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RESEARCH ARTICLE

# Induced Response of Sugarcane Variety Co 86032 for Thermotolerance

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**Abstract** A study was conducted to investigate the induced and non-induced response of sugarcane variety Co 86032 by heat acclimation both under in vivo and in vitro conditions. For in vivo condition, 30 days old sugarcane settlings (Co 86032) were subjected to optimum induction temperature (40 °C with 10 h time) followed by critical temperature condition of 48 °C with 10 h. For in vitro condition, calli of Co 86032 were subjected to optimum induction temperature condition of 42 °C with 10 h time followed by critical temperature condition of 48 °C with 15 h. Adaptive response of settlings and calli by heat acclimation were estimated in terms of soluble protein, total sugars, total phenolics, proline, glycine-betaine (GB) and ROS scavenging enzymes activities (SOD, POX and APX) and isozyme pattern (SOD and POX). Higher levels of soluble sugars, proteins, proline and GB were observed in pretreated settlings and calli. Significant differences in responses of SOD, POX and APX activities were observed in both induced and non induced settlings and calli. However heat acclimations (induced) led to higher activities of these enzymes and thereby protect the cells from oxidative damage. Exposure to high temperature caused a significant increase in lipid peroxidation (MDA content) and cell membrane injury (%), however pre treated settling and calli recorded lesser cell membrane damage. Formation of additional protein bands of different molecular weights (90, 70 and 27 kDa) showed the expression of these proteins upon heat acclimation particularly at 40 °C in settlings and 42 °C in calli.

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## Introduction

Several studies have shown that an acclimated plant survives when exposed to a temperature that would be lethal to a non-acclimated plant. This phenomenon is the major aspect of acclimation response termed as acquired thermo tolerance. This involves expression of diverse stress-responsive genes to maintain metabolic homeostasis during stress or to be able to re-establish, subsequent to the stress period (Hong et al. 2003). Thus, relevance of a physiological or biochemical trait for thermo tolerance can best be studied by pre-exposure of seedlings/plants to a sub-lethal acclimation temperature. The major principle in induction (acclimation) response technique is to initially expose seedlings/plants to a less severe temperature before they are challenged with severe temperature and subsequently recovery growth is measured. Since genetic variability is seen only upon acclimation, assessing stress responses on exposure to acclimation treatment could be a potential tool to screen for thermo tolerance or to identify thermo tolerant lines from germplasm or segregating populations.

Many researches have shown that thermotolerance might be acquired by heat acclimation, which can occur through exposure to a non-lethal heat treatment (Levitt 1980; Chen et al. 1982; Gong et al. 1997, 1998; Hatice and Atilla 2003). The processes involved in temperature acclimation are initiated by the perception of temperature signals and transduction of these signals into biochemical processes that finally lead to the development of heat

tolerance (Sangwan and Dhindsa 2002; Xu et al. 2006). These adaptation processes include adjustment of metabolism and gene expression at high temperatures (Vierling 1991), which enables plants to minimize heat injury. Heat acclimation also involves a considerable reorganization of thylakoid membrane, including adaptive changes of lipid composition (Larkindale and Huang 2004).

Elevated temperature affects the metabolic pathways mainly through oxidative damage to cells, thereby affecting the levels of both primary and secondary metabolites, which are of great biological significance. Of these, accumulation of free proline, glycine-betaine (GB) and soluble sugars is of great significance in regulating osmotic activities and protecting cellular structures from water, salt and other stresses that produce osmotic strain on the cells (Matysik et al. 2002; Wang and Li 2006; Bohnert et al. 2006). A high level of GB accumulation was reported in maize and sugarcane under water and heat stresses (Quan et al. 2004; Wahid and Close 2007). Similarly, accumulation of soluble sugars has been reported in sugarcane shoots, which had greater implications for heat tolerance (Wahid and Close 2007). There are many reports on the details of physiological metabolism, including the antioxidant metabolism in other plant species in response to heat stress, which clearly suggest that plant seedlings grown under high temperature could trigger the defense system. Very little is known, however, about the defense mechanism in sugarcane against high temperatures. The aims of the present study were to investigate comparatively the physiological changes in settlings and calli of popular sugarcane variety Co 86032 of under heat stress with heat acclimation and without heat acclimation, and to elucidate the possible biochemical mechanisms induced by high temperature stress in sugarcane.

## Materials and Methods

### Experimental Details and Stress Imposition

For in vivo study, healthy single budded chips of Co 86032 were sown in nursery tray (holding 50 bud chips in each tray) containing farmyard manure, coir pith and sand (1:1:1 ratio) and kept in mist chamber (28–29/21–24 °C). 30 days old settlings (2 trays for each treatment) were subjected to three different treatments ($T_0$ = control, $T_1$ = Non induced (directly subjected to 48 °C with 10 h), $T_2$ = Induced (40 °C with 10 h time followed by critical temperature condition of 48 °C with 10 h in BOD incubator (Make: Lab line Instrument). For in vitro study, calli of Co 86032 were subjected to three different treatments ($T_0$ = control, $T_1$ = Non induced (directly subjected to 48 °C with 15 h), $T_2$ = Induced (42 °C with 10 h time followed by critical temperature condition of 48 °C with 15 h) in BOD incubator. On completion of temperature treatment, calli and settlings were allowed to recovery for 24 h at room temperature (30 °C and 60 percent RH).

### Determination of Proline, Glycine-Betaine, Soluble Sugars and Proteins

Freshly excised leaves and calli tissues were immediately frozen in liquid N, ground to a fine powder, transferred to falcon tubes and stored at −80 °C until analysis. Free proline was determined from the frozen fresh leaf powder that was extracted with sulphosalicylic acid and the extracts reacted with acid-ninhydrin, as described by Bates et al. (1973). Glycine-betaine was determined using the method of Grieve and Grattan (1983). Tissue extracts prepared by vigorous shaking in 2N $H_2SO_4$ were cooled and mixed with an equal volume of periodide, vortexed and kept at 0–4 °C for 16 h. The mixture was centrifuged at 10,000 ×g at 4 °C for 15 min and the supernatant was aspirated while cool. The periodide crystals were dissolved in 1, 2-dichloroethane to measure the absorbance at 365 nm using a spectrophotometer (Make: Shimadzu, Model: UV-1601). For the determination of soluble sugars, the frozen powder was extracted in water at 80 °C by continuous shaking for 4 h and vacuum filtered. An aliquot of the filtrate was reacted with anthrone reagent by heating in a water bath at 100 °C for 20 min and the absorbance of the coloured complex was measured at 620 nm (Yoshida et al. 1976). Protein was estimated by the method as described by Lowry et al. (1951). Extraction was usually carried out with buffers used for the enzyme assay. Weighed 0.5 g of frozen powdered sample of heat treated settlings leaf and calli and homogenized with a pestle and mortar in 5–10 ml of the buffer. Centrifuged and used the supernatant for protein estimation. The blue color developed at the end of reaction was read at 660 nm.

### Determination of Antioxidant Enzyme Activities and Isozyme Pattern

Frozen leaf samples and calli were ground in a mortar with liquid nitrogen and extracted in 50 mM sodium phosphate buffer (pH 7.0) containing 2 mM EDTA. The homogenate was centrifuged at 20,000 rpm for 15 min at 4 °C in a refrigerated centrifuge. The supernatant was dispensed into aliquots for further analysis. Ascorbate peroxidase (APX) was extracted as above except that the buffer contained ascorbate (5 mM). Total soluble proteins were measured using bovine serum albumin (BSA) as a standard. POX activity was measured spectrophotometrically by monitoring the increase in absorbance (of the oxidized O-dianisidine), at 430 nm. POX isozymes were separated on 7 %

native PAGE and stained by the method of Malik and Singh (1980). APX activity was assayed following the method of Nakano and Asada (1981). Total SOD activity was measured based on inhibition in the photochemical reduction of nitro blue tetrazolium (NBT) (Beauchamp and Fridovich 1971). One unit of SOD was defined as the quantity of enzyme required to inhibit the reduction of NBT by 50 % in a 1 ml reaction volume. The reaction mixer contained 50 mM sodium phosphate buffer, pH 7.8, 58 mM NBT, 2.4 μM riboflavin, 9.9 mM methionine and 0.025 % Triton X-100. Isozymes of SOD were separated on a 10 % non-denaturing gel at 4 °C.

### Determination of MDA Content

Lipid peroxidation was estimated by measuring melondialdehyde (MDA) content of leaf and calli homogenate extracted in 20 % trichloro acetic acid (TCA) containing 0.5 %, thiobarbituric acid (TBA) and heated at 95 °C for 25 min (Heath and Packer 1968). The sample (0.5 g) was homogenized in 10 ml of 0.1 % TCA. The homogenate was centrifuged at 15,000 ×g for 5 min. To a one ml aliquot of the supernatant, 4 ml of 0.5 % TBA in 20 % TCA was added. The mixture was heated at 95 °C for 30 min and then quickly cooled in an ice bath. After centrifugation at 10,000 ×g for 10 min, the absorbance of the supernatant was recorded at 532 nm. The value for non-specific absorbance at 600 nm was subtracted. MDA content was determined by measuring absorbance at $A_{532}$ and corrected for non-specific absorbance at $A_{600}$ and it was calculated by its extinction coefficient of 155 $mM^{-1}$ $cm^{-1}$ and expressed as nmol MDA per gram fresh weight. To study membrane leakage, uniformly sized first fully opened leaf segments were floated on deionized water for 3 h and the extent of electrolyte leakage into the bathing medium was recorded using a conductivity meter (Elico-India, CM183, EC-TDS analyzer). Subsequently, the leaf segments were boiled for 30 min and allowed to cool. The final reading was recorded and the loss of membrane integrity was determined using the formula, % leakage = (Final reading − initial reading)/Final reading × 100. This protocol was modified from Leopold et al. (1981) and Tripathy et al. (2000).

### Protein Electrophoresis

Electrophoresis of protein was carried out through native and SDS-PAGE (Laemmli 1970). The collected heat treated settlings leaf and calli samples were homogenized with phosphate buffer 0.1 M (pH 7) containing ß-mercaptoethanol, in the ratio of 1:1 in pre-chilled pestle and mortar respectively. The slurry was centrifuged. The supernatant was collected and aliquots were frozen in small vials. The sample was analyzed by SDS-PAGE. The electrophoresis was carried out in 10 % gel containing 1 % SDS.

### Statistical Analysis

The experiment was conducted twice and all the determinations were made in triplicate. Data from both the experiments were pooled to perform statistical analysis using COSTAT software. LSD values were determined and Tukey's test (Steel et al. 1996) was used to ascertain the significance of temperature treatments and time points.

## Results and Discussion

### Soluble Protein of Settlings and Calli in Response to Heat Stress

The levels of total as well as soluble proteins are altered in plants growing under temperature-stressed environments compared with plants growing under non stressed conditions. In present study, a significant reduction of soluble protein was observed in both settlings (11.06 %) and calli (10.60 %) upon heat stress, however induced settling and calli were shown maximum accumulation of soluble protein content of 95.2 and 132.0 mg $g^{-1}$ respectively (Fig. 1a). The transient significant increase of soluble protein upon induction treatment might be due to the synthesis and accumulation of heat shock proteins as reported by Xu et al. (2006) in turf grass and Wahid and Close (2007) in sugarcane. Various workers have observed either a decrease or an increase in levels of total or soluble proteins in different organs of plants subjected to temperature stress. The increased or decreased levels of proteins depend on the plant species and organ studied as well as the severity of stress.

### Total Phenolics Content of Sugarcane Settlings and Calli in Response to Heat Stress

The phenolics are powerful antioxidants in plant tissues under stress (Sgherri et al. 2004). They are chemically heterogeneous compounds and include flavonoids, lignins and tannins. Settlings and calli, that are induced for sublethal temperature have shown higher total phenolics content of 125.1 and 142.5 μg $g^{-1}$ respectively compared to non induced (50.4 and 62.4 μg $g^{-1}$) and control (54.5 and 67.0 μg $g^{-1}$) with increase of 59.7 % for settlings and 56.2 % for calli over control (Fig. 1b). Similar pattern of accumulation of total phenolics under heat stress has been reported to be accompanied with increased polyamino lyase (PAL) but decreased peroxidase and polyphenol lyase

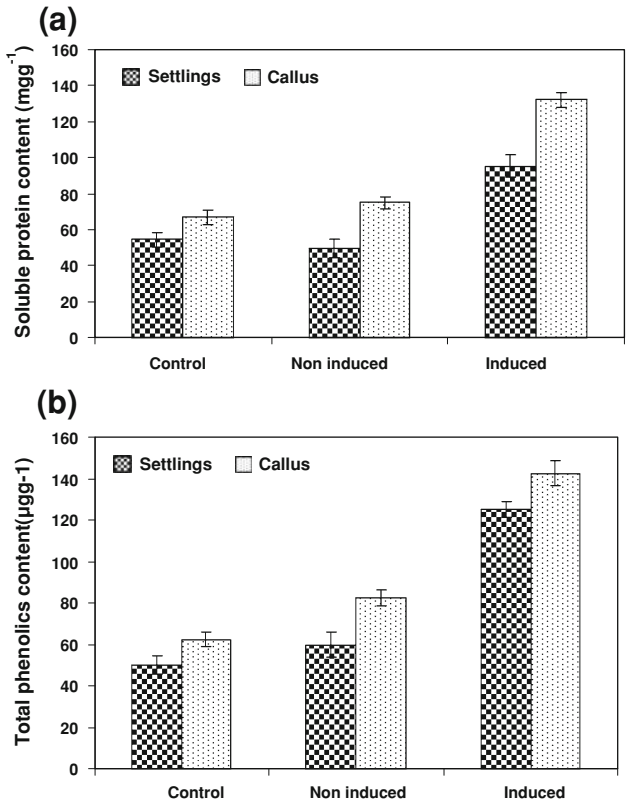


**Fig. 1** Effect of heat acclimation on soluble protein (**a**) and total phenolics (**b**) of sugarcane settlings and calli upon heat stress. *Error bars* represent the standard error (SE) of mean ($n = 4$)

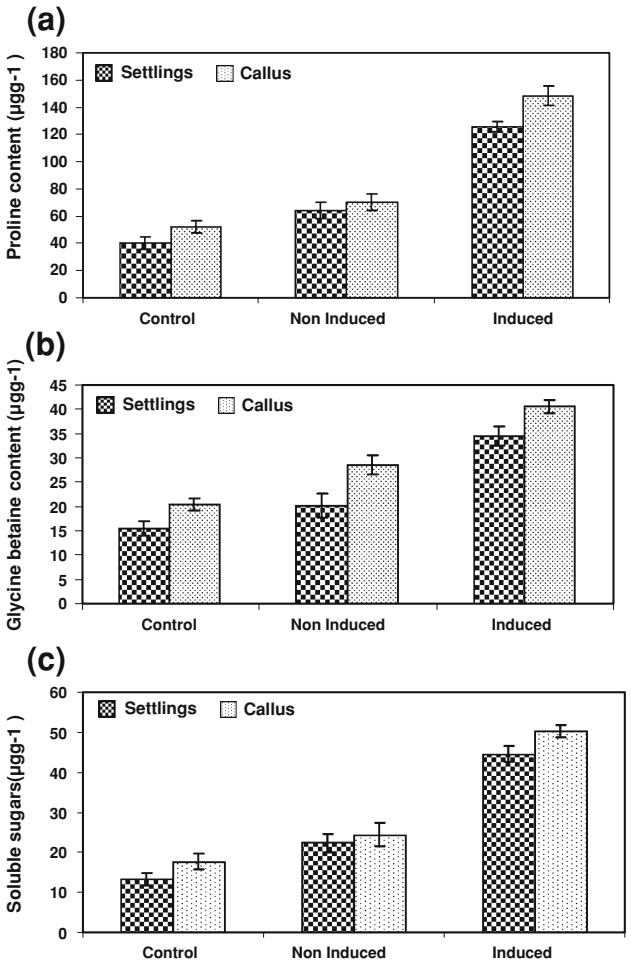


**Fig. 2** Effect of heat acclimation on proline content (**a**), glycine-betaine (**b**) and soluble sugars (**c**) of sugarcane settlings and calli upon heat stress. *Error bars* represent the standard error (SE) of mean ($n = 4$)

activities (Taiz and Zeiger 2006). Since the metabolism of phenolics takes place in the cytosol, it is believed that soluble phenolics themselves are the scavengers of ROS (Moyer et al. 2002). The role of phenolics has been recently reappraised as a protection from oxidative stress rather than protection from herbivores. Available data show that phenolics accumulate under a range of environmental stresses including temperature extremes and salinity (Wahid and Ghazanfar 2006). Hence increase in total phenolics upon induction treatment in both the tissues reveals that it plays crucial role in the heat tolerance of sugarcane settlings and calli.

## Osmolytes Concentration of Sugarcane Settlings and Calli in Response to Heat Stress

Under abiotic stresses including salinity, water deficit and extreme temperatures, different plant species may accumulate a variety of osmolytes such as sugars and sugar alcohols (polyols), proline, tertiary and quaternary ammonium compounds, and tertiary sulphonium compounds which play a role in mitigating the effect of osmotic and heat stresses (Sairam and Tyagi 2004). Proline accumulation in plant tissues under heat stress and desiccation has indicated that it is the most potent osmoprotectant. It has also been suggested to serve as an organic nitrogen reserve ready to be used after stress relief to sustain both amino acid and protein synthesis (Sairam and Tyagi 2004). Both in settlings and calli accumulation of free proline content were noticed upon heat stress treatment ($\sim$ 3 fold increase in both tissues). In settlings, proline content increased up to 125.5 µg $g^{-1}$ fr. wt. at 40 °C with 10 h stress treatment (induced), while in calli it reached up to 148.0 µg $g^{-1}$ fr. wt. The accumulation of proline was high in induced settlings (68.6 % over control) and calli (55.9 % over control) compared to non-induced settlings (37.6 % over control) and calli (34.6 % over control) (Fig. 2a). Accumulation of very large quantities of free proline (up to 200 % increase) was observed when sugarcane plants were subjected to heat and drought stress and this was suggested to be used as an index for screening sugarcane genotypes for abiotic stress tolerance (Wahid and Close 2007; Gomathi et al. 2011).

Glycine-betaine, an amphoteric quaternary amine, plays an important role as a compatible solute in plants under various stresses, such as salinity or high temperature. Capacity to synthesize GB under stress conditions differs from species to species (Ashraf and Foolad 2007). For example, high level of GB accumulation was reported in maize due to desiccating conditions of water deficit and high temperature (Quan et al. 2004). Data on GB content indicated that the induced settlings have shown maximum GB accumulation of 34.50 μg $g^{-1}$ with 41.50 % increase over control compared to non-induced settlings (15.50 μg $g^{-1}$) with 25.80 % increase over control. As that of settlings, induced calli have also shown maximum GB level of 40.50 μg $g^{-1}$ with 29.60 % over control compared to non-induced calli (20.40 μg $g^{-1}$) (Fig. 2b). Similar increase was reported in rice (Sakamoto and Murata 2002) and sugarcane upon heat acclimation (Wahid and Close 2007).

Data on total sugar content indicated that the temperature acclimated settlings and calli were recorded higher values of 44.5 and 50.2 μg $g^{-1}$ compared to non-acclimated settlings and calli (22.40 and 24.5 μg $g^{-1}$) respectively (Fig. 2c). Significant increase in total sugars upon heat stress treatment indicated that heat stress could contribute or induce a significant defense response resulting in better regulation ability in both sugarcane seedlings and calli under stress situation as reported by Yuan et al. (2010) in sugarcane. Similar accumulation of total sugars has been reported in sugarcane shoots, which had greater implications for heat tolerance (Wahid and Close 2007).

In present study, a maximum accumulation of total sugars, free proline and GB was evident in both sugarcane settlings and calli due to temperature induction treatment, although none of these metabolites accumulated under control conditions. Among the osmolytes, free proline indicated a sharper and prolonged accumulation only upon pre-treatment (acclimated) followed by GB and soluble sugars. Changes in the levels of these osmolytes indicated significant interactions of treatments and time points. Production of both the free proline and GB has been noted in various plant species under different stresses (Wang et al. 2003; Wahid and Shabbir 2005; Wahid and Close 2007); these osmolytes were believed to maintain cell water balance, membrane stability and buffer cellular redox potential.

## ROS Scavenging Enzyme Activity and Isozyme Pattern of Sugarcane Settlings and Calli in Response to Heat Stress

As an adaptation, plants have developed enzymatic and non-enzymatic detoxification systems to maintain ROS under control and protection from oxidative damage. The enzymatic mechanisms including SOD, APX, CAT, glutathione reductase (GR) and glutathione-synthesizing enzyme, and their activities were differentially expressed under high temperature (Chaitanya et al. 2001). Enhanced synthesis of antioxidants by plant tissues may increase heat tolerance plausibly by detoxification of the heavy load of AOS, which in turn provides protection. In this regard it is suggested that use of stress signaling molecules may enhance the antioxidant capacity of cells and produce thermotolerance (Sairam and Tyagi 2004). An apparent increase in POD and CAT during exposure to heat stress has been reported in several crops (Jiang and Huang 2001).

In present study, ROS enzyme activities APX, POX, SOD and isozyme pattern were estimated at different temperature condition of sugarcane settlings and calli. Results indicated that all the ROS enzyme activities significantly increased due to heat stress. In both the settlings and calli, all the ROS enzymes showed their maximum activity upon induction treatment (settlings and calli) and then declined gradually at temperature condition of 44 °C. APX is one of the most important antioxidant enzymes of plants that detoxify $H_2O_2$ using ascorbate for reduction. In present study, heat stress showed two fold average increases in APX activity over control both in sugarcane settlings and calli. However, induced settlings showed higher APX activity of 40.1 unit $g^{-1}$ $min^{-1}$ with 99.0 % over control, while non induced recorded as 28.50 unit $g^{-1}$ $min^{-1}$ with 39.8 % over control. Similar increase in APX activity upon heat acclimation was noticed in calli (Fig. 3a), suggesting that the ascorbate–glutathione cycle played a crucial role in mitigating the accumulation of $H_2O_2$ in sugarcane settlings and calli upon heat stress treatment. This is in agreement with data showing the up regulation of APX in *Lilium* and *Freesia* seedlings (Yin et al. 2008; Yuan et al. 2010).

Peroxidases (POX) catalyses oxidation of various substrates in the cell and significant roles of peroxidases have been suggested in plants. Previous studies have shown that POX activity increased during exposure to heat stress in bean (Ye et al. 2000), maize (Scandalios et al. 2000) and grass (Jiang and Huang 2001). Our results also suggest that heat acclimation could contribute to a significant increase in POX both in calli and settlings and maintained a high level even under lethal temperature. Induced settlings and calli are recorded higher POX activity of 36.50 and 41.0 Δ change OD $g^{-1}$ $min^{-1}$ respectively compared to non-induced (20.10 and 25.10 Δ change OD $g^{-1}$ $min^{-1}$ respectively) suggesting that the calli and settlings that are pretreated with sub-lethal temperature (induced) have an adaptation mechanism (increase in POX) when exposed to lethal temperature(Fig. 3b).

Among the antioxidants, SOD is an essential component of defense mechanism in plants under environmental

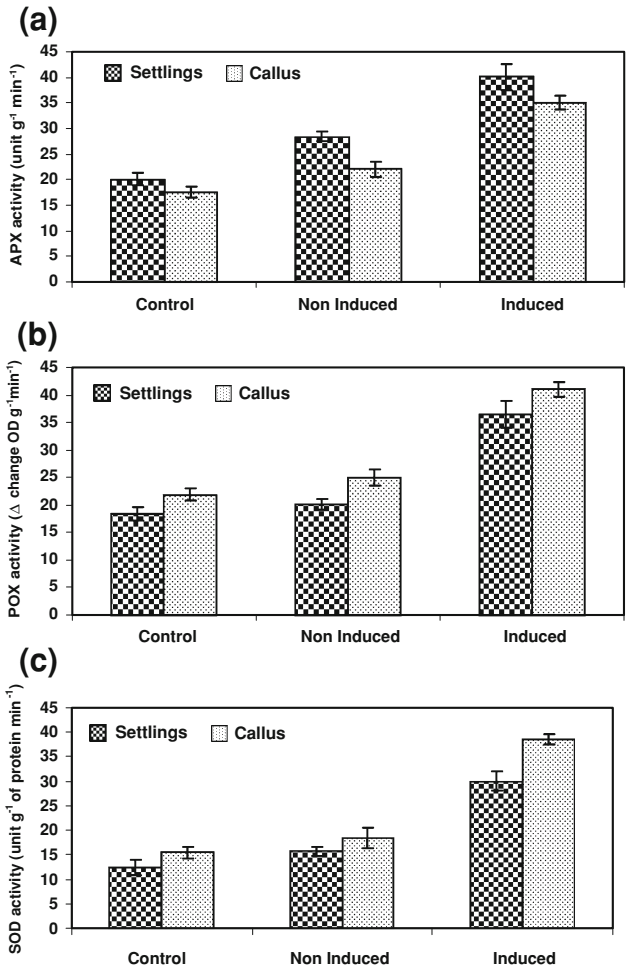


**Fig. 3** Effect of heat acclimation on APX activity (**a**), POX activity (**b**) and SOD activity (**c**) of sugarcane settlings and calli upon heat stress. *Error bars* represent the standard error (SE) of mean ($n = 4$)

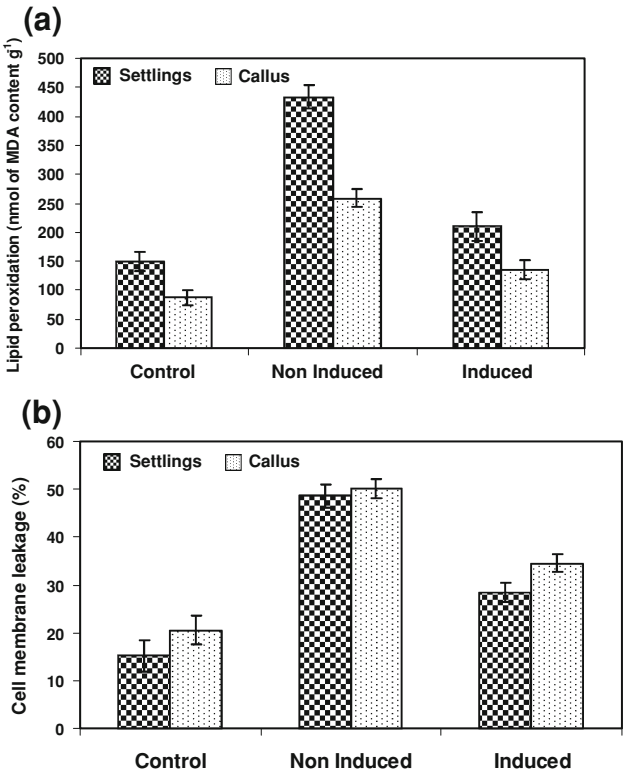


**Fig. 4** Effect of heat acclimation on lipid peroxidation (**a**) and cell membrane leakage (**b**) of sugarcane settlings and calli upon heat stress. *Error bars* represent the standard error (SE) of mean ($n = 4$)

adversity. A reduction in the activity of SOD under heat stress and its rapid increase following rewatering was reported in Kentucky blue grass (Zhaolong and Bingru, 2004). In present study heat acclimation pretreatment led to significant increase in SOD activity in both settlings and calli. In settlings, a much higher activity of SOD was noticed in induced (30.0 unit $g^{-1}$ $min^{-1}$) compared to non-induced (15.75 unit $g^{-1}$ $min^{-1}$) and control (12.5 unit $g^{-1}$ $min^{-1}$), respectively. As that of settlings, calli that are induced showed higher SOD activity of 38.50 unit $g^{-1}$ $min^{-1}$ compared to non-induced (18.50 unit $g^{-1}$ $min^{-1}$) and control (15.50 unit $g^{-1}$ $min^{-1}$) suggested a stronger ability to scavenge ROS caused by $O^{-2}$ as reported by Xu et al. (2006), Wahid and Close (2007), Yuan et al. (2010) in C4 species.

Isozyme studies on SOD and POX also support the fact that their activity is increased as evidenced by additional bands in heat stressed samples at 40 °C in settlings and 42 °C in calli. Results of isozyme pattern of POX indicated that, temperature induced over expression of the POX isoform was noticed only in heat acclimated settlings and calli at 40 and 42 °C respectively (Plate. 1a, b). Results of isoforms pattern of SOD indicated (Plate 2a, b) that different forms of isozymes (high and low molecular weights) were noticed in all the samples. However, these isoforms were over expressed at induction temperature of 40 °C (settlings) and 42 °C (calli). Higher degree of expression of these isoforms (POX and SOD) in their activity leads to protection of cells from oxidative damage suggesting that as an adaptation, plants have developed enzymatic control of scavenging ROS under stress condition. Inductive response to new isoforms or over expression results from modified metabolic rates of phytochemical reactions to scavenge ROS system. Similar inducible and up regulation of enzyme peroxidase and SOD in *Fressia* seedlings had been reported by Yuan et al. (2010).

## MDA Content and Cell Membrane Damage of Sugarcane Settlings and Calli in Response to Heat Stress

In general, the peroxidation of membrane lipid and the membrane damage were evaluated by MDA concentration and electrolyte leakage (EL), which therefore have been

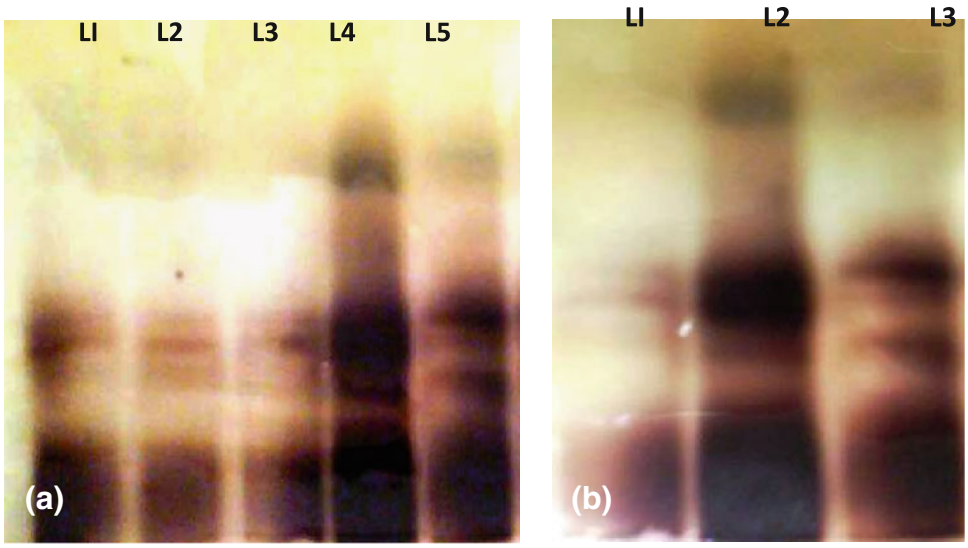


**Plate. 1** **a** POX isoforms (a) from settlings of Co 86032: *L1*, *L2* and *L3* = Control, *L4* = Induced, *L5* = Non induced; **b** POX isoforms (a) from calli of Co 86032: *L1* = Control, *L2* = Induced, *L3* = Non induced

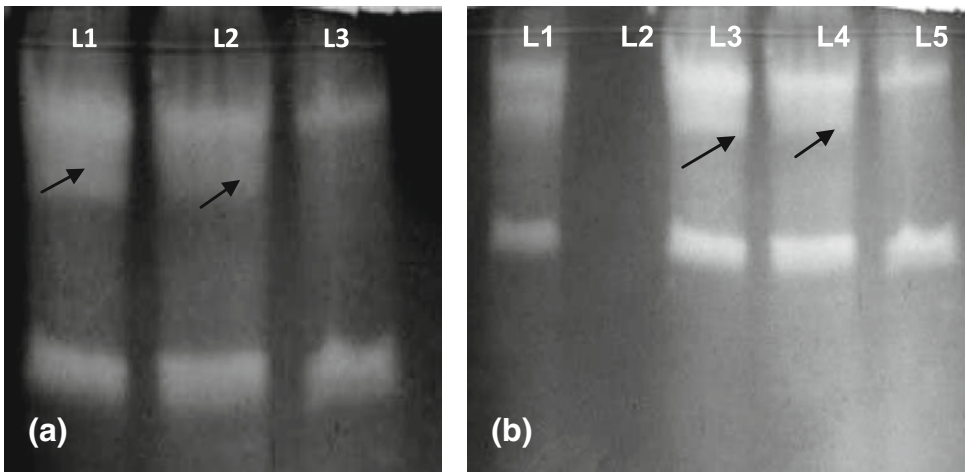


**Plate. 2** **a** SOD isoforms (a) from settlings of Co 86032: *L1* = induced, *L2* = non induced, *L3* = Control; **b** SOD isoforms (a) from calli of Co 86032: *L1* = Control, *L2* = blank, *L3* and *L4* = induced, *L5* = Non induced

widely used as a criterion to assess heat injury in various crops (Xu et al. 2006; Yin et al. 2008; Yuan et al. 2010). Membrane lipid saturation is considered as an important element in thermotolerance. In present study, in both the settlings and calli cell wall lipid peroxidation increased upon heat stress; however induced settlings and calli were recorded lower lipid peroxidation of 210.20 and 135.50 nmol MDA content $g^{-1}$ nmol MDA content $g^{-1}$ respectively compared to non-induced settlings (432.45 and control 150.0 nmol MDA content $g^{-1}$ respectively) and calli (258.40 nmol MDA content $g^{-1}$).

In both tissues, a significant influence on cell membrane damage was noticed under heat treatment. However severity of damage was higher in calli tissues compared to settlings, because the calli are the undifferentiated cells which are easily damageable under adverse situation. Acclimated settlings and calli showed comparatively less membrane damage of 28.50 and 34.50 % respectively than non-induced (48.50 and 50.03 % respectively (Fig. 4b.). Results suggest that, the increase of cell membrane leakage % is due to heat induced increase of MDA content as a result of higher peroxidation of membrane lipids. However, pretreated heat acclimated settlings and calli showed lower lipid peroxidation and cell membrane damage compared to non-induced tissues. The changes in MDA content and EL revealed that heat acclimation could contribute to prevent sugarcane settlings and calli from the membrane injury and indicated that sugarcane could endure heat stress. These results were also supported by the significantly enhanced activities of antioxidant enzymes (such as SOD, POX and APX) in inhibiting the accumulation of ROS upon heat stress. It is well known that ROS-induced peroxidation of membrane lipid is a reflection of stress induced damage at the cellular level (Jain et al. 2001; Xu et al. 2006). The increased solute leakage, as an indication of decreased cell membrane thermostability (CMT), has long been used as an indirect measure of heat-stress tolerance in diverse plant species, including potato and tomato (Chen et al. 1982),

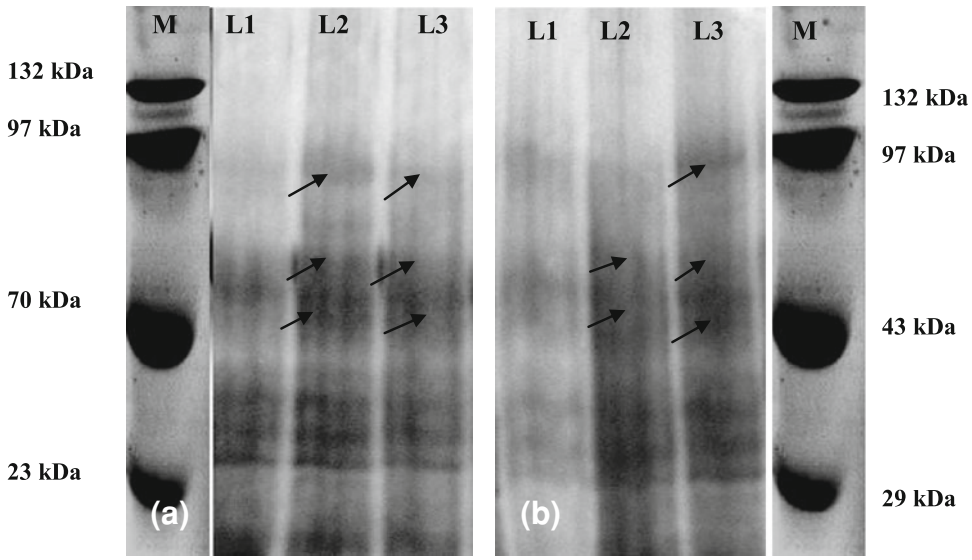


**Plate. 3** **a** SDS protein profile (a) for settlings of Co 86032: *M* = marker *L1* = control, *L2* = induced, *L3* = non induced; **b** SDS protein profile (a) for calli of Co 86032: *M* = marker *L1* = control, *L2* = non induced, *L3* = induced

wheat (Blum et al. 2001), cotton (Ashraf et al. 1994), sorghum (Marcum 1998), cowpea (Ismail and Hall 1999) and barley (Wahid and Shabbir 2005).

### Protein Profile of Sugarcane Settlings and Calli in Response to Heat Stress

One of the most widely studied aspects of thermotolerance is the enhanced expression of heat shock proteins (Hsps). Synthesis and localization of a few Hsps have been shown to trigger several physiological and biochemical processes (Cushman and Bohnert 2000) such as the maintenance of membrane integrity and chaperoning proteins. The expression of Hsps is primarily regulated by the heat-dependent activation of the heat shock transcription factors (HSFs) (Scharf et al. 1998). Changes in accumulation and synthesis of proteins have been observed in plants resulting from exposure to stress during growth. Plants have developed many strategies to tolerate stress that include expression of some novel proteins (Wang et al. 2003).

In the present study, temperature induced changes in the protein profile through SDS-PAGE was done in both the tissues of settlings and calli which revealed that the formation of additional protein bands of different molecular weights (90, 70 and 27 kDa) expressed well particularly at 40 °C in settlings ($L_2$) and 42 °C in calli ($L_3$) (Plate 3a, b). Similar expression of Hsps upon heat stress was recently reported Sanjam et al. (2010). The major role of Hsp70 is to protect heat-labile proteins from denaturation. Under normal conditions, Hsp70 facilitate folding of newly synthesized proteins, preventing undesirable interactions. Members of Hsp70 family play distinct roles in plant growth and development. Developmentally regulated Hsp70 genes have been described in *Arabidopsis* (Wu et al. 1988). In *Arabidopsis thaliana*, Hsp70 over expression has been shown to affect growth, development and thermotolerance (Sung et al. 2003). Hsp90 is one of the most abundant cytosolic proteins in eukaryotes and belongs to another highly conserved protein family. Hsp90 genes were isolated from numerous plant species, including *A. Thaliana* (Krishna and Gloor 2001), *Brassica napus* (Krishna et al. 1995), maize (Marrs et al. 1993) and tomato (Koning et al. 1992). In Arabidopsis, Hsp90 take part in various fundamental biological processes, such as cell cycle and growth control, gene expression, proteolysis and stress response (Rutherford et al. 2007). Hence, accumulation of heat inducible protein (Hsp90, Hsp70) and dehydrins (27 kDa) both in induced settlings and calli heat stress treatment play role in cell protection, survival and production of compatible solutes soluble sugars, proline and GB and thus protect the plant heat stress damage.

## Conclusion

In conclusion, heat acclimation pretreatment improved the thermotolerance of settlings and calli of Co 86032 compared with those without heat acclimation pretreatment under heat stress, which may result from decrease in membrane lipid peroxidation and accumulation of ROS, and increase in activities of antioxidant enzymes (APX, POX, SOD), accumulation of metabolites (total phenolics, soluble proteins and sugars, proline, GB etc.) and specific expression of heat inducible proteins (Hsp90, Hsp70) and dehydrins (27 kDa). Therefore, these physiological changes caused by heat acclimation may be helpful to improve the heat stress adaptation of sugarcane settlings and calli.

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