty *et al.*, 2002; Agarwal and Pandey, 2003). Total sugar contents of seedlings were found to decrease during the lethal temperature treatment, which was most significant in the susceptible genotypes. Pre-treatments could increase the contents to a certain degree. Similar trend was also observed in case of reducing sugars. Xu and Huang (2001) suggested that roots play more important role than shoots in the mediation of carbohydrate responses to high air temperatures or high soil temperatures. High soil temperature alone or combined with high air temperature causes imbalance between photosynthesis and respiration and decrease in carbohydrate availability, which could contribute to the decline in shoot and root growth under high temperature conditions. Analyses of phenols which are known in some cases to offer protection to various stresses were also carried out. Few of the phenolic acids commonly present were detected by HPLC analysis of

which Ferulic and Chlorogenic acids were recorded in tolerant genotypes subjected to SA and PGPR treatments. Sarma *et al.*, (2002) reported that PGPR could elicit alterations in phenolic profiles of chickpea subjected to biotic stress. Chakraborty *et al.* (2001) obtained increased levels of phenols in tea leaves subjected to temperature stress upto 45°C, and a decline thereafter.

Finally, induction of thermotolerance obtained in seedlings was confirmed in callus cultures. It was observed that temperatures of 36-40°C were lethal to callus formation but a prior acclimatization at 32°C for 2 hrs made the calli more tolerant to the lethal temperature. Similarly, supplementation of media with SA(10^{-5} and 10^{-6}M) also conferred tolerance for growth of calli. Lopez-Delgado (1998) also reported that low concentration of acetyl SA in culture medium improved tolerance of a 5 week high temperature (35°C) treatment.

In conclusion, it might be stated that exposure of chickpea seedlings to a lethal temperature of 46°C resulted in an overall change in several metabolic pathways. Temperature stress was observed to cause an oxidative stress that was however overcome by pre-treatment with SA, ABA, $CaCl_2$ as well as HA which could induce thermotolerance to the seedlings by exposure to a sub-lethal temperature. Among all treatments, SA and $CaCl_2$ were the most effective. This is not surprising since both SA and Ca^{2+} play important roles as secondary messengers. These might act in some signaling pathways limiting heat induced oxidative damage. It is clear that many protective pathways contribute to survival of plants at higher temperatures and probably the relative importance of the different pathways change throughout plant development. Depending on the stress applied and age of the plant it appears that different aspects of heat induced damage impact plant survival and different types of heat induced damage prevent or repair damage through different cellular systems. Probably signaling pathway may contribute to basal and acquired thermotolerance (Larkindale *et al.*,2005). Chen *et al.* (2006) revealed an important role of galactolipids in thermotolerance and suggested that the digalactosyldiacylglycerol (DGDG) level and/or the ratio of DGDG to monogalactosyldiacylglycerol (MGDG) may play an important role in basal as well as acquired thermotolerance in *Arabidopsis*. Signalling pathways involving SA and AOS are critical for events during both basal and acquired thermotolerance (Liu *et*

al.,2006). Overall results of the present study also indicate the possibility of selecting a few biochemical markers for temperature tolerance in chickpea. High constitutive POX activity, high membrane stability and high tolerance index was evident in all tolerant genotypes which were also enhanced during induced thermotolerance. This could therefore, be used as biochemical index for screening thermotolerant genotypes.