

# Exogenous Application of *Ethrel* and Gibberellic Acid Stimulates Physiological Growth of Late Planted Sugarcane with Short Growth Period in Sub-tropical India

Rama Kant Rai<sup>1</sup> · Nidhi Tripathi<sup>1</sup> · Deeksha Gautam<sup>1</sup> · Pushpa Singh<sup>1</sup>

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**Abstract** Physiological growth of late planted sugarcane crop is restricted by high temperature and a short growth period. This causes considerable reduction in crop and sucrose yields. Improving physiological growth within the short period is, therefore, highly desirable. Two field experiments were undertaken to determine the effect of exogenous applications of *Ethrel* and gibberellic acid (GA<sub>3</sub>) on sprouting, shoot population and physiological growth. Sugarcane setts were soaked overnight in *Ethrel* before planting. Foliar application of GA<sub>3</sub> was performed at 90, 120 and 150 days after planting (DAP). *Ethrel* soaking led to 100% sprouting and high settling population at 20 DAP, due to a significant increase in bud moisture and activities of acid invertase (AI), indole acetic acid oxidase (IAAO), adenosine triphosphatase (ATPase), superoxide dismutase (SOD) and nitrate reductase (NR) activity in vivo. Early sprouting increased the growth period to 245 days compared to 220 days in the unsoaked setts. The applications increased leaf area (57%), leaf area index (76%), leaf area ratio (71%), leaf area duration (48%), biomass duration (52%) and net assimilation rate (69.64%) at the grand growth stage. The changes led to increased shoot numbers (26.3%), internodal numbers stalk<sup>-1</sup> (40.74%), internodal length (40%), internodal girth (46.15%) and stalk length (42%) at the harvest stage. The stimulated physiological growth augmented dry matter content, °Brix and purity of cane juice by 24.2, 3 and 0.3%, respectively. The study demonstrates that the induction of higher shoot numbers together with increased leaf area index (LAI) and stalk elongation within a short

growth period through *Ethrel* soaking and gibberellic acid applications is positively associated with enhanced dry matter and sucrose contents.

**Keywords** Late planted sugarcane · *Ethrel* · Gibberellic acid · Leaf area index · Leaf area duration · Shoot numbers · Stalk elongation · Dry matter · Juice quality

## Introduction

Sugarcane (*Saccharum officinarum* L.) is one of the most productive plant species, represented by stout, jointed, fibrous stalks that potentially produce 40–70 tonnes of dry weight ha<sup>-1</sup>year<sup>-1</sup>, depending on crop growth duration (Bakker 1999). The crop is planted from culm pieces containing axillary buds positioned above each node. The phytomers originating from buds pass through four growth stages, that is, germination, tillering, grand growth and maturity, before the commercial component is harvested (Moore and Botha 2014). When the culms are cut from the plant, initiation of sprouting of axillary buds is not automatic. Sprouting of buds requires both the correct temperature (20–30 °C) and a moist environment (Donaldson 2009). The germination stage lasts until 45 days and is followed by the tillering stage. Tillering is the process of side shoots emerging from the axillary buds of the existing culm to form additional culms. Tillers arise at the base of the plant from the axillary buds on internodes that have undergone little expansive growth. Tillering is a major yield determining process as optimal yield depends on establishment of a sufficient tiller density (Bell and Gar-side 2005). The tillering stage lasts for around 120 days and vegetative growth involves several orders of tillering (van Dillewijn 1952). The tillering stage is followed by the

✉ Pushpa Singh  
parampushpa@yahoo.com

<sup>1</sup> Indian Council of Agricultural Research - Indian Institute of Sugarcane Research, Lucknow 226 002, India

grand growth stage of around 70–170 days, where internodes start their expansion until the leaf attached at its base is fully expanded. Elongation is completed at the individual cell and internode level by the time younger leaves have fully expanded (Rae and others 2006). The leaves on each internode serve as major source organs, whereas stalk internodes function as sink organs (Howell 1998). The structural development and elongation of culms is followed by the maturity stage, in which sucrose accumulation begins in the lower internodes while the internodes at the top of the culm expand, for about 90 days until harvest of the crop (Fernandis and Benda 1985).

In India, sugarcane is planted in October (autumn), February (spring) and May (late) with growth duration of 420, 360 and 270 days, respectively (Kapur and others 2011, Table 1). Though planting in October and February is favourable for obtaining high cane yield, farmers prefer to plant sugarcane in late May after the wheat harvest, as a part of the prominent crop rotation sequence of rice–wheat–sugarcane–ratoon–moong (Bhullar and others 2002). However, the temperature in late May is high and desiccating (40–43 °C, Table 1 ). The germination stage in late planted sugarcane, therefore, coincides with high temperature, exposing buds to temperature about 10–13 °C higher than their optimal growing conditions. This causes severe losses in soil and sett moisture contents (Yadav and others 1997). Plants exposed to temperatures about 5 °C above their optimal growing conditions exhibit a characteristic set of cellular and metabolic responses required for the

plants to survive under high-temperature conditions (Guy 1999). These effects include rapid and excessive accumulation of reactive oxygen species and abscisic acid (Maestri and others 2002), accompanied by a decrease in synthesis of normal proteins and accelerated transcription and translation of heat-shock proteins (HSPs; Bray and others 2000). The overproduced reactive oxygen species (ROS) react directly with lipids, proteins and nucleic acids and cause lipid peroxidation-mediated membrane injury, protein degradation, enzyme inactivation, deoxyribonucleic acid (DNA) strand disruption and moisture deficit in cells (Liu and Huang 2000; Maestri and others 2002). The cellular and metabolic changes prevailing at germination stage impose severe limitations on the early germination pattern, subsequent settling establishment and restrict the crop growth period to 220 days (Yadav and others 1997). The restricted crop growth period suppresses physiological growth of leaves and shoots at the tillering stage, stem elongation and dry matter accumulation during the grand growth stage and sucrose accumulation at the harvest stage (Lingle 1999).

High temperature also reduces CO<sub>2</sub> influx for photosynthesis, net photosynthetic rate and dry matter partitioning and the ability to utilize photosynthates and restricts tiller formation (Oh-e and others 2007). Under normal conditions, a synchronism exists between the mother shoot and tillers and further, between tillers themselves. High temperature, however, imposes adverse impacts on synchronism and mobilization of assimilates and nutrients amongst

**Table 1** Crop growth duration of sugarcane under autumn, spring and late planting seasons in sub-tropical India

Months	Oct	Nov	Dec	Jan	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec		
Temperatures	Low temperatures						High temperatures						Low temperatures				
Days after planting	0	30	60	90	120	150	180	210	240	270	300	330	360	390	420		
October (Autumn planting)	Sprouting		Tillering			Grand Growth						Maturity and Harvest Yield (100-120 tha <sup>-1</sup> )					
Months				Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	
Days after planting				0	30	60	90	120	150	180	210	240	270	300	330	360	
February (Spring planting)				Sprouting		Tillering			Grand Growth			Maturity and Harvest Yield (75-99 tha <sup>-1</sup> )					
Months								May	June	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb
Days after planting								0	30	60	90	120	150	180	210	240	270
May (Late planting)	<b>Smaller Time Window For Growth</b> Less by about 200 days against Autumn Less by about 75 days against Spring							Sprouting		Tillering + Grand Growth			Maturity and Harvest Yield (30-40 tha <sup>-1</sup> )				

tillers, causing a severe reduction in their numbers (Bita and Gerats 2013). Decreased tiller numbers reduce productivity, as tillers per plant at an early stage determine the number of millable canes, which is the key component of cane yield (Bell and Garside 2005). Physiologically, high temperatures also affect leaf development, leaf characteristics and internodal elongation at the tillering stage (Bonnett and others 2006). The base temperature for stem elongation has been calculated as 16–18 °C (Lingle 1999). Temperatures above 36 °C and reduced moisture availability led to shorter internodes and stalk length (Bonnett and others 2006). A short grand growth period imposes adverse effects on canopy coverage, amount of light interception, cumulative growth rate and dry matter accumulation (Table 1, Dhawan and others 1997; Moore and Botha 2014). After the grand growth stage, physiological growth again gets restricted from October onwards until January, due to sub-optimal temperatures (Rai and others 2008). Along with, tiller mortality reduces the ratio of mother shoot:tiller to merely 1:0.5 as compared with 1:2 in autumn and spring planted sugarcane (Kapur and others 2011; Moore and others 1997). This causes severe reduction in the number of millable canes per hectare (NMC ha<sup>-1</sup>), dry matter contents and cane yields (Bhullar and others 2002; Yadav and others 1997; Bonnett and others 2006).

Several attempts have been made for improving sprouting, shoot numbers and cane yield in late planted sugarcane. Reduction in moisture deficit in cane setts and buds due to high temperature has been attempted through irrigation between planting and the germination stage. However, irrigation causes crust formation in the upper layer of soil and blocks the emergence of young seedlings (Srivastava and Mahindra 2012; Yadav and others 1991). Improvement in sprouting has also been addressed through increased seed rate and altered plant geometry. A narrow row to row spacing of 60 cm was adopted with a huge seed requirement (7–8 tha<sup>-1</sup>), for accommodating increased seed rate, unlike row to row spacing of 75 cm in spring planting, where the seed requirement was 5–6 tha<sup>-1</sup> (Singh 2000; Bhullar and others 2002). However, no significant improvement in germination, shoot numbers, cane harvest index or sugar productivity was obtained. Low yields of late planted sugarcane thus continue to be a major problem in subtropical India (Yadav and others 1997; Dhawan and others 1997).

In sugarcane, studies are available that provide evidence on exogenous application of plant growth regulators (PGRs), which have altered the sprouting and growth processes. *Ethrel* application has improved sprouting in sugarcane (Li and others 2003). External application of GA<sub>3</sub> remarkably increased internodal length in sugarcane (Moore 1980; Pribil and others 2007). However, no information is available on combined application of *Ethrel* and

GA<sub>3</sub> at different growth stages for improving sprouting, enhancing shoot numbers and stimulating physiological growth within a short growth period. The objective of our study was to investigate the combined effects of *Ethrel* and GA<sub>3</sub> applied at critical growth stages on sprouting, shoot numbers and physiological growth within a short growth period in late planted sugarcane. The aims were threefold: (1) to test the ability of exogenously applied *Ethrel* to cane buds on earliness, enhanced sprouting and initial settling numbers during the germination phase; (2) to assess the effect of phasic GA<sub>3</sub> application on shoot numbers, leaf characteristics, internodal and stalk elongation during the tillering and grand growth phases; (3) to assess the combined effects of *Ethrel* and GA<sub>3</sub> application on total dry matter content and cane juice quality.

## Materials and Methods

### Experimental Site and Soil Climate

The experiment was conducted at the Indian Council of Agricultural Research (ICAR)-Indian Institute of Sugarcane Research, Lucknow, India, located at 26° 56'N, 80°52'E and 111 m above sea level. This falls in the Agro-Eco-region 4 (Northern plain and Central Highlands) and Hot Semi-arid Eco-region with Alluvial-derived (N8D2) soils (Sehgal and others 1990). The soil in the experimental field was sandy loam (13.3% clay, 24.5% silt and 62.2% sand) of Indo-Gangetic alluvial origin, very deep (>2 m), well drained, flat and classified as non-calcareous mixed hyperthermic *udic ustochrept*. The soil temperatures at 10 cm depth are given in Table 2. The climate of the experimental site is semi-arid, sub-tropical with hot dry summers and cold winters. The average monthly minimum and maximum temperatures during summer (April–June) range from 18.4 to 43 °C and in winter (November–February) from 7.4 to 29 °C. The average annual rainfall is 1045.5 mm and cumulative open pan evaporation is 1750 mm. Nearly 72% of the total rainfall is received through northwest monsoons during July to September (Table 2). The organic carbon (OC) content of soil was 0.48% with total nitrogen 0.069%. The available nitrogen (N), phosphorus (P), potassium (K) were 183.7, 18.7, 192 kg ha<sup>-1</sup> in 2012–2013 and 185.6, 18.2, 190 kg ha<sup>-1</sup> in 2013–2014.

Two crops were planted with sugarcane variety CoLk 94184 on 15th May 2012–13 and 2013–14 in different fields, prepared after wheat harvest on 6th May 2012 and 7th May in 2013, respectively, at the institute farm of the Indian Council of Agricultural Research-Indian Institute of Sugarcane Research (ICAR-IISR), Lucknow, India. Both fields with left-over wheat stubbles were irrigated and later on prepared with cultivator (once) and harrow (twice).

**Table 2** Climatologic data of mean temperature (Temp), total rainfall (Rainf.) and relative humidity (R.H.) in the period from 2012 to 2014

Months	May	June	July	August	September	October	November	December	January	February
2012										
Maximum Temp. (°C)	40.1	34.3	33.2	32.8	33.8	30.6	27.4	23.6	19.1	23.7
Minimum Temp (°C)	25.6	26	25.9	25.3	24.8	20.2	11.4	10.6	5.8	11.6
Rainf. (mm)	0.0	286.7	173.8	242.4	30.9	55.4	0.0	1.2	47.0	23.2
R.H. (%)	44	76	82	85	81	81	68	68	83	72
Soil temp (°C)	42.8	41.7	35.5	33.7	34.6	31.2	25.7	21.3	15.1	27.8
2013										
Maximum Temp. (°C)	39.5	40.3	33.8	34.5	33.2	31.1	28.2	20.4	18.1	22.3
Minimum Temp (°C)	22.8	26.4	22.9	24.2	22.1	15.4	9.7	7.8	9.8	10.8
Rainf (mm)	21.2	22.2	264	85.7	88.8	70.4	0.0	12.4	47	23.2
R.H. (%)	42	54	79	74	77	77	68	78	82	70
Soil temp (°C)	41.2	42.4	35.3	36.4	33.9	31.3	25.9	19.1	17.4	22.0
2014										
Maximum Temp. (°C)	40.1	39.6	34.2	34.1	36.0	34.3	30.7	24.5	18.2	26.2
Minimum Temp (°C)	25.6	27.2	26.7	26.3	25.4	19.8	13.9	9.7	8.5	12.3
Rainf. (mm)	3.0	48	230.8	185.7	26.2	1.2	0.8	2.1	29.4	15.6
R.H. (%)	47	59	79	80	83	89	93	75	82	70
Soil temp (°C)	42.2	43.0	36.2	36.7	34.1	32.1	29.3	20.1	17.1	24.4

Soil moisture of 16% was maintained in both fields during planting. Ridges and furrows were laid out at 75 cm spacing with a tractor-mounted furrow opener. The opened furrows were treated with chlorpyrifos (20% emulsifiable concentrate) for termite control.

**Crop Culture and Exogenous Applications of Ethrel and GA<sub>3</sub> at Critical Growth Stages**

The sugarcane variety CoLk 94184 was planted under seven treatments in a randomized block design. Each plot (20 m × 12 m) contained 16 rows with a row to row spacing of 75 cm. In each row, five three-budded setts m<sup>-1</sup> row length, were planted in three replications. The setts were placed in furrows with ends overlapping on each other. The treatments were T1: Unsoaked (Control), T2:

Unsoaked + Water application, T3: Unsoaked + GA<sub>3</sub> application, T4: Water soaked, T5: Water soaked + GA<sub>3</sub> application, T6: Ethrel soaked, T7: Ethrel soaked + GA<sub>3</sub> application (Table 3). Approximately 33,600 three-budded setts of sugarcane variety CoLk 94184 were planted in an area of 5040 m<sup>2</sup>. Prior to planting, 14,400 setts were covered with trash and left overnight for T1, T2 and T3. A total of 9600 setts were soaked in water for T4 and T5 (2400 L of water) and 9600 setts were soaked in Ethrel for T6 and T7 (2400 L of 100 ppm Ethrel). The setts were left overnight and taken out the next morning for planting. They were rinsed in Bavastine (@ 2 g L<sup>-1</sup>) prior to their planting in the furrows.

The foliar application of GA<sub>3</sub> was performed at 90, 120 and 150 days after planting (DAP) in T3, T5 and T7. GA<sub>3</sub> was dissolved in 0.5 cm<sup>3</sup> of ethanol and diluted with distilled water to a concentration of 100 m mol m<sup>-3</sup> and

**Table 3** Treatments details during the experiments

Treatments (sett priming + foliar application across growth cycle)	Sett priming done prior to planting	Foliar applications performed at 90, 120 and 150 Days after planting (DAP)
T1 Unsoaked + no foliar application (Control)	Unsoaked	No foliar application
T2 Unsoaked + water application	Unsoaked	Water application @ 5 mL/plant
T3 Unsoaked + GA <sub>3</sub> application	Unsoaked	GA <sub>3</sub> application @ 5 mL/plant
T4 Water soaked + no foliar application	Water (Overnight soaking)	No foliar application
T5 Water soaked + GA <sub>3</sub> application	Water (Overnight soaking)	GA <sub>3</sub> application @ 5 mL/plant
T6 Ethrel soaked @ 100 ppm + no foliar application	Ethrel (Overnight soaking)	No foliar application
T7 Ethrel soaked + GA <sub>3</sub> application	Ethrel (Overnight soaking)	GA <sub>3</sub> application @ 5 mL/plant

applied with knap sac (5 mL/plant) between 8.00 and 9.00 AM, while T2 was applied with an equal quantity of distilled water. The concentration of dissolving solvents was too low for any physiological effect on plants. The total quantity of water application and water used in the GA<sub>3</sub> solution varied with the number of plants in every row. Ethrel and gibberellic acid were purchased from Chemical Drug House (CDH) Biochemicals, Analytical Reagent (AR) grade, with minimum assay of about 39 and 99.9%, respectively.

The crops were raised with standard agronomic cultivation practices and recommended doses of N, P, K (150:80:80 Kg ha<sup>-1</sup>). Fertilizers used were urea (46% N), single super-phosphate (6.8% P) and Muriate of Potash (46.2% K). One third of N, P and K was applied as a basal dressing in furrows at the time of planting. The remaining N was top-dressed in two equal splits at 45 and 90 DAP. Both the crops received a total of four irrigations and three inter-cultural operations. Application of insecticides was made as per recommendation for the region. The plants in all the treatments were free of pests and diseases during the experiments.

#### Bud Sprouting and Initial Shoot Number Determination at 20 and 45 DAP

Sprouting % was calculated by counting the number of sprouted buds out of the total planted buds. Buds with initial shoot protrusion of at least 2 mm in length were considered to be sprouted (Rai and others 2008). Bud moisture, bud dry weight and relative growth rate (RGR) were recorded with 45 buds scooped from 15 setts from respective treatments. The freshly removed buds were washed thoroughly and dried with Whatman No.1 filter paper for recording fresh weight. Bud dry weight was recorded by drying buds in a hot air oven, at 102 °C for 24 h and 80 °C for 72 h, to constant weight. RGR was computed using the formula

$$= \ln W_2 - \ln W_1 / t,$$

where  $\ln W_1$  is initial bud dry weight and  $\ln W_2$  is bud dry weight attained after time ( $t$ ) in days, at 20 and 45 DAP. The numbers of seedlings sprouted per plot were counted manually for recording initial plant population.

#### Biochemical Analysis of Cane Buds at 20 and 45 DAP

Freshly sampled bud tissues were chopped and homogenized to prepare a 10% homogenate in a chilled pestle and mortar with chilled distilled water. The homogenate was filtered through four layers of cheese cloth and then centrifuged at 8000g for 20 min at 4 °C. The supernatant obtained after centrifugation was used for estimation

of reducing sugars, sucrose and total phenolic contents. Estimation of reducing sugar was done according to the method of Nelson (1944) and Somogyi (1945). Sucrose was estimated by the resorcinol thiourea method described by Roe and Papadopoulos (1954). Protein was estimated by the method of Lowry and others (1951) and acid invertase activity was assayed by the method of Hatch and Glasziou (1963). Total phenolic contents were estimated by the method described by Swain and Hillis (1999). Indole acetic acid (IAA) was determined as described by Nagar (1995) and IAAO activity was assayed by the method of Gordon and Weber (1951). The ATPase activity was assayed by the method of Fischer and Hodges (1959). Phosphorus estimation was done by following the method of Fiske and Subbarow (1925). NR activity in vivo and SOD activity were assayed by the method of Jaworski and others (1971) and Beauchamp and Fridovich (1971), respectively.

#### Determination of Leaf Characteristics and Shoot Development at 180 and 270 DAP

The area per leaf was calculated by the method described by Lerch and others (1977). The total leaf area of individual stalks was obtained by summation of leaf area on each stalk. Leaf area index (LAI) was calculated by multiplying the mean value of leaf area per stalk by number of stalks present in a known area. The growth parameters were individually calculated using formulae of Kvet and others (1971).

Net assimilation rate (NAR) =  $(W_2 - W_1) (\ln L_2 - \ln L_1) / [(t_2 - t_1) (L_2 - L_1)]$ , dry matter produced per leaf area and time units (mg cm<sup>-2</sup> day<sup>-1</sup>)<sup>-2</sup>. With these values and shoot numbers, NAR was calculated on a land area basis.

Leaf area ratio (LAR) =  $L/W$ , relates leaf area with total stalk dry matter (cm<sup>2</sup>g<sup>-1</sup>).

Leaf area duration (LAD) =  $L_2 + L_1(t_2 - t_1)/2$  (cm<sup>2</sup>day) 10<sup>4</sup>.

Biomass duration (Z) =  $W_2 + W_1(t_2 - t_1)/2$  (g day) 10<sup>3</sup>.

where  $W$  and  $L$  are mean values of dry weight and leaf area at a specific time, respectively.  $W_1$  and  $W_2$  represent initial and final mean values of total dry weight of a stalk.  $L_1$  and  $L_2$  are the initial and final mean values of leaf area belonging to a stalk over the period  $t_2 - t_1$ . The growth parameters were calculated at 180 and 270 DAP. The shoot numbers in each plot were counted at 30-day intervals until 270 DAP. Stalk length, internodal length, internodal girth and internodal weight were measured with a metre scale, vernier caliper and electronic balance in 21 stalks sampled at 180 and 270 DAP.

### Determination of Total Dry Matter and Juice Quality at 270 DAP

Stalk weight was recorded by chopping the stalk into small pieces. The chopped pieces were dried in an oven at 80 °C, until constant weight was achieved. Dry matter content m<sup>-2</sup> was quantified from total number of stalks in an area of 16 m<sup>2</sup> selected from each plot at 180 and 270 DAP. Cane juice quality was analysed from total number of stalks harvested from an area of 16 m<sup>2</sup> at 270 DAP (Meade and Chen 1977).

### Statistical Analysis

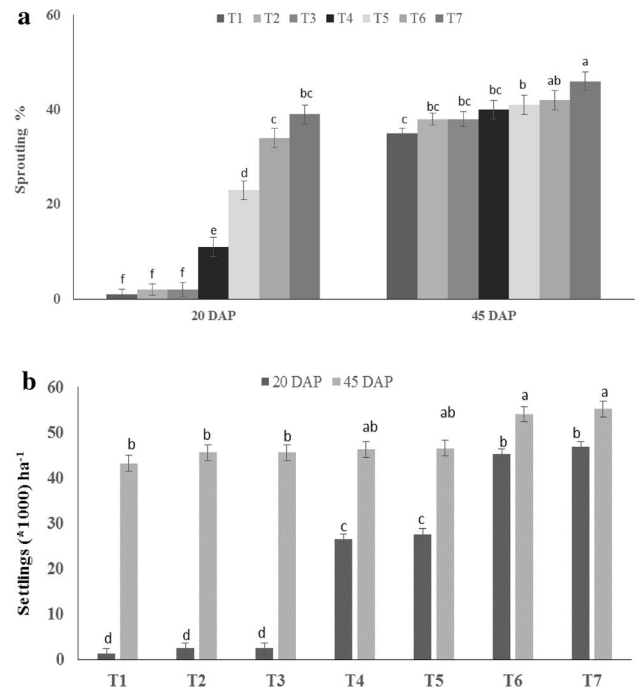
Data were analysed using the statistical product and service solution version 16.0 software (SPSS Inc, Chicago, IL). One-way analysis of variance with Duncan’s Multiple Range Test (DMRT) as post hoc analysis was used to compare the means (Snedecor and Cochran 1967). Graphics were generated using Sigma Plot version 10.0 (Systat software, Inc., Point Richmond, CA). Regression analysis and correlation coefficients were calculated using MS Excel statistical tools to assess the interrelationships between treatment means across temperature among different parameters.

### Results

#### Effect of Exogenous Application of Ethrel on Sprouting and Relative Growth Rate of Buds

Ethrel soaking increased bud dry weight and relative growth rate by 61 and 44%, respectively, against unsoaked setts at 20 DAP. Bud moisture, bud dry weight and RGR in unsoaked and water-soaked setts were significantly less compared to Ethrel-soaked setts (Table 4). Maximum sprouting with 54,000 settlings ha<sup>-1</sup> was achieved with Ethrel soaking against no settling in

unsoaked setts at 20 DAP (Fig. 1a, b). Later at 45 DAP also, there was a significant increase in bud moisture, bud dry weight, RGR and settling numbers with Ethrel soaking (Table 4). Bud moisture, bud dry weight and relative growth rate were significantly less in unsoaked setts. Sprouting of buds, therefore, was delayed up to 45 DAP in unsoaked setts (Fig. 1b). At 20 DAP, Ethrel soaking led to 100% sprouting and high settling numbers while



**Fig. 1** a Effect of treatments on sprouting % at 20 and 45 DAP. b Effect of treatments on settling numbers at 20 and 45 DAP. T1: Unsoaked (Control), T2: Unsoaked+Water application, T3: Unsoaked+GA<sub>3</sub> application, T4: Water soaked, T5: Water soaked+GA<sub>3</sub> application, T6: Ethrel soaked, T7: Ethrel soaked+GA<sub>3</sub> application. Vertical bars represent ± SE of mean values of three replicates (n=45). Means followed by same superscript in each parameter do not differ significantly at p=0.05 by Duncan’s Multiple Range Test (DMRT). DAP Days after planting

**Table 4** Effect of exogenous application of Ethrel on relative growth rate of buds and sprouting to 45 DAP

Treatments	Bud Moisture (%)			Bud dry weight (g)			RGR of buds (mg g <sup>-1</sup> day <sup>-1</sup> )	
	DAP			DAP			DAP	
	0	20	45	0	20	45	20	45
Unsoaked	38 ± 1.20 <sup>a</sup>	38 ± 1.2 <sup>c</sup>	45 ± 1.3 <sup>d</sup>	2.5 ± 0.23 <sup>a</sup>	3.4 <sup>c</sup> ± 0.21 <sup>c</sup>	3.6 ± 0.11 <sup>b</sup>	524.2 ± .3 <sup>c</sup>	554.1 ± 1.2 <sup>c</sup>
Water Soaked	38 ± 0.75 <sup>a</sup>	56 ± 1.2 <sup>b</sup>	60 ± 1.2 <sup>b</sup>	2.7 ± 0.22 <sup>a</sup>	3.9 ± 0.26 <sup>b</sup>	4.6 ± 0.12 <sup>b</sup>	569.6 ± 1.2 <sup>b</sup>	623.4 ± 1.4 <sup>b</sup>
Ethrel Soaked	38 ± 0.87 <sup>a</sup>	75 ± 1.1 <sup>a</sup>	87 ± 1.5 <sup>a</sup>	2.7 ± 0.20 <sup>a</sup>	5.9 ± 0.20 <sup>a</sup>	8.1 ± 0.16 <sup>a</sup>	749.4 ± 1.0 <sup>a</sup>	857.1 ± 1.5 <sup>a</sup>

Values are means of three replicates ±SE (n=45). Means followed by same superscript in each parameter do not differ significantly at p=0.05 by Duncan’s Multiple Range Test (DMRT). DAP Days after planting

sprouting was significantly less with unsoaked setts, even at 45 DAP (Fig. 2 a,b,c). At 45 DAP, the settling numbers increased to 55,200 ha<sup>-1</sup> with *Ethrel*-soaked setts against 42,000 ha<sup>-1</sup> in unsoaked setts. *Ethrel* soaking thus reduced the duration of germination to 20 days compared to 45 days in unsoaked setts and provided an additional 25 days for crop growth.

### Effect of Exogenous Application of *Ethrel* on Biochemical Changes During Sprouting and Shoot Numbers

*Ethrel* soaking of buds led to a fourfold increase in acid invertase (AI) activity at 20 DAP. This caused significant increase in reducing sugars and a decrease in sucrose contents. An increase of 53 and 81% in reducing sugar content and a decrease in sucrose contents by 37 and 40% were recorded in *Ethrel*-soaked setts at 20 and 45 DAP, respectively, against unsoaked setts. The increased reducing sugars and decreased sucrose contents due to higher AI activities at 20 DAP enhanced growth of buds and emergence of settlings in *Ethrel*-soaked setts (Table 5). Low AI activity, less reducing sugars and higher sucrose contents in unsoaked setts delayed sprouting and settling emergence to 45 days. NR activity in vivo, SOD and IAAO activities increased at 20 and 45 DAP in *Ethrel*-soaked setts against unsoaked setts. NR activity in vivo increased by 76 and 59% at 20 and 45 DAP, respectively. IAAO activity increased by 74.7 and 107% at 20 and 45 DAP, respectively. An increase of 79 and 77% in SOD activity was recorded at 20 and 45 DAP, respectively (Table 6). Although enzyme activities increased significantly in water-soaked setts, they were 20% less than in *Ethrel*-soaked setts. The IAA contents decreased by 42 and 25% whereas total phenolic contents decreased by 32 and 51% in *Ethrel*-soaked setts at 20 and 45 DAP against unsoaked setts, respectively (Table 6).

### Effect of Exogenous Application of *Ethrel* and GA<sub>3</sub> on Leaf Characteristics and Canopy Coverage at 180 and 270 DAP

Foliar application of GA<sub>3</sub> on shoots from *Ethrel*-soaked setts at 90, 120 and 150 DAP increased foliage numbers, leaf area (LA), leaf area index (LAI), leaf area duration (LAD), leaf area ratio (LAR), net assimilation rate (NAR) and biomass duration (Z) significantly, both at 180 and 270 DAP. The highest foliage numbers were recorded with *Ethrel* soaking and GA<sub>3</sub> applications at 180 DAP (Fig. 3a). LA and LAI increased by 52 and 23% with *Ethrel* soaking and GA<sub>3</sub> applications against unsoaked setts at 180 DAP (Fig. 3b, c). LAD, LAR, and Z increased by 48, 52 and 71%, respectively, at 180 DAP (Fig. 4a, b, d). At 270 DAP, LA, LAI, LAD and LAR were at a maximum with *Ethrel* soaking and GA<sub>3</sub> applications against unsoaked setts (Figs. 3a, b, 4a, b). Net assimilation rate (NAR), which indicates the rate of increase in biomass per unit leaf area per day, was highest with *Ethrel* soaking and GA<sub>3</sub> applications at 180 and 270 DAP (Fig. 4c). Biomass duration (Z, gd×10<sup>3</sup>) increased by 57 and 63% at 180 and 270 DAP, respectively (Fig. 4d).

### Effect of Exogenous Application of *Ethrel* and GA<sub>3</sub> on Shoot and Cane Juice Characteristics at 180 and 270 DAP

A significant increase in mean internodal number, internodal length and internodal weight was recorded with *Ethrel* soaking and GA<sub>3</sub> applications against unsoaked setts (Fig. 5a–c). Similarly, an increase in internodal number, length and weight was recorded with water soaking and GA<sub>3</sub> application but the shoot numbers were significantly less than with *Ethrel* soaking. Mean internodal numbers per stalk increased by 58% with *Ethrel* soaking and GA<sub>3</sub> applications at 270 DAP (Fig. 5a). At 180 and 270 DAP,

**Fig. 2** Effect of treatments on sugarcane sett sprouting at 20 DAP. **a** With *Ethrel* soaking. **b** With water soaking. **c** In an unsoaked sett.



**Table 5** Effect of exogenous application of *Ethrel* on biochemical changes during sprouting to 45 DAP

Treatments	Reducing sugars (mg g <sup>-1</sup> fwt)		Sucrose (mg g <sup>-1</sup> fwt)		Acid invertase activity (mmolmin <sup>-1</sup> mg <sup>-1</sup> protein)		ATPase activity (μgPi liberated mg <sup>-1</sup> protein 10 min <sup>-1</sup> )		Nitrate reductase activity in vivo (μmol of NO <sub>2</sub> g <sup>-1</sup> fwth <sup>-1</sup> )						
	DAP	0	DAP	0	DAP	0	DAP	0	DAP	0	DAP				
Unsoaked	8.8±0.12 <sup>a</sup>	9±1.1 <sup>c</sup>	10±1.2 <sup>c</sup>	35±1.03 <sup>a</sup>	29±1.4 <sup>a</sup>	27±1.5 <sup>a</sup>	0.11±0.01 <sup>a</sup>	0.16±0.02 <sup>c</sup>	0.18±0.05 <sup>c</sup>	0.14±0.02 <sup>a</sup>	0.19±0.01 <sup>c</sup>	0.27±0.01 <sup>c</sup>	45.3±1.1 <sup>a</sup>	45.4±1.2 <sup>c</sup>	51.2±1.3 <sup>c</sup>
Water Soaked	8.8±0.11 <sup>a</sup>	12±1.2 <sup>b</sup>	13±1.2 <sup>ab</sup>	37±1.03 <sup>a</sup>	27±1.4 <sup>b</sup>	23±1.1 <sup>b</sup>	0.12±0.03 <sup>a</sup>	0.30±0.01 <sup>b</sup>	0.38±0.02 <sup>b</sup>	0.15±0.02 <sup>a</sup>	0.54±0.01 <sup>b</sup>	0.44±0.04 <sup>b</sup>	47.3±1.3 <sup>a</sup>	57.5±1.3 <sup>b</sup>	60.6±1.3 <sup>b</sup>
<i>Ethrel</i> Soaked	8.8±0.13 <sup>a</sup>	14±1.4 <sup>a</sup>	16±1.3 <sup>a</sup>	36±1.3 <sup>a</sup>	22±1.3 <sup>c</sup>	20±1.3 <sup>c</sup>	0.12 <sup>ab</sup> ±0.02a	0.56±0.02 <sup>a</sup>	0.68±0.02 <sup>a</sup>	0.15±0.03 <sup>a</sup>	0.68±0.04 <sup>a</sup>	0.76±0.01 <sup>ab</sup>	45.3±1.2 <sup>a</sup>	87.2±1.1 <sup>a</sup>	82.9±1.2 <sup>a</sup>

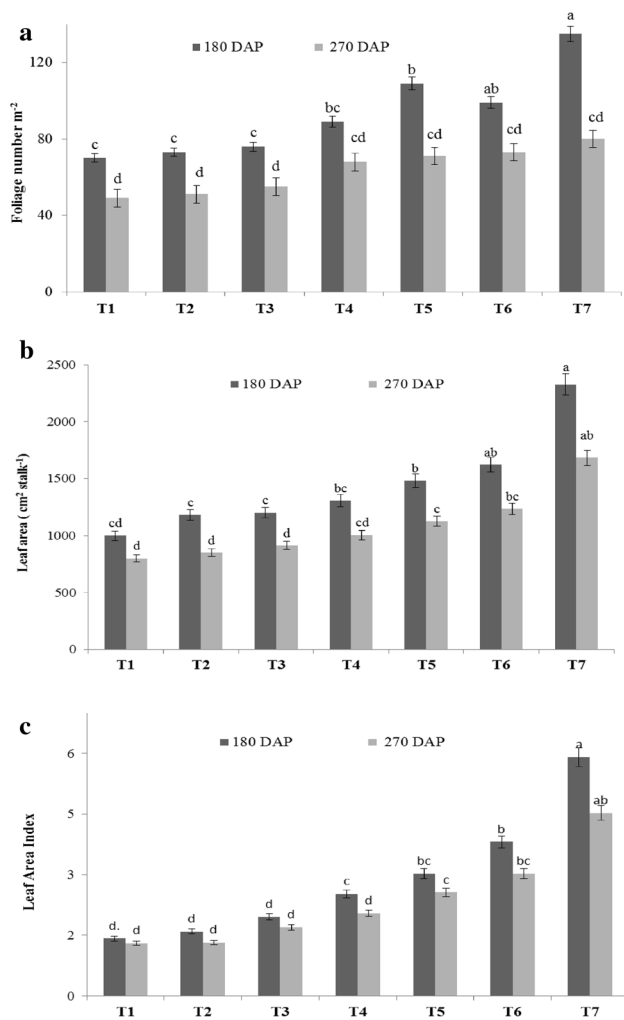
Values are means of three replicates±SE (n=15). Means followed by same superscript in each parameter do not differ significantly at p=0.05 by Duncan's Multiple Range Test (DMRT).DAP Days after planting

**Table 6** Effect of exogenous application of *Ethrel* on stress-induced biochemical changes during sprouting to 45 DAP

Treatments	IAA (μg g <sup>-1</sup> fwt)		TPC (mg g <sup>-1</sup> fwt)		IAAO activity (μg IAA oxidized mg <sup>-1</sup> protein)		SOD activity (EU mg <sup>-1</sup> protein)					
	DAP	0	DAP	0	DAP	0	DAP	0				
Unsoaked	0.83±0.01 <sup>a</sup>	0.77±0.10 <sup>a</sup>	0.75±0.10 <sup>a</sup>	3.7±0.01 <sup>a</sup>	3.29±0.01 <sup>a</sup>	3.04±0.04 <sup>a</sup>	9.12±0.05 <sup>a</sup>	9.45±0.12 <sup>a</sup>	9.76±0.05 <sup>c</sup>	0.42±0.01 <sup>a</sup>	0.53±0.03 <sup>c</sup>	0.56±0.02 <sup>c</sup>
Water Soaked	0.83±0.01 <sup>a</sup>	0.59±0.11 <sup>b</sup>	0.52±0.12 <sup>b</sup>	3.7±0.04 <sup>a</sup>	2.78±0.04 <sup>b</sup>	2.64±0.01 <sup>b</sup>	9.12±0.09 <sup>a</sup>	10.12±0.12 <sup>b</sup>	12.23±0.08 <sup>b</sup>	0.42±0.03 <sup>a</sup>	0.65±0.01 <sup>b</sup>	0.68±0.02 <sup>b</sup>
<i>Ethrel</i> soaked	0.8±0.02 <sup>a</sup>	0.40±0.11 <sup>c</sup>	0.35±0.08 <sup>c</sup>	3.7±0.01 <sup>a</sup>	2.23±0.02 <sup>c</sup>	1.89±0.01 <sup>c</sup>	9.12±0.06 <sup>a</sup>	13.51±0.14 <sup>a</sup>	14.89±0.04 <sup>a</sup>	0.42±0.02 <sup>a</sup>	0.75±0.02 <sup>a</sup>	0.86±0.02 <sup>a</sup>

T1: Unsoaked (Control), T2: Unsoaked+ Water application, T3: Unsoaked+ GA<sub>3</sub> application, T4: Water soaked, T5: Water soaked+ GA<sub>3</sub> application, T6: *Ethrel* soaked, T7: *Ethrel* soaked+ GA<sub>3</sub> application, Values are means of three replicates±SE (n=15). Means followed by same superscript in each parameter do not differ significantly at p=0.05 by Duncan's Multiple Range Test (DMRT). DAP Days after planting





**Fig. 3** Effect of treatments on growth parameters of sugarcane during growth cycle. **a** Foliage number, **b** Leaf area; **c** Leaf area index (LAI). T1: Unsoaked (Control), T2: Unsoaked+Water application, T3: Unsoaked+GA<sub>3</sub> application, T4: Water soaked, T5: Water soaked+GA<sub>3</sub> application, T6: *Ethrel* soaked, T7: *Ethrel* soaked+GA<sub>3</sub> application. Vertical bars represent  $\pm$  SE of mean values of three replicates ( $n=21$ ). Means followed by same superscript in each parameter do not differ significantly at  $p=0.05$  by Duncan's Multiple Range Test (DMRT). DAP Days after planting

internodal length increased by 66 and 62%, respectively (Figs. 5b, 6a). Mean internodal weight was at a maximum at 180 DAP and it increased by 120% at 270 DAP. There was a significant reduction in mean internodal length with unsoaked setts without GA<sub>3</sub> at 270 DAP (Fig. 6b).

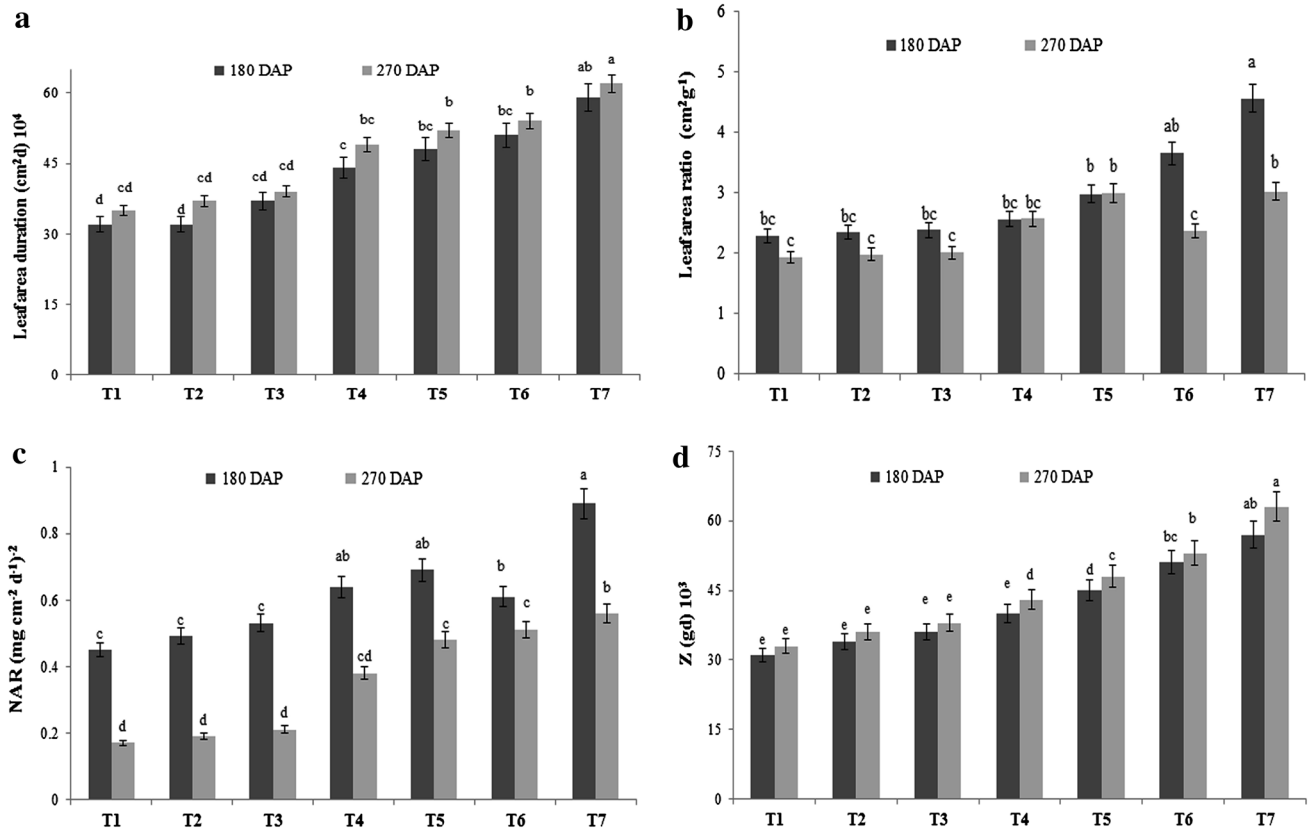
Cane stalk length, stalk and root dry weight increased significantly with *Ethrel* soaking and GA<sub>3</sub> applications both at 180 and 270 DAP. Stalk length increased by 46 and 42% and stalk dry weight increased by 77 and 78% at 180 and 270 DAP, respectively (Fig. 7a, b). Shoot numbers increased significantly by 55 and 47% at 180 and 270 DAP with *Ethrel* soaking and GA<sub>3</sub> applications against unsoaked

setts (Fig. 7c). *Ethrel* soaking and GA<sub>3</sub> applications led to a threefold increase in root weight. Root weight was at a maximum with GA<sub>3</sub> application against control at 180 and 270 DAP (Fig. 7d). Maximum shoot numbers in a clump were recorded with *Ethrel* soaking and GA<sub>3</sub> application whereas minimum shoot numbers in a clump of sugarcane were observed with unsoaked setts without GA<sub>3</sub> application at 270 DAP (Fig. 8a, b). The dry matter content, °Brix and purity of cane juice increased by 24.2, 3 and 0.3% in *Ethrel*-soaked setts with GA<sub>3</sub> application against control at 270 DAP (Table 7).

## Discussion

Impaired sprouting of sugarcane buds under high and desiccating temperatures (40–43 °C) affected dry matter and sucrose contents in late planted sugarcane (Yadav and others 1997). The setts containing axillary buds are highly sensitive to high temperatures which cause severe moisture loss and dysfunctional cell membranes (Finch-Savage and others 2006; Wahid and Close 2007). Moisture deficit in buds of unsoaked setts suppressed sprouting significantly. The decreased moisture level suppresses sprouting due to adverse effects on integrity and functioning of cell membranes, accelerated kinetic energy and loosening of chemical bonds within the molecules. The lipid bilayers of the cell membrane thereby become more fluid either by denaturation of proteins or by increased unsaturated fatty acids (Savchenko and others 2002). This enhances the permeability of membranes and causes severe loss of water and electrolytes (Rai and others 2008). Low bud moisture levels decreased the activities of acid invertase, IAAO, ATPase and NR activity in vivo. Poor sucrose hydrolysis, low reducing sugars and high IAA levels suppressed sprouting in unsoaked setts at 20 DAP. Singh and others (2003) reported that low enzyme activities and high IAA levels suppressed bud sprouting in sugarcane.

*Ethrel* soaking was very effective in increasing moisture levels and enhancing enzyme activities in buds at high temperatures. This led to 100% bud sprouting at 20 DAP. Although the buds were exposed to temperatures about 10–13 °C higher than optimal growing conditions, *Ethrel* minimized moisture loss in buds and maintained the optimum moisture level required for sprouting (Zhang and others 2001). Increased bud moisture improved the integrity and functions of cell membranes and provided an optimum environment required for sprouting (Rai and others 2008). *Ethrel* is believed to overcome the inhibitory effect of high temperatures by activating cell wall enzymes responsible for substrate digestion in several species (Hasegawa and Maruyama 1995). High bud moisture in *Ethrel*-soaked setts enhanced acid invertase activity, sucrose hydrolysis



**Fig. 4** Effect of treatments on growth parameters of sugarcane during growth cycle. **a** Leaf area duration (LAD), **b** leaf area ratio (LAR), **c** net assimilation rate (NAR), **d** biomass duration (Z). T1: Unsoaked (Control), T2: Unsoaked+Water application, T3: Unsoaked+GA<sub>3</sub> application, T4: Water soaked, T5:

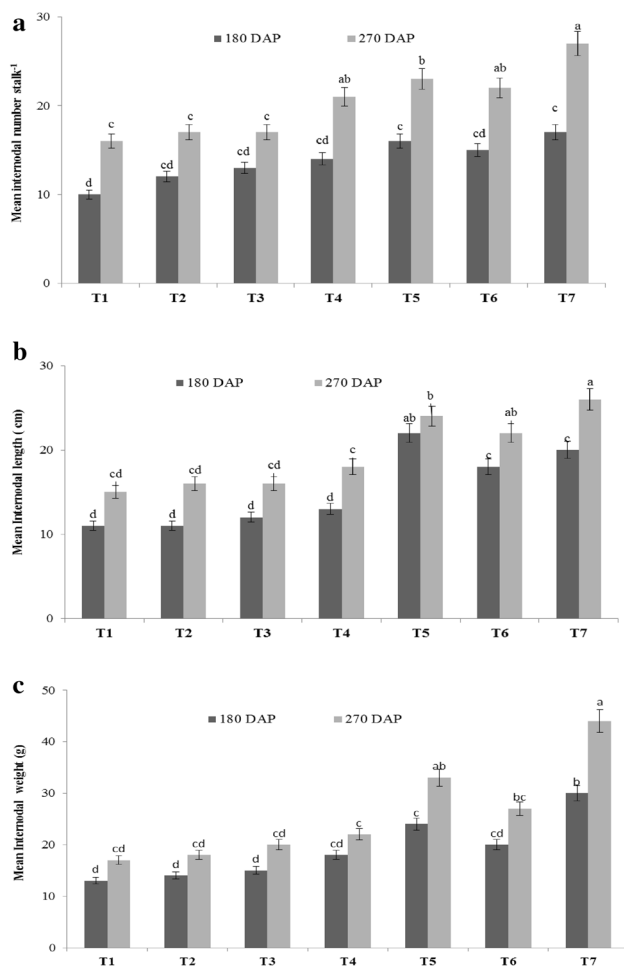
Water soaked+GA<sub>3</sub> application, T6: *Ethrel* soaked, T7: *Ethrel* soaked+GA<sub>3</sub> application. Vertical bars represent ±SE of mean values of three replicates (*n*=21). Means followed by same superscript in each parameter do not differ significantly at *p*=0.05 by Duncan's Multiple Range Test (DMRT). DAP Days after planting

and reducing sugars at 20 DAP. Acid invertase activity and reducing sugars have been reported to increase in sugarcane buds that received *Ethrel* treatment (Li and Solomon 2003). Increased ATPase activity and NR activity in vivo improved adenosine triphosphate (ATP) and nitrogen availability for bud growth (Ye and others 2003). High IAAO and SOD activities reduced IAA contents and detoxified hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which helped the buds to survive high-temperature stress (Zhao and others 2001). Li and Solomon (2003) reported *Ethrel* to be most effective at improving sugarcane bud sprouting and its growth.

Delayed and poor sprouting with unsoaked setts suggested a problem in establishment of initial shoot numbers at an early stage. Initial shoot numbers depend on adequate temperature and moisture (Singh and others 2003). Insufficient external moisture results in poor initial shoot establishment in sugarcane and several other crops (Inman-Bamber and Smith 2005). Although there was no sprouting at 20 DAP, the initial shoot numbers were limited to 42,000 shoots ha<sup>-1</sup> at 45 DAP. The physiological growth of shoots was suppressed. Low LAI, LAD and Z resulted in poor leaf

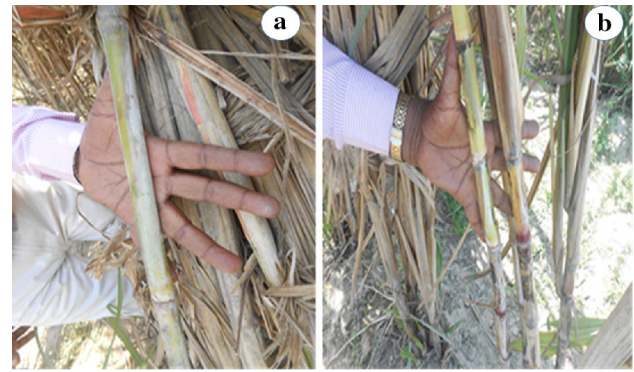
expansion and canopy development. This caused a poor balance between photosynthesis and respiration. Yadav and Prasad (1988) reported that when temperatures are not adequate, plants are compelled to go through a series of drought reactions which result in poor leaf emergence, leaf expansion, canopy development and formation of smaller internodes. Leuning and others (1991) reported that low LAI reduces light interception and radiation use efficiency in crops.

*Ethrel* soaking improved the initial shoot numbers both at 20 and 45 DAP. *Ethrel* induced early and enhanced bud sprouting, resulting in establishment of 54,000 shoots ha<sup>-1</sup> compared to no shoots with unsoaked setts at 20 DAP. There was a significant increase in shoot numbers at 45 DAP. The shoot numbers increased to 55,200 shoots ha<sup>-1</sup> compared to 42,000 shoots ha<sup>-1</sup> with unsoaked setts. Our results are in agreement with Rao and others (2005), who reported that exogenous application of *Ethrel* has the ability to promote the axillary bud break and increases initial shoot numbers in several species. The early and enhanced sprouting with *Ethrel*-soaked setts



**Fig. 5** Effect of treatments on intermodal growth parameters of sugarcane during growth cycle. **a** Mean internodal number; **b** mean internodal length; **c**. mean internodal weight. T1: Unsoaked (Control), T2: Unsoaked + Water application, T3: Unsoaked + GA<sub>3</sub> application, T4: Water soaked, T5: Water soaked + GA<sub>3</sub> application, T6: *Ethrel* soaked, T7: *Ethrel* soaked + GA<sub>3</sub> application. Vertical bars represent  $\pm$ SE of mean values of three replicates ( $n=21$  stalks). Means followed by same superscript in each parameter do not differ significantly at  $p=0.05$  by Duncan's Multiple Range Test (DMRT). DAP Days after planting

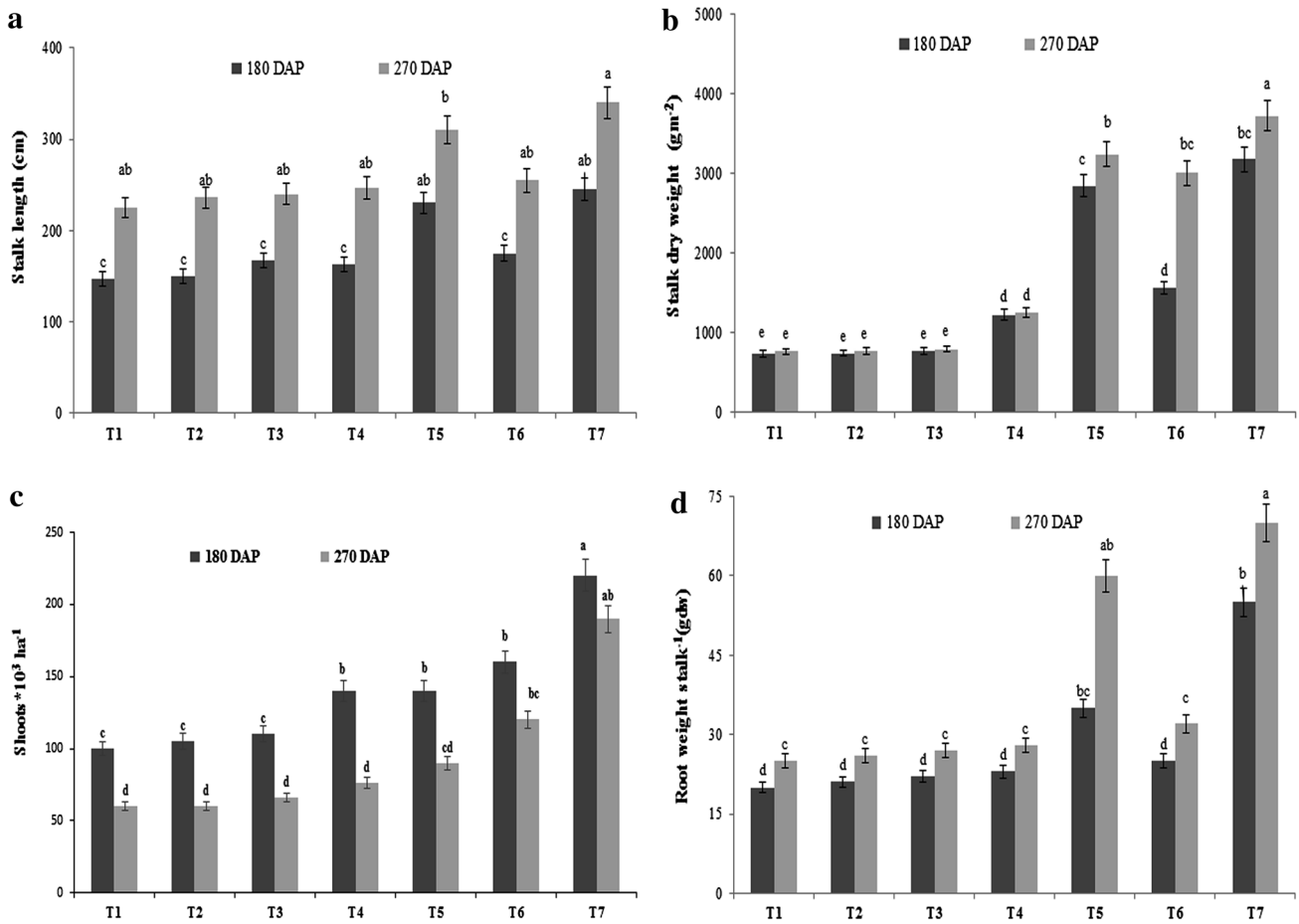
provided an additional 25 days for physiological growth of initial shoots against unsoaked setts. The additional time period of 25 days with *Ethrel* soaking led to a significant increase in physiological growth of shoots at the tillering stage. LAI was 2.54 in shoots from *Ethrel*-soaked setts against 0.46 in shoots from unsoaked setts at the tillering stage (90 DAP). High LAI during the tillering stage led to development of a vast canopy and provided greater green leaf surfaces for increased light interception, radiation use efficiency and carbon fixation. van Andel (1973) reported a significant improvement in canopy development in plants treated with *Ethrel*. It was reported by Maddonni and others (2001) that because



**Fig. 6** **a** Twofold increase in internodal length in sugarcane with *Ethrel*-soaked setts + GA<sub>3</sub> applications. **b** Reduced internodal length in sugarcane with unsoaked setts without GA<sub>3</sub> applications at 270 DAP (Days after planting)

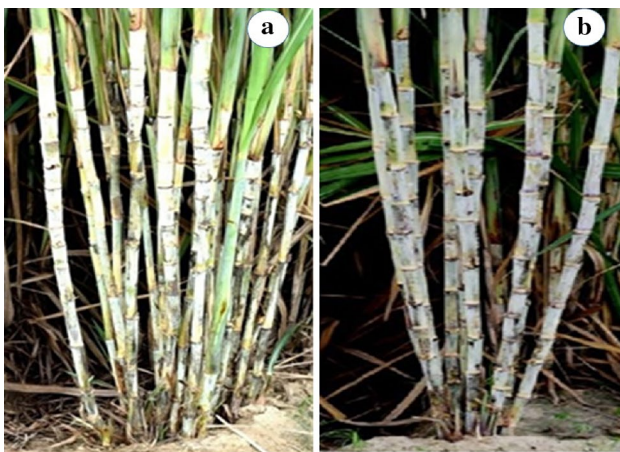
canopy structure is strongly related to total amount of intercepted radiation, a vast canopy structure favours dry matter accumulation for maximizing crop yield. Raji and others (1999) reported that high LAI and vast canopy at the initial growth stage improved sugarcane yield. A positive effect of early growth and positive correlation among LAI and yield in several varieties has been reported by Shimabuku and others (1980).

GA<sub>3</sub> applications led to a substantial increase in shoot numbers and foliage numbers at 180 DAP. Leaf area ratio, leaf area duration, biomass duration and net assimilation rate increased by 71, 48, 52 and 69.64 per cent, respectively. Shoot numbers increased to 2, 20,000 shoots ha<sup>-1</sup> and LAI increased to 6.03 against 1, 00,000 shoots ha<sup>-1</sup> with LAI limited to 2.22 with unsoaked setts. Increased LAI enhanced photosynthetic activity and assimilate production in leaves (source). This increased source activity led to increased internodal (sink) demand for assimilates for preventing its catastrophic overproduction. The enhanced sink demand is fulfilled by unloading of assimilates from leaves into internodes (Zamski and Schaffer 1996). The translocated assimilates into internodes are utilized in cell division, cell elongation and internodal elongation (Moore 1980). The enhanced assimilate translocation from leaf phloem to internodes thus increased internodal numbers, internodal length, internodal girth and stalk length by 40.74, 40, 46.15 and 42%, respectively, against control. It was reported by Ho and Vasil (1983) that GA<sub>3</sub> applications enhanced assimilate translocation from leaf phloem to internodes. Shimabuku and others (1980) reported that GA<sub>3</sub> applications induced a significant increase in leaf sheath length for increasing assimilate translocation into internodes. High LAD maintained the leaves for a longer duration and increased assimilate production and translocation to internodes for a longer duration (Raji and others 1999). High LAD at the grand growth stage thus promoted



**Fig. 7** Effect of treatments on stalks and roots of sugarcane. **a** Stalk length; **b** stalk dry weight; **c** shoot numbers **d** root weight. T1: Unsoaked (Control), T2: Unsoaked+ Water application, T3: Unsoaked+ GA<sub>3</sub> application, T4 : Water soaked, T5: Water soaked+ GA<sub>3</sub> application, T6 : Ethrel soaked, T7: Ethrel

soaked+GA<sub>3</sub> application. Vertical bars represent ±SE of mean values of three replicates (n=21 stalks). Means followed by same super-script in each parameter do not differ significantly at p=0.05 by Duncan’s Multiple Range Test (DMRT). DAP Days after planting



**Fig. 8** **a** Increased number of shoots in a clump of sugarcane with Ethrel soaking +GA<sub>3</sub> applications. **b** Less number of millable canes in a clump of sugarcane with unsoaked setts without GA<sub>3</sub> applications at 270 DAP (Days after planting)

faster and complete development of sinks for increasing dry matter accumulation within a short growth period.

The increased leaf activity and internodal length stimulated the physiological growth of shoots at 180 DAP. Moore (1980) reported a remarkable increase in internodal length through external application of GA<sub>3</sub> in sugarcane and suggested that it was a result of rapid cell division and cell elongation. Cell elongation with GA<sub>3</sub> applications is caused by the action of cell wall hydrolases which relax the cell wall and increase cell wall extensibility (Yang and others 1996). An additive effect of GA<sub>3</sub> applications on sugarcane internodal and stalk elongation is also reported by Moore and Botha (2014). Takahashi and others (1986) reported that GA<sub>3</sub> applications along with other hormones promoted rapid elongation and division of cells. Delayed and poor initial shoot numbers, low LAI, LAD, LAR, Z and NAR in unsoaked setts without GA<sub>3</sub> application during the grand growth stage led to poor photosynthetic activity

**Table 7** Effect of *Ethrel* and GA<sub>3</sub> application on dry matter contents and juice quality in late planted sugarcane

Treatments	DM (tha <sup>-1</sup> )	<sup>o</sup> Brix (B)	Sucrose % (S)	Purity
T1	22.4±0.5 <sup>c</sup>	19.7±0.02 <sup>a</sup>	17.1±0.01 <sup>a</sup>	86.9±0.01 <sup>a</sup>
T2	22.5±0.6 <sup>c</sup>	19.7±0.01 <sup>a</sup>	17.1±0.02 <sup>a</sup>	86.9±0.02 <sup>a</sup>
T3	22.8±0.4 <sup>c</sup>	19.7±0.03 <sup>a</sup>	17.1±0.01 <sup>a</sup>	86.9±0.01 <sup>a</sup>
T4	23.1±0.3 <sup>bc</sup>	20.0±0.02 <sup>a</sup>	17.2±0.03 <sup>a</sup>	87.0±0.03 <sup>a</sup>
T5	23.4±0.4 <sup>b</sup>	20.0±0.01 <sup>a</sup>	17.2±0.02 <sup>a</sup>	87.0±0.02 <sup>a</sup>
T6	25.3±0.3 <sup>bc</sup>	20.3±0.03 <sup>a</sup>	17.7±0.01 <sup>a</sup>	87.2±0.01 <sup>a</sup>
T7	28.3±0.2 <sup>a</sup>	20.3±0.01 <sup>a</sup>	17.7±0.02 <sup>a</sup>	87.2±0.02 <sup>a</sup>

Values are means of three replicates ±SE ( $n=21$ ). Means followed by same superscript in each parameter do not differ significantly at  $p=0.05$  by Duncan's Multiple Range Test (DMRT). DAP; Days after planting

<sup>a</sup>F-Interaction analysis for DM (dry matter): T1 (Unsoaked), NS; T6 (*Ethrel* soaked), S; T7 (*Ethrel* + GA<sub>3</sub> application), S. <sup>o</sup>Brix: T1 (Unsoaked), NS; T6 (*Ethrel* soaked), S; T7 (*Ethrel* + GA<sub>3</sub> application), S. Sucrose %: T1 (Unsoaked), NS; T6 (*Ethrel* soaked), S; T7 (*Ethrel* + GA<sub>3</sub> application), S. Purity: T1 (Unsoaked), NS; T6 (*Ethrel* soaked), S; T7 (*Ethrel* + GA<sub>3</sub> application), S

and assimilate production. Low LAI was responsible for smaller canopy development which affected the physiological growth of shoots adversely. Less green leaf surfaces decreased light interception, radiation use efficiency and carbon fixation. Low LAD caused assimilate losses through respiration resulting in shorter internodes with less weight. Shimabuku and others (1980) reported that low LAI, LAD, LAR, biomass duration and NAR are responsible for low energy conversion and suppression of physiological growth in several crops.

The stimulated physiological growth with *Ethrel* soaking and GA<sub>3</sub> led to an increase of 26.3% in shoot numbers against controls at 270 DAP. This was due to a significant decrease in shoot mortality. The reduced shoot mortality and increased shoot numbers with GA<sub>3</sub> application were due to development of a robust root system as indicated by a threefold increase in root weight. This reduced the competition for water and nutrients amongst shoots which is generally responsible for their mortality. The increased root weight improves absorption of water and mineral nutrients required for stimulated growth of shoots (van Antwerpen 1999). The shoot numbers, however, decreased significantly in controls due to 40% shoot mortality. Bell and Garside (2005) reported that about 50% of shoot mortality is due to competition for light and nutrients. Higher shoot numbers together with increased LAI and stalk elongation and reduced shoot mortality within a short growth period led to a significant increase in total dry matter, sucrose content and cane juice quality. Increased LAI and stalk elongation with GA<sub>3</sub> applications were thus positively associated with enhanced dry matter and sucrose contents. Kromer (2000) reported that total dry matter productivity relies on

the initial plant population, an appropriate carbon assimilation rate and its enhanced export from the source to sink organ.

In conclusion, our results indicate that *Ethrel* application exerted a protective effect on buds against high temperature and had beneficial effects on different parameters required for optimum sprouting during the germination stage (bud moisture level, activities of acid invertase, ATPase, NR activity in vivo, SOD and IAAO). The phenomenon of *Ethrel* action on sugarcane buds is associated with activation of cell wall enzymes responsible for maintaining the integrity and functions of cell membranes, which has positive effects on growth enzymes and metabolites required for optimum sprouting. The beneficial effect of *Ethrel* under high temperature also involved improvement in antioxidant capacity of buds through removal of reactive oxygen species with increased SOD activities. The results also indicated that phasic application of gibberellic acid on increased shoot numbers stimulated faster physiological growth of source and sink organs for increasing the dry matter accumulation within a short growth period, during the tillering and grand growth phases. The positive effects of GA<sub>3</sub> application on LAI, internodal elongation and root growth within a short growth period increased the dry matter production and improved cane juice quality at the harvest stage. The combined effects of *Ethrel* and GA<sub>3</sub> applications, thus precluded the adverse impacts of high temperature and a short growth period in late planted crops and increased total dry matter production. The finding suggests that use of *Ethrel* and gibberellic acid in late planted sugarcane crop holds strong potential to improve cane yield in sub-tropical India. Six field impact assessment trials at different locations are in progress for assessing the effects of exogenous application of *Ethrel* and gibberellic acid on cane yield.

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