SUMMARY

1. A brief review of literature pertaining to the line of investigation has been presented which mainly deals with biochemical responses of plants to elevated temperature stress and metabolic changes associated with induction of thermotolerance.

2. Materials and methods used in this investigation and experimental procedure followed have been discussed in detail.

3. Different genotypes of chickpea (*Cicer arietinum* L.) were screened for thermotolerance by performing cell membrane stability (CMS) test and testing their tolerance index (TI). The test revealed a distinct genotypic variation for heat tolerance amongst the fifteen genotypes tested.

4. Seed germination percentage of different genotypes was tested following elevated temperature treatments ranging from 35-55°C for 2 hrs duration. Germination percentage was also recorded in case of seeds imbibed in 100 µM/L of SA, 50 µM/L of ABA, 100 mM $CaCl_2$ solution overnight and seeds bacterized with Plant Growth Promoting Rhizobacteria (PGPR)-*Bacillus megaterium* following exposure to lethal temperature of 55°C for the same duration.

5. Some of the important biochemical parameters such as changes in enzymes related to defense and antioxidative stress response like peroxidase (POX), ascorbate peroxidase (APOX), superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR), variations in non-enzymatic antioxidants like ascorbate and carotenoids, changes in stress amino acid proline, sugar content, chlorophyll pigment content, Hill activity and quantitative and qualitative alteration of proteins were tested in seedlings following lethal temperature treatment of 46°C for 2 hrs duration.

6. Peroxidation of cell membranes due to heat stress injury and changes in phenolic profile of seedlings subjected to lethal temperature were also analyzed separately by spectrophotometric and High Performance Liquid Chromatography (HPLC) analysis respectively.

7. Desi types of chickpea with dark coloured seed coats were found to be comparatively more tolerant to to heat stress than the kabuli types. However, kabuli types were found to contain more protein per mg fresh weight.

8. Germination of seeds following elevated temperature treatments revealed decrease in germination percentage with increase in temperature. At a lethal temperature of 55°C only 10% germination occurred in thermotolerant genotypes while no germination occurred in susceptible genotypes.

9. Salicylic acid (SA) and $CaCl_2$ pre-treatments and bacterization of seeds with *Bacillus megaterium* enhanced the rate of germination in most genotypes. Abscisic acid (ABA) on the other hand exhibited inhibitory effects on germination in early stages which declined with the onset of germination.

10. Quantification of protein contents of different plant parts revealed maximum protein content in seeds followed by leaves, stem and roots.

11. Protein contents of both seeds and seedlings increased gradually following moderate heat stress but showed a rapid decline at lethal temperature in all genotypes.

12. SDS-PAGE analysis of seed proteins of various genotypes subjected to pre-treatments followed by lethal temperature revealed the appearance of new proteins having molecular masses of 18, 25, 26 and 82 kDa (HA); 13.3,18, 20, 25, 26, 26.5, 44, 82, 84 and 96 kDa (SA) and 18, 25, 26, 27.5, 82 and 96 kDa in ABA pre-treatments. Pre-treated seedlings exposed to lethal temperature also revealed the expression of some new proteins. Low molecular masses proteins of 15.6 and 17.3 k Da (approx.) and other proteins having molecular masses of 21.2, 22.3, 25.1, 39.8, 42.1, 44.6, 55, 66 and 70.7 k Da. (approx.) were observed in SA pre-treated seedlings challenged with lethal temperature. ABA and $CaCl_2$ pre-treatmnents also led to the expression of new proteins of molecular masses 10.6, 21.1, 22.3, 29.4, 39.8, 45.3 and 55 kDa and 11.2, 22.3, 33.5, 35.4, 39.8, 44.6, 45.3, 55 and 66 kDa respectively. Exposure to lethal temperature directly without any pre-treatments led to loss of some protein bands.

13. Changes in activities of antioxidative enzymes like POX, APOX, SOD and GR in pre-treated seedlings revealed that enzymatic activities gradually increase with increase in temperature and reach peak activity after which the activity declines sharply recording the lowest activity at lethal temperature. Quantum of increase in enzymatic activity was however highest in thermotolerant and lowest in heat susceptible genotypes. CAT activity, in contrast, showed a remarkable decline in its activity in pre-treated seedlings following exposure to lethal temperature.

14. POX isozyme analysis by native PAGE revealed the presence of five isozyme bands having R_m values of 0.166, 0.300, 0.336, 0.500 and 0.566 in control and pre-treated seedlings subjected to elevated temperatures (35-45°C) for 2 hrs SA, $CaCl_2$ pre-treatments and seed bacterization led to the induction of new isozymes of R_m values 0.083 and 0.316. Intense isozyme bands having R_m 0.166 and 0.300 were recorded only in thermotolerant genotypes following lethal temperature treatment.

15. CAT isozyme analysis, in contrast, revealed no induction of new isoforms in pre-treated seedlings challenged with a lethal temperature. Both control and pre-treated seedlings revealed the presence of only a single isozyme band of R_m value 0.466.

16. Cell Membrane Stability (CMS) test revealed 45% (C_a) and 83% (C_a) relative injury respectively in thermotolerant and susceptible genotypes subjected to lethal temperature treatment. Pre-treatments of seedlings however led to a remarkable reduction in membrane injury.

17. Pre-treatment of seedlings before exposure to lethal temperature treatment also led to a considerable reduction in peroxidation of cell membranes in all genotypes.

18. High accumulation of free proline and significant increase in sugar contents were recorded in pre-treated seedlings in comparison to untreated control samples.

19. Analysis of photosynthetic pigments (total chlorophyll, chlorophyll a and b) and Hill activity showed a high degree of thermosensitivity of chlorophyll molecules and a rapid decline in Hill activity following lethal temperature treatment. Pre-treatment of seedlings however showed a reasonable reduction in the impact of heat stress.

20. Biochemical analysis of non-enzymatic antioxidants like asorbate and carotenoid contents also revealed similar increasing trend in pre-treated seedlings followed by a drastic decline at lethal temperature.

21. HPLC analysis of pre-treated and control seedlings showed the presence of five phenolic acids viz. ferulic, chlorogenic, salicylic, cinnamic and gallic acids. Among the five phenolics, gallic acid was found to be present in all treatments including controls while chlorogenic acid was found to be completely absent in untreated samples.

22. Calli raised from various explants (shoot tip with a portion of leaf, cotyledonory node, hypocotyls and internode) in three differently amended MS medium supplemented with different concentrations and combinations of growth regulators (NAA+BAP, NAA+Kinetin and IAA+BAP) when subjected to elevated temperatures (30-40°C) showed rapid browning and decline in growth rate in both thermotolerant and susceptible genotypes. Calli raised in MS media supplemented with 10^{-5} and 10^{-6} M of SA and heat acclimatization (32°C- 2 hrs) however showed a much higher level of thermotolerance and comparatively much lower reduction in growth rate and browning of tissues.

23. Foliar spray treatments and seed bacterization with *Bacillus megaterium* offered a certain degree of thermoprotection. Thermoprotection induced were found to be concentration dependent up to a certain level. Very high concentration of foliar spray led to scorching of leaves and induction of oxidative stress.

24. Thermopotection provided by various pre-treatments may be due to coordinated action of antioxidative enzymes like POX, APOX, CAT, SOD and GR and non- enymatic antioxidants like ascorbate and carotenoids.

25. The high antioxidative enzymatic activity, low relative injury and peroxidation of membranes, lower sensitivity of pigment molecules and increased accumulation of non-enzymatic antioxidants like ascorbate and carotenoids could be directly linked with enhanced tolerance to heat induced oxidative damage. This could therefore, be used as biochemical markers for screening thermotolerant genotypes of chickpea.

26. Results of the investigation have been properly analyzed and their implications have been thoroughly discussed.