Pak. J. Bot., 54(2): DOI: http://dx.doi.org/10.30848/PJB2022-2(34)

SUGARCANE VARIETAL RESPONSE FOR SUCROSE ACCUMULATION SUBJECTED TO THERMAL STRESS AT FORMATIVE STAGE

MUHAMMAD KAZIM ALI[1,3*], FAISAL MEHDI[2], YAWEN ZENG[3], ABID AZHAR[1] AND SADDIA GALANI[1]

[1]Dr. A. Q. Khan Institute of Biotechnology and Genetic Engineering University of Karachi, Karachi Pakistan 75270
[2]Department of Agriculture and Agribusiness Management, University of Karachi, Karachi Pakistan 75270
[3]Biotechnology and Genetic Resource Institute, Yunnan Academy of Agricultural Sciences (YYAS), Kunming China 650205
**Corresponding author's email: ali.kazimm@gmail.com*

Abstract

Development and improvement of renewable energy sources is a vital task to cope with fossil fuel associated consequences on nature. Recently, green energy has been emerged as an alternative for the said challenge. Sugarcane (*Saccharum officinarum)* is the principal source of sucrose and bioethanol worldwide. However, unfavorable climatic condition particularly episodes of heat waves cause significant reduction in sugarcane biomass and sucrose accumulation. Selection of appropriate variety at formative stage is one of the vital approaches to cope with global warming and may reduce economic loss in cultivation of this annual crop. So, the study was formulated to a) elucidate the varietal differences for sucrose accumulation under thermal stress and b) understand the physiological and biochemical response towards high temperature. Therefore, two sugarcane cultivars viz S-2003-US-633 (high sucrose accumulation) and SPF-238 (low sucrose accumulation) were subjected to heat stress at formative stage. Cell membrane thermostability (CMT), lipid per-oxidation (LPO) and level of oxidative stress, were monitored as stress damage indicators while variation in proline accumulation showed osmoregulatory potentials. Non-reducing and reducing sugars proportion in the total sugar assessed and correlated with soluble protein concentration to mimic the activities of sugar metabolizing enzymes. Results revealed positive relationship of proline and protein content with total sugars in S-2003-US-633 as compared to SPF-238. The later crop had low sucrose may be due to low CMT, higher MDA, and H_2O_2. It is evident that cultivar S-2003-US-633 tolerated heat stress based on its physio-chemical managements and ensures high sucrose recovery. The study suggests potential application of the indices in field evaluation and selection of climate resilient sugarcane plant at formative stage to cope with yield and economic loss of this annual cash crop in times of climate change.

Key words: Sugarcane, Sucrose, Thermotolerance, Formative stage, Markers.

Introduction

Sugarcane (*Saccharum officinarum* L.) is an important cash crop due to high sucrose accumulation in its stalk (Ali *et al*., 2019; Tana *et al*., 2014). In Pakistan sugarcane is one of the foremost agricultural supplies because it produces 73.40 million tones sugarcane which produce 5.588 million tons sucrose cultivated in 1.22 million hectares land. In addition, the share of sugarcane in the value addition of agriculture and gross demotic production (GDP) is 3.4% and 0.7%, respectively. Therefore, it performs a vital role in the financial system of the state as well as furnishes unprocessed material to 81 sugar mills (Khan *et al*., 2019; Mehdi *et al*., 2020; Anon., 2014). It is reported that approximately, worldwide total sugar production and sugar consumption accounted 37% and 46% for Asian continent, respectively however, unprecedented climate change, particularly episodes of heat waves due to global warming severely hampered sugar production in Asia. Moreover, it has been reported the current mean temperature of the glob (earth) showed increment by 0.99°C (Anon., 2016) and 1.5°C (Anon., 2014) and projected to rise more. Therefore, high temperature stress is the most important impediments for sugarcane high yield and high sucrose recovery rate. Because it causes overproduction of free radicals (Tammy *et al*., 2015; Ali *et al*., 2018) which are highly reactive and hampers plant metabolism through damaging cell membrane, denaturing cellular protein and nucleic acid (Rezaei *et al*., 2015; Ali *et al*., 2017). H_2O_2 disrupts various metabolic activities like Calvin cycle and photosynthesis (Ali *et al*., 2016; Akram *et al*., 2012). In addition, extreme high/low temperature stress adversely affect cellular organelles like, cell wall, cell membrane, chloroplast, and nucleus which leads to interruption of cellular activities, denature of membrane protein, melting of membrane lipids, rupture of cell and leakage of cellar contents (Prasad & Jagadish, 2015; Rezaei *et al*., 2015; Sun *et al*., 2019). While high osmolyte (proline) content and soluble sugars are necessary to protect the cellular structure in stress condition by maintaining the membrane stability (Alves *et al*., 2019; Ali *et al*., 2018) and water balance in cell (Farooq *et al*., 2011; Alves *et al*., 2019). Moreover, high glucose and sucrose availability is important for physiologically regulate plant and development (Couee *et al*., 2006; Roitsch & González, 2004). Because high temperature adversely affects sucrose metabolizing enzymes such as, sucrose synthase and sucrose phosphate synthase (Joshi *et al*., 2013; Mehdi *et al*., 2020; Ali *et al*., 2019) while some studies showed divers effect on sucrose metabolizing enzymes during different growth stages of sugarcane genotypes (Mehdi *et al*., 2020; Tana *et al*., 2014). This diversity in storage of sucrose in the sink tissues of sugarcane is largely due to variation in the actions of sucrose phosphate synthase (SPS) and sucrose invertase (SAI) (Ansari *et al*., 2013; Joshi *et al*., 2013; Mehdi *et al*., 2020).

Major climatic factors that influence quality, yield, and growth of cane are moisture availability, sunlight, and temperature. Sugarcane plant flourishes finest in sunny and tropical hot areas. The ‘‘perfect’’ weather for production of highest sucrose from cane is depicted as a

warm and long growing period with elevated frequency of solar radiation and suitable rainfall (Pathak *et al.,* 2018). Usually, sucrose accumulation in the stalks happens from the basal internodes to the apex are called maturity, and this process (ripening) varies among sugarcane genotypes due to variation in sink strength. So, the large amount of sucrose is accumulated in sugarcane stalks which act as reservoir, but the capacity of this reservoir depends on several factors including the climate conditions. However, its response to climatic factors fluctuates with phonological stages due to its long growth period but sugarcane entails moist and warm climate for better yield and growth. Interestingly, low sucrose recovery rate correlated to humid and warm climate of coastal belts in tropics while some studies suggest chilling is undesirable for maturity (Pathak *et al*., 2018). By and large climate conditions determine the potential productivity and quality of the crop, therefore variation in sugarcane crop growth and yield under various climatic condition should explore at all phenological stages.

The optimum temperature for sugarcane normal growth and optimum concentration of sucrose accumulation was reported 27°C or 30°C. While 15°C and 45°C was considered for sub- and supra-optimal temperature of sugarcane growth and sugar storage and it was also observed that the concentration of sucrose was lower at 45°C than at both 27 and 15°C (Ebrahim *et al*., 1998). However, Sund & Clements (1974) stated that sugarcane cultivated in Iran had no negative effect at average temperature approximately 45°C which clearly indicated the diversification of sugar germplasms for thermotolerance. On the other hand some studies showed that temperature at 40°C triggered substantial deterioration in shoot dry mass, smaller internodes, increased number of tillers (Kohila & Gomathi, 2018; Ebrahim *et al*., 1998; Wahid, 2007; Bonnett *et al*., 2006). Therefore, the ideal temperature for sugarcane growth is 32-33°C (Kohila & Gomathi 2018; Wahid, 2007), but shown substantial yield reduction above this range (Kohila & Gomathi 2018; Ebrahim *et al*., 1998 and Robertson, 1998). Shrivastava *et al*., (2010) observed reduction in crop productivity and sugar recovery percentage with increment in temperature. However, sugar improvement in mills differs on the quantity of stored sugar in cane tissue as well as the storage temperature (Joshi *et al*., 2013).

Beside the average temperature, sugarcane yield also depends on crop cultivars, other biotic and abiotic factors, and management practices (Kohila & Gomathi, 2018; Kaushal *et al*., 2016). For increment in total yield and high sugar recovery under climate change, thermotolerant sugarcane will be required. Because the identification and selection of thermotolerant varieties are the important strategy in adaptation of climate change. Therefore, understanding of biochemical and physiological response of sugarcane crop towards heat stress is imperative. It is hypothesized that the change in temperature may cause physiological, morphological, and biochemical changes in sugarcane plant. The purpose of this research was to explore better yielding sugarcane genotypes subjected to heat stress at formative stage followed by improving sugarcane thermotolerance at later stages.

Materials Methods

Two genotypes (S-2003-US-633 and SPF-238) of sugarcane were grown in containers packed with 20 kg loamy topsoil and 5 kg farmyard manure (FYM) at the Agriculture Biotechnology Lab of KIBGE, University of Karachi, Karachi Pakistan. All agronomic practice, application of fertilizers (NPK) was applied regularly, added with partial potency nutrient in the whole research. For heat stress, all pots were shifted to heat Shock room, for photosynthesis the fluorescent tubes light ranging from 700-1200 µmol m^{-2} s^{-1}, heat was maintained (45±2°C) and humidity ranged from 60-70%. Heat shock treatment was applied after 50 days of planting at different episodes. The temperature 30±2°C was maintained for control treatments and 45±2°C was set for heat shock treatments while normal (30±2°C) temperature was applied to heat stressed plants for recovery treatments. Samples were collected after one day (24h), two days (48h) and three days (72h) of heat stress and recovery condition while the samples collected 24 h before the heat stress were control plants. Cell membrane permeability (RMP%) and Hydrogen peroxide (H_2O_2) were assayed on the day of sampling while remaining tissues were kept at -80°C for further biochemical experiments.

Relative Membrane permeability (RMP%): The permeability of cell membrane of stressed and unstressed plants leaves was determined by measuring electrolyte leakage in terms of percentage (%) according to the protocol of Yang *et al*., 1996. For which approximately 1 gm of leaf samples were collected, weighed, and cut into small chunks, followed by soaking in 20ml distilled water. The initial electrical conductivity (EC_0) was determined after mixing the tubes for 5 seconds. For second (EC_1) reading the tubes were incubated at 4℃ for overnight while the final (EC_2) reading was recorded after autoclaving of the tubes and cooling at room temperature. RMP was determined in terms of percentage through applying the subsequent equation

$$\text{RMP (\%)} = \frac{EC_1 - EC_0}{EC_2 - EC_0} \times 100$$

Malondialdehyde content (MDA): The chemical byproduct of lipid peroxidation mechanism is malondialdehyde (MDA), quantified by the protocol of Heath & Packer (1973). For which about 0.1gm of leaf sample was homogenized in 5% TCA (trichloroacetic acid, 2ml). The homogenate then subjected to centrifugation at 12000 rpm for 15 minutes. Approximately, 1ml thiobarbituric acid (TBA, 0.5%) was added in 0.5 ml of supernatant and boiled for 30 min in water-bath at 95℃ followed by centrifugation at 7500g for 5 minutes. The change in absorbance was determined at 532nm and 600nm while 5% TCA was used as blank. The content of MDA was calculated using the below given equation in which 15500 represents the coefficient of absorbance while A532 and A600 is absorbance values of wavelength at 532nm and 600nm, respectively.

$$\text{MDA} \left(\frac{\text{nmol}}{1}\right) = \frac{\text{A532 - A600}}{15500} \times (10)^6$$

Oxidative stress (H_2O_2): The level of oxidative stress followed by heat stress application was determined through the qualification of hydrogen peroxide (H_2O_2) according to standard protocol (Jessup *et al*., 1994) (Fig. 1). About 0.1 gm of fresh rice leaves was homogenized in 0.1% TCA (2ml) followed by centrifugation at 4℃ and 12000 rpm for 15 minutes. Approximately 0.5 ml of the supernatant mixed with about 0.5 ml potassium phosphate buffer (pH 7.0) and 1 ml of potassium iodide (KI) followed by gentle vertexing. The change in absorbance was determined at 390nm. For blank approximately1ml potassium iodide and 1ml potassium phosphate buffer was used. The amount of (H_2O_2) was calculated using plotted standard curve and the following formula:

$$\text{DF (Dilution factor} = \frac{\text{Extraction volume}}{\text{Sample volume}} \times 1$$

$$\text{CF = (Correction factor)} = \frac{\text{Standard curve of } H_2O_2 \text{ content}}{\text{Optical density of standard}} \times 1$$

$$H_2O_2\ \mu M\ (g/FW) = DF \times CF \times OD \text{ of sample}$$

Quantitative analysis of reducing, non-reducing, and total sugar: The method of Lu *et al*., (2011) were followed to extract total sugars content from the samples. While the protocol of Miller, 1959 were followed to explore the amount of reducing sugar content using dinitro salicylic acid (DNSA) reagent. Approximately 5 ml of 80% ethanol were used to homogenize the tissue (100mg) followed by phase separation through centrifugation at 12000rpm for 20 minutes. The reaction mixture (1ml) mixed with 1 ml DNS reagent were boiled for 5 minutes in boiling water. Approximately, 9 ml distill water was added in the mixture after cooling to room temperature and absorbance at 546 nm was measured. While the amount of non-reducing sugars was calculated using the following formula and expressed as mg/ml.

Non-reducing sugar =Total sugar-reducing sugar

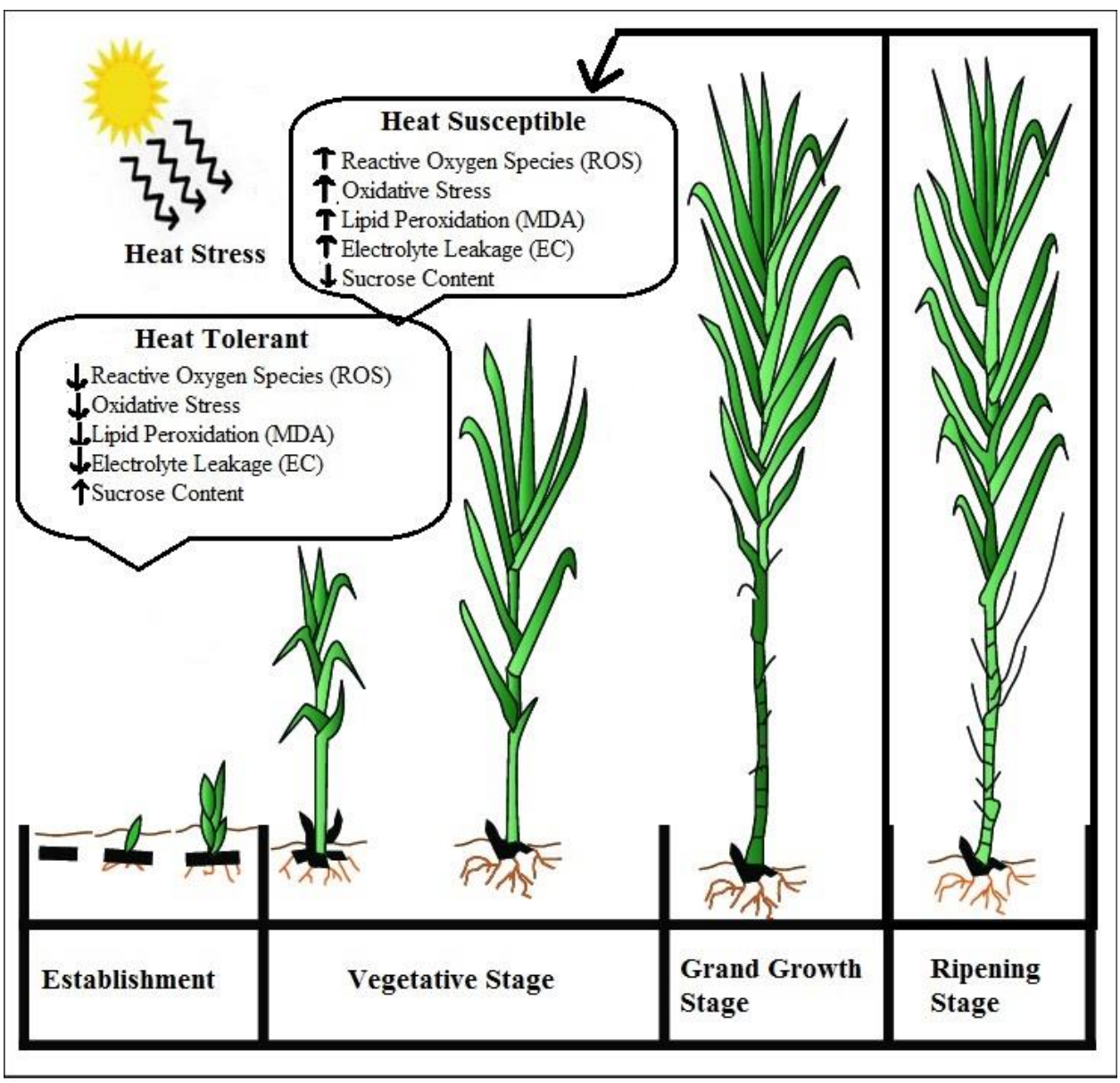

Fig. 1. Oxidative stress under heat stress and sucrose content of sugarcane (ROS, reactive oxygen species).

Table 1. Response of biochemical attributes and stress damage indicators of sugarcane subjected to high temperature stress at formative stage.

Biochemical attributes of sugarcane at formative stage											
Parameters	**Varieties**	**Mean ± SEM**							**P Value**		
		Control	**Heat shock**			**Recovery**			**Cultivar**	**Treatment**	**Interaction**
		C	**T24**	**T48**	**T72**	**R24**	**R48**	**R72**	**C**	**T**	**V×T**
		712.6±15.2	391.8±15.4	345.02±5.8	263.15±44.2	309.9±15.4	584.8±42.2	608.2±45.7	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	573.0±25.5	333.3±36.5	286.54±32.5	228.07±10.1	304.1±46.8	415.2±25.5	578.9±20.2			
Reducing sugar	S2003-US-633	0.25±0.00	0.15±0.00	0.13±0.00	0.12±0.00	0.19±0.01	0.206±0.01	0.21±0.02	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	0.19±0.01	0.15±0.00	0.12±0.00	0.11±0.00	0.18±0.00	0.18±0.01	0.18±0.01			
Non-reducing sugar	S2003-US-633	0.45±0.01	0.23±0.01	0.21±0.00	0.14±0.04	0.11±0.00	0.37±0.04	0.39±0.06	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	0.37±0.03	0.18±0.03	0.16±0.03	0.11±0.01	0.12±0.04	0.23±0.02	0.39±0.03			
Total soluble protein	S2003-US-633	1.18±0.00	1.11±0.04	0.93±0.05	0.81±0.05	0.77±0.01	0.92±0.14	0.88±0.13	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	1.00±0.09	0.87±0.17	0.65±0.05	0.63±0.04	0.83±0.09	0.72±0.05	0.56±0.16			
Stress damage indicators analysis at formative stage											
	S2003-US-633	14.70±1.06	20.00±0.60	21.66±1.62	24.30±0.22	22.28±0.09	21.35±1.62	19.23±1.66	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	16.68±1.91	27.36±1.24	27.17±0.75	28.71±0.77	23.41±0.87	20.75±0.90	18.59±0.14			
H_2O_2	S2003-US-633	64.39±5.89	94.38±7.37	126.10±6.24	187.8±6.13	181.3±5.21	162.1±6.38	125.1±5.80	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	92.42±6.24	97.72±5.89	141.2±14.41	208.2±6.53	193.3±8.38	156.8±11.1	129.7±7.80			
MDA	S2003-US-633	11.78±2.41	18.75±4.05	30.83±1.90	38.36±0.60	26.92±0.11	30.40±3.89	32.12±1.07	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	12.77±1.08	21.33±0.79	36.17±1.01	42.40±1.11	37.37±3.28	33.80±0.58	33.80±0.68			
Proline	S2003-US-633	97.10±2.85	168.5±32.9	242.8±28.16	293.6±3.71	177.1±5.71	187.1±13.85	179.4±0.75	*p*<0.05	*p*<0.05	*p*>0.05
	SPF-238	98.24±0.28	151.4±7.56	228.5±15.13	264.2±1.50	165.6±5.71	182.8±7.56	119.9±4.95			

For estimation of total sugars content, Hedge & Hofreiter, (1962) protocol was followed in which anthrone reagent was used, followed centrifugation at 1000 rpm for 10 minutes. About 25ul of the supernatant were mixed with 0.975 ml distilled water while 5ml volume was made up by the addition of anthrone reagent. The reaction mixture was heated for 15 minutes, and the absorbance was taken at 620 nm by using spectrophotometer.

Total protein analysis: 2ml phosphate buffer (pH 7.4) was used to extract total soluble proteins from 0.1 g leaf tissues and centrifuged at 12000 rpm for 20 minutes. Total soluble protein was calculated using protocol of Bradford (Bradford, 1976). Known concentration (10-100ug/ul) of bovine serum albumin (BSA) was used to construct protein standard curve. Protein sample (50ul), Bradford dye (1ml) and 0.15 N NaCl (0.95ml) were mixed with 2ml reaction mixture for protein quantification, after 15-20 minutes incubation at room temperature and read at 595nm.

Statistical assessment: All the data were examined for ANOVA using the SPSS, version 17.0.0. The considerable impacts were further than estimated, and means were equated using LSD test. Statistical significance was determined at α=0.05.

Results and Discussion

This study was designed to explore the comparative profiling of biochemical attributes of two sugarcane cultivars viz. SPF-238 (low sucrose accumulation) and S-2003-US-633 (high sucrose accumulation) under high temperature stress at formative stage. Significance of data was also validated through statistical test like, correlation and ANOVA (Table 1). The findings are discussed with objectives of the study in following paragraph. The core product of sugarcane crop is sucrose content and are heavily invested to improve sugar recovery percentage during sugar crushing in industries. Therefore, adverse effect of high temperature stress on percentage of sucrose recovery rate was determined. Results revealed reduction in accumulation of sucrose with increment in temperature stress which shows negative impact of thermal stress on this agronomic attribute (Alves *et al*., 2019; Kaushal *et al*., 2016). At normal growth condition (C), cultivar US-633 showed 0.70 mg/ml sucrose while cultivar SPF-238 had 0.58 mg/ml. Upon application of thermal stress both cultivars showed significant reduction of this attribute. After prolong application of heat stress (27h, T72), S-2003-US-633 and SPF-238 had 0.28 and 0.21 mg/ml sucrose, respectively. Which showed that former had more content of total sucrose as compared to the later variety. Secondly, heat stress declined sugar accumulation, however, regain of sugar loss upon recovery treatment shows positive adaptation of the crop to thermal stress condition (Rezaei *et al*., 2015). This response was observed in both sugarcane cultivars however significant variation was noticed depending on duration of stress and recovery conditions. Comparatively, cultivar S-2003-US-633 had maximum as well as consistent sucrose production for all types of temperature treatments specifically prominent variation was found during recovery treatments. Statistical analysis demonstrated that variety (V) and Treatment (T) showed no significant (p>0.05) while their interaction (V×T) showed significant (p<0.05) difference in total sugar content throughout the growth conditions. However, it is evident that there are clear differences in total sugar under control and recovery growth condition. It is very important to cope with stress condition in the field particularly heat stress because unlike drought and salinity heat stress magnitude varies from dawn to dusk (Teixeira *et al*., 2013). So, the survival of the plant under stress condition depends on the ability of plant to recover heat induced damages after peak of the stress condition (Fahad *et al*., 2017). In this context the cultivar S-2003-US-633 had thermotolerance capacity which may improve through different approaches. In addition of total sucrose, quantification of reducing sugar reduced under stress condition however both varieties recovered the damages upon lifting thermal stress condition and experiencing optimum temperature (recovery treatments). Concludingly, reducing sugar content of S-2003-US-633 was higher than SPF-238. Although the cultivars had significant difference in reducing sugar content at control condition but under stress the difference was not prominent specifically after 72 h of heat stress it seems both had similar response for this attribute. Interestingly, the difference was clear in recovery condition, showing S-2003-US-633 had better recovery mechanisms (Fahad *et al*., 2017). Statistical analysis indicated that variety (V), Treatment (T) and their interaction (V×T) had no significant (p>0.05) difference in reducing sugars (Table 1). Similarly, non-reducing sugar analysis revealed same pattern of response for thermal stress as total and reducing sugar had however, cultivars had significant variation for this attribute under stress condition. While statistical analysis demonstrated that variety (V), Treatment (T) and their interaction (V×T) revealed no meaningful (p>0.05) variation in non-reducing sugars.

Total protein (mg/ml) quantification was carried out after each treatment, and it was observed that heat stress declined total protein content. Compared to control, heat stress manifested significant reduction for this attribute however, significant variation was observed between them. This varietal difference in total protein analysis were clearer under stress while less variation was found upon recovery. Comparatively, S-2003-US-633 had maximum accumulation of total soluble protein in all condition showing better condition of growth. Statistical analysis demonstrated that variety (V), Treatment (T) and their interaction (V×T) showed considerable (p<0.05) variation in total protein content. Concludingly, it is evident that total soluble protein concentration was declined considerably (p<0.05) by high temperature in both sugarcane germplasms. Likewise, proline content was also determined as stress tolerance indicators because proline played key role as osmoregulatory agent as well as antioxidant to certify cellular homeostasis under harsh conditions (Ali *et al*., 2017; Fahad *et al*., 2017). Statistical analysis revealed that cultivars (C) and treatments (T) showed significant difference (p<0.05) in proline accumulation at formative stage however, there interaction (C x T) showed non-significant differences in proline content. A significant rise in the concentration of proline content was observed in both sugarcane varieties under heat shock conditions (24h, 48h and 72 h) while declined upon recovery. For proline

quantification, the tolerant variety (S-2003-US-633) had maximum accumulation compared to SPF-238 as evident in previous attributes. Interaction (V×T) showed non-significant ($p>0.05$) difference in proline contents while in control condition both varieties showed a normal amount (0.96-0.97 uM/gFW). Accumulation of proline triggered by biotic and abiotic stress behave as an electron acceptor and shield the key cellular components including membranes (Ain-Lhout *et al*., 2001; Abrahám *et al*., 2010). In addition, it protects photosynthetic distresses caused by reactive oxygen species (Ali *et al*., 2017; Hare *et al*., 1998). Results revealed that S-2003-US-633 performed better under stress conditions owing to maximum accumulation of proline, as compared to SPF-238.

MDA is believed as stress damage indicator usually determine to evaluate the level of lipid peroxidation under heat stress conditions (Ali *et al*., 2017 and 2018; Goel & Sheoran, 2003). The increase in lipid peroxidation is also a marker of oxidative stress (Ali *et al*. 2018; Schopfer *et al*., 2001). In response to a variety of biotic and abiotic stresses the increment in accumulation of MDA due to lipid peroxidation has been reported (Ali *et al*., 2018; Apel & Hirt, 2004). A marked increment in the MDA contents was observed in both sugarcane cultivars subjected to heat stress condition suggesting that substantial lipid peroxidation of lipids took place (Ali *et al*., 2019; Mehdi *et al*., 2020). While the amount of malondialdehyde (nM/gFW) were minimum in both varieties S-2003-US-633 and SPF-238 under control condition. Heat stress (T24, T48 and T72) showed increase in MDA suggesting that thermal stress caused successful damaging of membrane lipids. SPF-238 showed more content of MDA than S-2003-US-633 upon exposure to heat stress. After 72 h of heat stress both cultivars had similar response for MDA but S-2003-US-633 showed better recovery potential compared to SPF-238. Interaction (V×T) showed no major ($p>0.05$) variation in MDA contents. Which clearly suggested that the level of lipid peroxidation was much lower in S-2003-US-633 compared to SPF-238.

Relative membrane permeability (RPM%) of two varieties (S2003-US-633 and SPF-238) at formative stage was determined after exposure to temperature stress and recovery treatments. Statistical analysis demonstrated that variety (V), Treatment (T) and their interaction (V×T) showed significant ($p>0.05$) difference in cell membrane permeability. Under normal growth condition EC leakage was found 14.70% and 25.97% in Us-633 and SPF-238 respectively. Upon exposure to heat stress EC content was increased many folds giving 24.30% and 28.7 % in SPF-238 and US-633 respectively. Maximum EC (24.30%) leakage exhibited by SPF-238 at T72 while recovery condition exhibited 18.5-19.2% leakage. High temperature damage cell membrane or membrane integrity hence increases membrane permeability which disturbs all other physiological and biochemical processes owing to shift in electrolyte proportions and the pH of the cell (Kaur *et al*., 2010; Singh *et al*., 2005; Ali *et al*., 2018) These results indicate that normal membrane permeability characteristics are seriously affected of sugarcane plant when heat stress is imposed.

In heat shock conditions H_2O_2 contents of both varieties were increased many folds with the passage of time of heat stress (24h,48h and 72h) compared to control conditions. Although production of H_2O_2 was increased upon heat stress application in both cultivars but cultivar US-633 showed minimum accumulation of hydrogen peroxide content compared to SPF-238. Recovery treatments showed improvement in stress damages in both cultivars. Statistical analysis that variety (V), Treatment (T) and their interaction (V×T) had significant ($p<0.05$) difference in hydrogen peroxide content. Surplus hydrogen peroxide (H_2O_2) increase can develop oxidative stress condition in plants, which then causes cell death.

Plant can defend themselves by biotic and abiotic stress by gathering compatible solute such as proline (Saddia *et al*., 2012) which can stabilize protein, maintain the osmotic potential and cellular structure, protect ROS scavenging enzyme (Mirzaei *et al*., 2012; Kaushal *et al*., 2016; Schopfer *et al*., 2001) activate alternative detoxification pathways in plant under stress condition (Zhao *et al*., 2015; Seckin *et al*., 2009) that is why it acts as a direct anti-oxidant and activator of mechanism that act as antioxidants (Ali *et al*., 2017; Mafakhari *et al*., 2010; Bartels & Sunkar, 2005; Khedr *et al*., 2003). In conclusion more proline accumulation was observed in S-2003-US-633 along with higher concentration of protein and total sugars including non-reducing and reducing sugars. Moreover, less MDA content, H_2O_2 content and less electrolytes leakage (EC) suggest that S-2003-US-633 is resistant cultivar for high temperature and can be suggested to select for cultivation in hot areas. Thus, this study can be used as a useful approach to increase sugar cane yield providing new avenues towards the economic development of the country.

Conclusion

Identification and selection of thermotolerant sugarcane crop at early growth (formative) stage is imperative to cope with global warming because sugarcane is an annual crop, and any false identification may have consequences on sugar industries and national income. Therefore, an insight into the responses of sugarcane growth and yield attributes towards thermal stress will explore tolerance mechanisms. Particularly, key element investigation based on physiological and biochemical heat tolerance indexes could evidently differentiate sugarcane genotypes for thermotolerance. Notably in contrast to the heat-prone variety (SPF-238), sugarcane genotype S-2003-US-633 that had higher degrees of thermotolerance exhibited higher soluble sugar content, reducing sugar content, total protein, total proline content and lower level of oxidative stress, lipid peroxidation hence had least membrane injury indices.

Acknowledgment

The authors are thankful to Higher Education Commission (HEC) Pakistan for providing fiscal funding through Start-up Research Grant Project (SRGP). The SRGP No. was 21-483/2015.

References

Abrahám, E., C. Hourton-Cabassa, L. Erdei and L. Szabados. 2010. Methods for determination of proline in plants. *P. Str. Tol.,* 639: 317-331.

Ain-Lhout, F., F.A. Zunzunegui, M.C. Diaz-Barradas, R. Tirado, A. Clavijio and F. Garcia-Novo. 2001. Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. *Plant Soil,* 230: 175-183.

Akram, N.A., M. Ashraf and F. Al-Qurainy. 2012. Aminolevulinic acid-induced changes in some keyphysiological attributes and activities of antioxidant enzymes in sunflower (*Helianthus annuus* L.) plants under saline regimes. *Sci. Hort.,* 142: 143-148.

Ali, A., M. Khan, R. Sharif, M. Mujtaba and S.J. Gao. 2019. Sugarcane Omics: An Update on the Current Status of Research and Crop Improvement. *Plants,* 8: 344.

Ali, K.M., A. Azhar and S. Galani. 2016. Oxidative stress damage amelioration by antioxidant enzymes: Inducing thermotolerance in rice seedlings. *Pure & App. Sci.,* 5(4): 1131-1141.

Ali, K.M., A. Azhar, I. Salam and S. Galani. 2017. Differential expression of molecular chaperon (HSP70) and antioxidant enzymes: Inducing thermotolerance in Rice (*Oryza sativa* L.). *Pak. J. Bot.*, 49: 229-238.

Ali, K.M., A. Azhar, I. Salam, I.S. Ali and S. Galani. 2018. Mitigating heat induced damages in rice (*Oryza sativa* L.) by HSP70 modulation. *Pak. J. Bot.,* 50(6): 2181-2187.

Alves, L.C., J.P.P. Llerena, P. Mazzafera and R. Vicentini. 2019. Diel oscillations in cell wall components and soluble sugars as a response to short-day in sugarcane (*Saccharum* sp.). *BMC Plant Biol*., 19: 215.

Anonymous. 2014. "Climate Change 2014: Synthesis Report," in Proceedings of the Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, (Eds.): Core Writing Team, R. K. Pachauri, and L.A. Meyer (Geneva: IPCC), 151.

Anonymous. 2014. Pakistan sugar mill association. Annual report. http://www.psmacentre.com

Anonymous. 2016. National Aeronautics and Space Administration. https://climate.nasa.gov/vital-signs/global-temperature/https://www.ipcc.ch/report/-sr15/

Ansari, M.I., M. Yadav and R. Lal. 2013. An-overview on invertase in sugarcane. *Bioinform.,* 9(9): 464-465.

Apel, K. and H. Hirt. 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Ann. Rev. Plant Biol*., 55: 373-399.

Bartel, D. and R. Sunkar. 2005. Drought and Salt Tolerance in Plants. *Cri. Rev. Plant Sci*., 24: 1: 23-58.

Bates, L.S., R.P. Waldren and I.D. Teare. 1973. Rapid determination of free proline for water-stress studies. *Plant & Soil*, 39(1): 205-207.

Bonnett, G.D., M.L. Hewitt and D. Glassop. 2006. Effects of high temperature on the growth and composition of sugarcane internodes. *Aust. J. Agric. Res.,* 57: 1087-1095.

Bradford, M.M. 1976. A Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72: 248-254.

Couee, I., C. Sulmon, G. Gouesbet and A.E. Amran. 2006. Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants. *J. Exp. Bot.,* 57(3): 449-459.

Ebrahim, M.K., O. Zingsheim, M.N. El-Shourbagy, P.H. Moore and E. Komor. 1998. Growth and sugar storage in sugarcane grown at temperature below and above optimum. *J. Plant Physiol.,* 153: 593-602.

Fahad, S., A.A. Bajwa, U. Nazir, S.A. Anjum, A. Farooq, A. Zohaib, S. Sadia, W. Nasim, S. Adkins, S. Saud, M.Z. Ihsan, H. Alharby, C. Wu, D. Wang and J. Huang. 2017. Crop Production under drought and heat stress: plant responses and management options. *Front. Plant Sci.,* 8: 1147.

Farooq, M., H. Bramley, J.A. Palta and K.H.M. Siddique. 2011. Heat stress in wheat during reproductive and grain-filling phases. *Crit. Rev. Plant Sci*., 30: 491-507.

Farroq M., T. Aziz, S.M.A. Basra, M.A. Cheema and H. Rehman. 2008. Chilling tolerance in hybrid maize induced by seed priming with salicylic acid. *J. Agron. Crop Sci.,* 194: 161-168.

Goel, A. and I.S. Sheoran. 2003. Lipid peroxidation and peroxide-scavenging enzymes in cotton seeds under natural ageing. *Biol. Plant,* 46: 429-434.

Hare, P.D., W.A. Cress and J.V. Staden. 1998. Dissecting the roles of osmolyte accumulation during stress. *Plant Cell Environ.,* 21: 535-553.

Heath, R.L. and L. Packer. 1968. Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophysic.,* 125(1): 189-198.

Hedge, J.E. and B.T. Hofreiter. 1962. Estimation of starch by anthrone reagent. Methods in carbohydrate chemistry. Academic Press, New York. IPCC, 2007. In: (Eds.): Parry, M.L., O.F. Canziani, J.P. Palutikof, P.J. van der Linden & C.E. Hanson. Climate Change 2007: Impacts, Adaptation and Vulnerability. Contribution of Working Group II to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, UK, 976pp.

Jessup, W., R.T. Dean and J.M. Gebicki. 1994. Iodometric determination of hydroperoxides in lipids and proteins. *Meth. Enzym.,* 233, pp. 289-303.

Joshi, B.J., S. Krishnaveni, D. Vijayalakshmi, R. Sudhagar and M. Raveendran. 2013. Activities of enzymes involved in synthesis and degradation of sucrose in popular sugarcane varieties. Asian *J. Exp. Biol*., 4(2): 237-244.

Kaur, S., H.P. Singh, S. Mittal, D.R. Batish and R.K. Kohli. 2010. Phytotoxic effects of volatile oil from Artemisia scoparia against weeds and its possible use as a bioherbicide. *Ind. Crop Prod*., 32: 54-61.

Kaushal, N., K. Bhandari, K.H.M. Siddique and H. Nayyar. 2016. Food crops face rising temperatures: An overview of responses, adaptive mechanisms, and approaches of proline in plants. *Methods Mol. Biol.,* 639: 317-331.

Khan, M.T.I.A. Khan, S. Yasmeen, G.S. Nizamani and S. Afghan. 2019. Sugarcane biofuels and bioenergy production in pakistan: current scenario, potential, and future avenues. *Sugarcane Biofuel.,* 2019: 175-202.

Khedr A.H., M.A. Abbas, A.A. Wahid, W.P. Quick and G.M. Aboghdallah. 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancratium maritimum* L. to salt-stress. *J. Exp. Bot.,* 54: 2553-2562.

Kohila, S. and R. Gomathi. 2018. Adaptive physiological and biochemical response of sugarcane genotypes to high-temperature stress. *Ind. J. Plant Physiol*., 23(2): 245-260.

Loreto, F. and V. Velikova. 2001. Isoprene produced by leaves protects the photosynthetic apparatus against ozone damage, quenches ozone products, and reduces lipid peroxidation of cellular membranes. *Plant Physiol.,* 127(4): 1781-1787.

Lu, P.J., R.C. Dai, B.Y. Ye and R.H. Lin. 2011. HPLC determination of fructose, glucose and sucrose in sugarcane internode. *J. Food Sci.,* 32(2): 198-200.

Mehdi, F., M.K. Ali, N. Huma, I. Hussain, A. Azhar and S. Galani. 2020. Comparative biochemical analysis of high and low sucrose accumulating sugarcane varieties at formative stage under heat stress. *J. Agri. Sci.,* 26: 78-86.

Miller, G.L. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.*, 31(3): 426-428.

Mirzaei, M., D. Pascovici, B.J. Atwell and P.A. Haynes. 2012. Differential regulation of aquaporins, small GTPases and V-ATPases proteins in rice leaves subjected to drought stress and recovery. *Proteomics.*, 12: 864-877.

Mufakheri, A., A. Siosemardeh, B. Bahramnejad, P.C. Struik and E. Sohrabi. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Aust. J. Crop. Sci.*, 4: 580-585.

Pathak, S.K., P. Singh, M.M. Singh and B.L. Sharma. 2018. Impact of Temperature and Humidity on Sugar Recovery in Uttar Pradesh. *Sugar Tech*., 2018:1-6.

Prasad, P.V.V. and S.V.K. Jagadish. 2015. Field crops and the fear of heat stress – opportunities, challenges and future directions. *Proc. Environ. Sci.*, 29(2015): 36-37.

Rezaei, E.E., H. Webber, T. Gaiser, J. Naab and F. Ewert. 2015. Heat stress in cereals: Mechanisms and modelling. *Europ. J. Agron.*, 64: 98-113.

Robertson, M.J., G.D. Bonnett, R.M. Hughes, R.C. Muchow and J.A. Campbell. 1998. Temperature and leaf area expansion of sugarcane: Integration of controlled environment, field and model studies. *Aus. J. Agri. Res.*, 25: 819-828.

Roitsch and González. 2004. Function and regulation of plant invertases: Sweet sensations. *Trends Plant Sci.*, 9(12): 606-613.

Saddia M., A Jamil, N.A. Akram and M. Ashraf. 2012. A Study of proline metabolism in canola (*Brassica napus* L.) seedlings under salt stress. *Molecules*., 17: 5803-5815.

Schopfer, P., C. Plachy and G. Frahry. 2001. Release of reactive oxygen intermediates (superoxide radicals, hydrogen peroxide, and hydroxyl radicals) and peroxidase in germinating radish seeds controlled by light, gibberellin, and abscisic acid. *Plant Physiol*., 125: 1591-1602.

Seckin, B., A.H. Sekmen and I. Tbrkan. 2009. An enhancing effect of exogenous mannitol on the antioxidant enzyme activities in roots of wheat under salt stress. *J. Plant Growth Regul*., 2: 12-20.

Shrivastava, P.K., K.A. Kaleriya, Parmar, Vipul and R.G. Patil. 2010. Time series analysis of temperature variations and its impact on sugarcane crop, national symposium on climate change and rainfed agriculture, organized by central research institute for dryland agriculture at hyderabad, India. p 88- 90.

Singh, H.P., D.R. Batish, N. Setia and R.K. Kohli. 2005. Herbicidal activity of volatile essential oils from *Eucalyptus citriodora* against *Parthenium hysterophorus*. *Ann. Appl. Biol.*, 146: 89-94.

Sun, Z., D. Juan, P. Xiaoying, K.M. Ali, Y. Xiaomeng, D. Chengli, R. Meirong, L. Xia and Z. Yawen. 2019. Near-Isogenic Lines (NILs) of Japonica Rice Revealed New QTLs for cold-tolerance at booting stage. *Agron.*, 9(1): 40-54.

Sund, K.A. and H.F. Clements. 1974. Production of sugarcane under saline desert conditions in Iran. *Hawaii Agri. Exp. Stat. Res. Bullet.*, 160: 3-64.

Tammy, L.S., B. Shaheen, L.N. Vanessa, A.B. Haley, S. Stefanie and F.S. Rowan. 2015. The effect of high temperature stress on male and female reproduction in plants. *Field Crop. Res.*, 182: 30-42.

Tana, B., S. Chanprame, N. Tienseree and S. Tadakittisarn. 2014. Relationship between invertase enzyme activities and sucrose accumulation in sugarcane (*Saccharum* spp.). *Kasetsart J. Nat. Sci.*, 48: 869-879.

Teixeira, E.I., G. Fischer, H. van-Velthuizen, C. Walter and F. Ewert. 2013. Global hot-spots of heat stress on agricultural crops due to climate change. *Agri. Forest Met.*, 170: 206-215.

Wahid, A. 2007. Physiological implications of metabolite biosynthesis for net assimilation and heat-stress tolerance of sugarcane (*Saccharum officinarum*) sprouts. *J. Plant Res*., 120: 219-228.

Yang, G., D. Rhodes and R.J. Joly. 1996. Effect of high temperature on membrane stability and chlorophyll fluorescence in glycinebetaine-containing maize lines. *Aust. J. Pl. Phys.*, 23: 431-443.

Zhao, D. and Y.R. Li. 2015. Climate change and sugarcane production: Potential impact and mitigation strategies. *Int. J. Agron*., https://doi.org/10.1155/2015/ 547386.

(Received for publication 3 September 2020)