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# EFFECT OF HEAT SHOCK TREATMENTS ON SOME BIOCHEMICAL PARAMETERSIN DIFFERENT VARIETIES OF SOYBEAN *(GLYCINE MAX)*

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**Abstract**

The present study was undertaken to screen the thermotolerant varieties of soybean and analyse the changes brought about by heat shock conditions in least resistant variety. Four varieties of soybean viz., Williams, Chippewa, S-73 and Improved Pelican were subjected to 7 different heat shock conditions. 'Chippewa' was found to be the most theremotolerant and 'Williams' was the least. It was observed that 45°C-2 h had lethal effect on growth of soybean seedlings. A short pulse of 10 minutes at 45°C triggers the synthesis of some heat shock proteins which were accumulated at 45°C-2 h and then gradually decreased during recovery period. Altered levels of RNA, DNA, total phenols and anthocyanins were also observed in cv. Williams. An increase in temperature had adverse effect on growth however, Hsps-induced during shock may help in the recovery of growth and provide thermal tolerance in soybean seedlings.

## Introduction

A wide range of organisms from bacteria to higher plants and animals, respond to high temperature (heat shock) by syntheses of heat shock proteins (Hsps) and repressing the synthesis of normal proteins (Schlesinger *et al.,* 1982). Experimental evidence from etiolated soybean seedlings suggest that induction and accumulation of heat shock proteins are necessary for organisms to acquire thermotolerance to potentially lethal temperatures (Line *et al.,* 1984) by protecting the cell from the stressful condition in which they find themselves (Pelham, 1985). In plants, the non-heat shock stresses that will induce a typical HS response are more limited. In soybean seedlings osmotic stress, salt stress, anaerobiosis, ABA, 2,4-D, ethylene and cold shock have either no effect or are only very weak inducers of Hsps (Czarencka *et al.,* 1984).

The object of the present investigation was to find out the most thermotolerent variety of soybean and to analyse the biochemical changes brought about by heat shock conditions in the least resistant variety.

## Material and Methods

Fifty seeds each of four varieties of soybean (*Glycine max*) viz., Williams, Chippewa, S-73 and Improved Pelican were grown in rolls of moist filter paper at 28°C in a dark chamber for 24 h. The experiment was set in split plot design with three replications. Twenty four hours old uniform length germinating seeds were subjected to various heat shock conditions (Table 1). After each treatment, the seedlings were placed in moist tissue paper rolls and grown at 28°C for further 24, 48 and 72 h after which whole seedlings (roots and shoots) were measured (Fig.1). For biochemical assays and PAGE analysis another lot of seeds were germinated for 72 h at 28°C and then subjected to heat shock (Table 2). After each treatment seedlings were freezed at -4°C till further processing.

**Table 1. Details of different heat shock treatments in *Glycine max*.**

| | | | | | | |
|---|---|---|---|---|---|---|
| $T_1$ | 28°C | (Control) | | | | |
| $T_2$ | 45°C | (10 Min.) | | | | |
| $T_3$ | 45°C | (2 h) | | | | |
| $T_4$ | 45°C | (10 Min.) | 28°C | (30 Min.) | 45°C | (2 h) |
| $T_5$ | 45°C | (10 Min.) | 28°C | (1 h) | 45°C | (2 h) |
| $T_6$ | 45°C | (10 Min.) | 28°C | (2 h) | 45°C | (2 h) |
| $T_7$ | 45°C | (10 Min.) | 28°C | (3 h) | 45°C | (2 h) |

Growth of seedlings were analysed after a growth period of 24, 48 and 72 after HS treatments.

Total proteins were estimated by the method of Lowry *et al.*, (1951). Total Phenolic content were determined by reaction with Folin CioCalteus reagent (1927). Anthocyanins were extracted and estimated by the method of Rengel & Kordan (1987). For qualitative separation of proteins, polyacrylamide gel electrophoresis (PAGE) was performed in the manner described by Davis (1964).

**Table 2. Details of heat shock treatments in *Glycine max* for polyacrylamide gel eletrophoresis.**

| | | | | | | |
|---|---|---|---|---|---|---|
| $T_1$ | 28°C | | | | | |
| $T_2$ | 45°C | (10 Min.) | | | | |
| $T_3$ | 45°C | (2 Hrs.) | | | | |
| $T_4a$ | 45° | (10 Min.) | 28°C | (30 Min.) | | |
| $T_4b$ | 45°C | (10 Min.) | 28°C | (30 Min.) | 45°C | (2 h) |
| $T_5a$ | 45°C | (10 Min.) | 28°C | (1 h) | | |
| $T_5b$ | 45°C | (10 Min.) | 28°C | (1 h) | 45°C | (2 h) |
| $T_6a$ | 45°C | (10 Min.) | 28°C | (2 h) | | |
| $T_6b$ | 45°C | (10 Min.) | 28°C | (2 h) | 45°C | (2 h) |
| $T_7a$ | 45°C | (10 Min.) | 28°C | (3 h) | | |
| $T_7b$ | 45°C | (10 Min.) | 28°C | (3 h) | 45°C | (2 h) |

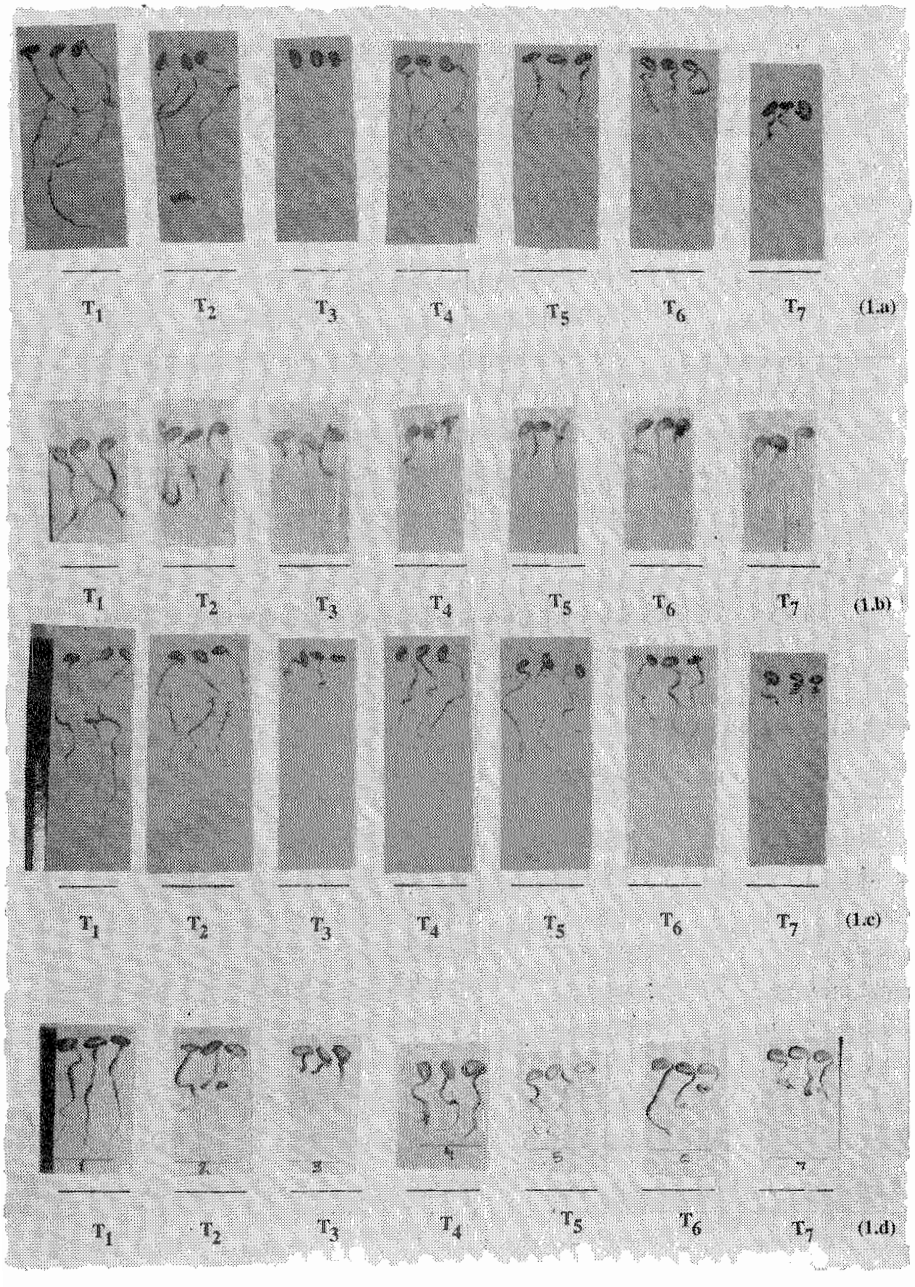


Fig.1. Photographs of seedlings under different heat shock conditions.
Williams - 1-a, Chippewa - 1-b, S-73 - 1-c, Improved Pelican - 1-a.

**Table 3. Growth (length of seedlings in cms.) of different cultivars of Soybean as affected by heat shock conditions.**

| Treatments | Soybean Cultivars | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Williams | | | Chippewa | | | S-73 | | | Improved Pelican | | |
| | Hours of incubation | | | | | | | | | | | |
| | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 | 24 | 48 | 72 |
| $T_1$ | 5.84 | 8.75 | 14.75 | 7.17 | 11.42 | 14.58 | 6.29 | 10.32 | 15.85 | 4.62 | 8.04 | 10.67 |
| $T_2$ | 4.30 | 7.35 | 11.90 | 5.72 | 09.48 | 12.97 | 5.12 | 07.63 | 12.29 | 4.10 | 6.23 | 08.34 |
| $T_3$ | 1.66 | 2.11 | 03.55 | 3.42 | 04.51 | 05.55 | 2.94 | 03.67 | 04.18 | 2.10 | 2.76 | 03.69 |
| $T_4$ | 3.43 | 6.05 | 09.10 | 3.53 | 05.85 | 07.41 | 4.59 | 07.44 | 11.02 | 2.51 | 3.73 | 05.57 |
| $T_5$ | 2.89 | 4.34 | 06.18 | 3.07 | 04.56 | 06.89 | 3.02 | 04.05 | 07.83 | 3.83 | 4.57 | 06.37 |
| $T_6$ | 2.99 | 4.40 | 06.45 | 2.11 | 04.20 | 05.59 | 3.38 | 04.53 | 06.20 | 2.61 | 3.95 | 04.97 |
| $T_7$ | 2.37 | 3.49 | 05.72 | 3.10 | 04.94 | 06.39 | 3.75 | 06.20 | 08.73 | 2.30 | 3.11 | 04.39 |

**Table 4. ANOVA showing growth (length of seedlings in cms.) of four varieties of Soybean in 7 heat shock treatments.**

| Sources of Variation | | M.S | | | |
|---|---|---|---|---|---|
| | df | Williams | Chippewa | S-73 | Improved Pelican |
| Replication | 2 | 0.668 | 0.913 | 0.739 | 0.786 |
| Time (A) | 2 | 127.575* | 112.540** | 145.367** | 50.02** |
| Error a | 4 | 4.99 | 0.098 | 1.324 | 0.161 |
| Whole plot | 8 | 261.477 | 28.412 | 37.1887 | 12.78 |
| Temperature (B) | 6 | 343.931** | 76.48** | 55.284** | 27.109** |
| Interaction A X B | 12 | 67.289** | 4.72** | 1.999n.s | 1.697** |
| Error b | 36 | 10.539 | 0.181 | 3.486 | 0.167 |
| Subplot | 54 | 421.751 | 9.66 | 8.911 | 3.501 |
| Total | 62 | 683.228 | 12.08 | 12.560 | 4.700 |

*Significant at 0.05 level of probability, **Significant at 0.01 level of probability, n.s. Non Significant

## Results and Discussion

In the present work soybean seedlings were raised at 28°C as reported to be the optimal temperature for germination of soybean (Key *et al.*, 1983); Line *et al.*, 1984; Schoff *et al.*, 1987). It was observed that the lethal temperature for etiolated soybean seedlings is 45°C (Table 3) for all the 4 varieties. A pretreatment at 45°C (10 minutes), 28°C (30 minutes) before exposure to 45°C (2 hours) allowed the seedlings to become thermotolerant. It has been suggested that a heat shock treatment for 10 minutes at 45°C followed by 2 h at 28°C provides thermal protection for seedling growth (Line *et al.*, 1984). In the present work three varieties of soybean viz., 'Williams', 'Chippewa' and S-73 recovered from lethal shock in T4, whereas 'Improved Pelican' showed recovery in T5. However when compared with control seedlings (T1) the growth of thermoprotected seedlings was less (Table 3). ANOVA for both length of root and shoot indicates that growth of seedlings in all the varieties of soybean is significantly ( $< 0.01$) affected by heat shock treatments (Table 4).

Total protein exhibited a varietion in different HS treatments. A lower concentration of protein was evident in T2 (45°C-10 min.) as compared to control followed by an increase in T3 and then a decline from T4 - T7 (Table 5). It would appear that a shock at 45°C for 10 min, triggers the synthesis of Hsps, which are accumulated at 45°C (2 hours) and later utilized by seedlings for the recovery of growth. It has been reported by Schoffl *et al.*, (1986) that a short heat pulse (45°C-10 min.) initiates a transient synthesis of Hsps to high levels. Thermotolerance may develop during the recovery period where heat shock proteins are being utilized (Pelham, 1985).

The electrophoretic pattern of proteins in control and treated seedlings clearly exhibit the qualitative difference (Fig.2). Several bands are consistent in control and treated seedlings, however disappearance of control bands and appearance of new bands in treated seedlings was also noticed. At T2 two bands of control P-4 and P-6 were missing which reappear in T3. It would appear that an increase in temperature was responsible for the supression of some normal proteins. T5-T7 also shows disappearance of some bands (P1, P2, P3, P4, P5, P6 and P7). In early treatments (4a, 5a, 6a and 7a) at least one new band was observed which may reflect the induction of Hsps. These proteins HSP-1, 3,4,5 do not persist during later treatment. The Hsps induced during early treatments are being utilized in late hours for survival of the seedlings. In *Escherichia coli*, yeast and *Dictyostelium* it has been reported that synthesis of certain Hsps is essential for cell growth at elevated temperature (Schoffl *et al.*, 1986). In the present study the pattern of protein shifts back to normal in T7b [45°C (10 Min.) - 28°C (3 hours), 45°C (2 hours)]. Continuous exposure of maize seedlings to elevated temperature resulted in a depression in the synthesis of Hsp after 8-12 h and then the pattern shifts to the protein synthesized at 25°C (Atkinson *et al.*, 1989).

A 10 minute pulse at 45°C showed an increase in total RNA content (Table 5) while a decline was observed at 2 h. Results of recovery periods shows continuous increase uptil 2 h recovery (T4 - T6) but 3 h recovery (T 7) a fall in RNA content was observed. Schoffl & Key (1982) found a rapid increase of hs-specific RNA sequences when the temperature was raised from 28°C - 40°C (or 42°C) and within 2 h it reached to the maximum level. The results of the present studies would suggest that in soybean seedlings maximum level of hs RNA is obtained between 1-2 h at 45°C and then the pattern shifts to normal. Atkinson *et al.*, (1989) revealed a concomitant accumulation of hsmRNA with

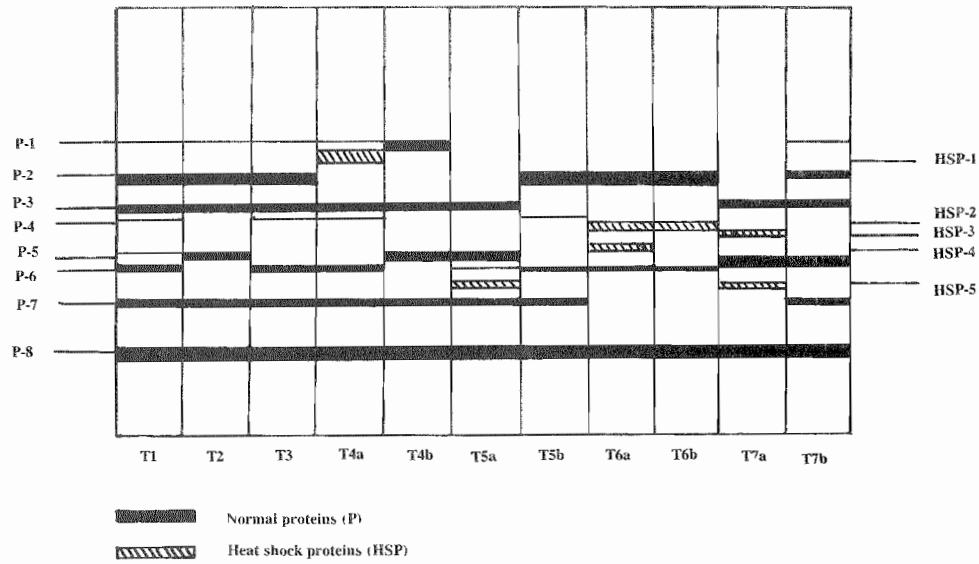


Fig.2. Zymogram showing qualitative separation of buffer soluble proteins in williams' under different heat shock conditions.

an increase in polyribosomes in maize seedlings which did not return to a steady state control (25°C) level for at least 24 h.

A rapid accumulation of DNA content (Table 5) was observed at 45°C (10 min. - 2h). It reflects the activation or may be amplification of certain heat shock genes as reported by Nagao *et al.*, (1985) and Schoffl *et al.*, (1987). A decline in T4 shows the return of normal gene activity or repression of heat shock genes.

Comparative studies of phenolic content in control and treated seedlings reveals a gradual increase from 10 minutes to 2 h, however the quantitiy decreases in T4 and returns to normal levels during recovery period (Table 5). A lethal temperature may induce synthesis of some heat shock phenols which may have inhibitory effect on growth of seedlings. Earlier, phenols have been studied under stress conditions (Wender, 1970) and an increase was noticed under cold treatment in tobacco stems and roots.

Anthocyanins are found to be greatly influenced by heat shock treatments, as a loss in pigmentation is noticed during elevated temperature. A decrease was observed in T2 as compared to control while anthocyanins are completely absent in T3, with a complete recovery in T4. During recovery period (T4 - T6) a gradual decrease was found. These results indicate that a 2 h incubation at 45°C completely inhibits the synthesis of anthocyanin. Light dependent anthocyanins, resulting from high irradiance reaction has been observed to be influenced by physical, chemical and biological factors (Mancinelli, 1985).

**Table 5. Biochemical parameters in seedlings of cv "Williams" under different heat shock conditions.**

| Treatment | Total Proteins (ug/g. F.W) | Total RNA Content O.D. at 290 nm | Total DNA Content O.D at 290 nm | Total Phenols (ug/g. F.W | Total Anthycyanin O.D at 530 nm |
|---|---|---|---|---|---|
| $T_1$ | 3480 a | 0.54 a | 0.42 a | 248.33 a | 0.020 ac |
| $T_2$ | 3110 b | 0.56 b | 0.5826 b | 0.00 b | 0.016 ad |
| $T_3$ | 3570 c | 0.55 c | 0.78 c | 306.67 c | 0.000 b |
| $T_4$ | 3442 d | 0.57 d | 0.47 d | 261.67 b | 0.026 c |
| $T_5$ | 3480 a | 0.67 e | 0.60 e | 255.00 d | 0.016 de |
| $T_6$ | 2655 f | 0.69 f | 0.80 f | 255.00 d | 0.015 e |
| $T_7$ | 2655 f | 0.60 g | 0.83 g | 307.50 e | 0.04 b |

Means followed by the same letters do not differ significantly at one percent probability level.

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