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Comparative Biochemical Analysis of High and Low Sucrose Accumulating Sugarcane Varieties at Formative Stage under Heat Stress

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ABSTRACT

Sugarcane (*Saccharum officinarum* L) is a valuable cash crop which plays an imperative role in the worldwide economy. However, high temperature has significantly retarded the crop growth and yield by alteration of biochemical pathways. Therefore, the biochemical activities of two sugarcane varieties were explored under heat stress condition. The sugarcane cultivars S2003-US-633 (high sucrose accumulation) and SPF-238 (low sucrose accumulation) were cultivated and subjected to different temperature regimes i.e. control at 30±2 °C, heat stress at 45±2 °C and recovery at 30±2 °C for 24, 48 and 72 hours at formative stage. Detailed profiling of physiochemical attributes, sugar analysis linked with sucrose metabolism enzymes and

thermotolerance indicators were investigated. S2003-US-633 exhibited better response in terms of sugar accumulation regulated by sucrose synthase, sucrose phosphate synthase and invertase activities along with more proline accumulation, total soluble protein contents with response to high temperature exposure. While S2003-US-633 is ranked as tolerant variety due to less MDA, H₂O₂ content and electrolytes leakage exhibiting its efficient tolerance mechanism, giving high sugar recovery rate despite harsh environmental conditions. Thus, these findings can be helpful in providing information for engineering sugar improvement along with thermotolerance in sugarcane varieties and providing new avenues towards the economic development of the country.

Keywords: Metabolizing enzymes; Oxidative markers; Sugar recovery; Stress indicators; Thermotolerant; Yield

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1. Introduction

Sugarcane is an important crop due to the presence of high sucrose content in its stalk. It plays an important role towards the economic development of the country. In spite of extension in sugarcane production in Pakistan, average sugar recovery is reported 8-9% only which is far beyond from other developed countries (PSMA Report 2007). On the other hand, altering climate conditions along with low sugar recovery potential of sugarcane poses a challenge to sugarcane industry. High glucose, sucrose availability

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is important for physiologically regulating plant development, sugar signaling and water balance in cell (Liu & Huang 2000; Roitsch & González 2004). Sugar recovery depends on the amount of stored sugar in cane tissue (Joshi et al 2013) involving sucrose metabolizing enzymes such as sucrose synthase, sucrose phosphate synthase, neutral, vacuolar and cell wall invertases. These sucrose metabolizing enzymes in sugarcane genotypes are diversely affected during different growth stages (Tana et al 2014). It has been investigated that accumulation of sucrose in sugarcane tissue is mainly dependent on invertase and SPS activities which are adversely affected by climatic alterations (Ansari et al 2013). However, these climate changes are reported to have a substantial impact on crop production ultimately leading to yield losses with great risk for future global food security (Christensen & Christensen 2007). Rise in current global mean temperature has increased by 0.99 °C (NASA 2017) which is projected to have severe implications on plant growth and development by altering the underlying molecular mechanisms. It has been reported that there are substantial yield reductions observed at temperature more than 45 °C, also affecting the sugar recovery in sugarcane leading to the huge economic losses (Shrivastava et al 2010). This reduction is due to the down regulation of specific genes in carbohydrate metabolism which may lead to the altered activities of carbon metabolism enzymes, compromised starch accumulation and sucrose synthesis due to heat stress (Ruan et al 2010). Like other abiotic stresses, heat stress results in the production of ROS (Potters et al 2007) such as, superoxide anion (O_2^-), H_2O_2 hydroxyl radical (OH^{\cdot}), which are highly reactive and can alter the metabolism of plant through oxidative damage to membrane, denaturing of protein and nucleic acids leading to cell death. Moreover, H_2O_2 is involved in disruption of various metabolic activities like calvin cycle (Akram et al 2012). Due to the current scenario, development of thermotolerant varieties are the important strategy in adaptation of climate change. In this regard, biochemical characterization is crucial step to recognize varieties with desirable agronomical traits to meet sugar industry requirements. In this study, the effect of heat stress on sugarcane was studied to determine the physiological and biochemical analysis in sugarcane at formative stage to better understand the biochemical response of plants in heat stress condition for improving sugarcane thermotolerance.

2. Material and Methods

In this study, the two cultivars (S2003-US-633 and SPF-238) of sugarcane were cultivated in 20 kg capacity pots having loam soil at net house, The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE) University of Karachi, Karachi, Pakistan in February 2016. Experiment was conducted in Completely Randomized Design (CRD) with three replicates per each treatment group. Half strength Hoagland solution was supplemented weekly for whole experiment. Heat stress was applied by shifting all pots to heat shock room, where white fluorescent tube lights/mercury lamps were used for maintaining photosynthesis active radiation (PAR) ranging from 650-700 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Temperature was set at 45 ± 2 °C and 34 ± 2 °C for day and night respectively while humidity was maintained at 60-70%. For air circulation, fans were adjusted. For recovery experiment, pots were again shifted to growth room at 30 ± 2 °C. Samples were collected for control at 30 ± 2 °C, heat stress treatment at 45 ± 2 °C for 24 h (T24), 48 h (T48) and 72 h (T72) and recovery treatments at 30 ± 2 °C for 24 h (R24), 48 h (R48) and 72 h (R72) and preserved at -80 °C for biochemical analysis.

Extraction and quantification of sugars: For this, 0.5 g fresh sugarcane leaf was homogenized in 2.5 mL ethanol (80%) immediately after sampling. The samples were centrifuged at 1000 rpm for 10 min. Reducing sugar was estimated using dinitrosalicylic acid DNSA reagent (Miller 1959) and total sugar was quantified by anthrone reagent (Hedge & Hofreiter 1962). Whereas non-reducing sugar was calculated by following formula;

$$\text{Non-reducing sugar (mg mL}^{-1}\text{)} = \text{Total sugar} - \text{Reducing sugar} \quad (1)$$

Quantification of sugar metabolizing enzymes: For enzyme extraction, 0.5 g leaf tissues were homogenized in 2 mL MOPS-NaOH buffer (pH 7.5) and then centrifuged at 12000 rpm for 30 min. The reaction mixture for Sucrose Synthase (SS) contained; enzymes (10 μL), (0.2 mM) UDPG substrate (25 μL), (4 mM) Fru 6-P, (40 mM) Glu 6-P, (5 mM) MOPS-NaOH (pH -7.5), (5 mM) MgCl_2 and (0.1 mM) EDTA. All tubes were placed at 37 °C for 15 min and reaction was stopped by adding 70 μL 30% KOH. The reaction mixture was then placed at 100 °C for 10 min. Then 5 mL 0.14% anthrone in sulphuric acid

was added in reaction. All tubes were again heated at 100 °C for 15 min and OD was taken at 620 nm. For SPS quantification, above-mentioned reaction mixture was used having 40 mM fructose instead of fructose 6 p and Glucose 6 p (Hubbard et al 1989). The activity of cell wall bound invertase was analyzed by using some modification in the procedure of (Vorster & Botha 1999). The assay medium consisted of 250 µL K-Phosphate buffer (pH 7.0) for neutral invertase while for cell wall invertase, acetate buffer (pH 3.5) consisting sucrose (4%), 250 µL supernatants and 500 µL deionized water was used and incubated at 37 °C for 60 min. Reaction was terminated by adding 1ml of 1.6% NaOH with DNSA reagent, heated at 100 °C for 10 min. Absorbance was read at 540 nm using known glucose standard curve.

Thermotolerance indicators: For proline estimation, 100 mg leaf tissues were extracted in sulphosalicylic acid (3%) and centrifuged at 10000 rpm for 15 min. In 1ml of supernatant, 1 mL ninhydrin reagent and 1 mL acetic acid were added. The reaction was boiled for 60 min and was placed on ice bath followed by addition of toluene (4 mL). It was vortexed and placed at room temperature for 20 min. Optical density was measured at 520 nm (Bates et al 1973). Total soluble protein was quantified through Bradford assay (Bradford 1976). Reaction mixture (3 mL) contained plant extract (50 µL), Bradford dye (150 µL) and 0.15 N NaCl (2800 µL), incubated at room temperature for 20 min. Absorbance was measured at 595 nm using known protein standard curve.

Stress induced oxidative markers analysis: Relative membrane permeability (RMP) in terms of percentage was measured by assessing electrolytes leakage using the method by (Yang et al 1996) with the help of electrical conductivity (EC) meter. For H₂O₂ quantification, 100 mg sugarcane leaf tissues were homogenized in 2 mL (0.1%) TCA. After centrifugation, 0.5 mL supernatant was added in (1 mL) phosphate buffer (pH 7.0) and (2 mL) potassium iodide. Then OD was measured at 390 nm. Potassium iodide (2 mL) and potassium buffer (1 mL) were used for blank in the absence of leaf extract. Standard curve of H₂O₂ was constructed by using different concentration of H₂O₂ (Loreto & Velikova 2001).

Determination of lipid peroxidation: Malondialdehyde (MDA) contents were assessed by Heath & Packer (1968). Sugarcane leaf tissues (0.1g) were extracted in 2 mL of tetrachloroacetic acid (5%) and the mixture was centrifuged at 14000 rpm for 10 min. The supernatant (1 mL) was separated and 1 mL thiobarbituric acid (0.5%) was added and the mixture was heated for 20 min. After cooling, the reaction was re-centrifuged at 10,000 rpm for 10 min and then first OD was taken at 532 nm and at 600 nm.

Protein profiling:

SDS-PAGE

Extracted proteins from leaf tissues were resolved on SDS-PAGE gel (Laemmli 1970). Resolving (10%) and stacking gels (4%) were prepared by combination of acrylamide and bis-acrylamide solution, resolving (pH 8.8), stacking buffers (pH 6.8), SDS, fresh APS (5%), TEMED and double distilled water. The gel was run at 100 Volt for 2 hours. The gel was then stained by Coomassie dye (G-250) with shaking for overnight at room temperature. Next day the gel was destained by using destaining solution. Then the gel was scanned and documented.

Statistical analysis: Data was statistically analyzed for analysis of variance (ANOVA) using the SPSS package program, version 17.0. Test for normality of data were done using Least Significant Difference (LSD). Statistical significance was determined at P<0.05.

3. Results

Sugar analysis: Results revealed that total sugar, reducing and non-reducing sugars had statistically significant differences (P<0.05) between cultivars and treatments, while no significant differences (P>0.05) between interaction (C x T) were observed. Although, there were varietal differences for all these parameters, but increase in temperature episode caused reduction in total sugar profile (Figure 1a). After 72 hours of thermal stress (T72), pronounced reduction in sucrose was evident in S2003-US-633 (263 µg mL⁻¹) and SPF-238 (228 µg mL⁻¹) as compared to control (712 µg mL⁻¹) and (573 µg mL⁻¹) respectively.

However, recovery treatments greatly triggered regain of sugar loss with passage of recovery time. Same pattern of accumulation upon thermal stress was also observed in reducing and non-reducing sugars (Figure 1b-1c). S2003-US-633 exhibited decline up to 0.12 mg mL⁻¹ at T72 from control 0.25 mg mL⁻¹ for reducing sugars and 0.45 mg mL⁻¹ at T72 from control 0.14 mg mL⁻¹ for non-reducing sugars respectively.

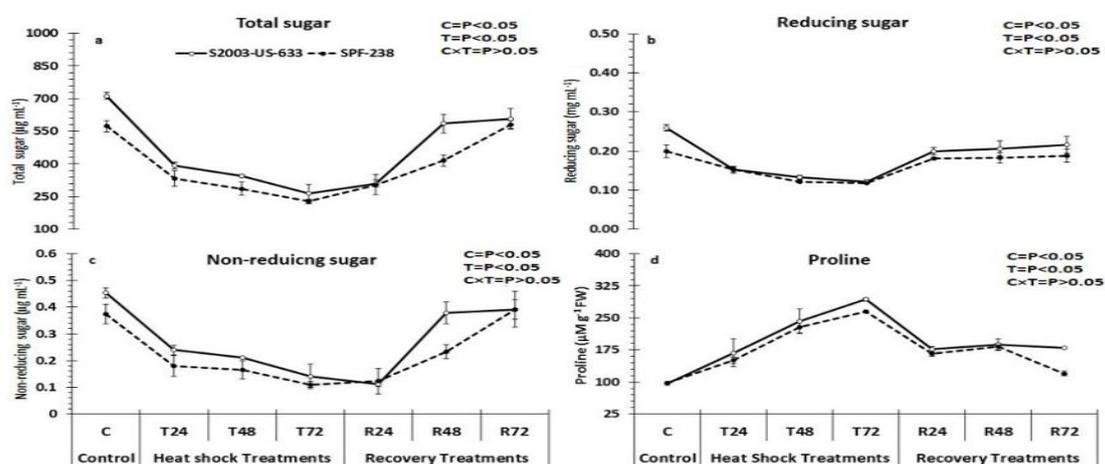


Figure 1- a, Total sugar; b, reducing sugar; c, non-reducing sugar and d, proline of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at 30±2 °C, heat shock treatment at 45±2 °C for 24, 48 and 72hours and recovery treatment at 30±2 °C after 24, 48 and 72 hours

Thermotolerance indicators: Statistical analysis showed significant differences ($P < 0.05$) for proline and total soluble protein contents for cultivars (C) and treatments (T) but there was no statistically significant difference ($P > 0.05$) for interaction (C x T) between them. It is clear from the results that free proline accumulation increased by high temperature in both cultivars S2003-US-633 and SPF-238 (Figure 1d). Comparatively, S2003-US-633 had maximum accumulation of total soluble protein in all condition showing better condition of growth. A significant rise in the concentration of proline content observed in both sugarcane varieties under heat shock conditions (T24, T48 and T72), while declined upon recovery. Between these varieties, S2003-US-633 had maximum accumulation of proline (293.5 $\mu\text{mol g}^{-1}$ FW) under heat shock treatment (T72).

Stress induced oxidative markers: There were statistically significant differences for all stress induced damages such as RMP and malondialdehyde contents among cultivars and treatments at the ($P < 0.05$) levels respectively. Upon exposure to heat stress, increased EC content was evident at T72 in SPF-238 (28.7%) and S2003-US-633 (24.30%) respectively. During recovery conditions, both cultivars exhibited same pattern for electrolyte leakage (Figure 2b). Similarly, MDA (malondialdehyde) (nmol mL⁻¹ FW) was maximum with the progression of heat stress (T24, T48 and T72) in SPF-238. But statistically non-significant differences were found for this attribute only between varieties and treatments (Figure 2c). For hydrogen peroxide contents, there were statistically significant differences ($P < 0.05$) between cultivars and treatments while in interaction (C x T) no significant differences were observed. Although heat stress increased production of H₂O₂ in both cultivars but cultivar S2003-US-633 showed minimum accumulation of hydrogen peroxide content as compared to SPF-238. In heat shock conditions H₂O₂ contents of both varieties increased many folds (Figure 2d).

Sugar metabolizing enzymes analysis: The activities of key sucrose metabolizing enzymes (SPS, SS, NIV and CWIN) of two cultivars at vegetative stage under heat stresses are presented in (Figure 3a-3d). Statistical analysis of sugar metabolizing enzymes activities exhibited significant differences ($P < 0.05$) for sucrose phosphate synthase and neutral invertase among cultivars (C), treatments (T) but no significant difference ($P > 0.05$) was observed for their interaction (C x T) in neutral invertase. The exposure of heat stress declined the enzymes activities in both varieties as compared to the control. Regarding SPS activity, drastic reduction was observed after 72 hours of heat stress in S2003-US-633 (2257 U mL⁻¹ min⁻¹) and SPF-238 (2407 U mL⁻¹ min⁻¹) and was increased in recovery treatments (R72) (Figure 3a). While, SS activity

exhibited non-significant differences ($P>0.05$) for cultivars (C) and treatments (T) and significant differences ($P<0.05$) for interaction (Figure 3b). It is evident from the results that SS activity was sequentially decreased as episodes of heat stress progressed, maximum reduction ($69.68 \text{ U mL}^{-1} \text{ min}^{-1}$) was observed at heat shock treatment (T72) in SPF-238. While both cultivars recovered the maximum SS activity after 72 hours of recovery treatment (R72). For cell wall invertase and sucrose synthase activity, significant differences ($P<0.05$) were found for treatments (T) while cultivar and their interaction (C x T) were non-significant ($P>0.05$). Among, heat shock treatments, only 72 hours of heat shock (T72) exhibited decreased activity of SS as compared to other treatments. After 72 hours of heat stress, neutral invertase activity declined in both cultivars S2003-US-633 ($0.91 \text{ U mL}^{-1} \text{ min}^{-1}$) and SPF-238 ($0.90 \text{ U mL}^{-1} \text{ min}^{-1}$), moreover quick recovery was observed in SPF-238 ($1.36 \text{ U mL}^{-1} \text{ min}^{-1}$) as compared to S2003-US-633 ($1.11 \text{ U mL}^{-1} \text{ min}^{-1}$) (Figure 3c). Whereas, cell wall invertase activity declined in heat stress during T24, T48 and T72 hours but there was no significant difference observed in both cultivars (Figure 3d).

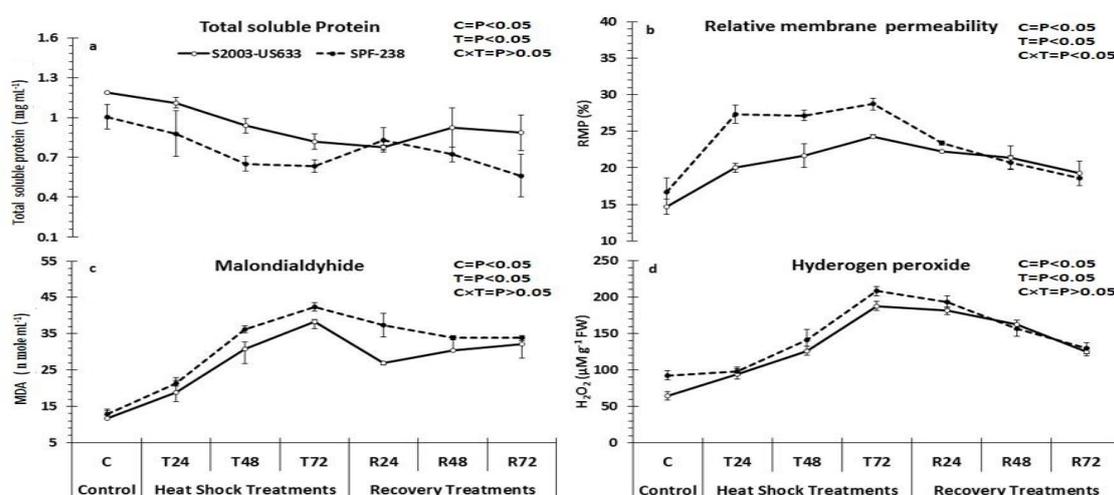


Figure 2- a, Total soluble protein; b, MDA; c, RMP and d, H_2O_2 of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at $30\pm 2^\circ\text{C}$, heat shock treatment at $45\pm 2^\circ\text{C}$ for T24, T48 and T72 hours and recovery treatment at $30\pm 2^\circ\text{C}$ after T24, T48 and T72 hours

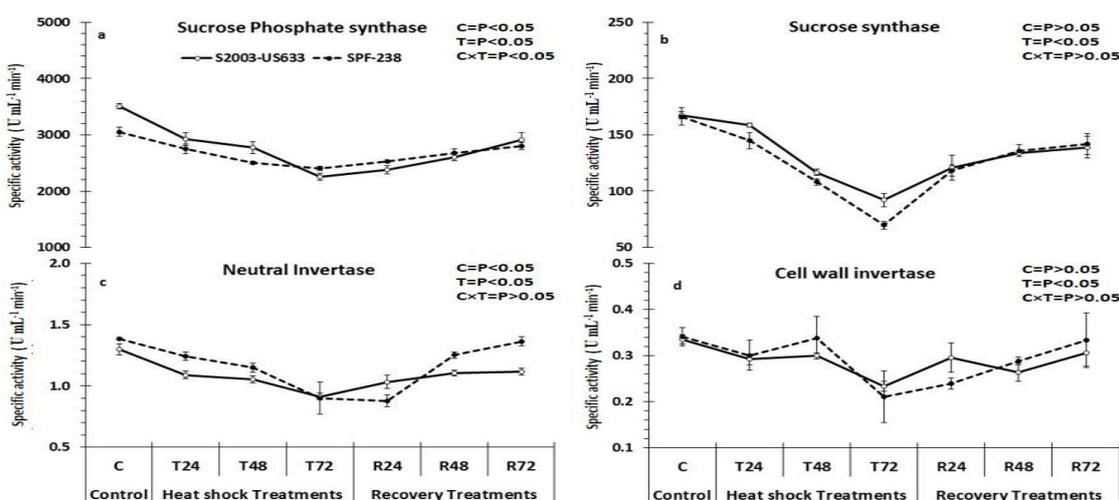


Figure 3- a, SPS; b, SS; c, NIV and d, CWIN of both cultivars (S2003-US-633 & SPF-238) at formative stage under control at $30\pm 2^\circ\text{C}$, heat shock treatment at $45\pm 2^\circ\text{C}$ for T24, T48 and T72 hours and recovery treatment at $30\pm 2^\circ\text{C}$ after R24, R48 and R72 hours

Protein profiling:

SDS PAGE

At formative stage, protein expression was analyzed through SDS-PAGE for both the varieties (Figure 4). Proteins of different molecular weights were differentially expressed during heat stress. The highest molecular weight protein (≈ 150 kDa) was clearly expressed upon heat stress and recovery treatments. The band intensity of the protein remained stable during all the episodes of heat stress (T24, T48 and T72) but during the recovery phase it was diminished (R24, R48) and then reappeared at R72 hours. Similarly, among high molecular weight proteins 90, 70 and 60 kDa protein bands were consistently expressed during the heat shock treatments but at the initial stages of recovery (R24-R48) these were not visible. Thus, in both varieties same pattern of protein expression was observed in high molecular weight protein but in case of low molecular weight proteins there was a sharp high intensity band observed at approx. 15 kDa (green) in variety S2003-US-633 which was not present in variety SPF-238. During the SDS PAGE analysis 15 kDa protein might be differentially expressed in S2003-US-633 as compared to SPF-238 during the heat shock and recovery phases.

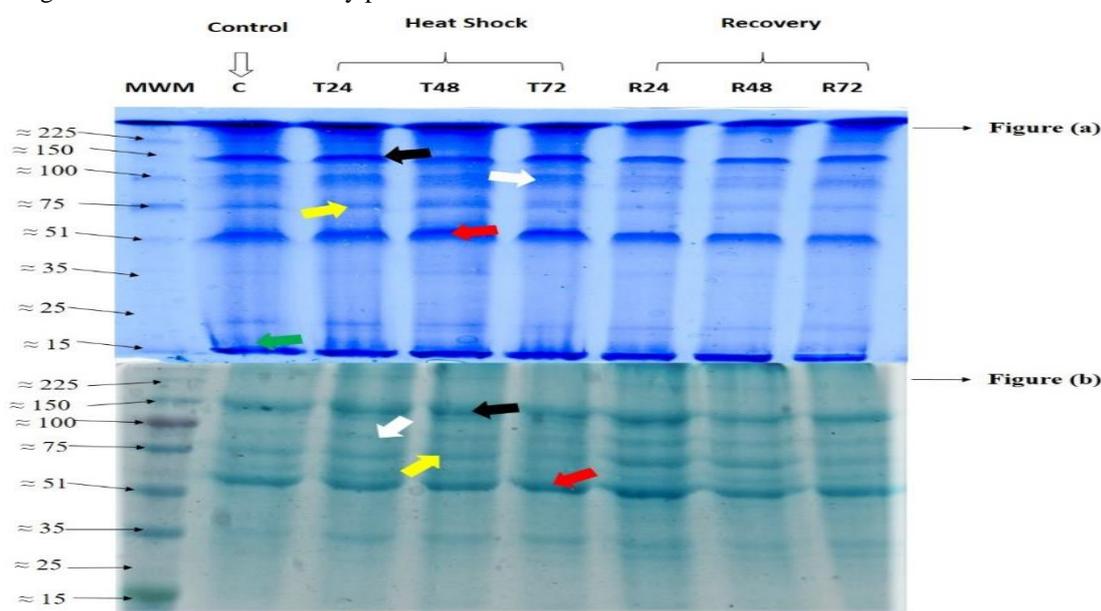


Figure 4- SDS-PAGE protein profiling a, S2003-US-633; b, SPF-238 at formative stage under control at 30 ± 2 °C, heat shock treatment at 45 ± 2 °C for T24, T48 and T72 hours and recovery treatment at 30 ± 2 °C after R24, R48 and R72 hours

4. Discussion

Present study was planned to unravel the sugar metabolism pathways in sugarcane cultivars, for reprogramming its resources to cope with unprecedented heat waves without any compromise in its core product (sucrose) accumulation. This differential accumulation of sucrose in sugarcane, found multifarious, not only cultivaral, phenological and temporal (McCormick et al 2008) but also affected by several biotic and abiotic factors (Albacete et al 2011). Regarding sucrose analysis, both sugarcane cultivars showed significant variations depending on duration of stress and recovery treatment. While, reducing and non-reducing sugars analysis revealed same pattern of response for thermal stress by significant decrease. This reduction in sucrose concentration is attributed due to less carbon assimilation and subsequent partitioning of carbon derived energy products including sucrose, from source (leaves) to sink (stem), tissues (Ebrahim et al 1998) or increased respiratory demand. On the other hand, sugar metabolism is regulated by sucrose synthase (SS), sucrose phosphate synthase (SPS) and invertases. Sucrose is catabolized or resynthesized either by sucrose synthase or sucrose phosphate synthase, while invertases are involved in its hydrolyzation,

into smaller sugars. The exposure of heat stresses hampered the activities of SPS, SS and invertases during the exposure of heat stresses in different episodes (T24, T48 and T72) in both sugarcane varieties. But S2003-US-633 exhibited more SPS activity suggesting that SPS may play central role in accumulating of sucrose in sugarcane plants. The high sucrose phosphate synthase activities were associated with high level of sucrose in sugarcane (Botha & Black 2000). A positive correlation of SS, SPS activities with sucrose accumulation was evident in both sugarcane genotypes. In addition, SS, SPS and acid invertase activities were observed in S2003-US-633 (high sucrose accumulation variety) under heat stress. Regarding heat tolerance, S2003-US-633 showed better performance under heat stress conditions by high accumulation of proline as compared to SPF-238. Proline accumulation triggered by biotic and abiotic stresses, act as an electron acceptor, osmolyte and protect the membrane (Abrahám et al 2010) and photosynthetic machinery induced by reactive oxygen species (Hare et al 1998). The increase in lipid peroxidation is also a marker of oxidative stress (Goel & Sheoran 2003) for abiotic and biotic stresses (Apel & Hirt 2004). In this study, marked increase in MDA contents was reported in both sugarcane cultivars under heat shock conditions showing considerable lipid peroxidation of biological membranes leading to the production of ROS along with losing membrane integrity (Boaretto et al 2014). While high temperature also damages cell membrane by losing membrane integrity affecting all other physiological and biochemical processes (Kaur et al 2010). Under heat stress conditions, electrolytes leakage increased many folds as compared to the normal growth condition as well as same recovery pattern were observed of both varieties. This enhanced permeability of membranes severely damaged the mesophyll cells (Zhang et al 2005) and led to the increased electrolytes leakage at high temperature in leaves sugarcane (Savchenko et al 2002). More proline accumulation, total protein contents, total sugars, reducing sugars, non-reducing sugars content while less MDA content, H₂O₂ content and less electrolytes leakage (EC) with response to high temperature exposure in S2003-US-633, ranked as tolerant variety. SDS-PAGE analysis revealed that high and low molecular weight proteins bands ranging from 15kDa to 150 kDa protein in S2003-US-633. It is assumed that HSPs (60, 70 and 90 kDa) might be play role in development of stress tolerance without inhibiting the activities of sugar metabolizing enzymes. On the other hand, accumulation of proline content that act as an osmolyte and the oxidative makers, responsible to stabilize the protein in stressed condition. It can be concluded that S2003-US-633 proved more thermotolerant variety with great sugar accumulation under different regimes. Thus, these biochemical attributes can index the degree of tolerance of sugarcane crop to exhibit adaptability under stressful conditions providing the insights to molecular breeders to identify the thermotolerant sugarcane varieties with improved recovery of sugarcane.

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References

- Abrahám E, Hourton-Cabassa C, Erdei L & Szabados L (2010). Methods for determination of proline in plants. In *Plant Stress Tolerance* pp. 317-331
- Akram N A, Ashraf M & Al-Qurainy F (2012). Aminolevulinic acid-induced changes in some key physiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus L.*) plants under saline regimes. *Scientia Horticulturae* 142: 143-148
- Albacete A, Grosskinsky D K & Roitsch T (2011). Trick and treat: a review on the function and regulation of plant invertases in the abiotic stress response. *Phyton* 50(2): 181-204
- Ansari M I, Yadav A & Lal R (2013). An-overview on invertase in sugarcane. *Bioinformation* 9(9): 464
- Apel K & Hirt H (2004). Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* 55: 373-399
- Bates L S, Waldren R P & Teare I D (1973). Rapid determination of free proline for water-stress studies. *Plant and Soil* 39(1): 205-207

- Bradford M M (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1-2): 248-254
- Boaretto L F, Carvalho G, Borgo L, Creste S, Landell M G, Mazzafera P & Azevedo R A (2014). Water stress reveals differential antioxidant responses of tolerant and non-tolerant sugarcane genotypes. *Plant Physiology and Biochemistry* 74: 165-175
- Botha F C & Black K G (2000). Sucrose phosphate synthase and sucrose synthase activity during maturation of internodal tissue in sugarcane. *Functional Plant Biology* 27(1): 81-85
- Christensen J H & Christensen O B (2007). A summary of the prudence model projections of changes in European climate by the end of this century. *Climatic Change* 81(1): 7-30
- Ebrahim M K, Zingsheim O, El-Shourbagy M N, Moore P H & Komor E (1998). Growth and sugar storage in sugarcane grown at temperatures below and above optimum. *Journal of Plant Physiology* 153(5-6): 593-602
- Goel A & Sheoran I S (2003). Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biologia Plantarum* 46(3): 429-434
- Hare P D, Cress W A & Van Staden J (1998). Dissecting the roles of osmolyte accumulation during stress. *Plant, Cell & Environment* 21(6): 535-553
- Heath R L & Packer L (1968). Photo peroxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Archives of Biochemistry and Biophysics* 125(1): 189-198
- Hedge J E & Hofreiter B T (1962). Estimation of starch by anthrone reagent. *Methods in Carbohydrate Chemistry* pp. 9-10
- Hubbard N L, Huber S C & Pharr D M (1989). Sucrose phosphate synthase and acid invertase as determinants of sucrose concentration in developing muskmelon (*Cucumis melo* L.) fruits. *Plant Physiology* 91(4): 1527-1534
- Joshi B J, Krishnaveni S, Vijayalakshmi D, Sudhagar R & Raveendran M (2013). Activities of enzymes involved in synthesis and degradation of sucrose in popular sugarcane varieties. *Asian Journal of Experimental Biology* 4(2): 237-244
- Kaur S, Singh H P, Mittal S, Batish D R & Kohli R K (2010). Phytotoxic effects of volatile oil from *Artemisia scoparia* against weeds and its possible use as a bioherbicide. *Industrial Crops and Products* 32(1): 54-61
- Laemmli U K (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227(5259): 680
- Liu X, Huang B (2000). Heat stress injury in relation to membrane lipid peroxidation in creeping bentgrass. *Crop Science* 40: 503-513
- Loreto F & Velikova V (2001). Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiology* 127(4): 1781-1787
- McCormick A J, Watt D A & Cramer M D (2008). Supply and demand: sink regulation of sugar accumulation in sugarcane. *Journal of Experimental Botany* 60(2): 357-364
- Miller G L (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Analytical Chemistry* 31(3): 426-428
- NASA (2017). NASA's Goddard Institute for Space studies (GISS). <https://climate.nasa.gov/vital-signs/global-temperature/> (July 2017)
- PSMA (2007) (Pakistan sugar mill association). *Annual Report* pp. 4
- Potters G, Pasternak T P, Guisez Y, Palme K J & Jansen M A (2007). Stress-induced morphogenic responses: growing out of trouble? *Trends in Plant Science* 12(3): 98-105

- Roitsch T & González M C (2004). Function and regulation of plant invertases: sweet sensations. *Trends in Plant Science* 9(12): 606-613
- Ruan Y L, Jin Y, Yang Y J, Li G J & Boyer J S (2010). Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Molecular Plant* 3(6): 942-955
- Savchenko G E, Klyuchareva E A, Abramchik L M & Serdyuchenko E V (2002). Effect of periodic heat shock on the inner membrane system of etioplasts. *Russian Journal of Plant Physiology* 49(3): 349-359
- Shrivastava P K, Kaleriya K A, Parmar V & Patil R G (2010). Time Series Analysis of Temperature Variations and its Impact on Sugarcane Crop. In *National Symposium on Climate Change and Rainfed Agriculture, Organized by Central Research Institute for Dryland Agriculture at Hyderabad February 18-20*
- Tana B, Chanprame S, Tienseree N & Tadakittisarn S (2014). Relationship between invertase enzyme activities and sucrose accumulation in sugarcane (*Saccharum spp.*). *Kasetsart Journal: Natural Science* 48: 869-879
- Vorster D J & Botha F C (1999). Sugarcane internodal invertases and tissue maturity. *Journal of Plant Physiology* 155: 470-477
- Yang G, Rhodes D & Joly R J (1996). Effects of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-deficient and glycinebetaine-containing maize lines. *Functional Plant Biology* 23(4): 437-443
- Zhang J H, Huang W D, Liu Y P & Pan Q H (2005). Effects of temperature acclimation pretreatment on the ultrastructure of mesophyll cells in young grape plants (*Vitis vinifera L. cv. Jingxiu*) under cross-temperature stresses. *Journal of Integrative Plant Biology* 47(8): 959-970