

Summary

1. A review of literature has been presented on previous work done in the line of the investigation. The review has been presented on oxidative stress in plants and response of plants in general to temperature stress (cold temperature stress and elevated temperature stress) and metabolic changes associated with induction of thermotolerance.
2. Experimental procedure and protocol used in this investigation have been presented in detail.
3. Four varieties of soybean (*Glycine max.* (L.) Merrill) and six varieties of lentil (*Lens culinaris* Medikus) seedlings have been subjected to low (5-25°C) and high (20-50°C) temperature stress respectively. Further at 5°C soybean seedlings were treated for different hours from 0-24h for 2h intervals. For amelioration of heat stress, seedlings of lentils were pre-treated with chemicals like 100µM SA, 50µM ABA and 10mM CaCl₂ before exposure to lethal temperature.
4. Tolerant and susceptible varieties of lentil were screened by performing cell membrane stability test and tolerance index.
5. Seed germination percentage of different varieties of lentil seeds were tested at elevated temperatures ranging from 20-50 °C.
6. Lipid peroxidation was determined as the amount of malondialdehyde (MDA) in the membrane in both soybean and lentil seedlings. In all cases temperature stress led to increased MDA accumulation.
7. Among the antioxidative enzymes assayed in soybean, catalase showed a decline in activity from 25° C to 5° C while peroxidase, ascorbate peroxidase, glutathione reductase and superoxide dismutase showed an initial increase before declining. Further analysis at 5° C for 24h revealed that activity declined in all varieties upto 4 h, with the decline being very steep in Rossio and NRC 37. In case of peroxidase activity, Rossio showed enhanced activity till 12 h.
8. In case of lentil , catalase, ascorbate peroxidase and superoxide dismutase activities showed an initial increase before declining at 50°C, while peroxidase and glutathione reductase activities declined at all temperatures. Activities of CAT, POX, APOX, SOD and GR, which had decreased to very low levels at 50°C, were enhanced after pre-treatments to levels near normal. Among the

- three chemicals, SA was most effective in case of CAT, SOD and APOX, while CaCl_2 was most effective in enhancement of POX and GR.
9. The effect of elevated and low temperatures on total antioxidant activities was measured in terms of percentage inhibition of DPPH and the effect of lethal temperature after pre-treatments were also studied. Total antioxidant activity increased initially during early period of stress, but declined thereafter. Activity was high after pre-treatments with SA, ABA and CaCl_2 .
 10. Isozyme analysis was done on native PAGE. Peroxidase isozyme analysis revealed the presence of new bands at high temperature (40-45°C) in lentil leaves. In soybean nine bands of peroxidase isozymes was observed in JS 335 and five in Rossio at 10 °C, there was a suppression of bands at 5°C. Expression of peroxidase isozymes was further tested in soybean (var. Rossio) at 5°C for different time interval (0-24h). The result revealed that a band of R_m value 0.63 was not expressed initially and again expressed at 6h and 10h in leaves of treated seedlings.
 11. Catalase isozymes analysis revealed that Cat 2 and Cat 3 isozyme were expressed at 40-45 °C in lentil leaves. In JS 335 in control itself CAT 3 was very prominent which became lesser expressed at 5°C and 10°C. Expression of catalase isozymes by native PAGE was further tested in soybean (var. Rossio) at 5°C for different time interval (0-24h). The result revealed that CAT 3 band was much expressed at 5°C.
 12. Biochemical parameters such as change in proline accumulation, chlorophyll content, phenolics and proteins were tested in leaves of soybean and lentil at low and high temperature treatments and also after pre-treatments.
 13. Protein content in seed of different varieties of lentil and soybean showed varietal difference. Highest protein content was found in IPL 406 and lowest in that of Lv varieties of lentil. Rossio and NRC 35 showed high protein content of soybean. Protein content in seedlings of soybean shows decline at low temperature except in Rossio variety. In case of lentil protein content in seedlings initially increase then declined at lethal temperature which was again increased by pre treatments.
 14. Protein profile was analysed by SDS-PAGE. Protein synthesis in seeds of all six varieties of lentil showed similar type of protein profile in all five varieties

(Subrata, Lv, Sehore, IPL 81 and IPL 406) except in Asha where protein of molecular mass 98 Kda was not expressed.

15. Seedlings of lentil subjected to elevated temperatures showed expression of new protein bands and suppression of certain existing bands at elevated temperatures.
16. Seedlings of soybean treated at 5°C for different time period showed increased in expression of some new proteins having molecular masses of 85.6 and 16 at 16h and 22h respectively.
17. Phenol content was initially increased then declined except in Asha and subrata there was a slight increase at 50°C. Phenol content was increased in leaves of seedlings pre-treated with SA, ABA and CaCl₂. Quantification of phenolics in soybean at low temperature revealed varietal difference.
18. HPLC analysis showed the presence of salicylic acid, catechol, chlorogenic acid in lentil.
19. Proline content showed increase at high temperature which declined in leaves of seedlings pre-treated with SA, ABA, CaCl₂ prior to lethal temperature treatment. At low temperature in soybean proline content was increased with the decrease of temperature.
20. Chlorophyll content declined at both high and low temperatures which was higher in susceptible varieties than in tolerant varieties. Slight increase in chlorophyll content was observed in leaves of seedlings treated with SA, ABA and CaCl₂.
21. Results of all experiments revealed that in lentil, of the six varieties, Sehore and Lv were susceptible, while the other four were more or less tolerant. In case of soybean, Rossio was more tolerant than the other three.
22. Statistical analysis of data has been done by Anova and Co-relation studies. Correlation between total antioxidant activity of % DPPH inhibition and accumulation of antioxidants in both high and low temperatures stress have been studied, in between catalase and hydrogen peroxide negative co-relation was obtained in two varieties and more or less positive two susceptible varieties at low and high temperature stress.