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Modulation of Low-Temperature-Induced Biochemical Changes in Bud and Root Band Zones of Sugar Cane Sets by Potassium, Zinc, and Ethrel for Improving Sprouting

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Low-temperature-induced biochemical changes in bud and root band zone of the sugar cane set suppress sprouting, which is responsible for drastic yield decline in ratoon crops. This study was undertaken to modulate these low-temperature-induced biochemical changes using potassium, zinc, and Ethrel to enhance the sprouting of buds at 5 and 10 °C. Potassium, zinc, and Ethrel led to 80, 50, and 40% improvement in bud sprouting at 5 °C, respectively. An increase in reducing sugars and a decrease in sucrose contents were recorded with treatment of potassium, zinc, and Ethrel. Acid invertase, adenosine triphosphatase, indoleacetic acid oxidase, and nitrate reductase in vivo activities were also enhanced. However, treatments led to a significant decline in indoleacetic acid, total phenols, and superoxide dismutase activity, which rendered the in situ toxicity buildup in sets at low temperatures.

KEYWORDS: Low temperature; biochemical changes; sugar cane; bud sprouting; potassium; Ethrel; zinc

INTRODUCTION

Ratooning in sugar cane farming is a practice of cultivating underground stubble buds left in the field after harvest of the plant crop. In tropical regions, about six to eight ratoons are cultivated, whereas in subtropical parts of India, only one or two ratoons are harvested due to drastic reduction in productivity of successive ratoons. Reduction in yields is due to harvesting of plant crop during the month of December and January when stubble buds are exposed to temperatures as low as 1−2 °C and fail to sprout (*1*). Suppression in sprouting has been explained due to low-temperature stress, which induces several biochemical changes in the buds and root band zone of stubbles. Low temperatures are known to reduce the availability of reducing sugars concomitant with a reduced activity of acid invertase and increased levels of indoleacetic acid (IAA) together with phenols. The in situ toxicity build up due to these compounds in stubble buds led to dormancy of stubble buds at low temperatures (*2*). Several attempts have been made to improve stubble bud sprouting by using plant growth promoters. These applications were made on either on the cut ends of millable canes or as foliar spray on standing cane. However, the problem with these compounds was their cost and difficult mode of application in fields by the cane growers. Besides these two factors, uptake of these compounds through cut ends of millable cane was not sufficient to bring about a perceptible change in stubble bud sprouting (*3*).

It is known that plants exposed to chilling temperature suffer from oxidative damage that impairs the cellular functions and growth. Evidence suggested that improvement in the potassium nutritional status of plants could greatly lower the oxidative damage. Potassium-sufficient plants are associated with enhanced activity of enzymes involved in detoxification of H_2O_2 (ascorbate peroxidase) and utilization of H_2O_2 in oxidative processes (*4*). Potassium also enhances membrane permeability and adenosine triphosphatase (ATPase) activity that is required for energy generation in cells under low temperature. These effects were modified with potassium in several crops. Potassium treatment also changes IAA contents inside cells and regulates nitrate reductase (NR) activity in vivo, which is essential for nitrogen requirement during the germination of seeds (*5*).

Preharvest foliar application of Ethrel has shown the desiccation effect on sugar cane stalks and enhances sucrose contents in internodes and sprouting of buds. The sets prepared from preharvest Ethrel treated cane showed improved bud sprouting owing to a change in the cell membrane permeability, increase of moisture percent, uptake of electrolytes in buds, and induction of NR, acid invertase (AI), and peroxidase activities in sugar cane cultivars (*4*).

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Zinc is involved in the synthesis of IAA through tryptophan and plays a significant role in regulating IAA content, affecting germination process (*6*), and it is involved in superoxide dismutase (SOD) synthesis; hence, it reduces the oxidative stress generated due to low temperature (*7*). It is also a prosthetic group of carbonic anhydrase enzyme, which regulates growth processes in various crops. The widespread zinc deficiency and low temperature (1−2 °C) in northern India in winter months resulted in poor ratoon yield, which was alleviated through zinc application for increased cane and sugar yields.

Our earlier studies have indicated that low temperatures suppressed sugar cane bud sprouting by inducing several biochemical changes involving reduced availability of reducing sugars, relatively higher levels of IAA, and decreased acid invertase, ATPase, and IAA oxidase activities (which otherwise are relatively higher under favorable temperatures, in this case, 25 °C) in the buds and the adjacent root band zone (*1*). With the role of potassium, Ethrel, and zinc in reducing oxidative stress and their involvement in modulating biochemical activities during low temperatures in mind, the present work was carried out to study whether low-temperature-induced biochemical changes might be modulated by potassium, zinc (postharvest application), and Ethrel (preharvest foliar application) in the bud and root band zone of sets so that sugar cane bud sprouting might be improved.

MATERIALS AND METHODS

Site. The experiment was conducted at the Indian Institute of Sugarcane Research, Lucknow, India, located at 26 ° 56′ N, 80 °52′ E, and 111 m above sea level, which falls in the Agro-Ecoregion 4 [Northern Plain and Central Highlands, Hot Semiarid Ecoregion with Alluvial-derived (N8D2) soils] of India (*8*).

Plant Material. Single bud sets (approximately 5 cm in length) were cut from the middle portion of stalks of the sugar cane variety CoSe 92423 (*Saccharum* spp. hybrids) (after 10 months of growth), cultivated at the farm of the Institute. Care was taken while cutting to obtain sets of the same girth (approximately 2.3 cm). The cut sets were washed initially in running water to remove extraneous particles and then with 5% Clorox solution for 10 min.

Planting of Single Bud Sets. Washed sterile sets were layered in acid-washed sand (particle size = 0.25−0.84 mm) in plastic trays (45 cm × 30 cm) with equidistant holes plugged with glass wool. Thirty sets were placed in each tray and covered with a thick layer (1.5 cm) of sand. For Ethrel treatment, sets were prepared from cane stalks, which were treated with Ethrel (1000 ppm) 15 days earlier on their foliage by spray. The rest of the sets obtained after cutting untreated canes and application of potassium (at 80 kg/ha as Muriate of potash) and zinc (at 25 kg/ha as zinc sulfate) were made in triplicates along with control, to which only distilled water was added. Trays were laid out in a completely randomized design (CRD) with three replications maintained at two temperature regimens, 5 and 10 °C, separately in different test chambers for each replication (Complab, India), with 14 h light period, light intensity of 5292 μW cm^{-2}, and 73% relative humidity in three replications for 12 days. Sterile water was applied to sand culture to maintain 15−22% moisture in the planted sets. Chloramphenicol and amphotericin B (30 and 100 μg mL^{-1}, respectively) were added in sand to avoid microbial contamination. Hoagland's nutrient solution (hafl-strength, 100 mL) was added in the sand on alternate days until the 12th day of analysis.

Sprouting of Buds and Biochemical Analysis. After removal of the sets from the trays, sprouting of the buds was recorded. The buds, which showed excessive bulging (swollen), had broken scales, and the initial shoot protrusion at least 2 mm in length (*9*) was considered to have sprouted. To determine percent sprouting, the numbers of sprouted and unsprouted buds were recorded. Freshly sampled buds and root band zone tissues were chopped and homogenized to prepare 10% homogenate in a chilled pestle and mortar using chilled distilled water. This was filtered through four layers of cheesecloth and then centrifuged at 8000*g* for 20 min at 4 °C. The supernatant obtained after centrifugation was used for estimation of reducing sugars, sucrose, and total phenolic contents.

Reducing Sugars, Sucrose, and AI Activity. Estimation of reducing sugars was made according to the method adopted from the procedure described by Nelson (*10*) and Somogyi (*11*). An aliquot of 0.5 mL of 10% homogenate was taken in sugar tubes. Distilled water (1.5 mL) was added to make the final volume of 2 mL, to which 2 mL of copper reagent was mixed and kept in a boiling water bath for 20 min. After cooling, 2 mL of arsenomolybdate reagent was added and final volume was made up to 25 mL with distilled water; the absorbance was recorded at 540 nm. The results are expressed as milligrams per gram of fresh weight (mg/g of fwt). The optical density of the reduced blue color solution was taken at 540 nm against a reagent blank.

Sucrose contents were estimated according to the resorcinol−thiourea method as described by Roe and Papadopoulos (*12*). An aliquot of 0.01 mL of 10% homogenate was taken in a test tube. Distilled water was added to make the final volume of 2 mL, to which 2.0 mL of resorcinol thiourea was added. After proper mixing, 6 mL of concentrated HCl was added, and the tubes were shaken carefully. The tubes were then transferred to a water bath maintained at 80 °C for 20 min and then cooled under running water. The absorbance was recorded within 30 min at 490 nm against a reagent blank. The results are expressed as mg/g of fwt.

Estimation of Soluble Protein and AI Activity. Protein was precipitated from the 10% homogenate with 10% (w/v) chilled trichloroacetic acid (TCA), and the clear residue was dissolved in 1.0 mL of 0.1 N NaOH at 80 °C for 10 min. Protein was estimated in a 0.5 mL of aliquot, according to the method of Lowry et al. (*14*). The intensity of the blue color was measured at 660 nm using bovine serum as the calibration standard. The activity of the acid invertase was determined through extraction with 1.0 mL of citrate buffer (pH 5.4) according to the method of Hatch and Glasziou (*13*). The reaction was initiated by adding 1 mL of 0.2 M sucrose to a reaction mixture containing 1.5 mL of 0.1 M citrate buffer (pH 5.4) and 1 mL of 10% homogenate. The reaction was set in a water bath at 37 °C for 1 h. The reaction was stopped by boiling the tubes for 5 min. Tubes were centrifuged at 8000*g* for 15 min. The amount of invert sugars was estimated in a 0.5 mL aliquot of the supernatant according to the method of Nelson (*10*). The acid invertase activity was expressed as micromoles per milligram of proteins per minute (μmol/mg of protein/min).

Total Phenolic Content (TPC), IAA, and IAAO Activity. The TPC was estimated according to the method described by Swains and Hillis (*15*) and determined by Folin−Ciocalteu reagent. Half a milliliter of 10% homogenate was mixed with 0.5 mL of Folin−Ciocalteu reagent (CDH, New Delhi) and 1.0 mL of supersaturated carbonate solution, and the volume of the reaction mixture was made up to 10 mL with distilled water. After 1 h, the color intensity was measured at 725 nm in a spectrophotometer (Systronics-2202, Ahmedabad, India), and the results are expressed as mg/g of fwt.

IAA was determined according to the method of Nagar (*16*) using the freshly sampled buds and RBZ. A sample 5 g of fwt was separately homogenized in chilled 80% methanol three times. The homogenates were centrifuged at 10000*g* at 5 °C for 30 min, and the pH of the extract was maintained at 6.5 by checking it on a pH-meter. The supernatant were concentrated in vacuo at 30 °C and then applied to a polyvinypyrrolidine (PVP) column. The column (20 cm × 1.5 cm internal diameter) was eluted with phosphate buffer (pH 8.0), and the resulting eluates were again adjusted to pH 8.0 with 1 N HCl and partitioned against peroxide free diethyl ether (three times). The ether phases were discharged. The remaining aqueous fractions were adjusted to pH 3.0 and partitioned against diethyl ether (three times). The ether phases were evacuated in vacuo and taken up in methanol (HPLC grade) for estimation of IAA.

The partially purified methanolic extracts were filtered through 0.54 μm Millipore filters and injected into a 20 μL injector loop fitted over the Lichrosorb RP 18 (10 um) (250 × 4.6 mm i.d.) protected by a guard column. Elution was carried out by a gradient of 30−70% methanol (5 min) followed by 70−100% methanol (5 min) and finally with pure methanol for 15 min at the flow rate of 1 mL/min. The column eluates were passed through an ultraviolet (UV) detector set at 254

Table 1. Effects of Treatments on Bud Sprouting and Growth Parameters at 12 Days after Planting[a]

	5 °C				10 °C			
treatment	sprouting (%)	moisture (%)	bud dry wt (g)	RGR (mg/g/day)	sprouting (%)	moisture (%)	bud dry wt (g)	RGR (mg/g/day)
control	0	51.6 ± 3.0	3.1 ± 0.7		10 ± 3	42.3 ± 3.3	3.7 ± 0.3	
Ethrel	40 ± 5	72.6 ± 3.0	4.5 ± 0.5	4.3 ± 0.3	60 ± 9	67.5 ± 2.0	4.9 ± 0.4	4.61 ± 0.01
K	80 ± 6	89.3 ± 4.0	6.3 ± 0.3	6.0 ± 0.2	90 ± 10	81.5 ± 3.1	6.9 ± 0.2	6.62 ± 0.03
Zn	50 ± 3	79.2 ± 4.0	4.8 ± 0.3	4.5 ± 0.2	75 ± 7	75.1 ± 4.3	5.3 ± 0.1	5.00 ± 0.12
LSD ($p = 0.05$)	4.5	4.2	0.08	0.11	10.6	8.5	0.27	0.12

[a] *F*-interaction analysis for bud sprouting: treatment (Tr), S (significant); temperature (T), NS (nonsignificant); Tr × T, S. Moisture: (Tr), S; (T), NS; Tr × T, S. Bud dry weight: (Tr), S; (T), NS; Tr × T, S. RGR: (Tr), S; (T), NS; Tr × T, S ± SD (standard deviation of the mean of three replicates).

nm, and the IAA was estimated measuring the peak area and comparing it with standard curve of indole-3 -acetic acid (Sigma Chemical Co., St. Louis, MO). The IAA content was expressed as mg/g of fwt.

For IAAO activity assay, tissues from fresh buds and RBZ (500 mg) were homogenized with a mortar and pestle in 5 mL of 0.05 M sodium phosphate, pH 4.0, containing 0.01 M 2-mercaptoethanol. The homogenate was squeezed through four layers of cheesecloth, and the filtrate was centrifuged at 5000*g* for 5 min. Then the supernatant solution was centrifuged at 20000*g* for 20 min. Protein was fractionated by ammonium sulfate precipitation. A 100% saturated ammonium sulfate solution (pH 4.0) was added dropwise to the supernatant solution until the desired concentration was reached. The salt solution was allowed to stand for 2 h before the precipitate was isolated. For the final fractionation step, solid ammonium sulfate was added over 4 h to give 100% saturation. Following each step the protein precipitate was collected by centrifugation at 10000*g* for 30 min. The billets were resuspended individually in extracting buffer and dialyzed for 18 h against 1 L of 0.005 M sodium phosphate, pH 4.0. The dialyzed protein was centrifuged at 10000*g* for 30 min, and the supernatant was used for assaying IAAO activity. The IAAO activity was assayed by measuring the degradation of IAA according to the method described by Gordon and Weber (*17*). The assay mixture (3 mL) consisted of 0.2 mM 2,4-dichlorophenol, 0.2 mM $MnCl_2$, 0.02 M phosphate buffer (pH 6.4), and 0.2 mM IAA. The reaction was started by adding 1 mL of enzyme extract (10% homogenate). After 30 min of incubation at 30 °C, a 2 mL aliquot was placed in test tubes with an equal volume of Salkowski reagent (40 mL of 35% perchloric acid, 1 mL of 0.5 M $FeCl_3$). A simultaneous blank was also run for each tube, where the aliquots were placed in tubes with Salkowski reagent at 0 time. The absorbance was taken at 530 nm after 0.5 h of color development. The activity was expressed as μg/mg of protein.

ATPase, NR in Vivo, and SOD Activity. The ATPase activity was assayed according to the method described by Fischer and Hodges (*17*). The enzyme homogenate was prepared by grinding 500 mg of fresh buds and root band zone tissues in 4 mL of extraction buffer containing 0.05 M Tris (pH 7.5), 0.03 M EDTA, and 0.25 M sucrose. The extract was centrifuged at 10000*g* for 20 min. The supernatant was collected and used for enzyme assay. The reaction mixture (2 mL) contained 0.1 M Tris (pH 7.5), 0.5 M KCl, and 0.05 M ATP. The reaction was initiated by adding 1 mL of suitably diluted enzyme extract. After 10 min of incubation at 38 °C, the reaction was stopped by adding 2 mL of 20% TCA. The tubes were immediately transferred to an ice bath. The phosphorus estimation was done according to the method described by Fiske and Subbarow (*20*). The enzyme activity was expressed as micrograms of P_i liberated per milligram of protein per 10 min.

NR activity in vivo was assayed according to the method described by Jaworski (*19*). The bud and the root band zone tissues were washed with distilled water, blotted, and chopped into fine pieces. Five hundred milligrams of chopped tissue was transferred immediately into a conical flask (beaked at neck), and then 5.0 mL of phosphate buffer (pH 7.5, 0.2M) and 0.1% isopropyl alcohol were added to the flask. The flasks were covered with black cloth. The reaction was initiated by adding 5.0 mL of 0.1 M potassium nitrate. The tissue in the flask was infiltrated with the help of a vacuum pump for 1 min, and then the flask was incubated at 30 °C for 1 h. Afterward, the assay solution was boiled for 10 min in a boiling water bath to stop the reaction. An aliquot was taken from the supernatant, and 1 mL of 1% sulfanilamide and 1 mL of 0.02% NDD [*N*-(1-naphthyl)ethylenediamine dihydrochloride] were added to it. The volume was made up to 4.6 mL, and absorbance was measured at 540 nm with a spectrophotometer (Genesis 6). The values were compared with the standard curve made by using sodium nitrite in a graded concentration. The enzyme activity was expressed as nanomoles of NO_2 per gram of fresh weight per hour at 30 ± 1 °C.

The SOD activity was assayed by measuring the photochemical reduction of nitro blue tetrazolium chloride (NBT) as described by Beauchamp and Fridovich (*21*). A sample of 0.2 g of fwt of the chopped root band zone and the bud tissue was mixed with a pinch of acid-washed sand and 2 mL of chilled phosphate buffer (pH 7, 0.05 M) in a clean dry pestle and mortar kept in an ice bath. After grinding, it was transferred to the centrifuge tube containing buffer (pH 7). Later it was centrifuged at 30000*g* for 30 min at 4 °C. The supernatant was used for assay of the enzyme. The 3.0 mL reaction mixture contained 50 mM potassium phosphate buffer (pH 7.8), 10 mM methionine, 1.17 mM riboflavin, 56 mM NBT, and suitably diluted enzyme extract. Riboflavin was added in the last, and switching on the light started the reaction. The reaction was allowed to take place for 20 min and was stopped by switching off the light. The absorbance of the solution was measured at 560 nm. The absorbance of un-irradiated reaction mixture (corresponding blank) was deducted from this. A_{560} was plotted as a function of fresh matter equivalent of the enzyme extract used in the reaction mixture. From the resultant graph, fresh matter equivalent of enzyme extract corresponding to 50% inhibition of the reaction was read and considered as 1 enzyme unit (EU). The SOD activity is expressed as EUs per milligram of protein.

Statistical Analysis. Data are the mean of three replicates, and factorial analysis of the data was tested by analysis of variance (ANOVA). Regression analysis and correlation coefficients were calculated using MS Excel statistical tools to assess the interrelationships between treatment means across the temperatures among different parameters. Treatments were considered to be significant when $P < 0.05$.

RESULTS AND DISCUSSION

Effect on Relative Growth Rate (RGR) and Bud Weight of Sugar Cane Buds. The aim of the study was to evaluate the impact of potassium, Ethrel, and zinc on the sprouting of buds and the related biochemical parameters influencing the process of germination at 5 and 10 °C. The results indicated that there was significant improvement in sprouting against the control with all three treatments irrespective of temperature (**Table 1**). Eighty percent improvement was recorded with potassium at both temperatures, whereas 50 and 65% improvements with zinc and 40 and 50% improvements with Ethrel were recorded at 5 and 10 °C, respectively. It was observed that the treatments led to significant enhancement in the moisture contents and dry weight of the buds irrespective of the temperature. Potassium led to an increase in moisture by >37%, whereas with zinc and Ethrel, the increases were greater than 27 and 21%, respectively, against control.

Similarly there was an increase in bud weight by 45 and 35% with Ethrel, whereas by 102 and 89% with potassium and by

Table 2. Treatment-Induced Changes in Sugars and AI Activity of Bud and Root Band Zone[a]

		5 °C			10 °C		
plant part	treatment	reducing sugars (mg/g of fwt)	sucrose (mg/g of fwt)	AI activity (μmol/mg of proteins/min)	reducing sugar (mg/g of fwt)	sucrose (mg/g of fwt)	AI activity (μmol/mg of proteins/min)
bud	control	1.71 ± 0.10	21.3 ± 0.2	0.013 ± 0.002	1.3 ± 0.2	20.6 ± 1.1	0.014 ± 0.002
	Ethrel	2.33 ± 0.03	18.5 ± 0.1	0.042 ± 0.003	2.3 ± 0.1	15.2 ± 1.3	0.051 ± 0.001
	K	2.61 ± 0.02	12.4 ± 0.1	0.071 ± 0.001	2.5 ± 0.1	11.3 ± 0.1	0.090 ± 0.003
	Zn	2.30 ± 0.01	14.6 ± 0.1	0.044 ± 0.002	2.2 ± 0.1	13.2 ± 1.0	0.063 ± 0.002
	LSD ($p = 0.05$)	0.04	1.2	NS	0.03	1.02	0.005
RBZ	control	0.12 ± 0.04	73.4 ± 1.0	0.001 ± 0.003	0.5 ± 0.2	69.8 ± 2.1	0.002 ± 0.001
	Ethrel	0.17 ± 0.01	47.0 ± 2.3	0.006 ± 0.001	0.8 ± 0.3	38.8 ± 3.0	0.008 ± 0.002
	K	0.19 ± 0.02	48.6 ± 3.1	0.006 ± 0.002	0.9 ± 0.5	40.7 ± 2.4	0.015 ± 0.004
	Zn	0.17 ± 0.03	51.0 ± 3.4	0.005 ± 0.015	0.7 ± 0.3	42.3 ± 1.1	0.012 ± 0.001
	LSD ($p = 0.05$)	NS	1.0	NS	0.1	1.12	0.004

[a] *F*-interaction analysis for reducing sugars: plant part (PP), S; treatment (Tr), S; temperature (T), NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S. Sucrose: plant part (PP), S; treatment (Tr), S; temperature (T), NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S. AI activity: plant part (PP), S; treatment (Tr), S; temperature (T), NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S ± SD (standard deviation of mean of three replicates).

Table 3. Treatment-Induced Enzymatic Changes in Bud and Root Band Zone[a]

		5 °C				10 °C			
plant part	treatment	ATPase activity	NR activity in vivo	IAAO activity	SOD activity	ATPase activity	NR activity in vivo	IAAO activity	SOD activity
bud	control	0.15 ± 0.02	45.3 ± 2.1	0.23 ± 0.001	0.42 ± 0.03	0.17 ± 0.02	51.5 ± 1.2	0.20 ± 0.02	0.43 ± 0.03
	Ethrel	0.45 ± 0.03	61.3 ± 3.4	0.31 ± 0.03	0.31 ± 0.02	0.53 ± 0.01	85.6 ± 3.4	0.31 ± 0.01	0.29 ± 0.02
	K	0.49 ± 0.04	69.1 ± 3.2	0.52 ± 0.02	0.23 ± 0.01	0.85 ± 0.03	145.6 ± 7.8	0.48 ± 0.03	0.23 ± 0.01
	Zn	0.51 ± 0.02	68.3 ± 2.8	0.47 ± 0.01	0.31 ± 0.02	0.61 ± 0.03	95.0 ± 1.2	0.43 ± 0.02	0.36 ± 0.03
	LSD ($p = 0.05$)	0.02	0.46	0.05	0.02	0.06	7.4	0.03	0.01
RBZ	control	0.13 ± 0.02	67.5 ± 2.7	0.21 ± 0.01	0.79 ± 0.04	0.23 ± 0.02	71.2 ± 2.3	0.20 ± 0.01	0.87 ± 0.04
	Ethrel	0.31 ± 0.03	110.0 ± 4.8	0.35 ± 0.03	0.61 ± 0.03	0.39 ± 0.03	117.6 ± 3.1	0.40 ± 0.01	0.70 ± 0.03
	K	0.34 ± 0.01	112.3 ± 2.8	0.43 ± 0.02	0.41 ± 0.02	0.48 ± 0.02	123.5 ± 4.8	0.50 ± 0.02	0.45 ± 0.02
	Zn	0.32 ± 0.02	105.3 ± 3.2	0.36 ± 0.01	0.59 ± 0.01	0.42 ± 0.01	119.6 ± 5.2	0.54 ± 0 0.02	0.61 ± 0.01
	LSD ($p = 0.05$)	0.005	1.7	0.006	0.03	0.04	1.3	0.006	0.04

[a] ATPase, (μg of P_i liberated/mg of protein/10 min); NR activity (in vivo), nmol of NO_2/g of fwt/h); IAAO activity, μg/mg of protein; SOD activity, EU/mg of protein. *F*-interaction analysis for ATPase: PP, S; Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S. NR: PP, S; Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S. IAAO: PP, S; Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × T, S. SOD: PP, S; Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S ± SD (standard deviation of the mean of three replicates).

54 and 45% with zinc at 5 and 10 °C, respectively, after 12 days. The relative growth of buds was maximum with potassium (8 mg g^{-1} day^{-1}), whereas with Ethrel and zinc, it ranged between 7.75 and 7.9 mg g^{-1} day^{-1}, indicating that accumulation of the dry matter was maximum with potassium, although with the other two treatments, too, it was more than that of the control.

Sugars and Acid Invertase (AI) Activity in Bud and Root Band Zone. Preharvest application of Ethrel led to increases in the reducing sugars by 32.9 and 73.8% at 5 and 10 °C as compared to the control, respectively (**Table 2**). However, with potassium, the reducing sugars increased by 54.1 and 94.6%, whereas with zinc the percent increases were found to be 36.5 and 72.3% at 5 and 10%, respectively. The sucrose in the bud was found to undergo reduction in all of the treatments as compared to the control at both 5 and 10 °C. It was found that with Ethrel, the reductions in sucrose contents were by 31.14 and 26.4% at 5 and 10 °C, whereas the decreases were by 41.64 and 45.13% with potassium. In the case of the zinc, the reductions were by 31.7 and 36.1% at 5 and 10 °C, respectively. AI activity in buds increased with all of the treatments and was found to be maximum with potassium. About a 4-fold increase was observed with Ethrel-treated as well as with zinc-treated buds, whereas a 7-fold increase was observed with potassium irrespective of temperature. The reducing sugars in RBZ were increased by 41.7 and 75% with Ethrel, by 58 and 93.8% with potassium, and by 41.7 and 52.1% with zinc at 5 and 10 °C, respectively. As observed in the buds, the sucrose content in RBZ was decreased by 35.9 and 44.3% with the Ethrel treatment at 5 and 10 °C, whereas it was decreased by 33.8 and 41.6% with potassium and by 30.5 and 39.4% with zinc. About 4−7.5-fold increases in the AI activity were observed in the RBZ with Ethrel, potassium, and zinc irrespective of the temperature.

ATPase and NR Activities. There were significant changes in the growth regulating enzymes, and it was observed that the ATPase activity increased by about 3−3.4-fold compared with the control at 5 °C with the treatment of Ethrel, potassium, and zinc, respectively, whereas about 3.2−5.6-fold increases were observed at 10 °C with similar treatments in the buds. Furthermore, ATPase activity in RBZ also increased by 2.3−2.6-fold at 5 °C, whereas at 10 °C, it increased by 1.7−2.1-fold with Ethrel, potassium, and zinc, respectively (**Table 3**). The treatments led to significant increase in the NR activity (in vivo), which increased in buds with the treatment of Ethrel, potassium, and zinc at 5 °C by 35.3, 52.5, and 50.8%, respectively, whereas at 10 °C the increases were 66.2, 182.7, and 84.5% at 10 °C, respectively, compared with control. This activity was also increased by 62.9, 66.4, and 56.0% at 5 °C with Ethrel, potassium, and zinc, whereas at 10 °C it increased by 65.2, 73.5, and 68.0% in RBZ (**Table 3**).

TPC, IAA, IAAO, and SOD Activities. IAA contents decreased by 35.0, 48.8, and 18.7% at 5 °C with the treatment of Ethrel, potassium, and zinc, whereas at 10 °C the decreases were 36.9, 51.1, and 23.4% with Ethrel, potassium, and zinc, respectively (**Table 4**). Furthermore, IAA was decreased in RBZ by 37.8, 40.8, and 31.6% with Ethrel, potassium, and zinc at 5

Table 4. Treatment-Induced Changes in Indoleacetic Acid (IAA) and Total Phenolic Contents (TPC) in Bud and Root Band Zone[a]

		5 °C		10 °C	
plant part	treatment	IAA (mg/g of fwt)	TPC (mg/g of fwt)	IAA (mg/g of fwt)	TPC (mg/g of fwt)
bud	control	8.91 ± 0.12	3.70 ± 0.03	8.01 ± 0.04	3.94 ± 0.02
	Ethrel	5.80 ± 0.04	2.43 ± 0.02	5.02 ± 0.02	2.41 ± 0.01
	K	4.63 ± 0.02	2.14 ± 0.01	3.90 ± 0.01	2.20 ± 0.01
	Zn	7.31 ± 0.03	2.51 ± 0.02	6.13 ± 0.02	2.33 ± 0.02
	LSD ($p = 0.05$)	1.12	0.12	1.02	0.14
RBZ	control	9.64 ± 0.04	4.73 ± 0.03	10.24 ± 0.04	6.34 ± 0.02
	Ethrel	5.91 ± 0.02	1.71 ± 0.01	6.01 ± 0.02	1.92 ± 0.01
	K	5.33 ± 0.03	1.50 ± 0.02	3.83 ± 0.01	1.33 ± 0.01
	Zn	6.61 ± 0.03	2.13 ± 0.01	6.94 ± 0.03	2.50 ± 0.02
	LSD ($p = 0.05$)	0.56	0.10	0.62	0.54

[a] *F*-interaction analysis for IAA: PP, S; (Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S. TPC: PP, S; Tr, S; T, NS; PP × Tr, S; PP × T, S; Tr × T, S; PP × Tr × T, S ± SD (standard deviation of the mean of three replicates).

°C, whereas these decreases were 40.8, 52.4, and 32.7% at 10 °C. The decrease in IAA indicated the release of inhibitory effect of IAA so that buds may sprout earlier. IAAO activity was increased by 38.3-, 129.1-, and 107-fold with the treatment of Ethrel, potassium, and zinc compared with control (**Table 3**), whereas the increases were by 53.5-, 137-, and 112-fold at 10 °C. The activity of IAAO in RBZ was increased by 66.7, 104.8, and 71.4% with Ethrel, potassium, and zinc at 5 °C, and at 10 °C, these increases were by 76, 120, and 125%. The increase of IAAO activity indicated the degradation of IAA (*25*), leading to enhancement in bud sprouting.

TPC increased by 35.3% with Ethrel at 5 °C, whereas it decreased by 43.6 and 30.4% with treatment of potassium and zinc, respectively (**Table 4**), whereas at 10 °C, the decreases in TPC were by 37.6, 44, and 39.9% with Ethrel, potassium, and zinc, respectively. The increases in TPC with Ethrel were 0.02 and 0.01 μg/g of fwt/day at 5 and 10 °C, whereas the rates of reduction per day were 0.10 and 0.04 μg/g of fwt/day with potassium treatment and 0.01 and 0.19 μg/g of fwt/day with zinc treatment at 5 and 10 °C, respectively. This showed that at 5 °C, the rate of decrease was maximum with potassium, whereas it was maximum at 10 °C with zinc. TPC in RBZ decreased by >63.8% at 5 °C with Ethrel, potassium, and zinc, whereas these decreases were 58.9, 55.2, 40.9 and 69.9, 66.3 and 59.8% at 10 °C with treatments of Ethrel, potassium, and zinc, respectively (**Table 4**). Total phenols both in buds and in RBZ are indicative of the inhibitory role of phenolics in the sprouting of buds. SOD activity decreased by 25.3, 45.2, and 26.2% with the treatment of Ethrel, potassium, and zinc at 5 °C compared with control, whereas these decreases were 32.6, 46.5, and 16.3% at 10 °C with the same treatments in the buds and RBZ, respectively (**Table 3**). Decreases by 22.8, 48.1, and 25.3% at 5 °C with Ethrel, potassium, and zinc treatments in bud and by 19.5, 48.3, and 29.9% in RBZ were observed at 10 °C.

Low temperatures are known to generate reactive oxygen species (ROS), primarily responsible for impairment of cellular function and growth depression in plants. Improvement of sprouting due to potassium might be explained as being due to the fact that it plays a significant role in lowering ROS productions by reducing the activity of NAD(P)H oxidase. The production of ROS due to chilling temperatures causes oxidative damage catalyzed by superoxide radical, hydrogen peroxide, and hydroxyl radicals impairing cellular functions. Potassium is known to render the maintenance of the suppressed photosynthetic electron transport and the cellular functions. Furthermore, it is also reported that potassium application improves the stomatal conductance, decreases the mesophyll resistance, and increases the ribulose biphosphate carboxylase activity. This in turn leads to the detoxification of the H_2O_2 (ascorbate peroxidase) and utilization of H_2O_2 in oxidative processes and thus helps the plant to survive the temperature stress (*22*). It is also documented that compared to plants supplied adequately with potassium, there was a several-fold increase in the activities of enzymes involved in the detoxification of H_2O_2 (ascorbate peroxidase) and the utilization of H_2O_2 in oxidative processes (*5, 23*). Potassium supply has been reported to have reduced seedling death rate and broken the dormancy in tomato, pepper, and eggplant, leading to improvement in their germination and growth (*23*). Zinc significantly improved the sprouting as compared to control plants as it causes significant changes in the contents of cytokinins, IAA, and gibberellic acid (GA_3). It significantly improved the carbohydrates content and reduced fibers required for the metabolic process during germination (*24*).

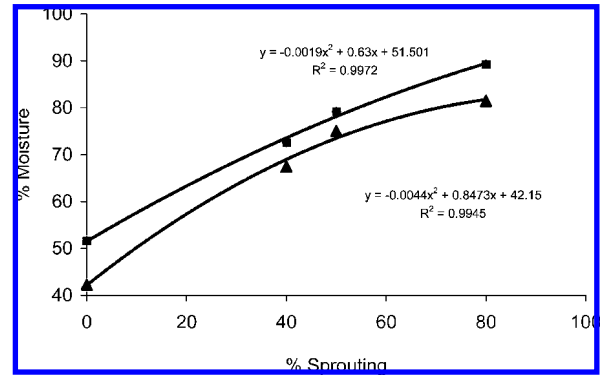

Figure 1. Correlation of moisture with sprouting in sugar cane bud at 5 °C (■) and 10 °C (▲) after 12 days of growth.

The increase in sprouting was associated with an increase in the moisture percent ($R^2 = 0.99$, **Figure 1**) in the buds with all three treatments, indicating that the membrane permeability increased and led to the influx of water and electrolytes inside the cells, leading to the increase of the bud weight at 5 and 10 °C. The maximum moisture percent was found with potassium followed by zinc and Ethrel. Furthermore, the relative increment of bud weight per day was again maximum in the case of potassium compared with zinc and Ethrel (**Table 1**). Potassium helps in improving the membrane permeability of the cell walls in plant tissues under abiotic stress (*25*). It is possible that potassium helped in breaking the barrier created by moisture deficit in the ratoon buds by improving the cell membrane permeability damaged by the low temperature. As a result of improved membrane permeability by potassium, the enhanced moisture content causes the activation of the enzymatic activities, which were suppressed under the low-temperature stress.

The growth of the buds, indicated by increased RGR, is a process that utilizes the reducing sugars for supplementing the sprouting phenomenon ($R^2 = 0.88$, **Table 5**). A reduced availability of reducing sugars concomitant with lower activity of acid invertase affects ratoon bud sprouting adversely in sugar cane. A positive correlation also existed between the percent sprouting and reducing sugar contents of bud and root band zone ($R^2 = 0.88$ and 0.55, respectively, **Table 5**). This was shown by the fact that there was significant ($p < 0.05$) enhancement in the reducing sugar contents of the buds and the RBZ with all three treatments. The maximum reducing sugars were formed by potassium followed by zinc and Ethrel. Opposite to the increase in the reducing sugar contents, it was

Table 5. Correlation of Sprouting and Relative Growth Rate (RGR) with Biochemical Parameters in Sugar Cane Bud and Root Band Zone

	sprouting (%)		RGR	
parameter	bud	root band zone	bud	root band zone
sucrose	−0.98	−0.89	−0.87	−0.20
reducing sugars	0.88	0.53	0.88	0.36
acid invertase activity	0.26	0.87	0.04	0.59
indoleacetic acid	−0.90	−0.90	−0.75	−0.80
total phenol content	−0.92	−0.83	−0.85	−0.58
indoleacetic acid oxidase activity	0.87	0.96	0.73	0.65
adenosine triphosphatase activity	0.93	0.94	0.70	0.65
nitrate reductase activity in vivo	0.80	0.93	0.69	0.57
superoxide dismutase activity	−0.45	−0.38	−0.19	−0.16

found that the sucrose contents declined significantly in the bud and root bad zone with all three treatments. A negative correlation existed between the percent sprouting and RGR with sucrose contents in bud and RBZ (R^2 = 0.98 and −0.89, respectively, **Table 5**). This indicated that the treatments led to the hydrolysis of sucrose, which had accumulated in the RBZ under low temperature and was unavailable for the utilization, causing the suppression of bud sprouting. The decrease in the sucrose contents with treatments indicated its mobilization into the buds through the root band zone. This was supported by Jain et al. (*3*), who reported the progressive decline in the sucrose contents during the sprouting of the cane buds in different sugar cane varieties grown in northern India. Furthermore, there was significant ($p < 0.05$) enhancement in the acid invertase activity of the RBZ as well as in the buds that was responsible for the hydrolysis of sucrose into reducing sugars for its utilization in the sprouting of buds. An enhanced AI activity associated with a decline in the sucrose contents and an increase in reducing sugars leading to enhancement in germination at low temperatures has been reported in potato and soybean (*26*). The maximum mobilization of sucrose occurred with the potassium treatment. Acid invertase hydrolyzes sucrose into hexoses (glucose and fructose) to provide the cells with carbon and energy for synthesis of different compounds essential for sprouting and subsequent growth. Besides this, hexoses may increase the osmotic equilibrium of the cells, suggesting a possible function of acid invertase in the plant growth and cell elongation.

Increase in the hexoses after hydrolysis of sucrose enhanced the metabolic process within the cells. This led to utilization of ATP through the electron transport chain in the mitochondria. A positive correlation between ATPase activity, percent sprouting, and RGR (R^2 = 0.93, 0.94; 0.77, 0.64) in bud and RBZ, respectively, indicated that potassium, zinc, and Ethrel supported the growth due to the availability of inorganic phosphorus and energy requirements of the cells. The low temperature suppressed the activity of nitrate reductase in vivo, affecting nitrogen assimilation and de novo protein synthesis in the cells. The reduced activity also contributed to the suppression of bud sprouting and RGR due to direct thermal effect on the enzyme or due to lack of substrate, as nitrate uptake is severely depressed (*27*). Potassium, zinc, and Ethrel, however, enhanced the NR activity as indicated by its positive correlation with percent sprouting and RGR in bud and RBZ (R^2 = 0.88, 0.93, 0.69, and 0.57, respectively, **Table 1**). The treatments thus leading to the increase in the reducing sugars, AI, ATPase, and NR activities in bud and RBZ might be responsible for improved sprouting and RGR of the sugar cane buds under low temperature.

In contrast, accumulation of phenols and IAA at low temperatures is known to be responsible for in situ buildup of toxicity, leading to maintenance of dormancy in buds at low temperatures. A higher accumulation of IAA and TPC at low temperature produces an inhibitory effect on growth of sugar cane buds (*2*), and an increased amount of IAA in shoots was observed in root cooling in wheat seedlings (*28*). Besides, IAA is known to induce the activity of cytokinin oxidase, thus adversely affecting cell division during sprouting. Our results, too, indicated a negative correlation between IAA and total phenols contents with percent sprouting and RGR in buds as well as the root band zone (R^2 = −0.90, −0.90, −0.75, and 0.80; and −0.92, −0.83, −0.85, and −0.58, respectively, **Table 5**). Accumulations of IAA and TPC were reduced with potassium, zinc, and Ethrel in both bud and RBZ, thus decreasing the in situ toxicity buildup and improving the bud sprouting and the RGR. The degradation of IAA can be explained on the basis that with treatments there was significant increase in IAAO activity in both bud and RBZ. A positive correlation between IAAO activity activity, percent sprouting, and RGR (**Table 5**) further supports that an increase in IAAO activity led to degradation of IAA and helped buds to sprout at these temperatures. The treatments led to a decline of accumulation of the endogenous IAA and phenol levels in buds and root band zones.

Low temperatures are known to enhance SOD activities for quenching ROS. Due to low temperature, the rate of enzymatic reactions falls (in control treatments), leading to a decrease in demand for ATP and accumulation of electrons in certain points of the respiratory chain. This situation promotes a sudden increase in the production of a number of ROS, which removes the burden of excess reducing potential. Hydrogen peroxide is one of the major metabolites produced in plants under cold stress. Increase in the hydrogen peroxide level of wheat leaves by cold treatment was evidenced earlier. To protect the cellular machinery from the deleterious effects of ROS, the antioxidant defense mechanism of cell is further activated (*7*). Cold stress is therefore associated with an increase in the intracellular oxidative stress, and an increase in the activity of antioxidants appears to be one of the features of cold adaptation. This activation was decreased with the treatment of potassium. SOD activity that plays a key role in the disposal of hydrogen peroxide was enhanced at both 5 and 10 °C. However, the treatments led to significant reduction, and a negative correlation was observed in sprouting percent and RGR (**Table 5**). The decrease in SOD helped in peroxidation of membrane lipids, which plays a decisive role in cold sensitivity, probably linked to a limitation in the functioning of the antioxidative system provoked by a decreased respiratory activity. The latter brings about a low availability of reducing power that in turn leads to failure of antioxidative enzymes to protect lipid membranes carrying higher root tissue damage and membrane rigidity (*28*). Maintenance of low activity of SOD especially during a cold exposure period is a mechanism to control oxidative stress (*29*). It is recognized that under cold conditions photochemical use of energy absorbed by chlorophyll is greatly diminished, leading to an accumulation of excited-state molecules in the pigment bed and to overproduction of ROS. Changes in redox potential are implicated in induction of gene expression of ROS scavenging enzymes, which agrees with the decisive role of these enzymes in cold conditions (*30*). The deactivation of SOD with treatments of potassium, zinc, and Ethrel provided a mechanism to protect buds from low temperature and rendered enhanced sprouting and RGR of sugar cane buds.

It is thus concluded that the application of potassium, Ethrel, and zinc modulated the oxidative stress and biochemical changes

caused due to low temperature and led to enhancement in bud sprouting. Their application caused an increase in reducing sugars, acid invertase, ATPase, IAAO, and NR activities in vivo and a decrease in sucrose contents in buds and root band zone. Besides, the treatments also led to a significant decline in IAA and TPC, which rendered the in situ toxicity buildup in sets at low temperatures. Furthermore, it also led to a decrease in SOD activity, which was enhanced in buds and root band zone at low temperatures. The inversion of sucrose mediated by an increased activity of acid invertase and a decrease of IAA content due to enhanced activity of IAAO were major causes for sprouting of buds due to these treatments. Further decrease in phenol content followed by reduced SOD activity provided a mechanism for removal of toxicity generated due to low temperatures. The enhanced NR activity in vivo due to the application of potassium, Ethrel, and zinc acted to stimulate the growth of buds by fulfilling the nitrogen requirement during bud sprouting.

ABBREVIATIONS USED

ATP, adenosine triphosphate; IAA, indoleacetic acid; IAAO, indoleacetic acid oxidase; NR, nitrate reductase; RGR, relative growth rate; ROS, reactive oxygen species; SOD, superoxide dismutase; TPC, total phenolic contents.

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