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HEAT STRESS TOLERANCE STUDIES IN CALLUS CULTURES OF *GOSSYPIUM HIRSUTUM* L. CV. ACALA SJ2.

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Abstract

Heat stress tolerance was studied in cotton (*Gossypium hirsutum* L.). Callus cultures of two cotton cultivars viz., Coker 312 (cotyledonary-derived) and Acala SJ2 (leaf and petiole-derived) showed significant stimulation in growth at stress temperature of 44°C for 2 hours after 2 days of pre-incubation at 28°C followed by 26 days of incubation at 28°C. Reduction in callus growth at 47 and 50°C was higher in Acala SJ2 compared to Coker 312. Petiole-derived callus exhibited more heat tolerance than leaf-derived callus. At 50°C callus tissue turned brown The relative significance of increases in growth rate at 44°C and drastic reduction in growth rate at temperatures above 44°C is discussed in the light of heat induced production of proline, glycinebetaine, heat-shock proteins and killing of some viruses which have been reported to protect plant tissues from damage caused by heat stress.

Introduction

Environmental stresses reduce crop yield. Of these, temperature stress has been most intensively studied at cellular and molecular level (Sachs & Ho, 1986). Cotton (*Gossypium* spp.) a major crop grown in areas of the world is subject to recurring periods of high temperature and drought stress. Trolinder & Shang (1991) found that pollen fertility is often reduced by excessive temperatures and drought stress affects both quantity and quality of fibre produced. Long exposure of cotton callus to high temperature reduces the biomass production (Dani, 1992). The calli are more sensitive to growth environment alterations in the early stages of growth. Plants of *Gossypium hirsutum* cv. Coker 312 tolerant to 45°C have been regenerated through somatic embryogenesis in suspension cultures (Trolinder & Shang, 1991) where 75% cell viability after 1 hour and 30% after 10 hours exposure to 50°C was observed. An exposure of cells of Coker 312 to 45°C for 24 hours resulted in complete death of the cells. The present report describes the effect of different stress temperatures on growth of callus cultures of cotton cvs. Coker 312 and Acala SJ2.

Material and Method

Callus Induction and Maintenance: The calli were induced from cotyledon, leaf and petiole explants of Coker 312 and Acala SJ2 on Murashige & Skoog (1962) basal salts

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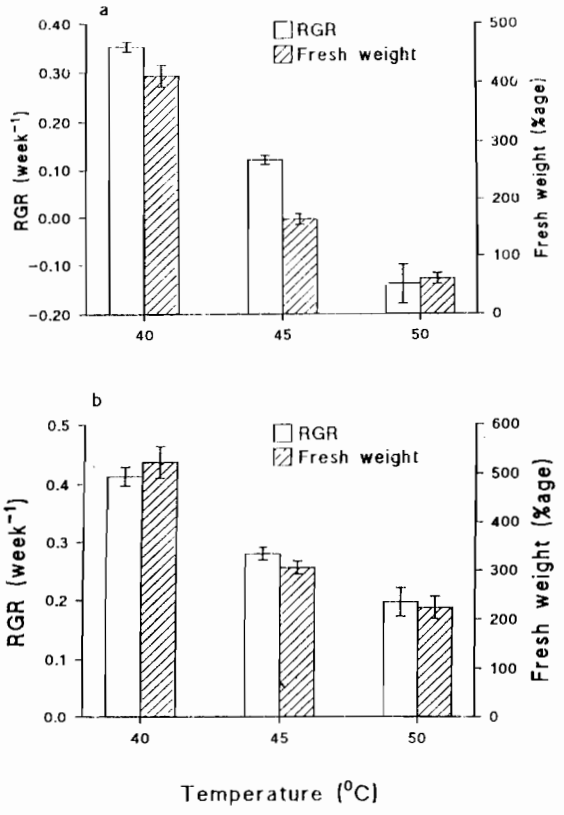

Fig. 1. Effect of heat stress for two hours on growth of leaf (a) and petiole (b) callus cultures of Acala SJ2 after 2 days of preincubation at 28°C. Data points are means of 5 replications. Vertical bars represent SE (Experiment No. 1).

medium plus 2,4-D and BAP (0.1 mg l^{-1} each), GA_3 (0.1 mg l^{-1}), *myo*-inositol (100 mg l^{-1}), thiamine (0.4 mg l^{-1}), phytagel (2 g l^{-1}) and glucose (30 g l^{-1}). These calli were subcultured and maintained to provide sufficient material for experimentation.

Culture Medium Used: The callus cultures of Coker 312 (cotyledonary-derived) and Acala SJ2 (leaf-derived) were used except otherwise stated. A series of experiments with different temperature and time intervals was conducted to investigate the effect of stress temperature on callus growth *in vitro*. The tissue culture medium used in all these experiments contained Murashige & Skoog (1962) inorganic salts, *Myo*-inositol (100 mg), thiamine (35 mg), 2,4-D and BAP (each 0.1 mg), glucose (45 g) and phytagel (2 g) per litre. *Myo*-inositol and thiamine concentrations were changed to 200 and 25 mg l^{-1} respectively, when petiole-derived callus of Acala SJ2 was used.

Application of Heat Stress: The calli were subjected to heat stress in the growth chamber in light at 90 μmol m^{-2} s^{-1} followed by dark incubation at 28°C. There were 5 replications/treatment.

Measurement of Growth: Pre-weighed disposable sterilized Petri dishes containing 15 cm^3 culture medium were inoculated with callus tissue and the inoculated dishes were re-weighed to obtain the initial weight of the callus inoculum. The cultures were incubated at $28 \pm 2^{\circ}C$. Callus fresh weight (%age) was calculated according to the following formula:

F. Wt. (%age) = (Final weight/Initial weight) x 100

and Relative Growth Rate (R.G.R) (per week) was recorded as described by Akhtar *et al.*, (1995) as follows:

R.G.R = [ln (Final weight) - ln (Initial weight)]/4 weeks

(where ln is natural log)

Data Analysis: An analysis of variance of the data and computation of standard error were performed by the minitab statistical package on computer.

Results

Expt.1: Leaf-derived calli of Acala SJ_2 subjected to 40, 45 and 50°C for 2 hours, responded differently to the stress conditions than petiole-derived callus. Although the relative growth rate and callus fresh weight was reduced at 45 and 50°C, the degree of reduction was less than that found in petiole-derived calli (Fig.1a). Mean R.G.R.s ($P=0.000$) and callus fresh weights (%age) ($P=0.000$) at different temperatures were significantly different from each other.

The petiole-derived calli of Acala SJ2 stressed at 40°C had maximum relative growth rate (0.352) (Fig.1b). Relative growth rate was drastically reduced at stress temperatures (45 & 50°C) to 0.122 and -0.138, respectively. Analysis of variance revealed highly significant differences between treatment means ($P=0.000$). Similar results were obtained when data were represented on fresh weight (%age) basis (Fig.1b).

Expt.2, Coker 312: The calli of Coker 312 subjected to 35, 38, 41, 44 and 47°C for 2 hours, showed an increase in relative growth rate and callus fresh weight at increasing temperatures up to 44°C which then reduced at 47°C. The relative growth rates at 35, 38, 41, 44°C were 2.6, 10.4, 14.0 and 36.1% higher than the control at 28°C (100 %), respectively. At 47°C, 4.5% reduction in relative growth rate was found compared to control (Fig.2a). Analysis of variance showed significant differences between treatment means ($P<0.032$). The results expressed on callus fresh weight (%age) basis showed the same trend (Fig.2a) showing an increase of 4.8, 20.9, 29.1 and 88.7 % compared to control. Callus fresh weight decreased to 5.4 % at 47°C compared to control.

Acala SJ2: Relative growth rate gradually increased with increasing temperature except at 47°C. Fig.2b indicates that there was 12.7, 17.2, 19.2 and 59.6% increase in relative growth rate at 35, 38, 41 and 44°C compared to the control (°C). High temperature stress at 47°C for 2 hours reduced the relative growth rate (18.5%) compared to control (100%). Analysis of variance gave probability values of 0.000 for the effects of temperature on both relative growth rate and callus fresh weight. Data regarding fresh weight (%age) showed 12.4, 16.5, 17.7 and 74.4% increase at 35, 38, 41 and 44°C, respectively and 17.3% decrease at 47°C compared to control (Fig.2b).

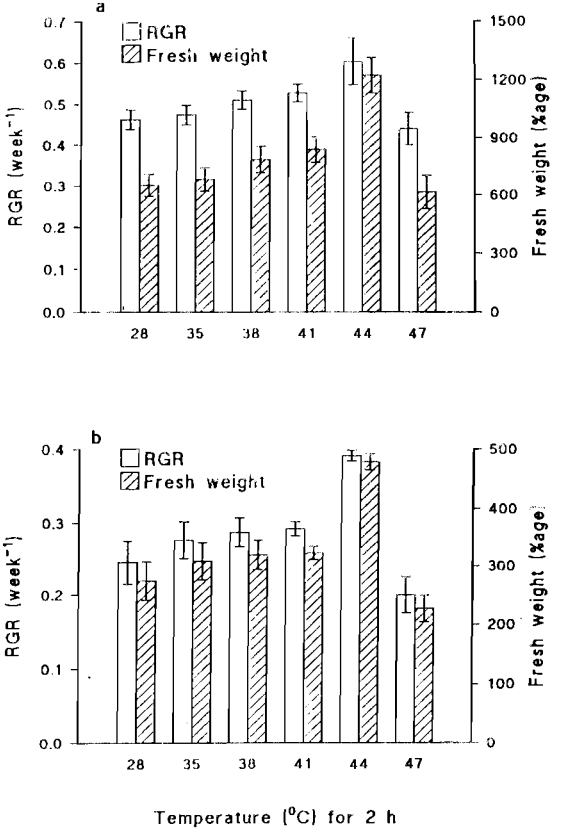

Fig.2. Effect of heat stress for two hours on growth of callus cultures of Coker 312 (a) and Acala SJ2 (b) after 2 days of preincubation at 28°C. Data points are means of 5 replications. Vertical bars represent SE (Experiment No.2).

These results indicated that increasing temperature between 28-44°C for 2 hours stimulated the growth of callus. Higher temperature (47°C) was inhibitory to growth. The reduction in relative growth rate and callus fresh weight in Acala SJ2 was greater than that in Coker 312 suggesting that Acala SJ2 cannot withstand higher temperature stress while Coker 312 is slightly more tolerant to heat stress than Acala SJ2.

Exp.3: Coker 312: Keeping in view the stimulation of growth by heat stress as observed in Expt.2, the effect of one stress temperature of 44°C after 2 days of preincubation at °C was evaluated for different time intervals of 0, 1, 2, 4 and 8 hours. The objective was to find out whether a longer exposure of callus to higher temperatures had any effect on callus growth. The results showed that callus growth rate at various time intervals was not significantly different ($P < 0.046$) (Fig.3a). Although the temperature stress for 1 to 8 hours stimulated the growth of callus, the stimulation was greater in the 1 and 2 hours treatments (68% > control).

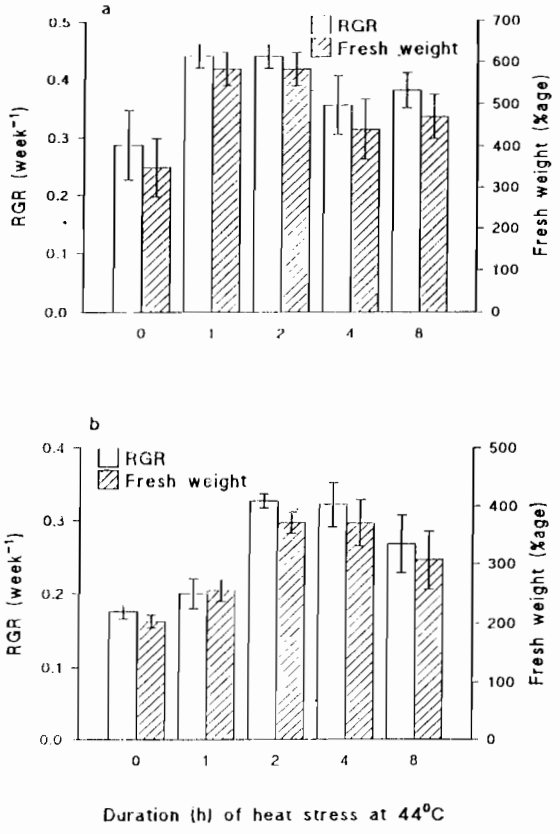

Fig 3. Effect of heat stress at 44°C for various time intervals on growth of callus cultures of Coker 312 (a) and Acala SJ2 (b) after 2 days of preincubation at 28°C. Data points are means of 5 replications. Vertical bars represent SE (Experiment No.3).

Acala SJ2: Relative growth rates and fresh weights of calli subjected to the stress temperature for various times were higher than those of untreated callus (Fig. 3b). The highest callus growth rate was found in 2 and 4 hours treatments (82% > control) but these two values were statistically the same. Temperature stress for 1 and 8 hours did not produce any significant effect on callus growth. Mean R.G.R. ($P<0.001$) and fresh weight ($P<0.002$) at different time intervals of stress were significantly different from each other. It can be concluded that a temperature stress of 44°C for 2 hours was the best condition for stimulating the growth of callus.

Exp. 4: Coker 312: In this experiment, the temperature stress (44°C) was applied after 2,4,8,12 and 16 days of preincubation. Highest relative growth rate (27.5% > control) and fresh weight (49.5% > control) were found in 2 days treatment (Fig.4a). The temperature stress after 4, 8, 12 and 16 days of preincubation resulted in a decreased relative growth rate and callus fresh weight compared to 2 hours stress and control.

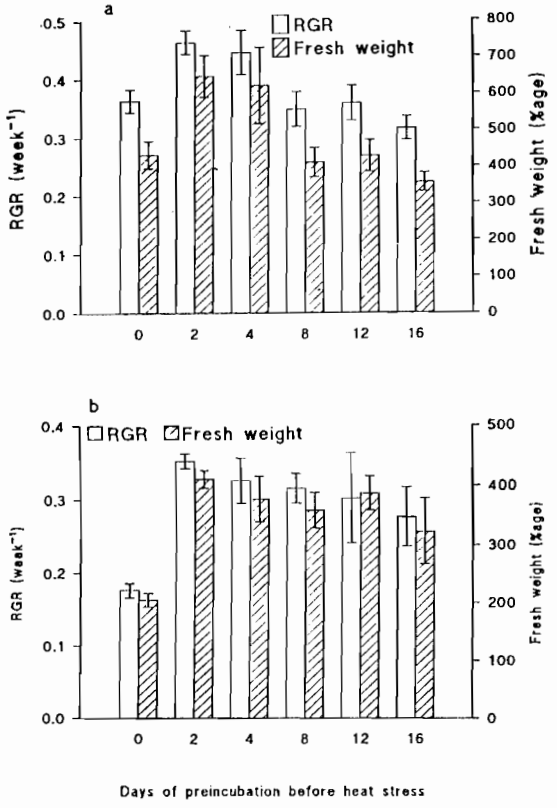

Fig.4. Effect of heat stress of 44°C for two hours on growth of callus cultures of Coker 312 (a) and Acala SJ2 (b) after 2,4,8,12 and 16 days of preincubation at 28°C. Total culture period is 28 days. Data points are means of 5 replications. Vertical bars represent SE (Experiment No.4).

Analysis of variance of the data regarding relative growth rate and callus fresh weight gave probability values of 0.004 and 0.005, respectively.

Acala SJ2: Relative growth rates in all treatments were significantly increased when calli were subjected to temperature stress at 44°C after 2 to 16 days of preincubation at 0C compared to control. Comparatively higher relative growth rate (85% > control) and fresh weight (85.2% > control) were obtained when calli were stressed after two days of preincubation (Fig.4b). Highly significant differences were found between mean R.G.R. ($P<0.022$) while there were no differences in mean fresh weights ($P=0.190$). Growth stimulation was greater when calli were stressed after 2 days of preincubation at °C. Any delay in the application of heat stress resulted in reduced callus growth compared to treatment after 2 days.

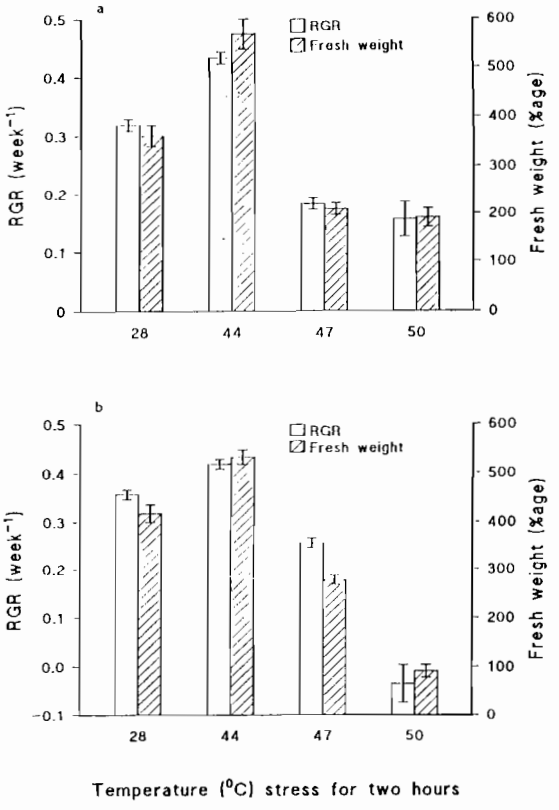

Fig.5. Effect of heat stress for two hours on growth of callus cultures of Coker 312 (a) and Acala SJ2 (b) after 2 days of preincubation at 28°C. Data points are means of 5 replications. Vertical bars represent SE (Experiment No.5).

Exp. 5: The calli of Coker 312 and Acala SJ2 were subjected to heat stress at 44, 47 and 50°C for 2 hours after 2 days of preincubation (28°C). In this experiment, the quantity of callus was almost three times more than that used in the previous experiments.

Coker 312: Relative growth rate and callus fresh weight were maximum at 44°C temperature stress (Fig.5a). Higher temperatures of 47 and 50°C, resulted in considerable reduction in both parameters compared to control (°C). Relative growth rate and callus fresh weight at 44°C was significantly different from control and from those at 47 and 50°C (P=0.000). Calli turned brown at 50°C (Fig.6a).

Acala SJ2: Temperature stress at 47 and 50°C resulted in a drastic reduction in relative growth rate and callus fresh weight (%age) (Fig.5b). Maximum growth was found when calli were stressed at 44°C for 2 hours. At 50°C, even the calli's original weight was reduced resulting in less growth rate. It can be concluded that the growth of calli from both cultivars was stimulated at 44°C only, while it was drastically reduced above this temperature. The calli of Acala SJ2 were more affected by higher temperatures than calli of Coker 312 (Fig.6b).

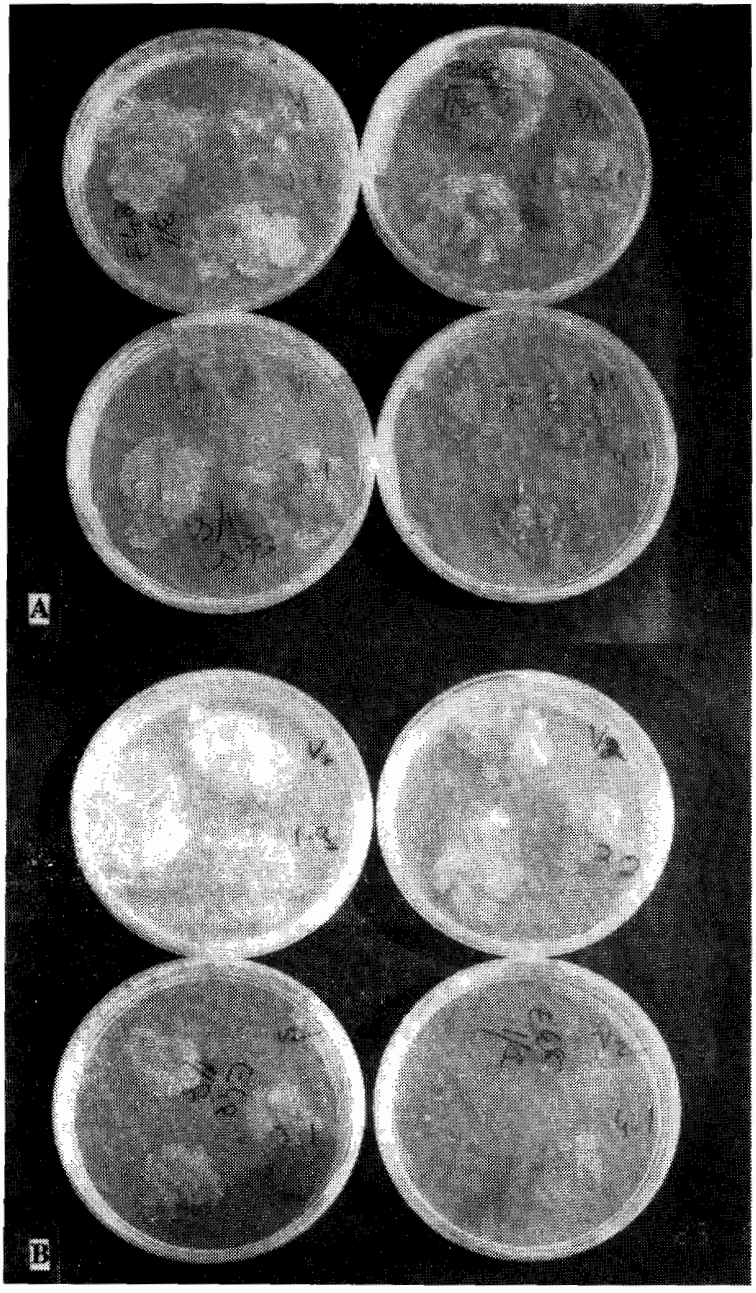

Fig.6. Effect of various temperature stress for two hours on callus cultures of cotton cvs. Coker 312 (a) and Acala SJ2 (b) after two days of preincubation at 28°C. The numbers 1,2,3 and 4 on the Petri dishes denote temperatures of 28, 44, 47 and 50°C.

Discussion

Heat stress at 44°C for 2 hours after two days of preincubation at 28°C, significantly stimulated the growth of calli of cotton cvs Coker 312 and Acala SJ2. At higher temperatures of 47 and 50°C the callus growth was severely reduced in both cultivars. Callus cultures of Coker 312 were less affected by higher temperature compared to callus of Acala SJ2. These results are supported by the findings of Trolinder & Shang (1991) who reported 75% and 30% cell viability in Coker 312 on exposure of callus cultures to 50°C for 1 and 10 hours, respectively. In the present studies the callus growth was reduced when the callus cultures were exposed to heat stress at 44°C for 8 hours. These findings agree with those of Dani (1992) who found that callus biomass production is reduced on exposure to high temperatures for longer periods in cotton. Acala SJ2 was earlier found to be severely affected by high temperature in the chlorophyll fluorescence experiments (Akhtar *et al.*, 1996). Degree of heat tolerance observed in the whole plant in Acala SJ2 was exhibited in callus tissues also. These results suggest a heat tolerance mechanism operating at cellular as well as whole plant level. The findings of Rodriguez-Garay *et al.*, (1986) also agree with our results.

Callus cultures of Acala SJ2 and Coker 312 subjected to high temperature stress revealed that at temperatures above 45°C severe injury to the callus tissue occurred leading to large reductions in growth rate in both cultivars. This reduction in growth rate was more pronounced in leaf callus of Acala SJ2 than in petiole-derived callus and callus cultures of Coker 312. This reduction may be due to dehydration of callus tissues or perturbation in cell division in the callus cultures. Sethar (1993) reported a large mortality of cotton roots at 45°C. Francis & Barlow (1988) reported that variation in temperature may change the duration of cell cycle resulting in a change in growth of plant organs. They further found that the changes in temperature disturb the cell division rate. This change may be of tissue- and species-specific manner. The nuclear division and cell growth are tightly coupled which may be disturbed by extreme stress temperature (Francis & Barlow, 1988). In plants, the loss of membrane integrity is basic response to higher temperature stress. Salisbury & Ross (1991) have mentioned that high temperature may result in leakage of water and other organic and inorganic substances from the cell ending in the death of the cell. Various stresses like heat, cold, toxins, removal of calcium ions and salinity may damage the cell membranes. The membrane structure can be changed by these situations by increasing the leakage of electrolytes. As the callus tissues are more vulnerable to environmental stresses, the heat shock of 45°C and above may have damaged the tissues/cells severely causing a substantial reduction in growth and ultimately the death of cells. Transportation of growth hormones (such as auxins) is also reduced by high temperature. Morris (1979) found reduced velocity of the auxins transport in sunflower, pea and cotton at 44°C while there was no transport above that temperature. This mechanism may have worked in callus cultures at temperature above 44°C in present studies producing deleterious effects on callus growth.

The question, which arises, is what makes the callus tissue grow more vigorously after a heat shock at 44°C for two hours? There can be few possibilities for this growth

stimulation. First, the heat shock may have killed some viruses present in the callus tissues which may otherwise be a source of slow growth. Cotton leaf curl virus is an example of growth reduction at whole plant level. Secondly, the accumulation of certain organic substances like proline (Ashraf *et al.*, 1994; Kuznetsov *et al.*, 1993) and glycinebetaine (not measured and applied in this case) may have protected the callus tissue from damage by heat shock at 44°C (Nash *et al.*, 1982; Nash & Wiskich, 1982). If this was the case then why did this mechanism not work at temperatures above 44°C. The possible explanation may be that the higher temperatures (45°C and above) may have damaged the tissues to such an extent that there was no chance of recovery. Besides, the accumulation of heat shock proteins (not measured in this case) may have protected the callus tissue from heat injury resulting in higher growth rate. At temperatures above 44°C, a delayed heat shock protein synthesis might not be able to protect the callus tissues from heat injury as suggested by Cooper & Ho (1987) if this mechanism of protection had been involved.

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