

## Interaction Effects of 6-Benzylaminopurine, Indole-3-Butyricacid and Urea Fertilizer to Enhance Tillering Potential of Sugarcane (*Sacharum officinarum* L) Plants

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### Abstract

Even though tillering is a key yield attribute in sugarcane and determined by the genetic base of the genotype, it can also be affected by applications of plant growth regulators, phenolic compounds, nitrogen fertilizers and other agronomic management practices. With this view, the effects of BAP, IBA and Urea fertilizer on two sugarcane genotypes with early maturing and high sugar content but shy in tillering followed by few millable stalk populations and hence low in cane and sugar yield was investigated. Accordingly, three levels of BAP (0, 0.03 & 0.06 mg/l), three levels of IBA (0, 0.02 and 0.03 mg/l) and four levels of Urea (0, 300, 450 and 700 kg/ha) with two sugarcane genotypes in a completely randomized block design with 3\*3\*4\*2 factorial treatment combination arrangements was tested. Data on the number of tillers per shoot, average shoot length (cm) and number of active leaves per shoot were collected after 60 days of planting on Luvisol of Metahara Sugar Estate. Analysis of variance proved that the interaction effects of BAP\* IBA\*Urea\*Sugarcane genotypes was highly significant ( $P < 0.001$ ) on the response variables tested. In sugarcane genotype C132-81, the optimum number of tillers per shoot (11.43) was obtained at 0.03 mg/l IBA, 0.06 mg/l BAP and 300 kg/l Urea fertilizer with 30.47 cm average shoot length and 8.27 active leaves per shoot while C86-56 produced 10.97 tillers per shoot with 33 cm average shoot length and 6.57 active leaves per shoot on the same treatment combination. From this result, it can be deduced that, it is possible to double the key yield attribute; number of tillers per shoot in shy tillering sugarcane genotypes that can in turn increase cane and sugar yield.

**Keywords:** BAP; IBA; Urea fertilizer; C132-81; C86-56; Sugarcane yield attributes

### Introduction

Sugarcane belongs to the genus *Saccharum* and is a grass that stores energy as sugar (sucrose) in stalks rather than as starch in seed heads as compared to grasses cultivated for grain production [1]. It is grown all over the world from cultivars of complex genetic constitution ultimately coming from hybridization made between *Sacharum officinarum* and *Sacharum spontaneum* [2]. *Sacharum officinarum* is a cultivated tropical plant species requiring careful nurture and is characterized by low in fiber, thick diameter, juicy, colorful canes with good sugar content, moderate tillering with broad and short living leaves while *Sacharum spontaneum* is a wild species, tolerant to biotic and abiotic stresses with profuse tillering, small in diameter, fibrous stalks with narrow and long living leaves [3-7]. Tillering and ratooning ability are two of them, among other sugar and cane yield attributing factors, much inherited from *Sacharum spontaneum* and of remarkable value in the profitability of the crop [3]. Tillering lays the foundation of the dominant yield determining attribute in sugarcane, i.e., stalk population [2]. Among the major attributes contributing for sugar and cane yield, the most significantly related factor is the number of millable canes (Stalks) per unit area of land at harvest [8]. Although, length and girth (thickness) of canes can also influence yield, but with lesser degree [9]. Among other yield attributes, the number of millable stalks and cane length could be altered by modifying the micro-environment and providing optimum conditions to plant for growth. The girth, being genetically controlled, cannot be changed substantially by environment [10] except it is due to the carryover effect of plant growth regulators in micropropagation followed by excessive tillering and hence reduced cane girth which can then be improved by fertilizer application. Although tillering ability is a genetically governed trait, a breeder tends to select the genotypes

with good tillering potential having better conversion efficiency of tillers in to optimal number of millable canes [2].

The typical segregating breeding populations show considerable variability for tillering ability, depending on the proportion of the chromosomes of the wild species, *S. spontaneum*. On account of the naturally stressful growing conditions, subtropical cane genotypes have more of the *spontaneum* complement compared with that in the tropical genotypes which are closer to *S. officinarum*. For this reason, it would appear that tropical genotypes would tiller less [2] and produce limited number of millable canes which will in turn limit yield in sugarcane production. Thus, in addition to selecting genotypes with good tillering potential, modification of the micro-environment, optimization of fertilizer rate and plant growth regulator types and concentration that can produce better tillering in tillering shy sugarcane genotypes is necessary to improve sugarcane and sugar yield. Many agronomical studies therefore aimed to improve the number of millable stalks at harvest than to alter girth and length of canes [9]. Although, many countries have successful cane breeding programs to produce their own cultivars having specific desirable characteristics suitable to

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their particular environmental conditions, Ethiopia has no facilities for sugarcane Breeding Research to develop its own genotypes that satisfy its specific requirements. Thus, since the first establishment of the Ethiopian Sugar Industry, the demand for improved sugarcane genotypes is entirely dependent on introduced genotypes having various limitations. In addition, obsolescence of productive commercial cane genotypes due to different biotic and abiotic stresses, most of the introduced genotypes have lesser performance than the standard checks used and, therefore; vast plantation area is covered with very few sugarcane genotypes over many years: among the major reasons for the current decline in cane and sugar yield and increased cost of production challenging the profitability and sustainability of the industry. Furthermore, early flowering (Tana-Beles and Wolkyte Sugar Development Projects), early lodging (Kuraz Sugar Development Project), lack of genotypes tolerant/resistant to drought and salt affected soils (Tendaho and Kessem Sugar Development Projects and Metahara Sugar Estate), lack of early maturing and high yielding genotypes are the other critical challenges beyond cultural practices for improvement calling for replacement or improvement of existing genotypes with suitable ones.

The only limiting barrier in sugarcane yield is the number of millable cane (stalks) per hectare and this could be increased by certain suitable agro-techniques which could easily help achieve the potential yield of sugarcane [9].

In the efforts made to evaluate the performance of sugarcane genotypes introduced from Cuba in 2003, some of them showed promising results to circumvent the current challenges. Even though, the selected genotypes have high sugar content and early maturing, they are constrained by low tillering potential and reduced stalk population which subsequently resulted in low cane and sugar yield. Thus, concerted research efforts to improve the tillering potential with increased millable stalk population are key to increase cane and sugar yield of these genotypes. Besides, breeding efforts to improve tillering potential of varieties with synchronised early tillering with rapid initiation of roots in tillers and proper display of leaves for better transmission of radiation through canopy, a search for compounds that would induce tillering was started several years ago and several compounds such as ethephone, phenolic compounds like chlorogenic, syringic and ferulic acid and plant growth regulators showed considerable improvement in tillering of sugarcane [3]. Furthermore, among all the 16 essential nutrient elements, nitrogen is the prime promoter of tillering in sugarcane [11] with added advantage in reducing tiller mortality and increasing millable stalk population. Therefore, the current study was carried out with the aim to evaluate the effects of different concentrations and combination of plant growth regulators and Urea fertilizer on field growth performance of micropropagated selected sugarcane genotypes to improve the tillering potential of the sugarcane genotypes and hence the seed cane yield.

## Materials and Methods

The study was carried out at Metahara sugar Estate, located at Eastern part of the country at about 200 kms away from Addis Ababa, Ethiopia. Metahara Sugar estate is situated at 8°53' N latitude and 39°52'E longitude at an altitude of 950 m.a.s. with semi arid climatic conditions. The experimental materials were micropropagated primary acclimatized sugarcane genotypes introduced from Cuba in 2003, tested for their adaptation under different sugar estates with different agro-ecologies. The experiment was laid out in Randomized Complete Block

Design (RCBD) consisting three levels of IBA (0, 0.02 and 0.03 mg/l), three levels of BAP (0, 0.04 and 0.06 mg/l), four levels of Urea fertilizer (0, 300, 450 and 750 kg/ha) and two sugarcane genotypes (C86-56 and C132-81) forming 3\*3\*4\*2=72 treatment combinations, each replicated three times. Treatments were applied after one month of planting on survived plants. Then, after one month of treatment application, data on the number of tillers per shoot, shoot length and number of active leaves per shoot were collected. The collected data were subjected to analysis of variance using SAS statistical software version 9.2 and Significant Treatments' means were separated using the procedure of REGWQ multiple range test.

## Results and Discussion

Analysis of variance proved that the interaction effects of 6-benzylaminopurine (BAP), Indole-3-butyric acid (IBA), Diammonium phosphate (Urea) fertilizer and sugarcane genotypes have a highly significant (BAP\*IBA\*Urea\*Genotype= $p < 0.001$ ) effect on the number of tillers per shoot, average shoot length and number of leaves per shoot (Table 1). The two sugarcane genotypes (C132-81 and C86-56) also showed marked variation in the number of tillers per shoot, average shoot length and number of leaves per shoot. Regardless of the other treatments, the pooled value of the genotypes showed that C132-81 gave better number of tillers per shoot and number of leaves per shoot than C86-56 while C86-56 was better in average shoot length than C132-81 (Table 2).

In sugarcane genotype C132-81, the lowest number of tillers per shoot (5.00), average shoot length (19.00 cm) and number of leaves

Source of Variations	DF	Mean squares		
		Number of Tillers/Shoot	Average shoot length	Number of leaves/shoot
IBA	1	25.56*	11.20 <sup>ns</sup>	1.53*
BAP	1	0.31 <sup>ns</sup>	16.63 <sup>ns</sup>	0.01 <sup>ns</sup>
IBA*BAP	1	10.50 <sup>ns</sup>	6.48 <sup>ns</sup>	0.10 <sup>ns</sup>
Urea	2	8.09 <sup>ns</sup>	1.32 <sup>ns</sup>	0.33 <sup>ns</sup>
IBA*Urea	2	0.72 <sup>ns</sup>	2.63 <sup>ns</sup>	0.32 <sup>ns</sup>
BAP*Urea	2	10.13 <sup>ns</sup>	0.78 <sup>ns</sup>	0.27 <sup>ns</sup>
IBA*BAP*Urea	2	14.03**	36.97 <sup>ns</sup>	3.08**
Genotype	1	19.70*	198.76**	27.9**
IBA*Genotype	1	6.42 <sup>ns</sup>	0.76 <sup>ns</sup>	0.52 <sup>ns</sup>
BAP*Genotype	1	0.95 <sup>ns</sup>	0.96 <sup>ns</sup>	0.78 <sup>ns</sup>
IBA*BAP*Genotype	1	11.25**	10.28 <sup>ns</sup>	0.01 <sup>ns</sup>
Urea*Genotype	2	3.72 <sup>ns</sup>	7.94 <sup>ns</sup>	0.34 <sup>ns</sup>
IBA*Urea*Genotype	2	11.39**	6.40 <sup>ns</sup>	1.45*
BAP*Urea*Genotype	2	9.95**	16.48*	2.14*
IBA*BAP*Urea*Genotype	2	21.37**	42.09**	2.46**

Remark: IBA: Indole-3-butyric acid; BAP: 6-Benzylaminopurine; Urea: Urea Fertilizer; DF: Degrees of Freedom; \*: Significant ( $p \leq 0.05$  but  $> 0.01$ ); \*\*: Very Significant ( $p \leq 0.01$  but  $> 0.001$ ); \*\*\*: Very Highly Significant ( $p \leq 0.001$ ); ns: Non-Significant; P: Alpha Value at 5% Probability Level

**Table 1:** ANOVA Summary for Response of Sugarcane Genotypes to BAP, IBA and Urea fertilizer.

Sugarcane Genotype	Number of tillers/shoot	Average shoot length (cm)	Number of leaves/shoot
C132-81	8.97a	27.03b	8.32a
C86-56	7.72b	31.94a	6.85b

Remark: Means with the same letter are not significantly different

**Table 2:** Comparison of sugarcane genotypes for the Pooled effects of BAP, IBA and Urea fertilizer.

per shoot (7.57) was obtained at 0 mg/l IBA, 0 mg/l BAP and 0 kg/ha Urea fertilizer (control treatment). At 0.02 mg/l IBA and 0.04 mg/l BAP, increasing the level of Urea fertilizer from 0 kg/ha to 300 kg/ha, increased the number of tillers per shoot from 5.00 to 8.83, average shoot length from 19.00 cm to 28.00 cm and number of leaves per shoot from 7.57 to 8.17 in genotype C132-81. Similarly, for the same genotype, further increase in the level of Urea fertilizer from 300 kg/ha to 450 kg/ha increased the number of tillers per shoot, average shoot length and number of leaves per shoot to 9.90, 29.33 cm and 8.70, respectively. However, further increase of Urea fertilizer to 700 kg/ha reduced the number of tillers per shoot from 9.90 to 7.40 and average shoot length from 29.33 cm to 26.67 cm while the number of leaves per shoot remains the same (Table 3).

The maximum number of tillers per shoot (11.43) was obtained at 0.03 mg/l IBA, 0.06 mg/l BAP and 300 kg/ha Urea fertilizer with 30.47 cm average shoot length and 8.27 leaves per shoot. Similarly, in sugarcane genotype C86-165, the lowest number of tillers per shoot (4.47) and average shoot length (19.40 cm) was obtained on control treatment while the lowest number of leaves per shoot was obtained at 0.03 mg/l IBA with 0.04 mg/l BAP and 450 kg/ha Urea fertilizer. In this genotype (C86-165), the increase in the number of tillers per shoot from 4.47 to 8.73 and average shoot length from 19.40 cm to 34.23 cm is due to 0.02 mg/l IBA, 0.04 mg/l BAP and 300 kg/ha Urea fertilizer (Table 3). The maximum number of tillers per shoot (10.97) was obtained at 0.03 mg/l IBA, 0.06 mg/l BAP and 300 kg/ha Urea fertilizer. However, the optimum average shoot length (34.97 cm) was obtained at 0.03 mg/l IBA, 0.04 mg/l BAP and 450 kg/ha Urea fertilizer. At 0.03 mg/l IBA and 0.06 mg/l BAP, in both sugarcane genotypes (C132-81 and C86-56), increase in the levels of Urea fertilizer beyond the optimum level showed a declining trend in the number of tillers per shoot: a key response variable. Generally, 0.03 mg/l IBA, 0.06 mg/l BAP and 300 kg/ha Urea fertilizer was found to be the optimum treatment combination for the number of leaves per shoot. Urea fertilizer being a source of Nitrogen is the most important plant nutrient for crop production. It is a constituent of the building blocks of almost all plant structures. It is an essential component of chlorophyll, enzymes and proteins. It stimulates root growth and crop development as well as uptake of the

other nutrients [12]. However, high doses of urea fertilizer are toxic and deleterious to plant cells. Dose-response curves for all of the known plant growth substances are bell shaped. At lower concentrations the effects are typically stimulatory reaching a maximum beyond which they become inhibitory [13]. Moderate concentrations of cytokinins increased the shoot proliferation rate, but very high concentrations decreased multiplication and especially depressed shoot elongation [14]. Although cytokinins are known to stimulate cell division, but does not induce DNA synthesis. Nevertheless, the presence of auxin promotes DNA synthesis. Hence, the presence of auxin together with Cytokinin stimulates cell division and control morphogenesis thereby influences shoot multiplication [15].

### Conclusion

The application of 0.03 mg/l IBA, 0.06 mg/l BAP and 300 kg/ha Urea fertilizer increased the key yield attributing character: number of tillers per shoot more than double as compared to the free check (untreated control) in both sugarcane genotypes tested. It is generally known that not all tillers grow in to the adult millable cane stalks due to physiological, pathological, entomological, agronomic (planting time, planting density, planting technique, planting geometry, availability of nutrition, soil moisture, endogenous and exogenous plant growth regulators and genotype. Evidences show that 30-50% of the total tillers that emerge survive till harvest. Based on this evidence, from the current result, it can be deduced that the optimum treatment combination obtained to double the number of tillering per shoot can substantially increase the number of harvestable stalks for seed cane, increase seed cane yield and propagation ratio; and hence can solve the limitation (shy tillering) of the selected genotypes. Similarly, use of the current result can increase tillering ability of the genotypes with subsequent increase in the number of millable canes for commercial purpose that can help improve the productive potential of the varieties. Finally, research efforts to improve the key sugarcane yield attributing factors (tillering and ratooning ability) using different compounds (plant growth regulators), fertilizers and other agronomic management practices for selected high sugar containing genotypes will be the future line of work.

PGRs (mg/l)			Response variables of Sugarcane Genotypes					
IBA	BAP	Urea (kg/ha)	C132-81			C86-56		
			Number of Tillers/ Shoot	Average Shoot Length (cm)	Number of Leaves/ Shoot	Number of Tillers/ Shoot	Average Shoot Length (cm)	Number of Leaves/ Shoot
0	0	0	5.00 <sup>a</sup>	19.00 <sup>k</sup>	7.57 <sup>h</sup>	4.47 <sup>h</sup>	19.40 <sup>k</sup>	6.77 <sup>i</sup>
0.02	0.04	300	8.83 <sup>c</sup>	28.00 <sup>h</sup>	8.17 <sup>e</sup>	8.73 <sup>b</sup>	34.23 <sup>b</sup>	6.63 <sup>m</sup>
0.02	0.04	450	9.90 <sup>b</sup>	29.33 <sup>g</sup>	8.70 <sup>c</sup>	8.40 <sup>c</sup>	32.37 <sup>e</sup>	7.33 <sup>j</sup>
0.02	0.04	750	7.40 <sup>e</sup>	26.67 <sup>i</sup>	8.60 <sup>c</sup>	6.93 <sup>f</sup>	32.37 <sup>e</sup>	6.97 <sup>k</sup>
0.02	0.06	300	8.67 <sup>c</sup>	26.33 <sup>j</sup>	8.40 <sup>d</sup>	7.57 <sup>d</sup>	31.33 <sup>f</sup>	6.83 <sup>k</sup>
0.02	0.06	450	8.20 <sup>d</sup>	28.67 <sup>h</sup>	8.73 <sup>b</sup>	7.10 <sup>e</sup>	31.23 <sup>f</sup>	6.73 <sup>j</sup>
0.02	0.06	750	7.47 <sup>f</sup>	30.33 <sup>g</sup>	9.10 <sup>a</sup>	7.40 <sup>e</sup>	32.90 <sup>d</sup>	7.03 <sup>j</sup>
0.03	0.04	300	7.73 <sup>e</sup>	27.63 <sup>j</sup>	7.87 <sup>f</sup>	6.83 <sup>f</sup>	33.50 <sup>c</sup>	7.23 <sup>j</sup>
0.03	0.04	450	8.40 <sup>c</sup>	29.70 <sup>g</sup>	8.60 <sup>c</sup>	8.17 <sup>c</sup>	34.97 <sup>a</sup>	6.43 <sup>o</sup>
0.03	0.04	750	9.87 <sup>b</sup>	31.97 <sup>e</sup>	7.77 <sup>g</sup>	7.77 <sup>d</sup>	33.53 <sup>c</sup>	7.20 <sup>j</sup>
0.03	0.06	300	11.43 <sup>a</sup>	30.47 <sup>g</sup>	8.27 <sup>e</sup>	10.97 <sup>ab</sup>	33.03 <sup>d</sup>	6.57 <sup>n</sup>
0.03	0.06	450	9.27 <sup>b</sup>	29.67 <sup>g</sup>	8.17 <sup>e</sup>	8.37 <sup>c</sup>	31.90 <sup>ef</sup>	6.50 <sup>n</sup>
0.03	0.06	750	8.50 <sup>c</sup>	22.37 <sup>l</sup>	8.27 <sup>e</sup>	7.60 <sup>d</sup>	34.50 <sup>ab</sup>	6.87 <sup>k</sup>
CV			14.20	17.51	7.16	14.20	17.51	7.16
SE			1.16	3.01	0.31	1.16	3.01	0.31

Remark: IBA: Indole-3-Butyric Acid; BAP: 6-Benzylaminopurine; UREA: Diammonium Phosphate; Kg/ha: Kilogram per Hectare; CV: Coefficient of Variation; SE: Standard Error

Table 3. Interaction effects of IBA, BAP and urea fertilizer on field growth responses of sugarcane.

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