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Effect of Benzyladenine (BA) and Thidiazuron (TDZ) Application on the Bud Emergence and Seedling Uniformity of Sugarcane Setts

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ABSTRACT

Benzyladenine (BA) and thidiazuron (TDZ) are widely recognized as plant growth regulators that can stimulate bud activation in sugarcane. However, information regarding the synergistic effects of BA and TDZ, particularly in field-level sett treatment, remains limited. This study aimed to evaluate the effect of benzyladenine (BA) and its combination with thidiazuron (BA+TDZ) on bud emergence and seedling uniformity of sugarcane (Saccharum officinarum L.) setts. The experiment was arranged in a randomized complete block design with three replications, involving four sugarcane varieties (GP 99-8009, GP 05-17, GP 08-132, and TC 09) and three treatments: control (0 ppm), BA 50 ppm, and BA 50 ppm + TDZ 5 mg/L. Each experimental unit consisted of 20 buds from 10 two-budded setts. The results showed that the combination of BA 50 ppm + TDZ 5 mg/L significantly enhanced bud emerging percentage, plant height, and fresh and dry stem weights compared to BA alone and control. Variety had no significant effect, and no interaction was observed between variety and hormonal treatment. It can be concluded that the combination of BA and TDZ effectively stimulates bud emergence and early growth of sugarcane, indicating its potential use in seedling establishment.

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L.) is a major industrial crop that plays a crucial role in national sugar production and serves as a raw material for bioenergy. Sugarcane production in Indonesia still faces challenges of low productivity, partly due to delayed and irregular seedling growth during the early vegetative phase. Slow-growing seedlings lead to non-uniform plant populations in the field, which ultimately reduce sugar yield per hectare. Buds may sprout within 24 hours after planting, although this varies among varieties (Smith *et al*., 2005), yet germination often occurs unevenly and inconsistently (Purlani *et al*., 2019). Therefore, efforts to improve seedling efficiency are essential, particularly through physiological technologies involving the application of plant growth regulators (PGRs).

Cytokinin is one group of PGRs that functions in cell division, differentiation, and the formation of shoots and apical growth (Taiz *et al*., 2015). Benzyladenine (BA) is a synthetic cytokinin widely used for its effectiveness in stimulating meristem activity, breaking bud dormancy, and promoting young leaf formation (Hapsoro *et al*., 2012). In addition, BA enhances protein and RNA synthesis in young tissues, which is closely related to accelerated early vegetative growth (Werner *et al*., 2010).

Thidiazuron (TDZ) is also known for its strong cytokinin-like activity. This phenylurea derivative mimics and prolongs the effects of endogenous cytokinins and enhances the efficiency of BA in activating apical meristem growth (Huetteman & Preece, 1993). TDZ functions by inhibiting cytokinin oxidase, an enzyme responsible for degrading active cytokinins in plant tissues. Thus, the combination of BA and TDZ potentially produces a synergistic effect in accelerating bud break and shoot growth (Mutunga, 1998; Nowakowska *et al*., 2022).

Several studies have shown that combining two cytokinins results in faster shoot regeneration compared to a single application. In Rhododendron, the combination of BA and TDZ increased the number of shoots by two to three times compared to BA alone (Nowakowska *et al*., 2022). Similarly, in sugarcane, cytokinin combinations have been reported to enhance the rate and uniformity of shoot emergence from setts (Kumari *et al*., 2018). However, the effectiveness of BA–TDZ combinations may vary among varieties, depending on their cytokinin responsiveness and physiological condition (Gallo-Meagher *et al*., 2000).

Based on these considerations, this study aimed to determine the effects of single benzyladenine (BA) application and its combination with thidiazuron (TDZ) on bud break time and early vegetative growth of four sugarcane varieties. Although cytokinins have been widely studied, information on the synergistic effects of BA–TDZ applied through field-scale sett soaking across multiple varieties remains limited. The results are expected to provide an alternative technique for efficient, rapid, and uniform seedling stimulation to support the production of healthy planting material in the field.

2. MATERIALS AND METHODS

2.1. Experimental Site

The study was conducted at the sugarcane seedling nursery unit of PT Gula Putih Mataram, Central Lampung Regency, from February to July 2025. The study area was characterized by an average monthly rainfall of 264.5 mm and a mean air temperature of 28.0 °C, with maximum and minimum temperatures of 33.0 °C and 23.6 °C, respectively. The soil type at the study site was classified as podsolic soil. The plant materials used were four sugarcane varieties, namely GP 99-8009, GP 05-17, GP 08-132, and TC 09, each at 8 months of age. The plant growth regulators applied were benzyladenine (BA) and thidiazuron (TDZ). The planting method used two-budded setts, with 20 buds per experimental unit (equivalent to 10 setts) arranged in 3 m rows, and planted at a depth of approximately 30–40 cm. No supplemental irrigation was applied, as soil moisture at planting exceeded 70%.

2.2. Experimental Design

The experiment was arranged in a two-factor randomized complete block design (RCBD) with three replications. The first factor was the variety, and the second factor was the PGR treatment: control (0 ppm), BA 50 ppm, and BA 50 ppm + TDZ 5 mg/L. Each experimental unit was established with a uniform planting arrangement. Sugarcane stalks were sectioned into two-budded setts. A total of 20 buds (10 setts) were planted per unit in rows measuring 3 m in length. The concentration of BA at 50 ppm was selected based on preliminary experiments, in which this level showed a significant effect on bud break and early growth. The addition of TDZ at 5 mg/L was based on its high physiological activity, as TDZ is known to exhibit strong cytokinin-like effects even at low concentrations (Dey *et al*., 2012). Observations were conducted on three randomly selected buds/plants from each experimental unit.

PGR application was carried out by soaking the sugarcane setts in BA or BA + TDZ solution for 30 minutes before planting. Standard fertilization practices were followed, and weed control was performed using pre- and post-emergence herbicides.

2.3. Data Analysis

The observed parameters included: (1) germination percentage, (2) plant height, (3) number of internodes, leaves, tillers, and roots, (4) root length, and (5) fresh and dry weights of roots, stems, and leaves. The field experiment was conducted over a period of four months, with observations recorded at two-week intervals. Data were analyzed using analysis of variance (ANOVA), and significant differences among treatments were further tested using the Least Significant Difference (LSD) test at the 5% level. Statistical analyses were performed using RStudio software.

3. RESULTS AND DISCUSSION

The ANOVA results showed that cytokinin treatments significantly affected several growth parameters, including germination percentage, plant height, fresh stem weight, and dry stem weight at 16 weeks after planting (WAP). The variety factor only significantly influenced the number of internodes, while no significant interaction was observed between variety and treatment. This indicates that the four varieties exhibited relatively similar responses to cytokinin application. Tables 1 and 2 respectively summarize the effect of PGR treatment and variety on the growth of sugarcane setts.

Bud emergence percentage increased with the application of BA and the combination of BA + TDZ. The control treatment resulted in an average of 64.2%, which increased to 72.5% with BA 50 ppm and reached 81.7% with the BA 50 ppm + TDZ 5 mg/L combination. This increase indicates a synergistic effect between the two cytokinins in promoting cell division (Cahyani *et al*., 2025). These results are consistent with reports stating that cytokinins play a crucial role in stimulating shoot initiation (Schaller *et al*., 2014).

The ANOVA results showed that cytokinin treatments significantly affected several growth parameters, including germination percentage, plant height, fresh stem weight, and dry stem weight at 16 weeks after planting (WAP). The variety factor significantly influenced the number of internodes and the rate of germination increase, while no significant interaction was observed between variety and treatment. This indicates that the four varieties exhibited relatively similar physiological responses to cytokinin application.

Bud emergence percentage increased with the application of BA and the combination of BA + TDZ. The control treatment resulted in an average of 64.2%, which increased to 72.5% with BA 50 ppm and reached 81.7% with the BA 50 ppm + TDZ 5 mg/L combination. This enhancement suggests a synergistic interaction between BA and TDZ in promoting bud activation (Cahyani *et al*., 2025). Mechanistically, cytokinins such as BA play a key role in breaking bud

Table 1. Effects of benzyladenine or benzyladenine + thidiazuron concentrations on the growth of sugarcane setts

Variable	Control	BA 50 ppm	BA 50 ppm + TDZ 5 mg/l	Significance
Germination (%)	64.2 ± 4.52 c	72.5 ± 9.30 b	81.7 ± 14.64 a	8.59*
Plant height (cm)	204.5 ± 22.93 b	228.6 ± 17.87 a	232.2 ± 10.28 a	14.46*
Number of leaves (blades)	13.7 ± 0.77	14.1 ± 1.24	13.9 ± 1.48	tn
Number of roots (pieces)	84.3 ± 22.22	92.6 ± 17.17	109.6 ± 16.13	tn
Number of tillers (stalks)	2.2 ± 0.98	2.4 ± 0.78	2.8 ± 1.28	tn
Number of internodes (segments)	11.4 ± 1.55	12.4 ± 1.77	12.5 ± 1.79	tn
Root length (cm)	22.1 ± 3.28	23.6 ± 3.33	21.5 ± 5.21	tn
Fresh root weight (g)	20.2 ± 5.35	22.2 ± 5.78	25.0 ± 6.32	tn
Fresh stem weight (g)	368.3 ± 91.79 b	419.8 ± 117.36 b	522.2 ± 127.87a	94.74*
Fresh leaf weight (g)	169.5 ± 64.75	199.2 ± 86.70	224.5 ± 72.50	tn
Dry root weight (g)	6.1 ± 3.86	8.6 ± 6.68	7.5 ± 2.30	tn
Dry stem weight (g)	56.9 ± 16.27 b	68.2 ± 18.26 b	84.6 ± 24.63 a	16.81*
Dry leaf weight (g)	46.7 ± 12.89	53.3 ± 13.62	54.8 ± 9.92	tn

Note: Mean values followed by different letters in the same row indicate significant differences at the 5% level (α = 0.05). Data are presented as means of three replications (*n* = 3).

Table 2. Effects of variety on the growth of sugarcane setts

Variable	GP 99-8009	GP 05-17	GP 08-132	TC 09	Significance
Germination (%)	73.3 ± 11.18	67.8 ± 7.92	73.9 ± 16.94	76.1 ± 13.64	tn
Plant height (cm)	221.8 ± 20.53	214.0 ± 17.14	232.7 ± 15.78	218.6 ± 25.60	tn
Number of leaves (blades)	13.7 ± 0.78	13.8 ± 1.28	14.3 ± 1.32	13.7 ± 1.43	tn
Number of roots (pieces)	99.4 ± 30.73	97.0 ± 20.78	99.1 ± 17.98	86.6 ± 18.43	tn
Number of tillers (stalks)	3.0 ± 1.18	2.4 ± 0.73	1.8 ± 0.93	2.6 ± 1.04	tn
Number of internodes (segments)	11.1 ± 1.60 b	10.8 ± 0.88 b	13.6 ± 1.48 a	12.8 ± 1.37 a	1.24*
Root length (cm)	22.9 ± 4.30	22.1 ± 3.60	21.7 ± 3.80	22.8 ± 5.23	tn
Fresh root weight (g)	23.8 ± 8.17	22.5 ± 5.57	23.1 ± 5.56	20.5 ± 5.49	tn
Fresh stem weight (g)	490.4 ± 153.59	391.0 ± 95.68	468.6 ± 57.64	397.1 ± 153.78	tn
Fresh leaf weight (g)	212.9 ± 68.35	184.9 ± 63.78	233.9 ± 64.83	159.3 ± 94.88	tn
Dry root weight (g)	5.3 ± 2.29	7.1 ± 3.65	7.5 ± 3.01	9.8 ± 7.60	tn
Dry stem weight (g)	70.2 ± 23.11	59.7 ± 12.39	79.2 ± 14.74	70.5 ± 32.82	tn
Dry leaf weight (g)	48.6 ± 8.74	53.5 ± 14.60	56.5 ± 11.69	47.8 ±12.19	tn

Note: Mean values followed by different letters in the same row indicate significant differences at the 5% level (α = 0.05). Data are presented as means of three replications (*n* = 3).

dormancy by stimulating cell division in the meristematic tissue and enhancing sink strength in developing buds, thereby accelerating bud outgrowth. In addition, BA can counteract apical dominance by modulating auxin–cytokinin balance, allowing lateral buds to grow more rapidly.

The addition of TDZ further amplified this response due to its high cytokinin-like activity and its ability to modulate endogenous hormone levels. TDZ has been reported to increase endogenous cytokinin accumulation while also influencing auxin metabolism, resulting in enhanced cell division and differentiation even at low concentrations. This dual effect likely explains the higher bud emergence observed in the BA + TDZ treatment compared to BA alone. Furthermore, TDZ is known to induce persistent physiological responses due to its relatively slow degradation, prolonging its activity within plant tissues. Therefore, the higher germination percentage in the combined treatment is not only a result of increased cytokinin availability but also due to hormonal balance regulation and sustained stimulation of meristematic activity, leading to faster and more uniform bud emergence.

The increase in plant height (Figure 1 and 2a) and stem weight followed a similar pattern to the germination percentage increase. Treatments with BA immersion alone (228.6 cm) and the combination of BA + TDZ (232.2 cm) produced significantly faster plant growth compared to the control (204.5 cm). Cytokinins are known to enhance young tissue expansion and accelerate cell division in the intercalary meristem (Werner *et al*., 2010; Mok & Mok, 2001; Zhang *et al*., 2019), as well as facilitate the translocation of nutrients to actively growing plant parts, including stems (Sorour & El-Shanhorey, 2016). The increase in the number of new leaves may be attributed to cytokinin stimulation that triggers cell division, leading to tissue differentiation (Hussain *et al*., 2021; Kumari *et al*., 2017). The number of leaves (Figure 2b), roots, tillers, and internodes did not differ significantly among treatments. Root primordia in sugarcane are pre-formed structures located around the nodes within the sett tissue, and only a portion of them develop into roots depending on environmental and physiological conditions (van Antwerpen *et al*., 2022). The application of plant growth regulators such as auxin has been shown to promote root primordia initiation and development (Zhang *et al*., 2025). However, the effectiveness of PGRs is influenced by factors such as soaking duration, where insufficient exposure time may limit hormone absorption and subsequent rooting response (Kurnia *et al*., 2022).

However, the soaking duration applied in this study was based on common field practices to avoid potential negative effects, such as tissue damage or hormonal imbalance, resulting from prolonged exposure. Therefore, the applied duration may have been sufficient to influence shoot growth responses but not adequate to significantly stimulate root formation. Further studies involving variations in soaking duration and PGR concentrations are required to better understand their role in enhancing root development in sugarcane setts.

Figure 1. Plant height and root growth of sugarcane setts at 16 WAP under various BA and TDZ treatments (from left: V4T0, V4T1, V4T2, V1T0, V1T1, V1T2)

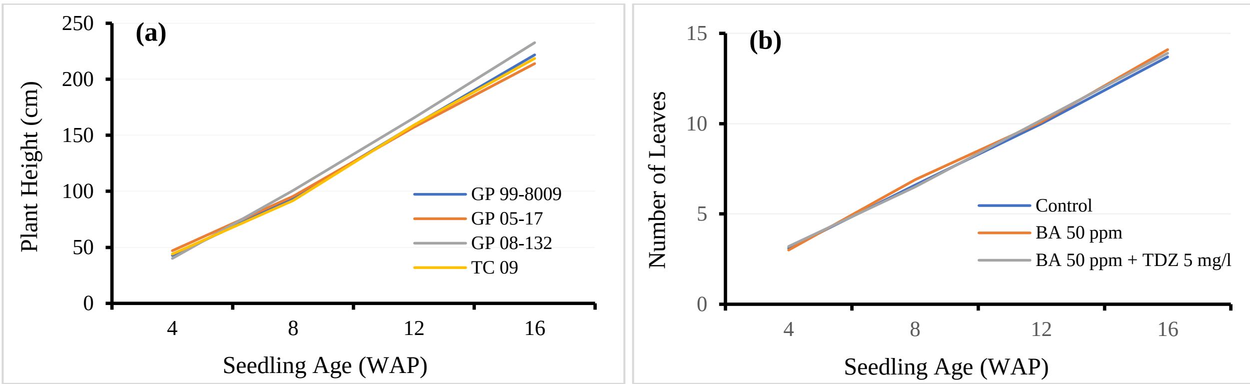

Figure 2. (a) Effect of varieties on the plant height of sugarcane seedlings, and (b) Effect of BA and TDZ on the development leaf number of sugarcane seedling for 16 weeks after planting (WAP)

Immersion treatments with BA alone or in combination with TDZ did not have a significant effect on root length or fresh root weight. Root length is more strongly influenced by the auxin–cytokinin balance within root tissues; therefore, exogenous cytokinin application alone is insufficient to induce root elongation (Rivas *et al*., 2022).

Fresh and dry weights of roots and leaves also showed no significant differences among treatments. This indicates that cytokinin effects are more dominant in the shoot than in the root, as the hormonal balance between cytokinin and auxin determines organ growth dominance in plants (Hopkins & Hüner, 2008; Taiz *et al*., 2015).

Thus, the application of BA and TDZ was more effective in stimulating the growth of the upper plant parts during the early vegetative phase. Genetic variation only began to show a significant effect when the plants entered more complex growth stages, such as tissue differentiation and the formation of storage organs (McIntyre *et al*., 2021).

The ANOVA results showed that varietal treatments did not significantly affect most of the observed parameters, including germination percentage, plant height, and stem biomass, while only limited effects were observed on specific traits such as the number of internodes and germination rate. This indicates that the four sugarcane varieties responded relatively similarly to BA application during the early growth phase, as also reflected by the absence of significant interaction between variety and cytokinin treatment.

This uniform response can be explained by the physiological nature of early sugarcane growth. During the initial stage, shoot development primarily depends on the activation of pre-formed axillary buds and the mobilization of carbohydrate reserves stored in the setts. Under these conditions, the application of benzyladenine (BA) likely plays a dominant role by stimulating cell division and breaking bud dormancy, thereby minimizing the expression of genotypic differences among varieties. As a result, the effect of the plant growth regulator becomes more pronounced than the genetic variability at this stage.

Nevertheless, the significant effect of variety on the number of internodes and germination rate suggests that certain traits remain under partial genetic control even in early growth. These parameters are closely related to intrinsic varietal characteristics such as bud vigor and metabolic activity, which may differ among genotypes. This indicates that although BA can standardize early shoot emergence, it does not completely eliminate varietal differences in all growth aspects. Furthermore, the absence of significant differences in fresh leaf weight among varieties suggests that leaf development at this stage is still largely influenced by internal reserves rather than fully expressed genetic potential. In sugarcane, genotypic differences in traits such as leaf area expansion and biomass partitioning generally become more evident after the transition from heterotrophic growth (sett-dependent) to autotrophic growth (photosynthesis-dependent) (Liu *et al*., 2020).

These findings highlight that the lack of strong varietal effects in early growth should not be interpreted as genetic uniformity, but rather as a temporal limitation in trait expression. The dominance of BA response at this stage may mask underlying genetic variability, which is likely to emerge more clearly at later developmental stages. Therefore, further evaluation across extended growth periods is necessary to better understand the interaction between cytokinin application and varietal performance, particularly in relation to biomass accumulation and yield components.

The absence of a significant interaction between variety and treatment indicates that the physiological responses to cytokinin were general during the early growth stage of sugarcane (Gallo-Meagher *et al*., 2000). This response is primarily controlled by similar fundamental physiological mechanisms, namely the activation of dormant buds and the formation of new vegetative tissues. However, in later growth stages, genetic differences among varieties may influence stem formation and biomass accumulation (Mutunga, 1998). Punpee (2015) also reported that the application of BA at various concentrations and through different methods did not result in significant interactions with environmental factors or leaf conditions for several major physiological parameters, such as chlorophyll content, photosynthetic rate, and leaf protein degradation. This finding suggests that the effects of BA tend to be general and not dependent on genotype differences or the tested environmental conditions.

Overall, the combination of 50 ppm BA + 5 mg L^{-1} TDZ produced the best effects on shoot emergence rate, early vegetative growth, and stem biomass accumulation. These results indicate the potential of applying the BA–TDZ combination as a simple technology to improve sugarcane seedling quality and propagation efficiency in the field.

4. CONCLUSION AND RECOMMENDATION

This study demonstrates that the combined application of BA and TDZ exerts a synergistic effect in promoting bud emergence and early vegetative growth of sugarcane under field conditions. The optimal treatment (50 ppm BA + 5 mg L^{-1} TDZ) significantly outperformed single BA application, indicating that TDZ enhances cytokinin activity during early growth stages. These findings contribute to the limited field-based evidence on BA–TDZ interactions in sett treatment. From a practical perspective, this combination offers a feasible pre-planting strategy to improve germination uniformity and early stand establishment, thereby supporting better crop management and yield optimization.

AUTHOR CONTRIBUTION STATEMENT

Author	C	M	So	Va	Fo	I	R	D	O	E	Vi	Su	P	Fu
PN	✓		✓		✓		✓	✓	✓	✓	✓		✓	✓
Yus	✓	✓		✓		✓				✓		✓		
DH	✓	✓		✓		✓				✓		✓		
KS	✓	✓		✓		✓				✓		✓		
RE	✓			✓		✓				✓		✓		

C: Conceptualization; M: Methodology; So: Software; Va: Validation; Fo: Formal Analysis; I: Investigation; D: Data Curation; R: Resources; O: Writing - Original Draft; E: Writing - Review & Editing; Vi: Visualization; Su: Supervision; Fu: Funding Acquisition; P: Project Administration

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