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The Effect of Gibberellic Acid 3 and Benzylamino Purine Combination in Sugar Cane Development and Sugar Accumulation

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Abstract
Sugarcane is an important agricultural crop that belongs to the family Poaceae. An experiment was carried out to study the effect of different concentrations of Gibberellic Acid (GA3) and Benzyl-amino purine (BAP) on the development and sucrose accumulation of two sugarcane varieties; N-41 and R-579. Mother shoots were initiated from the bud chips that had been soaked for 24 hours in a solution made from a mixture of various concentrations of GA3 and BAP (0 mg/L GA3 + 0 mg/L BAP (T0,0), 1 mg/L GA3 + 0.5 mg/L BAP (T1,0.5), 2 mg/L GA3 + 1 mg/L BAP (T2,1), 3 mg/L GA3 + 1.5 mg/L BAP (T3,1.5), 4 mg/L GA3 + 2 mg/L BAP(T4,2)); per liter of water. These shoots were grown in nursery pots and, after 15 days, were transplanted into an experimental plot which was laid out in a randomized complete block design with four replications. The highest average number of cane stalks, internode length, and Pol values of 16.1, Brix values of 21.17 and 20.9 were observed in treatment T4,2 for sugarcane varieties N41 and R579, respectively. Purity was significantly higher, 86.32% for variety N-41 from seedlings regenerated in treatment T4,2 and 84.40 % obtained in variety R-579. The results of this study recommended the use of 4 mg GA3 combined with 2 mg BAP per liter for optimal development of the sugarcane plant and sucrose accumulation.

Key words: Saccharum officinarum, Sucrose accumulation, Sugarcane development.

1.0 Introduction

Sugarcane (Saccharum officinarum L.) is an economic crop of worldwide importance which provide over 80% of the sugar requirement. It is cultivated in tropical and subtropical climatic regions of the world, producing up to 1.5 billion metric tons of canes to be crushed [1]. Many years ago, extractable sugarcane juice have been used as a source of sugar and other by products like molasses, ethanol, and bagasse being the energy source to sugar industries. However, over 110 countries to date cultivating sugarcane [7]. In 2019, 1,683 million metric tons was harvested from estimated 26.2 million hectares worldwide which amounts to 22.4% of the total world agricultural production by weight [8]. The world

largest producer of sugarcane is Brazil and India about 50 %., Brazil being the leader followed by India, China, Thailand and Pakistan in hectares' coverage [6]. About 70 % of sugar produced originates from the sugarcane and 30 % of sugar, produced from sugar beet [7, 11, 20]. In sub Saharan Africa, the largest sugarcane producers are South Africa, Mozambique and Cameroon [1, 2]. Contribution of the continent to the world's sugarcane production is 5 %, majority coming from sub-Saharan Africa, with 1.5 % originates from East Africa and 1 % from Tanzania, whose annual sugar production is approximately 500 000 tons [18].

The sugar industry in Tanzania contributing significantly to the social economic development through foreign exchange earnings, providing source of income and employment [29]. The sugar industry generated annual revenue of about Tanzania Shillings 94.7 billion from exports to the rest of the world. It also generates estimated foreign exchange earnings of dollar 3.17 million in 2023 from exports of sugars and sugar confectionery [33]. Sugarcane production in Tanzania is done by commercial and smallholder farmers in Kilimanjaro, Morogoro, Manyara and Kagera whereby 90 % practiced rain fed and 10 % rely on irrigation [17]. In Morogoro sugarcane is one of the cash crop and majority of people living around sugar industries depend on its production for their survival. The region is having three sugar mill companies, Mtibwa Sugar Company at Mvomero District and Kilombero Sugar Company found at Kilombero District, being the largest followed by the newly government owned called Mkulazi Holding Company Limited located at Kilosa District, in Morogoro region.

However, apart from the importance of the sugarcane sector to the economy of Tanzania, smallholder farmers face production declining time after time [1, 29]. This decline not only affecting directly farmers but also sugar industries due to low supply of raw materials. The challenges such as accessibility of quality seed cane with technology to optimize its multiplication and drought due to weather changes, late or no gap filling, absence of cane spacing and loss of cane following use of unsustainable seed cane preparation techniques, attributing to the decrease in sugarcane yield. The changes in weather conditions have affected to high extent sugarcane farmers. Current fluctuations in rainfall and longer sun shine period within production season, place up to 90% of sugarcane farmers who depend on rain-fed for production in risk [17]. As a result, the prolonged drought is the major factor damaging sugarcane specifically due to the heightened water requirement during vegetative stage. In Tanzania, formal seed cane system contributes 20% while informal supply 80% of seed cane in sugar cane industry [30, 32]. This means most of the smallholder farmers who are the larger producer and supplier of sugarcane to sugar industries, accessing seed cane from large farmers or nearby sugarcane estates (informal sector). The quality of these seeds is almost upon the hands of farmers themselves. Lack of knowledge and down involvement of experts at rural areas, led to the establishment of seeds which infested by disease and pest, buds destroyed as a result of poor management during harvest and transportation, leading to poor sugarcane crop development. Continuing using conventional method where one bud produces 4-5 shoots leading to poor stand establishment and hence more gaps created [14]. These gaps if not timely repaired rendering to lower crushable cane and affect farmer's and national income. The huge amount of seed cane, 6 to 8 tons/hectare under traditional model is too much to a small scale farmer with more than 10-hectare farm and cannot afford due cost [26]. Under bud chip technique large amount of cane to be crushed is served, this is different from cane sett where the cane stalk into pieces of 3 to 4 buds planted, nothing is served while in noddle seedling is only nodes taken and internodes thrown away rather than been sent for crushing, therefore the two methods practiced has no sustainability [3]. Nursery production

of seedling will enable selection of good and healthier seedling to plant, while ensuring proper crop establishment and projected sugarcane yield.

To improve sugarcane production and accounting for conventional method limitations, suggestion on application of plant growth regulators have been proposed [20]. Plant growth regulators are organic compounds that influence physiological processes in plants in small concentrations. Normally, they can either be natural or synthetic compounds which applied directly into plant cell to change its life structure in some way beneficial to enhance yield, improve quality and facilitate harvesting [32]. The application of Plant growth regulators in facilitating sugarcane stalk development and sucrose accumulation have been described with many scientist such as, [12, 11, 20], reported that phyto-hormones like GA3 causes hyper elongation of stem by stimulating both cell division and cell elongation in sugarcane crop, which results to large sink size and strength hence influencing sucrose accumulation. The mature sink, serves as a significant reservoir of sucrose in the unique source-sink system of sugarcane, with the leaves serving as the source of photosynthetic sugar [37]. This provides more evidence in favor of optimizing the concentration of plant growth regulators in order to expand cell size, sink size, and sink capacity, which frees up more room for stalk development and sucrose accumulation. However, cytokines such as BAP in active tissue like bud chip induce cell division, enlargement and promote shoot and root initiation [6]. As the amount of BAP reaching the shoot will reflect the extent of the root system [12].

In the present study, mother shoot generated from plastic pot after soaking of sugarcane bud chip into GA3 and BAP solution in various concentrations transplanted to assess the effect of plant growth regulators on sugarcane development and sucrose accumulation.

2.0 Methodology

Study was conducted at Kilosa District, in Morogoro Region, Tanzania. The experiment was carried out in the rainy season from February, 2024. The study was assessed on impact of phyto-hormones in sugarcane development and sugar accumulation in two commercial varieties, R-579 and N-41. The bud chip from two sugarcane varieties were soaked for 24 hours into the solution made from combined treatment of GA3 + BAP at five concentrations; 0 mg/L GA3 + 0 mg/L BAP (T0,0), 1 mg/L GA3 + 0.5 mg/L BAP (T1,0.5), 2 mg/L GA3 + 1 mg/L BAP (T2,1), 3 mg/L GA3 + 1.5 mg/L BAP (T3,1.5), and 4 mg/L GA3 + 2 mg/L BAP (T4,2) and then planted into nursery pots to produce a single seedling termed as mother shoot. In 15 days' mother shoots were transplanted into prepared experimental plots at a spacing of 0.4 M. The experiment was laid out in Randomized complete block design with four replications, each consist of 10 experimental plots. The distance between margin of each block was 1.2 M. The plants in all the treatments were pest and disease free during experiment and no fertilizer was applied. The following data were collected and compared when sugarcane stalk reached 270 days; length of internodes (cm) determined by using meter rod on 3 selected sample of sugarcane stalk from each variety in experimental plot, measured from the bottom, middle and to the tip and average length of internode calculated. Number of sugarcane stalk counted from each experimental unit in replicate and their average number calculated. For sugarcane quality (Pol, Brix and Purity), all sugarcane sample, the base was cut off and the top part was removed along with the leaves before washing. Using a sugarcane crushing machine, sugarcane was crushed to extract juice, which was then filtered to obtain 200 ml. The juice was divided into two parts, each in 100 ml for measurement of Brix and Pol values. The brix value was determined by taking 100 ml of juice and then adding 2 grams of Cellite (Filter aid) to obtain a

clear filtrate, which was then subjected to a refractometer. Polarimeter was used to determine Pol level, a sample of 100 ml of juice mixed with 1.5 grams of lead II acetate (filter aid) to obtain a clear filtrate which was subjected to Polarimetry. Purity percentage were calculated by using the following formula; Purity= (Pol/Brix) x 100. Statistical analysis; the data were subjected to the analysis of variance (ANOVA) with the combining GA3 and BAP treatments forming the main fixed effects. Mean comparison between treatments was done by using Tukey's test (Honestly Significant Difference test) at 5% level of probability.

3.0 Results and Discussion

From the Analysis of Variance, the effects of varieties tested observed to be highly significant ($P<0.001$) different on the average number of cane stalk, length of internode, pol value, brix value, and purity percentage. From the results in Table 1; the number of cane stalks increases as the hormonal concentration is increased. For R-579, the number of stalks ranges from 9.50 (R-579 x T0,0) to 37.50 (R-579 x T4,2) and for N-41, the number of stalks ranges from 8.75 (N-41 x T0,0) to 38.75 (N-41 x T4,2). Treatment T4,2 (4 mg/L GA3 + 2 mg/L BAP) yields the highest number of stalks in both varieties. The findings supported by that of [1] on exogenous application of Gibberellic Acid 4 and 7 in combination to Benzyl amino-adenine in cane set at higher levels of plant hormones results to greater effect on the number of tillers in the selected sugarcane varieties. Furthermore, Hedden and Sponsel (2015) explained gibberellin treatment promotes stem elongation by stimulating cell division and enlargement in the stem tissues. This is supported by Daviere and Achard (2016), that the sensitivity of meristematic tissues in plant organs to the concentration of phyto-hormones determine the growth and development response of the plants (Daviere and Achard, 2016). This results in the characteristic elongation of plant stems observed in number of sugarcane stalks. The results of this study support the findings of (Nguyen et al., 2019), who found that applying gibberellin acid to plants can stimulate growth by affecting their appearance and reserves. Gibberellin also enhance the physical and physiological characteristics. Also study conducted by (Qiu et al. 2019) indicated that the external application of gibberellic acid results in an increased production of sugarcane stalks per plant.

The length of internodes follows a similar trend, with increasing values across treatments, Table 1. From variety R-579, the internode length increases from 3.075 cm (R-579 x T0,0) to 9.875 cm (R-579 x T4,2) while variety N-41 the length increases from 3.00 cm (N-41 x T0,0) to 10.050 cm (N41 x T4,2). Hormonal treatments positively influence elongation, with T4,2 being the most effective for both varieties. These findings are in correlation of that of Achola (2020) as reported that, the elongation and extension of cells, as a results of gibberellin and cytokine stimulation, are responsible for the increase in internode length observed in cane varieties, leading to a more pronounced development of longer internodes. However, study conducted by (Sujutha et al., 2018) it was found that, the application of gibberellic acid significantly increased the internode length, a similar result to that of the present study. Another study by (Roopendra et al., 2019) also indicated that cytokine and gibberellic acid could be utilized to enhance sucrose production from sugarcane by promoting elongation of internodes as one of the important parameter in source-sink system, aligning with the findings of the current research. Moreover, the length of internodes acts as a signal for the potential of sugar accumulation in different sugarcane varieties. This implies that longer internodes, achieved through the application of gibberellins

and cytokine contribute to increased yields. The present findings establish that hormonal treatments positively influence internode elongation, with treatment T4,2 being the most effective for both varieties.

Pol value an indicator of sucrose content, varies significantly among treatments, table 2. For R-579, it increases from 10.4 (R-579 x T0,0) to 15.3 (R-579 x T4,2). For N-41, it increases from 9.93 (N-41 x T0,0) to 16.1 (N-41 x T4,2). Treatment T4,2, consistently leads to the highest Pol value demonstrating its role in enhancing sucrose accumulation. Nonetheless, there was positive correlation between pol % and the concentration of gibberellic acid 3 and BAP. The higher Pol % may serve as an indicator of superior quality within a specific variety, reflecting the value of the sugarcane. Consistent, with the findings of (Roopendra et al. (2019), the application of GA3 and BAP resulted in a significant increase in Pol % aligning with the results of the present study. Also (Nguyen et al., 2019) conducted an experiment that demonstrated how gibberellic acid application influenced an enhancement in sucrose level in sugarcane, indicative of high quality in the harvested cane, which parallels the outcome observed in the current study.

Brix value also increases with hormonal treatments, Table 2. For R-579, it ranges from 16.45 (R-579 x T0) to 20.9 (R-579 x T4). For N41, it ranges from 16.67 (N-41 x T0) to 21.17 (N-41 x T4). The increase in brix value suggests improved overall sugar content, with T4 again being the most effective. The findings of this study agree with that of Achola (2020) and (Roopendra et al., 2019), both reported that application of gibberellin and cytokines resulted in significant increase of Brix value which aligns with the current findings. Brix measurement in sugarcane plays a significant role in determining the quality and sugar content of the crop, as it affects the production of sugar, molasses and ethanol.

Purity value which reflects the proportion of sucrose in the total soluble solids, improves with higher hormonal concentrations, Table 2. For R579, it increases from 61.59% (R-579 x T0,0) to 84.40% (R-579 x T4,2). For N-41, it increases from 59.24% (N-41 x T0,0) to 86.32% (N-41 x T4,2). Higher purity under T4,2 indicates improved efficiency of sucrose formation relative to total solids. According to Jain (2024), a higher purity percent indicates a higher concentration of sucrose in the juice, leading to a higher sugar recovery rate. This study agrees with the findings of Gupta (2020) and Brown, (2018) who reported that factors such as the variety of sugarcane, maturity at harvesting, climatic conditions, soil quality, and agricultural practices influence the purity percentage in sugarcane. Purity percent in sugarcane therefore, is a critical factor that influences the quality and efficiency of sugar production, maintaining high purity percent in sugarcane, leading to improved sugar recovery rates and higher quality sugar products

Table 1: Effect of different GA3 + BAP concentrations in growth of two sugarcane varieties, R-579 and N-41

S/NO.	Treatment	Number of cane stalk	Length of Internodes (cm)
1	R579 x T0,0	9.50 ab	3.075 a
2	R579 x T1,0.5	13.75 ab	4.075 b
3	R579 x T2,1	24.50 c	5.000 c
4	R579 x T3,1.5	32.00 d	7.875 de
5	R579 x T4,2	37.50 de	9.875 de
6	N41 x T0,0	8.75 a	3.00 a
7	N41 x T1,0.5	14.75 b	4.00 b

8	N41 x T2,1	20.75 c	7.75 c
9	N41 x T3,1.5	32.00 d	8.675 d
10	N41 x T4,2	38.75 e	10.050 e
Grand mean		**23.23**	**4.737**
Degree of freedom		**27**	**27**
L.S.D.		**3.535**	**0.2**
C.V. %		**3**	**0.6**

Mean with same letter in column not significantly (P<0.05) different; Mean separated by using Tukey's honest significant different test

Key: T 1,0.5 = 1.0 mg/l GA3 + 0.5 mg/l BAP, T 2,1 = 2.0 mg/l GA3 + 1.0 mg/l BAP, T 3,1.5 = 3 mg/l GA3 + 1.5 mg/l BAP, T 4,2 = 4 mg/l GA3 + 2.0 mg/l BAP, T 0,0 = 0.0 mg/l GA3 + 0.0 mg/l BA

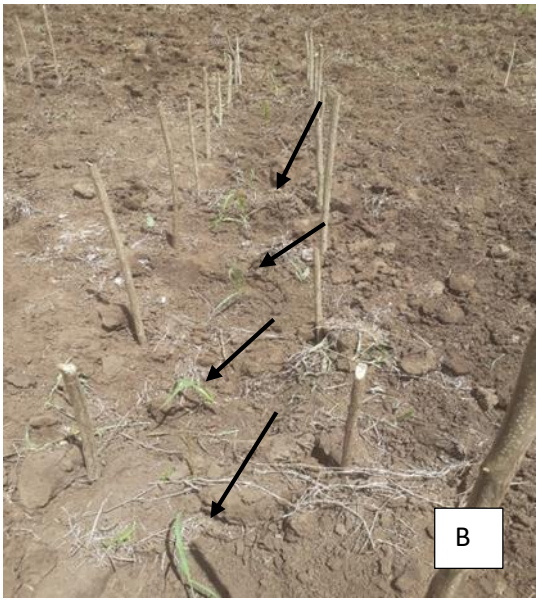
Table 2: Effect of different GA3 + BAP in quality development of two sugarcane varieties, V1 - R579 and V2 - N41

S/NO.	Treatment	Pol	Brix	Purity %
1	R579 x T0,0	10.4 a	16.45 a	61.59 ab
2	R579 x T1,0.5	11.35 bc	17.37 b	64.11 ab
3	R579 x T2,1	12.43 c	18.37 c	68.66 b
4	R579 x T3,1.5	14 d	20.15 d	80.46 c
5	R579 x T4,2	15.3 de	20.9 e	84.40 c
6	N41 x T0,0	9.93 a	16.67 a	59.24 a
7	N41 x T1,0.5	11.6 bc	17.30 b	62.79 ab
8	N41 x T2,1	12.18 c	19.02 c	66.16 ab
9	N41 x T3,1.5	14.55 d	20.67 d	82.31 c
10	N41 x T4,2	16.1 e	21.17 e	86.32 c
Grand mean		**12.783**	**19.527**	**72.00**
Degree of freedom		**27**	**27**	**27**
L.S.D.		**0.79**	**0.43**	**5.54**
C.V. %		**3.1**	**0.8**	**2**

Mean with same letter in column not significantly (P<0.05) different; Mean separated by using Tukey's honest significant different test Key: T 1,0.5 = 1.0 mg/l GA3 + 0.5 mg/l BAP, T 2,1 = 2.0 mg/l GA3 + 1.0 mg/l GA3, T 3,1.5 = 3.0 mg/l GA3 + 1.5 mg/l BAP, T 4,2 = 4.0 mg/l GA3 + 2.0 mg/l BAP, T 0,0 = 0.0 mg/l GA3 + 0.0 mg/l BAP

Figure 1: Effect of GA3 + BAP per liter in sugarcane development through bud chip technique.

A) Raised mother shoot (seedlings) of two sugarcane varieties; N-41 and R-579 from the treated bud chip into the plastic pots ready to be transplanted.



B) Transplanted seedlings into the experimental plot.

C & D) Multiple shoots in variety N-41 (left) and R-579 (right) from the seedling that generated from bud chip treated with T4,2.

E) Minimum cane stalk regenerated in variety R-579 (Control).

F) Maximum cane stalk regenerated in variety R-579 from the seedling that, its bud chip treated with T4,2.

G) Minimum cane stalk regenerated in variety N-41 (Control).

H) Maximum cane stalk in variety R-579 from the bud chip treated with T4,2.

I) Sample of cane stalk showing internode length, from the left side is variety N41 and R-579 with short internodes (Control), while at the middle and right hand side is N41 and R-579 cane stalks with longer internodes (T4,2).

5.0 Conclusion

From the results, it is clear that the application of GA3, had an improving effect on the sugarcane development and sugar accumulation through seedlings produced from treated sugarcane bud chips. GA3 and BAP combination increased number of sugarcane stalk, internode length, and enhances sugar accumulation. The study concluded that the optimal hormonal treatment for enhancing sugarcane productivity and quality is 4mg GA3 + 2mg BAP (T4,2) per liter which significantly improved development and sugar accumulation compared to control and other treatments. The findings underscore the potential of using specific plant growth regulators to enhance sugarcane yield and quality which is crucial for the sugar industry.

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