# Heat Tolerance in Sugarcane: Optimum Temperature and Phenological Stage to Determination of Thermotolerance as Selection Criteria

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## Abstract

Heat stress is the major abiotic stressor in agriculture which reduces crop productivity and yield. Six sugarcane (*Sacharum officinarum* L.) genotypes were studied to investigate the impact of three temperature levels at four phenological stages on tissue electrolyte production and the feasibility of using the cell thermostability method (CTM) for the identification and selection of heat tolerant sugarcane genotypes. The cell membrane thermostability was quantified by measuring relative cell injury percentage with a modification in the temperature treatment on four phenological stages in a field experiment. Our results suggest that heat tolerance based on cell membrane thermostability can be improved using the existing genetic variability available within the commercial or experimental sugarcane germplasm. We conclude that the cell membrane thermostability test can be a useful screening procedure for selecting sugarcane genotypes that tolerate high temperature stress. The test can be used in conjunction with a temperature trait of 60 °C during the maturity stage. This procedure predicts the ability of sugarcane genotypes to maintain yield and juice quality under stressful field conditions.

Keywords: heat stress, high temperature, relative cell injury, Sacharum officinarum L., sugarcane, thermostability

# 1. Introduction

In recent years, one of the important challenges facing crop physiologists is understanding and overcoming the major abiotic stresses in agriculture (Wahid et al., 2007). One of these stressors particularly predominant in the world is heat stress (Trivedi, 2015), which decreases plant growth and development and also crop productivity and yield (Wahid et al., 2007; Gomathi et al., 2014).

Ambient temperatures are rising at a considerable rate as part of the current global climate change; additionally, climatological extremes such as heat waves are likely to occur more frequently (IPCC, 2013). The intensity, duration, and rate of temperature change together determine the impact of high temperature on plant development and physiology (Wahid et al., 2007).

Sugarcane requires optimum temperature (32-33 °C) for growth, productivity and yield expression, and it is known to tolerate temperatures approaching 40 °C, while high temperatures around 45 °C are detrimental to sugarcane growth (Wahid et al., 2007). On the non-irrigated zones where the rain is the only source of water, sporadic days with heat extremes above 40 °C can occur at any development stage during the growing season (Chen et al., 2010).

Global increases in the ambient temperature is an agricultural problem in the world (Wahid, 2007). Transitory or constantly high temperatures cause an array of morpho-anatomical, physiological and biochemical changes in plants, which affect plant growth and development and may lead to a drastic reduction in economic yield (Wahid et al., 2007), as a function of many processes throughout the plant cycle (Masuka et al., 2012). The adverse effects of heat stress can be minimized by developing genetically improved plants using the thermotolerance as a selection strategy.

Heat stress greatly changes the physiological and biochemical phenomena of sugarcane leading to growth and yield suppression (Wahid et al., 2007). Recent research indicates that sugarcane grown under high temperature exhibited smaller internodes and early drying of leaves with increased tillering with reduced biomass (Gomathi et al., 2014). Furthermore, in spite of variation in susceptibility of different development stages to heat stress,

almost all stages of plant life are affected by heat stress. The developmental stages at which the crop plants are exposed to the stress, may determine the severity of possible damages experienced by the crop (Trivedi, 2015). During heat stress modifications in different plant processes take place in such a way to minimize the effect and develop tolerance to sustain stressful environment (Trivedi, 2015).

Physiological and biochemical screening techniques as a complement to empirical breeding methods could increase selection efficiency (Fischer, 1985). The genes securing heat tolerance may be lost in the breeding programs which rely mainly on only empirical selection (Reynolds et al., 1994).

Heat stress causes loss of integrity and functions of biological membranes due to alteration in the tertiary and quaternary structures of membranes proteins (Trivedi, 2015). Such alterations enhance the permeability of membranes and cause increased leakage of solutes, as an indication of decreased cell membrane thermostability (CMT). The CMT was developed by Sullivan (1972) to measure heat tolerance and determines the thermostability of the cell membrane through the measurement of the amount of electrolytes lost from the foliar tissue after exposure to a heat treatment (> 40 °C). Electrolyte leakage tests have been widely used to assess the level of plant tolerance to various stresses in different plant species such as *Glycine max* L. (Martineau et al., 1979); *Solanum tuberosum* L. and *Lycopersicon esculentum* Mill. (Chen et al., 1982); *Triticum aestivum* L. (Saadalla et al., 1990a; 1990b; Fokar et al., 1998; Blum et al., 2001); *Vigna unguiculata* L.(Ismail & Hall, 1999; Thiaw & Hall, 2004); *Gossypium hirsutum* L. (Rahman et al., 2004); *Sorghum bicolor* L. Moench (Sullivan & Ross, 1979); *Oryza sativa* L. (Tripathy et al., 2000); *Zea mays* L. and *Phaseolus vulgaris* L. (Castro-Nava et al., 2012; Castro, 2013; Castro-Nava et al., 2014); and *Sacharum officinarum* L. (Wahid & Close, 2007; Sudhakar et al., 2010; Gomathi et al., 2013). The technique is very simple, rapid, require inexpensive equipment, and can be used on plant material from a variety of crops and it is suitable for the analysis of large numbers of genotypes at the same time.

However, a disadvantage in the method is that the temperatures used vary across crops, ranging from 32 to 52 °C, along with differences in tissue exposure time (Sullivan, 1972; Martineau et al., 1979; Blum & Ebercon, 1981; Saadalla et al., 1990a; Saadalla et al., 1990b; Ibrahim & Quick, 2001; Rahman et al., 2004). To identify tolerant genotypes, the plant tissue should be exposed to a temperature where tolerance is indeed expressed; that is, it is necessary to define a temperature where high electrolyte values are induced. In addition, it is necessary to consider the phenological stage in which the methodology is applied to obtain the maximum response (Barnabás et al., 2008). The objective of the study was to investigate three temperature levels for tissue exposure using the thermostability method for six sugarcane cultivars, and determine which of these produced a greater quantity of electrolytes and the establish which is the best phenological stage to identify tolerant genotypes in a selection process.

## 2. Materials and Methods

## 2.1 Site, Genotypes and Weather

The experiment was carried out at the Facultad de Agronomía, Universidad Autónoma de Tamaulipas, Victoria, Tamaulipas, México. The experimental material were six commercial sugarcane genotypes (Mex 68-P23, CP 72-2086, Mex 68-1345, Mex 79-431, RD 75-11 and Mex 95-60). These genotypes were included because they are widely grown in dryland conditions in Mexico, have contrasting agronomic characteristics, and were expected to show different responses to CMT. The experimental material was evaluated for CMT under rainfed conditions. The field experiment was carried out during 2016 and 2017, and was planted on October 15, 2016.

## 2.2 Agronomic Management, Treatments and Data Collection

The general production practices recommended for sugarcane for the growing region were adopted. The experimental area was fertilized at a rate of 103-41-46 NPK per hectare as indicated by Castro et al. (2015). Weed and insect control applications were made when required. CMT was measured following the method proposed by Sullivan (1972) with a modification in the temperature treatment. The temperatures of 40, 50 and 60 °C were used, with a duration of 60 minutes. Treatment temperature was used as suggested Castro (2013) to determine the treatment conditions producing greatest sensitivity in detecting genetic differences. As leaves of different ages might show differential responses, CMT was measured on the second youngest fully expanded leaf at four phenological stages: leaf development, tillering, grand growth and maturity (Bonnett, 2014). Samples collected at each phenological stage from each plot consisted of a paired set (control and heat treated) of 10 leaf discs (10 mm diameter) cut from ten randomly selected plants within a central row. Excised samples were immediately placed in glass vials containing 10 ml deionized water to prevent desiccation of leaf tissue and were brought to the laboratory as quickly as possible. In the laboratory, leaf tissue was washed thoroughly with two changes of deionized water to remove electrolytes adhering to leaf tissue, as well as electrolytes released into the

water. After rinsing, vials were drained and 10 ml deionized water was added to each vial and capped to avoid desiccation and evaporation during heat treatment. Heat treatment vials were covered with aluminum foil. One set of vials was treated in a controlled temperature water bath (Boekel Grant BB-1400) maintained at each temperature treatment for 60 min. The controlled test vials were kept at 28 °C for the same period. After heat treatment, 10 ml deionized water was added to each vial and held at 10 °C for 24 h to allow diffusion of electrolytes. Vials were brought to 28 °C and shaken to mix the contents. Electrical conductivity (EC) was measured with an EC meter (Digital Conductivity Meter, VWR model CRB-10M). Vials were autoclaved (Felisa, Model FE 399) for 10 min. at 0.10 MPa pressure and 120 °C to completely kill tissues and release all the electrolytes. Vials were then brought to 28 °C and the final EC was measured with the same instrument. Percentage relative cell injury (RCI%), as indicator of CMT, was calculated with the following formula (Sullivan, 1972):

$$RCI(\%) = 1 - \{ [1 - (T_1/T_2)] / [1 - (C_1/C_2)] \} \times 100$$
(1)

where, T and C refer to EC values of heat treated and controlled vials, and subscripts 1 and 2 denote initial and final EC readings, respectively.

## 2.3 Statistical Analysis

The design was a randomized complete block with five replications. The plants were grown in one row of 5 m length with inter row spacing of 1.30 m. The plot size in each replication was of three rows and 50 plants each one. To assess treatment effects, genotypes, temperature treatment, and phenological stage were considered as fixed effects, and replicate block was considered a random effect. Combined analysis of variance were made using the GLM procedure (SAS, 2010) for RCI to determine variation among the six genotypes. Effects associated with genotype, temperature treatment, phenological stage and their interactions were identified. Mean comparison was performed on the factors using Tukey's Studentized Range Test at the P = 0.05 levels. Association among temperature treatment and RCI% were examined by simple correlation analysis.

## 3. Results and Discussion

Under field conditions, the evaluation of heat tolerance is probably the most relevant approach in a sugarcane breeding program to identify germplasm tolerance. However, meeting this goal under field conditions is extremely difficult because specific higher temperatures do not always occur at specific developmental stages or with right intensity or duration. To overcome this inherent difficulty, we evaluated a modificated CMT method at four phenological stages in six sugarcane genotypes under rainfed conditions, as a selection criterion in a breeding program.

## 3.1 Analysis of Variance

Analysis of variance carried out on relativity cell injury (RCI%) assessed under three temperature treatments and four phenological stages (Table 1), revealed highly significant effects ( $P \le 0.01$ ) for genotypes, temperature treatment, and phenological stages. Furthermore, genotype by phenological stage and temperature treatment by phenological stage interactions were highly significant ( $P \le 0.01$ ) for RCI%. Differences between genotypes were observed in previous studies in sugarcane (Sudhakar et al., 2010); wheat (Ibrahim et al., 2001; Yildirim et al., 2009; Saadalla et al., 1990a; Blum et al., 2001; Blum & Ebercon, 1981; Fokar et al., 1998); cotton (Rahman et al., 2004) and soybean (Martineau et al., 1979). However, reports in relation to the use of CMT in sugarcane using different temperatures and different phenological stages are very rare scanty.

Source of Variation	df	Mean Squares	Probability
Replication	4	214,887	0.209 ns
Genotypes (G)	5	505,874	0.005 **
Temperature (T)	2	86588,199	0.000 ***
Phenological stage (PhS)	3	1775,509	0.000 ***
$G \times T$	10	132,267	0.525 ns
$G \times PhS$	15	365,597	0.002 **
$T \times PhS$	6	3787,103	0.000 ***
$G \times T \times PhS$	30	350,890	0.000 ***
Residuals	284	145,406	
CV (%)	27.5		

Table 1. Analysis of variance for CMT as measured by RCI (%) of six sugarcane genotypes in an experiment conducted in the field under rainfed conditions

*Note*. ns: Non-significant at P > 0.05; \*\*: Significant at  $P \le 0.01$ ; \*\*\*: Significant at  $P \le 0.001$ .

## 3.2 Effect of Genotypes

RCI% is an indicator of cellular or tissue heat tolerance; low RCI% reflects high CMT, and high RCI% reflects low CMT (Rahman et al., 2004). Genotypes differed significantly (P < 0.01) among themselves for the expression of RCI% in the field (Figure 1). Among genotypes, RCI% ranged between 42 and 50%, and averaged 47%, considering temperature treatments and phenological stages. These RCI% values indicate a wide difference in heat tolerance among the genotypes, as assessed by the membrane thermostability. Genotype Mex 68 P-23 possessed the lowest RCI% and Mex 68-1345 the highest. Similar values of RCI% were observed by Sudhakar et al. (2010) in sugarcane under moisture stress.

## 3.3 Effect of Temperature

Sugarcane genotypes responded differently for RCI% across temperature treatments. Regardless of the genotype and the phenological stage, the impact of temperature treatment was significant, strongly related because as the temperature increased from 40 to 60 °C, the RCI% increased from 18 to 73% (Figure 2). The increase in the RCI% when increasing the temperature from 40 to 50 and 60°C was 170% and 283%, respectively. The increase in the RCI% as a result of the increase in temperature during the procedure represented 3.2% per each °C, when increasing from 40 to 50 °C, and 2.67 °C per each °C when the temperature increased from 40 at 60 °C. On the other hand, when the temperature increased from 50 to 60 °C, the rate of increase was 2.13 °C for each °C temperature increase. The RCI (%) caused by the high temperature occurs as a result of changes in the function (Barnabas et al., 2008) and the composition and structure of the cell membrane (Rahman et al., 2004). Exposure to the high temperature (60 °C) during heat treatment in the CMT test produced greater ability to discriminate between genotypes and safely identify the heat-tolerant genotypes. Our results demonstrate the ability of the genotypes to adjust their CMT under heat-stressed conditions. Furthermore, the research indicates sugarcane's physiological adaptation to heat stress or heat hardening as suggested by Blum and Ebercon (1981) for wheat. The differential response of sugarcane genotypes further suggests that this adaptation or hardening was under genetic control and should be amenable to genetic improvement. Although the impact of temperature heat stress is significant, it is also important to investigate the significance of phenological stage as suggested by Rahman et al. (2004) in cotton and Castro (2013) in corn and bean.



Genotypes

Figure 1. Mean performance of six sugarcane genotypes for RCI% at the four phenological stages in field grown sugarcane under three temperature treatments. Standard errors are indicated by vertical bars



Figure 2. Relationship between temperature treatment and RCI% under field of six sugarcane genotypes. Standard errors are indicated by vertical bars

#### 3.4 Effect of Phenological Stage

Contrary to what might be expected, in our study, the stage of leaf development was not the most susceptible to heat (Figure 3). Among phenological stages, the means of RCI% ranged between 43 and 54%. These RCI% values indicate a wide response in heat tolerance among phenological stages. At maturity, we found the highest RCI% values, and at the tillering stage the lowest RCI%. Vegetative stage plant cells were less sensitive than mature plant cells, which coincides with Castro (2013) in corn and beans, but differing from those obtained by Fokar et al. (1998) in wheat. This means that at more advanced phenological stages, there are more possibilities that with the application of CMT, higher values of RCI% are obtained and with this the possibility of identify genotypes with heat tolerance genes. However, results obtained by Saadalla et al. (1990a) indicate that CMT values obtained by Ismail and Hall (1999) in cowpea when using the CMT at the reproductive stage, and those obtained by Rahman et al. (2004) at the fruiting stage in cotton. In other words, the plant physiological processes differ in their response to heat stress from one phenological stage to another. Our data and results indicate that the determination of the CMT (RCI%) in a genetic improvement program for heat tolerance will depend on the crop, the objectives, the time available, and the number of genotypes to be evaluated.



Figure 3. Mean performance of four phenological stages for RCI% of six sugarcane genotypes in field grown sugarcane exposed to three temperature treatments. Standard errors are indicated by vertical bars

#### 3.5 Interactions

The genotypes studied had a significant interaction (Figure 4) with the phenological stage ( $P \le 0.01$ ). The RCI (%) in Mex 68-1345 and Mex 95-60 was higher in the grand growth stage, while in the rest of the genotypes this same condition was observed at the maturity stage. CP 72-2086, RD 75-11 and Mex 79-431 had a drastic increase in RCI% from the grand growth stage to maturation, and the highest RCI% values in the same stage. This suggests that these genotype cells would be more sensitive to temperature changes at the maturity stage. Gomathi et al. (2014) reported similar observations in sugarcane with the gradual increase in temperatures from 38, 40, 42 and 44°C, but with different time duration using the CMT technique. At the temperature levels studied, the plants were able to modify their cellular and metabolic response, which allows them to survive. The phenological stages, the RCI% was significantly lower at 40 °C. The greatest difference was in the leaf development stage (66%) compared to the 60 °C treatment. The tillering stage RCI% was consistently very similar to the grand growth and maturity stages at the 50 and 60 °C treatments. However, the greatest values were always at the 60 °C treatment. This indicates that there was a greater cell sensitivity in this phenological stage compared with the vegetative stage, where the response was a function of the exposure temperature.



Figure 4. Relative cell injury (%) as determined by the cell membrane thermostability (CMT) test on plants of six sugarcane genotypes at four phenological stages exposed to four treatment temperatures



Phenological stage

Figure 5. Relative cell injury (%) from leaf disks of three temperature treatments at four phenological stages. Standard errors are indicated by vertical bars

It was important that although sugarcane genotypes responded differentially for RCI%, their performance in temperature treatment and phenological stages were quite consistent. Our results suggest that heat tolerance based on CMT (RCI%) can be improved using the existing genetic variability available within the commercial or experimental sugarcane germplasm. The goodness of the test was demonstrated by Castro et al. (2011) and Castro et al. (2014) in maize; Gomathi et al. (2014), Sudhakar et al. (2010), Gomathi et al. (2013), in sugarcane; Rahman et al. (2004), Ibrahim and Quick (2001) and Blum et al. (2001), Shanahan et al. (1990) in wheat; and Ismail and Hall (1999) in cowpea.

#### 4. Conclusion

We conclude that the CMT test can be a useful screening procedure for selecting sugarcane genotypes that tolerate high temperature stress. It could be used in conjunction with a temperature trait of 60 °C during the maturity stage. This procedure predicts the ability of sugarcane genotypes to maintain yield and juice quality under stressful field conditions.

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