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Ethrel and GA_3 Induced Physio-biochemical Alterations in Sugarcane for Manoeuvring the Biometric Traits for Enhancing Cane and Sugar Yield

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ABSTRACT

Background: Physiological growth of sugarcane crop is restricted by high temperature and limited growth period. This causes considerable reduction in crop and sucrose yield. Improving physiological growth within the limited period is therefore highly desirable.
Methods: Field experiment was undertaken in 2021-2023 for determining the effect of exogenous applications of *Ethrel* (100 ppm) and Gibberellic acid (35 ppm) on germination, shoot population, physiological characteristics, dry matter and sucrose contents. Sugarcane setts were soaked overnight in *Ethrel* before planting and foliar application of GA_3 was conducted at 90, 120 and 150 days after planting (DAP).
Result: *Ethrel* soaking led to 100% sprouting and high settling population at 20 DAP. Early sprouting increased the growth period to 285 days as compared to 240 days in control. The applications increased leaf area (55%). The changes led to increased shoot numbers (28.3%), internodal numbers $stalk^{-1}$ (41.02%), internodal length (42%), internodal girth (45.15%) and stalk length (45%) at harvest stage. The stimulated physiological growth augmented the dry matter content, °Brix and purity of cane juice by 24.2%, 3% and 0.3% respectively. The study demonstrates that higher shoot population together with increased LAI and stalk elongation during the growth period, both induced through *Ethrel* soaking and Gibberellic acid applications, are positively associated with increased dry matter and sucrose contents.

Key words: Dry matter, *Ethrel,* Gibberellic acid, Juice quality, Leaf area, Shoot population, Stalk elongation, Sugarcane.

INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) production grew from around 1.71 billion tonnes in 2008 to 1.9 billion tonnes in 2023 from 26 million ha cultivated land worldwide (FAOSTAT, 2023). It goes through distinct stages during its growth cycle, namely germination, tillering, grand growth and maturity (Dillewijn, 1952). The leaves located on every internode serve as primary organs for photosynthesis while sections of the stem between the nodes with sucrose storing parenchyma cells and vascular tissues functions as sink organs (Moore and Maretzki, 2014). The key reasons identified for yield stagnancy are delayed germination, slow pace of early phase of development, poor tillering, limited internodal elongation and higher dry matter losses during the crop cycle (Rai *et al.,* 2017). Delay in germination reduces the growth period of sugarcane crop cycle (Rai *et al.,* 2017).

The canopy coverage and development of leaf area index (LAI) leads to poor accumulation of dry matter (Som-Ard *et al.,* 2021). The reduced leaf area index (LAI), low canopy coverage, rate of leaf area expansion, leaf number, shoot population, root density and total plant dry weight changes lead to poor accumulation of photosynthates (Som-Ard *et al.,* 2021). As growth phase is of 360 days, the crop experiences low temperature, high temperature, drought and rainy season. (Dillewijn, 1952; Moore and Maretzki, 2014). Its germination phase often coincides with low and high temperatures while the rest of the critical stages experience around 10-13°C above the ideal growth conditions. Such changes have exhibited severe loss in soil, sett moisture content and distinct array of cellular and metabolic reactions (Yadav *et al.,* 1997). This degrades proteins and inactivates enzymes in addition to damaging membranes. This process also causes pigment fading and DNA strand disruption, leading to cellular moisture deficit and hindering tiller formation (Maestri *et al.,* 2002). The cellular and metabolic changes have been reported to have imposed severe limitation on early germination pro-

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cess, establishment of seedlings and duration of crop growth (Park *et al.,* 2017; Rai *et al.,* 2017).

Higher temperatures have been reported to diminish CO_2 intake, decrease photosynthetic rate, reduce dry matter partitioning and thus impede plant's capacity for growth (Rai *et al.* 2017). High temperatures have also affected the synchronism existing between mother shoot and tillers, movement of metabolic by products and nutrients adversely resulting in a reduction in number of millable canes. The tiller numbers thus are reduced at tillering phase. Reduced tiller numbers affect the productivity adversely as tillers per plant determine the quantity of millable canes at harvest stage. The leaf development, canopy coverage, light interception level, stalk elongation, leaf area expansion and cumulative growth rate during tillering and grand growth phases too are impacted adversely and as a result, the adverse temperatures have restricted the tiller formation (Moore *et al.,* 2014). It lowers the availability of reducing sugars, simultaneously decreasing the activity of acid invertase, while raising the levels of indoleacetic acid (IAA) and phenols. The accumulation of these compounds in sugarcane buds in their natural environment results in bud dormancy (Rai *et al.,* 2017). Plant growth regulators are a wide range of organic compounds produced by plants for their own growth and development of their defense system (Rademacher, 2016; Kumar, 2020). The compounds are produced in small amounts and are primarily used on-site (Park *et al.,* 2017) or applied exogenously for their onsite usage (López-Salmerón *et al.,* 2019; Guleria *et al.,* 2021). As the impacts on developmental processes are dose dependent, their exogenous application has produced remarkable results on plant growth and stem elongation (Bagale *et al.,* 2022). *Ethrel* (ethylene releasing compound) and GA_3 are plant growth regulators which have been used in several crops and sugarcane since decades. In light of above, the current paper deals with advantages of applying *Ethrel* (ethylene releasing compound) and GA_3 exogenously at critical growth junctures in sugarcane crop cycle.

MATERIALS AND METHODS

The experiments were carried out at the ICAR-Indian Sugarcane Research Institute (ICAR-ISRI), located in Lucknow, India, in 2021-2023. The effect of exogenous applications of *Ethrel* and GA_3 on Sugarcane variety CoLk 94184 was assessed in a randomized block design Three-budded setts of sugarcane are cut from main canes talks. Before planting, setts are treated with *Ethrel* at a concentration of 100 ppm (Table 1). The setts are soaked overnight and removed the next morning for planting. The setts were treated with a fungicidal solution prior to planting to mitigate the risk of soil-borne diseases. Standard agronomic practices, including soil preparation, planting and subsequent crop management, were rigorously followed. The crop was treated with a recommended fertilization regime of 150 kg/ha nitrogen, 80 kg/ha phosphorus and 80 kg/ha potassium (NPK). GA_3 is dissolved in 0.5 cm^3 of ethanol and diluted with distilled water to a concentration of 100 $mmolm^{-3}$ and applied with knap sac (5 mL/plant) between 8.00 and 9.00 AM. The GA_3 treatment was administered at 90, 120 and 150 days after planting @ 35 ppm (Table 1). The volume of water used for the GA_3 solution depends on the number of plants in each row. It received six irrigation sessions at critical growth stages to ensure optimal water availability. Additionally, three intercultural operations, including weeding and soil aeration were uniformly conducted across all plots to maintain soil health and reduce competition from weeds. The sugarcane crop was manually harvested after a growth period of 12 months and observations on yield attributing traits were recorded. The data collected were subjected to analysis of variance (ANOVA) to assess the significance of differences among treatments. Mean comparisons were performed using the least significant difference (LSD) test at a 5% significance level. All statistical analyses were executed using appropriate MS excel statistical software.

RESULTS AND DISCUSSION

Effect of *Ethrel* and GA_3 application on germination

Field experiments were conducted with sugarcane variety CoLk 94184, employing a randomized complete block design with treatments applied during critical growth phases: Germination (45 DAP), tillering (150 DAP), grand growth (210 DAP), maturity (300 DAP) and harvesting (365 DAP). Treatments included optimized nutrient applications and growth regulators (*Ethrel* + GA_3) compared against untreated controls. Morpho-physiological parameters including plant height, root length, number of millable canes, internode count and length, as well as

Table 1: Treatments details during the experiment.

Treatments code	Treatments	Sett priming	Foliar applications performed at 90, 120 and 150 days after planting (DAP)
T_1	Unsoaked + No foliar application	Unsoaked	No foliar application
T_2	Water soaked + No foliar application	Water (Overnight soaking)	No foliar application
T_3	*Ethrel* soaked + No foliar application	*Ethrel* (Overnight soaking)	No foliar application
T_4	Unsoaked + GA_3	Unsoaked	GA_3 application @ 5 ml/plant
T_5	Water soaked + GA_3	Water (Overnight soaking)	GA_3 application @ 5 ml/plant
T_6	*Ethrel* soaked + GA_3	*Ethrel* (Overnight soaking)	GA_3 application @ 5 ml/plant

water use efficiency (WUE), nitrogen use efficiency (NUE), biomass accumulation and dry matter contents were measured. The impacts of *Ethrel* led to fourfold increase in germination % at 20 DAP, which was commensurate with high acid invertase (AI) activity that led to increase in reducing sugar content and decrease in sucrose levels (Rai *et al.,* 2017). In addition, a fourfold increment in reducing sugars and twofold decline in sucrose contents was recorded with *Ethrel* at 20 and 45 days after planting respectively, against untreated setts respectively (Fig 1). Increased reducing sugar and decreased sucrose caused by higher AI activities led to enhanced growth of buds and emergence of settlings at 20 DAP. NR activity *in vivo*, SOD and IAAO activities in *Ethrel* setts were elevated at 20 and 45 DAP respectively. Increased NR activity *in vivo*, IAAO and SOD activities supported the faster sink to source transition and growth of bud. There was a seven-fold increase in NR activity *in vivo* and fivefold increase in IAAO activity at 20 and 45 DAP respectively, in *Ethrel* treated setts. Also, a sevenfold increase in SOD activity was recorded in *Ethrel* treated setts at 20 and 45 DAP. Increase in IAAO activity resulted in decrease in IAA contents at 20 and 45 DAP in *Ethrel* treated setts. The fourfold and twofold decrease in IAA contents and total phenolic contents was obtained in *Ethrel* setts respectively at 20 and 45 DAP against untreated setts. *Ethrel* induced enzymatic and metabolite changes led to establishment of initial population of 55,000 settlings ha^{-1} at 20 DAP. Thus, it led to gain of 20 days in initial crop growth period due to early sprouting and rapid flush of shoots and leaves on young settlings (Rai *et al.* 2017).

Effect of *Ethrel* and GA_3 Application on Biometric Traits

Exogenous application of GA induces transverse reorientation of microtubules in cell wall of dwarf pea plants that results in longitudinal expansion changing dwarf mutants to tall ones (Yang *et al.* 1996). The basic mechanism of GA mediated elongations through exogenous GA_3 application leads to active transport of solutes into the vacuoles present in plant cells and causes passive influx of water and generates turgor pressure. This turgor pressure creates osmotic imbalance between the intracellular and extra cellular fluids and provides the driving force for cell expansion. The cellulose-hemicellulose network and matrix polysaccharides defines the shape of differentiated cells and determines the direction of cell elongation.

Effect of *Ethrel* and GA_3 application on leaf characteristics

After germination with exogenous application of *Ethrel*, GA_3 was applied through foliar spray at all the critical growth stages (90,120,150 DAP). Foliar applications of GA_3 at 90,

Fig 1: Establishment of uniform and robust settlings through exogenous *Ethrel* application.

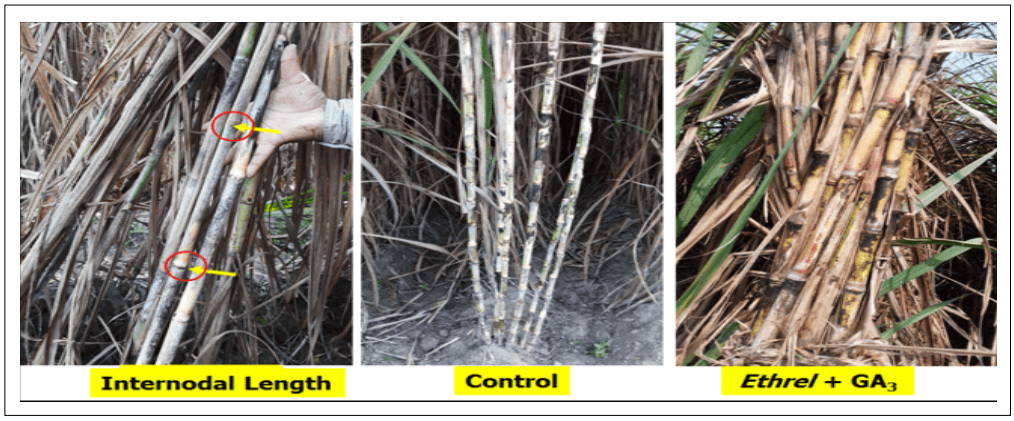

Fig 2: Exogenous combined application of *Ethrel* and GA_3 led increase in stalk lengths in plant and ratoon crops.

120 and 150 days after planting significantly enhanced various growth metrics, including the number of leaves, total leaf area, leaf area index, leaf area duration, biomass duration, leaf area ratio and net assimilation rates at 180 and 270 days after planting, especially in the *Ethrel*-treated setts. The applications led to highest foliage numbers at 180 DAP in *Ethrel* treated setts. There was sevenfold increase in leaf area index in *Ethrel* treated setts with GA_3 at 180 DAP. It led fivefold and twofold increase in leaf area and leaf area index in *Ethrel* treated setts with GA_3 application at 180 DAP. Duration of leaf area (LAD), ratio of leaf area (LAR) and period of biomass accumulation were elevated by four, five and seven-fold respectively at 180 DAP. At 270 DAP, despite a twofold decrease of leaf area, leaf area index, duration of leaf area and ratio of leaf area were maximum. Duration of Biomass accumulation ($gd*10^3$) increased by fivefold and six-fold at 180 and 270 DAP. The net assimilation rate, which measures the daily increase in biomass per unit of leaf area, was at its highest in *Ethrel* treated setts with GA_3 application at 180 and 270 DAP. Architectural modifications led to quicker transitions from heterotrophic to autotrophic growth at the planting stage (February). This resulted in a high initial plant population at 45 days after planting, followed by the development of a more efficient canopy with increased source activity and enhanced sink development both above and below ground by 60 days after planting. Adjustments in leaf angle also improved CO_2 utilization and radiation use efficiency (RUE). The application of GA_3 resulted in a more optimized canopy structure and better distribution of dry matter. The increased angle of leaf orientation reduced shading between leaves on the stalk, allowing the lower leaves to capture more light. Additionally, GA_3 stimulated the growth of roots with a steep angle (30°), significantly increasing root weight and enhancing root hair development, which supported the nutrient needs of the larger shoot population. As a result, improvements were observed in net assimilation rates (0.65 cm^2 per day), ratio of leaf area (16 cm^2 per gram) and duration of leaf area (55×10^4 cm^2 days), leading to greater internodal counts, lengths and weights. Exogenous application of GA_3 led to remarkable increase in internodal length in sugarcane (Moore 1980; Pribil *et al.* 2007; Chauhan *et al.* 2023). Exogenous foliar application of GA_3 has been found to be much effective in improving height, thickness and number of cane stalks ha^{-1}. Because sugarcane responds in this manner and because sugar yields depend largely upon the volume of internodes produced by the crop, the possible

Fig 3: Exogenous combined application of *Ethrel* and GA_3 led increase in shoot numbers/number of millable cane in plant and ratoon crops.

Table 2: Exogenous application of *Ethrel* + GA_3 - Impact on growth attributes and juice quality in sugarcane plant and ratoon crops.

PGR Treatment	Variety	Corrected °Brix	Sucrose %	Purity coefficient	Wt (g) per cane	Wt of juice (Kg/5 canes)
Sugarcane plant crop (360 days crop)						
Ethrel + GA_3	CoLk 94184	21.53	18.31	87.35	1038	2.390
Control	CoLk 94184	18.65	15.53	83.27	488	0.997
LSD ($p \leq 0.05$)	-	S	S	NS	S	S
I Ratoon crop (Initiated after harvest of plant crop)						
Ethrel + GA_3	CoLk 94184	22.54	19.60	87.03	877	2.120
Control	CoLk 94184	19.72	16.61	84.24	722	1.688
LSD ($p \leq 0.05$)			S	S	NS	S S

Values are mean of three replicates; * F interaction analysis for corrected °Brix, sucrose %, purity coefficient, Wt (g) per cane and Wt of juice (Kg/5 canes), LSD ($p \leq 0.05$); Least significant difference, S; Significant difference, NS; Non significant difference.

Table 3: Exogenous application of *Ethrel* + GA_3- Impact on initial plant population, Tmax cane and sugar yield.

Planting type	Initial plant Population (,000ha^{-1})		Tmax (,000ha^{-1})		NMC (,000ha^{-1})		Cane yield (tha^{-1})		Sugar yield (CCS tha^{-1})	
	C	*E*+GA_3	C	*E*+GA_3	C	*E*+GA_3	C	*E*+GA_3	C	*E*+GA_3
Sugarcane plant crop										
Spring planted crop	72.7	156.5	483.7	1160.4	152	443.3	129	333	13.2	34.2
	60.3	129	213	537	132	301	84.7	255	8.7	26.2
LSD (p≤0.05)	S	S	S	S	S	S	S	S	S	S
Ratoon crop initiated from respective crop										
Ratoon from spring	90	220	459	673	150	306	99.8	183.2	10.2	18.8
planted crop	30.5	67.4	377.5	546	134	156	78.69	96.63	8.1	9.96
LSD (p≤0.05)	S	NS	S	S	NS	S	NS	S	NS	S

Values are mean of three replicates; * F Interaction analysis for initial plant population, Tmax, NMC, cane yield, sugar yield, LSD (p≤0.05); Least significant difference, S; Significant difference, NS; Non significant difference C- Control; E + GA_3; *Ethrel* + GA_3.

use of gibberellins to increase sugarcane yields has been conducted since several decades (Alexander *et al.,* 1970; Mongelard and Mimura, 1972; Buren *et al.,* 1979, Thomas and Beena, 2024).

Effect on shoot, root architecture and cane juice characteristics

During the grand growth and harvest stages, the combination of *Ethrel* and GA_3 treatment in plants resulted in a maximum of 6.73 lakh shoots per hectare and a number of mature culms (NMC) of 3.01 lakh per hectare. In comparison, the untreated plants achieved a maximum of 4.59 lakh shoots per hectare and an NMC of 1.32 lakh per hectare. Increase in number of millable cane /clumps was recorded in plant as well as ratoon cane (Fig 3). The *Ethrel*-soaked setts treated with GA_3 showed a notable increase in the average number of internodes, their length and weight compared to the untreated setts. A fivefold increase was recorded in mean internodal number per stalk at 270 days after planting. At 180 and 270 days after planting, internodal lengths increased six fold respectively. Mean internodal weight was maximum at 180 DAP and increased by twelvefold at 270 days after planting. The applications led to increase in shoot population, stalk length, stalk and root dry weights. At 180 and 270 days after planting, the shoot numbers increased twofold and sixfold, respectively. Additionally, there was a fivefold and sevenfold rise in shoot numbers at these time points. Minimum shoot numbers were recorded in untreated setts without GA_3 application. Maximum increase in stalk length and stalk dry weight at 180 and 270 days after planting were recorded with combined application of *Ethrel* and GA_3. Stalk lengths increased by fourfold in *Ethrel* treated setts against untreated setts with GA_3 applications at 180 and 270 days after planting, respectively (Fig 2). A significant sevenfold and eightfold increase were recorded in stalk dry weight in *Ethrel* soaked setts with GA_3 application at 180 and 270 days after planting. Maximum dry matter content and root weights were recorded in *Ethrel* soaked setts against untreated setts with GA_3 application at 180 and 270 days after planting. At both the stages, a threefold increase was recorded in root weights. The dry matter content, Brix% and purity of cane juice was higher in *Ethrel* treated setts against untreated setts with GA_3 applications at 270 days after planting (Table 2). At the grand growth and harvest stages, the combination of *Ethrel* and GA_3 achieved a maximum of 5.37 lakh shoots per hectare In contrast, the control group had 2.13 lakh shoots per hectare. Additionally, there was a recorded increase in the number of millable canes and clumps in both plant and ratoon crops.

Effect of *Ethrel* and GA_3 application on ratoon crops

Foliar applications of *Ethrel* @ 100 ppm at 60 days after planting and GA_3 at 90, 120 and 150 days after planting in the first ratoon crop led to a higher sprouting percentage, a decrease in tiller cessation and a denser tiller population with enhanced stalk elongation rates. This resulted in a 66.5 tons per hectare increase in cane yield compared to untreated plants. Additionally, the *in situ* decomposition of sugarcane trash, applied at 12 tons per hectare after the harvest and treated with PUSA compost inoculant at 300 grams per ton of trash, led to a maximum of 5.37 lakh shoots per hectare, reduced tiller mortality to 54.5% and maintained the number of mature culms and cane yield at 3.06 lakh per hectare and 183.2 tons per hectare (with an average cane weight of 598 grams), respectively. In comparison, the untreated plants had a maximum of 2.13 lakh shoots per hectare, 66.7% tiller mortality, 1.53 lakh mature culms per hectare and a yield of 99.8 tons per hectare (with an average cane weight of 501 grams) (Table 3). The *in situ* decomposition of trash combined with foliar application of GA_3 results in an increase in ratoon cane yield by approximately 16.9 tons per hectare.

CONCLUSION

Germination, development and accumulation of dry matter in both sugarcane plants and ratoon crops were enhanced by combined exogenous application of *Ethrel* @100 ppm and GA_3 @35 ppm at critical growth phases under field conditions. *Ethrel* led to faster rate and enhanced germination in both plant and ratoon crops. This was due

to improved ethylene pool in buds that led to activation of membranes for improved rate of imbibition, reserve mobilisation and faster radical protrusion of the buds on the vegetative setts. *Ethrel* application saves about 20-25 days from 45 days of the germination phase. As most of the buds germinated, a gain was obtained in the number of shoots at an early stage (at about 90 DAP). *Ethrel* led to improvement in leaf characteristics, increase in number of shoots, improved photosynthetic process, canopy coverage and robust root system with better nitrogen and water use efficiency. GA_3 application influenced the cell membrane permeability that facilitated mineral nutrition, uptake and transport of photosynthates which led to improved biomass accumulation. GA_3 led to marked changes in morphological traits and promoted the biomass accumulation towards leaves, initially and later towards the stalks or cane stems. Inducing cell division and elongation, *Ethrel* along with GA_3 increased the height of the plant and enhanced the source and sink organs. The high density of stalks in a restricted ground area with *Ethrel* and GA_3 was attributed to the formation of upright canopies and strong root systems. This led to architectural alterations causing threefold increase in cane yield of plant and two fold increase in ratoon crop. *Ethrel* + GA_3 application across the crop cycle merely involved an additional input cost of Rs. 8,500 ha^{-1}. However qualitative and quantitative estimate of benefits to beneficiaries/ stakeholders indicated that combined exogenous application offered an additional return of about Rs 60,000 -65,000 ha^{-1}. Exogenous application of *Ethrel* and GA_3 at key stages of the crop growth cycle can thus be recommended to farmers and commercial growers as a technique for promoting sugarcane growth and yield.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

No animal were harmed during the study.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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