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Regular Article

Thermal Sensitivity and Sucrose Accumulation in Response to Chemical Ripener Application in Sugarcane

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ABSTRACT: Effect of two promising chemical ripeners (Ethrel and Glyphosate) was tested on the sucrose accumulation process of two popularly grown sugarcane varieties (Co 86032 and Co 94012) during maturity and ripening phase (240 days to 360 days). Invertase enzymes (acid and neutral invertase) responsible for sucrose accumulation, electrolyte leakage in relation to temperature, hormonal analysis through HPLC, sucrose % and Brix % of juice were studied in ethrel or glyphosate treated cane varieties. The acid and neutral invertases increased with moderate increase in temperature. However, 50 º C treatments were inhibitory for acid invertase particularly in Co 86032 at 360 days. At final harvest the sucrose% juice was 19.22% in Co 86032 and 21.98% in Co 94012. Ethrel has shown 0.7% improvement in Co 86032, while glyphosate treatment had shown 4.59% improvement in sucrose% juice.

Key words: Sugarcane, Ethrel, Glyphosate, Sucrose accumulation, Invertase enzyme

Introduction

Sugarcane (*Saccharum officinarum, L.* commercial hybrid), an annual crop of tropics is an important life sustaining crop cultivated in both tropical and subtropical climates. The crop passes through four distinct physiological growth phases. The first phase i.e. germination phase (0-60 days) denotes activation and sprouting of vegetative bud. The germination is followed by the formative phase (60 – 150 days) during which profuse tillering is initiated. The third important growth phase is the grand growth which starts around 150 days and ends by 240 days. During this phase the stalks grow rapidly almost at the rate of 4-5 internodes / month under favorable conditions. The grand growth is followed by maturity and ripening phase (240 to 360 days) during which the accumulation of sucrose in the cane stalk (Van Dillewijn, 1952) is at maximum. Sucrose accumulation is regulated by invertase enzymes which have been suggested to be the key regulators for accumulation of sucrose in parenchyma and also play a vital role in cane elongation and maturation of sugarcane stem. Two soluble invertases viz., acid invertase and neutral invertase along with cell wall bound acid invertase are known to be present in sugarcane storage tissue. Acid invertase is positively correlated with cane elongation, while neutral invertase is related to sucrose content. The acid invertase occurs in particulate cell membrane or in the cell wall free space. The neutral invertase is a soluble enzyme and hydrolyses sucrose at moderate levels (Zhu *et al.,* 1997). Moore (1987) accounted that the sugarcane varieties are capable of storing sucrose in parenchyma tissue of the stem up to 26%. The modern sugarcane hybrids have the potential of accumulating about 22 to 23% sucrose in juice. Ripening can be enhanced by drying off cane (Robertson and Donaldson, 1998), and by lowering of temperature (James, 1999). Artificial ripening of sugarcane has been made possible by the use of chemical ripeners that hasten sugarcane maturation and increase sugar yield (Nickell, 1984). Ripeners are chemical agents used to induce the ripening. Most chemical ripeners are used to increase the sucrose content at harvest in many countries like Australia, South Africa, Guyana, Swaziland, Hawaii, Mauritius, Florida and Louisiana and in Brazil. In order to study the influence of two chemical ripeners(Ethrel or Glyphosate), a field experiment was conducted during 2009-2010 crop season at Sugarcane Breeding Institute, Coimbatore, utilizing two popularly cultivated sugarcane varieties namely Co 86032 and Co 94012. These chemicals were sprayed at 200 ppm and 400 ppm concentration at the maturity initiation (240 days), and at 300 days.

Materials and Methods

A field experiment was conducted at Sugarcane Breeding Institute, Coimbatore (76.59º E longitude and 11.02º N latitude, 426.72 m TMSL), India, during 2009-2010, utilizing two sugarcane (*Saccharum officinarum, L.*) varieties Co 86032 and Co 94012 representing high sugar content at maturity. Healthy and disease free planting material was taken from 10 month old crop, and two bud cuttings taken from the middle and top portion of cane were planted (40 per row of 6.0m length, spaced 0.9m apart) using split-plot design replicated thrice. The gross and net plot sizes were 32.7m^2 and 21.8 m^2, respectively. The soil (Typic Haplustalf, Noyyal series) is of a sandy loam type and the fertility status is low: high: high with respect to N: P: K. the crop was fertilized with Urea, P2O5 and K2O (N: P: K) at 225:65:120 kg/ha. Nitrogen (Urea) and potash (K2O) were given in two split doses at 45 and 90 days age and P (Super phosphate) was applied at the time of planting.

Ripener application

During maturity phase (240-360 days age) the chemical ripeners (Ethrel or Glyphosate) were sprayed in split doses, i.e. at the commencement of maturity initiation (240 days) and at 360 days age. Ethrel or Glyphosate at 200 ppm and 400 ppm were prepared using commercial grade ethrel (39% a.i.) and glyphosate (41% a.i.). The chemicals were diluted with water and a few drops of surfactant (Triton X-100) were added to facilitate the entry of the applied chemicals. Three undisturbed rows in each set of treatment were sprayed with either of these two chemicals up to the full drenching of the top canopy. Water spray served as controls.

Acid and Neutral Invertase enzymes

Acid and neutral invertases were assayed at monthly intervals during maturity initiation (240 days) and at full maturity (360 days). A stem sample of each variety from each replication was cut, stripped and topped. The rind portion of stem was removed and different temperature treatments (30°C, 40°C, and 50°C) were given. A control was maintained where the stem tissue is kept at room temperature. Uniform discs weighing 1.0g were taken from the pooled top, middle, and bottom internodes of cane for the assay of acid or neutral invertase enzymes. The stem discs were treated with 5ml of cold ethyl acetate for 20 minutes and washed with distilled water until the smell goes off. Invertase activity was estimated in a reaction mixture (10ml) containing 0.05M citrate buffer (pH 5.0) for acid invertase or 0.05M sodium phosphate buffer (pH 7.0) for neutral invertase, 0.2M sucrose, distilled water and 0.1 ml of toluene along with stem discs. The tubes were incubated at 30°C for 30 minutes. An aliquot of 0.5 ml of the reaction mixture was taken to which 1ml copper reagent was added and the tubes were kept in water bath for 10 minutes and then cooled. The reducing sugar content was estimated using arsenomolybdate reagent, and the absorbance was read at 700nm using a UV-VIS Spectrophotometer (Shimadzu, Japan). The amount of reducing sugar formed was quantified against a standard curve prepared with known concentration of glucose in identical conditions.

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Cellular membrane thermostabilty

Young and actively growing leaf from the main shoot of the plant was cut, cleaned under running tap water, blotted dry. Leaf discs (0.5cm diameter) weighing 0.2g were cut(free from mid vein) and infiltrated in 10 ml distilled water in a test tube, and the initial electrical conductivity (Eca) was measured using a conductivity meter. The tissues were incubated in water bath (35°C, 45°C, and 55°C) for 30 min, and the electrical conductivity (Ecb) was recorded. Subsequently the tissue was incubated in boiling water bath (100°C) for 10 min and the electrical conductivity (Ecc) was measured. Membrane damage was calculated using the following formula.

% Electrolyte leakage = Eca-Ecb x100 / Ecc

Hormone extraction

The endogenous level of hormones in the shoot apices of stalk of clones Co 86032, Co 94012 were quantified using HPLC (Shimadzu VP- series). Twenty grams of shoot tips were weighed and macerated with 70% methanol along with 2% ascorbic acid in a pre chilled mortar and pestle. The standard procedures were followed for the extraction of hormones in shoot apices as per Kuhnle et al, (1983). The basic, acidic, and bound form of hormones were fractionated and combined in HPLC Grade methanol for HPLC analysis.

HPLC Analysis

Prior to use, the methanol fraction of the solvent was filtered through a membrane filter and degassed. The sample was run on a reverse phase C18 column with acetonitrile: water (1:3) as the mobile phase and the chromatogram was obtained at the flow rate of 1.0 ml/min and read in UV-VIS detector at 254 nm. The retention time (RT) for hormones was observed at 5-15 min. the identification of hormones was done by comparison of retention time with authentic standards of hormones (Sigma). The concentration of the hormones in plant samples was calculated using the peak area against the hormones standard values and expressed as µg/g.

Juice analysis

Sucrose %

The fresh cane juice was clarified by adding lead acetate and filtered through Whatman filter paper No: 1. the sucrose % juice was quantified through Polarimeter.

Brix %

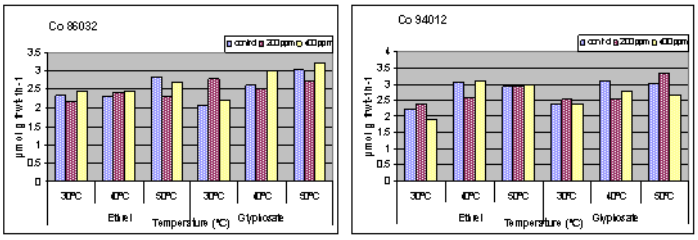
Total solids present in the juice were expressed in percentage. The brix% juice was quantified through Polarimeter.

Results and Discussion

Acid invertase

At 30 C, in both the varieties, the acid invertase activity was reduced at 200 ppm and a marginal increase was seen at 400 ppm in response to ethrel or glyphosate. At 40 C under ethrel treatment in, the enzyme activity was increased at all levels. Under glyphosate treatment (200 ppm), the activity was reduced while increased at 400 ppm in both varieties (Fig. 1). At 50 C the average enzyme activity was 3.02 µmol $gfrwt^{-1}h^{-1}$ in Co 86032, and 2.94 µmol g $frwt^{-1}h^{-1}$ in Co 94012 under ethrel treatment. Under glyphosate treatment the average activity was similar in both the varieties.

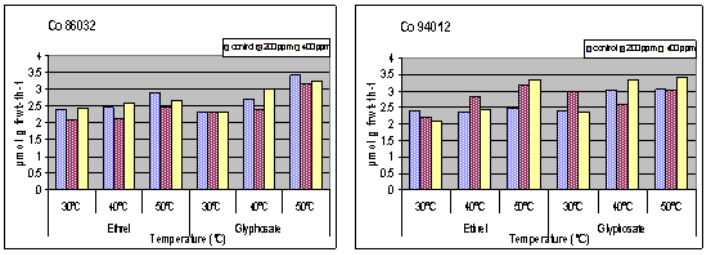
Fig.1. Acid Invertase activity at different temperatures in response to chemical ripener application



Neutral invertase

At 30 C, the neutral enzyme activity decreased at all levels of ethrel treatment in Co 94012. In Co 86032 at 200 ppm the activity was reduced (2.08 µmol $gfrwt^{-1}h^{-1}$) but increased at 400 ppm (2.78 µmol $gfrwt^{-1}h^{-1}$) as compared to control (2.37 µmol $gfrwt^{-1}h^{-1}$). Under glyphosate, in Co 94012, the activity was increased at 200 ppm and declined at 400 ppm. At 40 C under ethrel treatment, in both varieties, the neutral invertase activity declined as compared to control. Glyphosate at 400 ppm increased the activity in both varieties (Fig.2). At 50 C treatment, in Co 94012, the activity was increased in 200 ppm ethrel (3.10 µmol $gfrwt^{-1}h^{-1}$) and as well as in 400 ppm also (3.24 µmol g $frwt^{-1}h^{-1}$) as compared to control (2.47 µmol $gfrwt^{-1}h^{-1}$). Under glyphosate treatment the activity was decreased at 200 ppm but increased at 400 ppm in both the varieties.

Fig.2. Neutral invertase activity at different temperatures in response to chemical ripener application

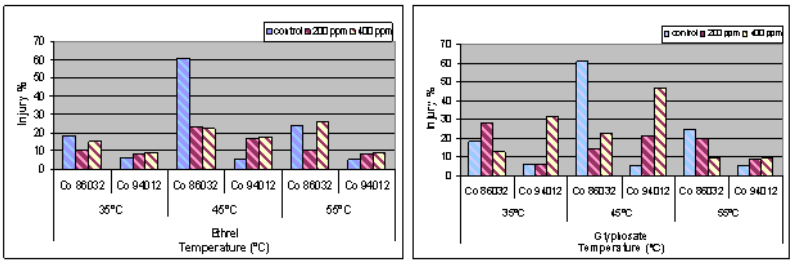


Cellular membrane thermo stability

The electrolyte leakage at 35 C under ethrel treatment showed a decline of 9.99 % at 200 ppm and 14.83 % at 400 ppm in variety Co 86032. In Co 94012, a slight improvement was noticed at all levels. Under glyphosate treatment in Co 86032 the leakage was 28.28 % at 200 ppm and 13.02 % at 400 ppm. At an elevated temperature of 45 C, in Co 86032, under both chemical treatments the leakage was comparable with control (60.72 %). In Co 94012, glyphosate treatment (400 ppm) showed high leakage (46.42 %) as compared to 200 ppm (21.23 %) and control (5.40 %). At 55 C under ethrel treatment(200 ppm), the leakage was 9.99 % as compared to the control (24.26 %) in Co 86032, and at 400 ppm the electrolyte

leakage was high (25.82 %). The electrolyte leakage was high in Co 94012 in response to both the chemicals. The linear increase in temperature treatment has influenced the leakage of cell contents in the leaf tissue. The cell leakage increased gradually with increase in the temperature (Fig.3). The maximum electrolyte leakage was observer at 45°C in both varieties under chemical ripener treatment, thus suggesting the vulnerability of leaf tissue to elevated temperature treatment and the consequent damage to the cellular membrane.

Fig. 3. Membrane injury% at different temperatures under the influence of ethrel or glyphosate



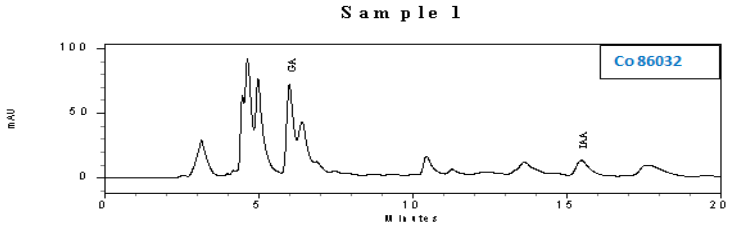
Hormones

Using HPLC, hormone content in meristem was estimated in both Co 86032 and Co 94012 in response to the ripener application during the maturity phase. The hormones were quantified against Indole Acetic Acid (IAA) and Gibberellic Acid (GA) which served as the standards.

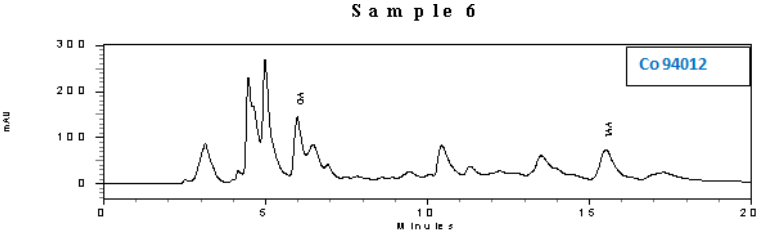
In Co 86032 (control) the gibberellic acid and indole acetic acid showed peaks having an area percent of 12.70 % and 4.82 % at retention time (RT) of 5.97 min and 15.45 min, respectively. In response to 200 ppm ethrel, the IAA peak showed maximum area percent of 10.23 % and the GA showed an area percent 2.95 % at RT of 5.96 min and 15.35 min, respectively(Fig.4). In 400 ppm ethrel, maximum area percent of 8.29 % in IAA at the RT of 15.36 min was noted, but the GA area percent was 4.35 % and the RT was 5.98 min. Under glyphosate treatment at 200 ppm the GA and IAA peaks had an area percent of 5.11 % and 4.19 % at RT of 5.98 min and 15.39 min, respectively. At 400 ppm, the GA and IAA peak had an area percent of 8.73 % and 7.50 % respectively. The RT of both hormones was 5.97 min and 15.37 min, respectively.

In Co 94012 under ethrel treatment the control plants had the GA and IAA peaks possessing the area percent of 7.89 % and 8.10 % at RT of 5.97 min and 15.50 min, respectively. At 200 ppm, both GA and IAA peaks resulted in an area percent of 5.08 % and 9.66 % at RT of 5.99 min and 15.52 min, respectively. The results showed that at 400 ppm, the GA area percent was 4.86 % at a RT of 6.01 min. The IAA area percent was 7.34 % at RT of 15.52 min. Under glyphosate (200 ppm) treatment, the GA and IAA peak area percents were 5.70 % and 5.89 % at RT of 6.02 min and 15.65 min, respectively. At 400 ppm the GA peak area percent was 6.42 % at RT of 6.01 min, while IAA peak area percent was 7.63 % at RT of 15.62 min. Many minor peaks at different retention times were noticed at all stages and only GA and IAA peaks only were prominent.

Fig. 4. Hormone content in sugarcane during maturity and ripening phase



Pk#	Name	Retention Time	Area	Area %	Height	Height %
9	GA	5.97	1164513	12.70	72438	14.99
23	IAA	15.45	442232	4.82	12824	2.65



Pk#	Name	Retention Time	Area	Area %	Height	Height %
7	GA	5.97	2569456	7.89	144213	10.87
22	IAA	15.50	2639770	8.10	71251	5.37

The hormone analysis through HPLC revealed the formation of various peaks at different retention times. Application of GA was known to increase the stalk fresh weight, total stalk length and the length of individual internodes. Earlier reports showed that Gibberellins (A1, A3, A4, A19, A20 and A36) were identified by gas chromatography selected ion monitoring (GC-SIM) in apices of sugarcane. GA3 increases the growth of cane which finally helps improve the yields.

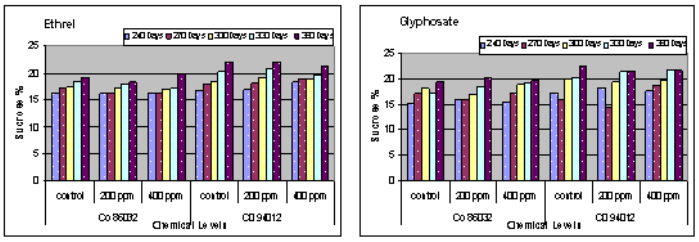
Gibberellins are also known for promoting cell growth through increased hydrolysis of starch and sucrose into glucose and fructose molecules. These hexoses provide energy via respiration and contribute to cell wall formation. As a result of the decrease in water potential, water enters more rapidly, causing cell expansion but diluting the sugars. In sugarcane stems gibberellin- promoted growth results in part from increased synthesis of invertase enzymes that hydrolyze incoming sucrose to glucose and fructose. Since, gibberellins are responsible for cell elongation process, in the present study also, the GA_3 were prominently present in both varieties.

The endogenous hormones particularly GA_3 and IAA might contribute for elongation of stem and cane growth i.e., higher level of GA_3 and lower level of IAA favors better stem growth particularly in Co 97008, Co 86032, Co 99004 and Co 2000-10. Also level of GA_3 and IAA might play a prominent role to mitigate the stunted growth in ratoon crop. In sugarcane genotypes with higher levels of IAA influence the tiller production (Vasantha *et al.,* 2004).

Sucrose % juice

Sucrose is the most important product in sugarcane. Under ethrel treatment, the average sucrose % was from 16.32 % at 240 days which has gone up to 19.12 % at 360 days in Co 86032(Fig.5). At 200 ppm treatment, the sucrose % showed a slight improvement and at 400 ppm at harvesting stage, sucrose% improved by 0.7% as compared to control in Co 86032. In Co 94012 the average sucrose % was from 17.38 % at 240 days which has reached to 21.79 % at 360 days. The maximum amount of sucrose % was in Co 94012 under ethrel (200 ppm) treatment (22.95 %). Under glyphosate treatment in Co 86032 the average sucrose % increased from 15.50 % at 240 days to 19.66 % at 360 days. At harvesting stage, the maximum sucrose % was 22.53 % in Co 94012.

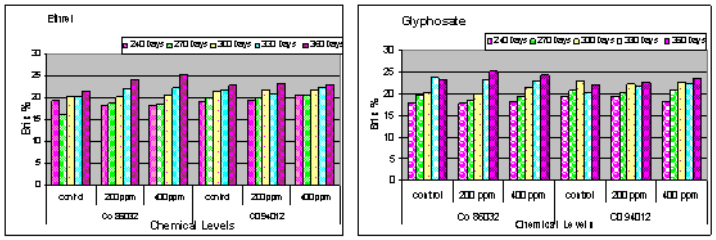
Fig. 5. Sucrose % at maturity phase in response to chemical ripener application



Brix %

Brix (total solids) estimated at monthly intervals during maturity showed gradual improvement in both varieties. Under ethrel treatment, the average brix % increased from 18.73 % at 240 days to 23.57 % at 360 days in Co 86032(Fig.6). But in Co 94012 at 200 ppm in 270 days the brix % was 20.55%, it was slightly declined (0.05 %) as compared to 240 days brix (20.60 %). In Co 86032 under ethrel treatment the brix % was slightly declined from control. Under glyphosate treatment, average improvement was in Co 86032 from 17.92 % at 240 days to 24.21 % at 360 days. Similarly in Co 94012 the brix improved from 19.01 % at 240 days to 22.76 % at 360days.

Fig. 6.Brix % at maturity phase in response to chemical ripener application



Sugarcane is known to tolerate temperatures up to 40°C. It is evident that high temperature beyond 40°C showed drop in various metabolic events. The temperature is a main factor which accelerates invertase activity and inhibits beyond a threshold. Bhowmik *et al.,* (2001) stated that the increased temperature up to a certain degree favorably alters the activity, while high temperatures have a detrimental effect. In the present study also, similar findings were observed in both varieties. The invertase activity increased at optimal and near super optimal temperature followed by a decline when the temperature increased beyond a threshold.

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