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In Vitro Axillary Shoot Growth of Sugarcane Cultivar 'AAS Agribun' under BAP and NAA

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Abstrak: Tebu (*Saccharum officinarum* L.) merupakan komoditas strategis penghasil gula di Indonesia, namun perbanyakan konvensionalnya terbatas oleh kecepatan dan risiko penyakit. Kultur jaringan *in vitro* menjadi alternatif untuk menghasilkan bibit unggul secara massal dan bebas patogen. Penelitian ini bertujuan menganalisis pengaruh kombinasi *Benzyl Amino Purine* (BAP) dan *Naphthalene Acetic Acid* (NAA) terhadap pertumbuhan tunas aksilar tebu kultivar '*Agriculture Acclimated Sugarcane* Agribun' yang disingkat dengan 'AAS Agribun' serta menentukan konsentrasi paling efektif pada tahap inisiasi. Penelitian dilaksanakan pada Oktober 2025 hingga Januari 2026 di Laboratorium Unit Pengelola Benih Unggul Pertanian (UPBUP), Pusat Perakitan dan Modernisasi Pertanian (BrMP) Perkebunan, Bogor, Jawa Barat menggunakan Rancangan Acak Lengkap faktorial dengan perlakuan BAP (0–2,0 mg/L) dan NAA (0–0,5 mg/L) pada media Murashige and Skoog. Parameter yang diamati meliputi persentase eksplan hidup, waktu muncul tunas, jumlah tunas, tinggi tunas, dan jumlah daun. Perlakuan BAP 0,5 mg/L tanpa penambahan NAA merupakan kombinasi paling efektif dalam menginduksi inisiasi tunas aksilar yang dibuktikan dengan waktu muncul tunas tercepat yaitu 1,75 MST serta jumlah daun terbanyak yaitu rata-rata 0,96 helai per eksplan.
Kata kunci: kultur jaringan, tebu, tunas aksilar, zat pengatur tumbuh.

Abstract: Sugarcane (*Saccharum officinarum* L.) is a strategic sugar-producing crop in Indonesia; however, conventional vegetative propagation is limited by slow multiplication rates and the risk of disease transmission. In vitro culture offers an alternative approach for rapid and disease free seedling production. This study aimed to evaluate the effect of *Benzyl Amino Purin*e (BAP) and *Naphthalene Acetic Acid* (NAA) combinations on axillary shoot growth of sugarcane cultivar 'Agriculture Acclimated Sugarcane Agribun' (abbreviated as

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'AAS Agribun') and to determine the most effective concentration for shoot initiation. The study was conducted from October 2025 to January 2026 at the Laboratorium Unit Pengelola Benih Unggul Pertanian (UPBUP), Pusat Perakitan dan Modernisasi Pertanian (BrMP) Perkebunan, Bogor, West Java. A completely randomized factorial design was applied using BAP (0–2.0 mg/L) and NAA (0–0.5 mg/L) on *Murashige and Skoog* (MS) medium. Observed parameters included percentage of surviving explants, time of shoot emergence, number of shoots, shoot height, and number of leaves. The results indicated that 0.5 mg/L BAP without NAA produced the most effective response for axillary shoot initiation, as reflected by higher explant survival and improved shoot growth compared to other treatments. This was evidenced by the earliest shoot emergence, observed at 1.75 Weeks After Culture (WAC), and the highest leaf number, averaging 0.96 leaves per plant. These findings suggest that low BAP concentration without auxin supplementation is optimal for early-stage shoot induction of sugarcane cultivar 'AAS Agribun'.
Keyword: axillary shoots, plant growth regulators, sugarcane, tissue culture.

1. Introduction

Sugarcane (*Saccharum officinarum* L.) is a major industrial crop that plays an essential role in fulfilling domestic sugar demand in Indonesia [1][2]. It serves as the primary source of national sugar production for household consumption and the food and beverage industry. However, increasing national sugar consumption has not been balanced by adequate domestic production capacity [3][4]. Domestic sugar production, which reaches approximately 2.23 million tons per year, remains far below the national demand exceeding 6.7 million tons, resulting in dependence on imports [5], [6]. This condition affects the sustainability of the national sugar industry and highlights the urgency of improving sugarcane productivity.

One important factor influencing sugarcane productivity is the propagation method. Conventional vegetative propagation using stem cuttings is still widely applied; however, this method requires large quantities of planting material and carries the risk of transmitting systemic diseases [7]. Therefore, an alternative propagation technique that is more efficient and capable of producing high quality, diseasefree seedlings is required. Plant tissue culture or *in vitro* culture is a vegetative propagation technique performed by isolating plant parts and growing them on artificial media under aseptic conditions [8]. The success of this technique

largely depends on the composition of the culture medium, which generally contains macro and micronutrients, vitamins, sucrose, and plant growth regulators (PGRs) [9]. Murashige and Skoog (MS) medium is widely used due to its comprehensive nutrient composition that supports regeneration in various plant species [10].

Plant growth regulators play a crucial role in directing morphogenic responses [11]. Cytokinins such as Benzyl Amino Purine (BAP) are effective in stimulating shoot formation and multiplication [12]. Whereas auxins such as Naphthalene Acetic Acid (NAA) are involved in cell division and elongation. The combination of BAP and NAA has been reported to support shoot initiation and growth, although the response depends on plant species and cultivar [13][14]. In sugarcane, different concentrations of BAP and NAA have been shown to influence shoot multiplication under *in vitro* conditions [15].

Sugarcane cultivar 'AAS Agribun' is a superior local cultivar officially released in Indonesia and derived from mutation breeding of the Bululawang cultivar. It has a yield potential exceeding 130 tons/ha with approximately 10% sugar recovery under optimal conditions and demonstrates good vigor and adaptability to suboptimal environments [16]. These characteristics make 'AAS Agribun' a promising candidate for micropropagation, particularly at the axillary shoot initiation stage.

Although several studies have reported the effects of BAP and NAA combinations on sugarcane shoot growth, information regarding the morphogenic response of cultivar 'AAS Agribun' during axillary shoot initiation remains limited. Therefore, this study aimed to analyze the effect of combined BAP and NAA concentrations on axillary shoot growth of sugarcane cultivar 'AAS Agribun' under *in vitro* conditions and to determine the most effective concentration for shoot initiation.

2. Research Method

a. Study Site and Plant Material

This study was conducted from October 2025 to January 2026 at the Laboratory of Unit Pengelola Benih Unggul Pertanian (UPBUP), Pusat Perakitan

dan Modernisasi Pertanian (BrMP) Perkebunan, Bogor, West Java. Axillary shoot explants of sugarcane cultivar 'AAS Agribun' were used as plant material.



Figure 1 Location map: A) Map of Indonesia, B) Map of West Java, C) Map of Bogor City, D) Map of the Agricultural Superior Seed Management Unit Laboratory (Source: Google Maps, 2025).

b. Experimental Design

The experiment was arranged in a Completely Randomized Design (CRD) consisting of 10 treatment combinations derived from two factors. The first factor was Benzyl Amino Purine (BAP) at concentrations of 0, 0.5, 1.0, 1.5, and 2.0 mg/L, while the second factor was Naphthalene Acetic Acid (NAA) at concentrations of 0 and 0.5 mg/L. Each treatment was replicated four times, resulting in a total of 40 experimental units.

Table 1. Factorial Experimental Design of BAP and NAA Concentrations in Axillary Shoot Culture of Sugarcane Cultivar 'AAS Agribun'.

Treatment	Concentration (mg/L)	Replication Number
P_0	BAP 0 + NAA 0	4
P_1	BAP 0 + NAA 0.5	4
P_2	BAP 0.5 + NAA 0	4
P_3	BAP 0.5 + NAA 0.5	4
P_4	BAP 1.0 + NAA 0	4
P_5	BAP 1.0 + NAA 0.5	4
P_6	BAP 1.5 + NAA 0	4
P_7	BAP 1.5 + NAA 0.5	4
P_8	BAP 2.0 + NAA 0	4
P_9	BAP 2.0 + NAA 0.5	4

c. Culture Media Preparation and Explant Sterilization

Murashige and Skoog (MS) medium was used as the basal medium and supplemented with 30 g/L sucrose and agar. The medium pH was adjusted to 5.7–5.8 before autoclaving at 121°C and 15 psi for 15 minutes. Culture vessels and

instruments were sterilized before use. Explants were washed under running water and surface-sterilized with appropriate disinfectants under aseptic conditions in a laminar airflow cabinet. The sterile explants were then inoculated onto MS medium containing the respective BAP and NAA combinations.

d. Culture Conditions and Observed Parameters

The research variables comprised independent, dependent, and controlled variables. The independent variables were BAP concentrations of 0, 0.5, 1.0, 1.5, and 2.0 mg/L and NAA concentrations of 0 and 0.5 mg/L. The dependent variables included explant survival, shoot emergence time, number of shoots, shoot height, and number of leaves. Controlled variables included sugarcane cultivar 'AAS Agribun' and explants derived from upper stem axillary buds, MS basal medium supplemented with 30 g/L sucrose and 7 g/L agar, and an incubation temperature of 24.2°C.

e. Statistical Analysis

The data included explant survival, shoot emergence time, number of shoots, shoot height, and number of leaves. All data were analyzed using SPSS version 25. Normality and homogeneity tests were performed before two-way Analysis of Variance (ANOVA) at a 95% confidence level. When significant differences were detected, Duncan's Multiple Range Test (DMRT) was conducted at the 5% significance level to compare treatment means.

3. Results and Discussion

a. Percentage of Surviving Explants

All treatment combinations resulted in 100% survival of axillary shoot explants (Table 2), indicating that 'AAS Agribun' explants maintained viability under all tested BAP and NAA concentrations. The absence of mortality suggests that the applied PGR levels were well tolerated during the initiation stage. The high survival rate also indicates that the MS basal medium provided suitable nutritional conditions to support explant viability under in vitro culture.

Table 2. Effect of BAP and NAA Combinations on Survival Percentage, Shoot Emergence, and Growth Parameters of Axillary Shoots of Sugarcane Cultivar 'AAS Agribun' in Vitro. WAC=Weeks After Culture.

Treatment	BAP + NAA (mg/L)	Survival (%)	Days to Shoot Emergence (WAC)	Number of Shoots	Shoot Height (cm)	Number of Leaves
P0	0 + 0	100	- e	0.00 a	0.00 d	0.00 c
P1	0 + 0.5	100	- e	0.00 a	0.00 d	0.00 c
P2	0.5 + 0	100	1.75 de	0.14 b	0.66 ab	0.96 a
P3	0.5 + 0.5	100	2.75 bcd	0.10 b	0.00 d	0.00 c
P4	1.0 + 0	100	4.75 abc	0.21 b	0.30 bcd	0.28 bc
P5	1.0 + 0.5	100	5.25 a	0.17 b	0.16 cd	0.28 bc
P6	1.5 + 0	100	2.50 cd	0.14 b	0.80 a	0.67 ab
P7	1.5 + 0.5	100	2.75 bcd	0.09 b	0.00 d	0.00 c
P8	2.0 + 0	100	5.00 ab	0.14 b	0.51 abc	0.39 bc
P9	2.0 + 0.5	100	5.25 a	0.14 b	0.17 cd	0.17 bc

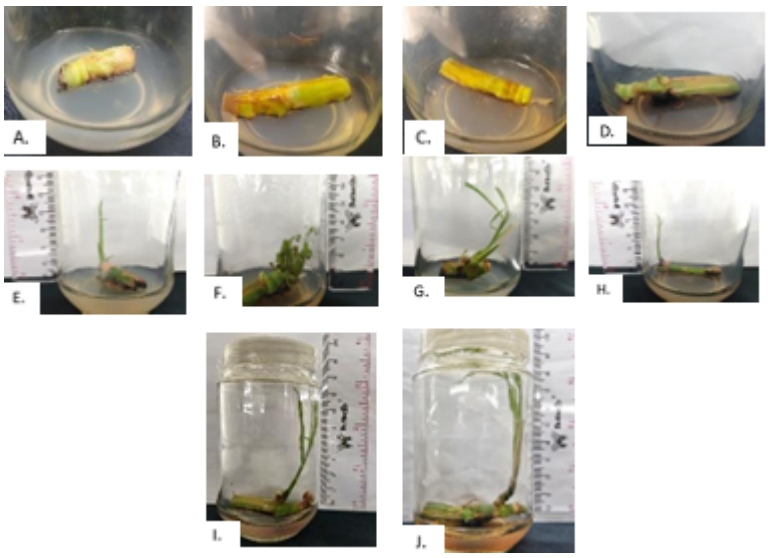
The high survival rate also reflects the suitability of the Murashige and Skoog (MS) basal medium in supporting early explant development. MS medium provides essential macro and micronutrients required for cellular metabolism and tissue maintenance under *in vitro* conditions [16]. In addition, proper sterilization and handling procedures likely minimized contamination and physiological stress, which are common factors causing explant failure in tissue culture systems.

Furthermore, the concentrations of BAP (0–2.0 mg/L) and NAA (0–0.5 mg/L) used in this study remained within the physiological tolerance range of sugarcane tissues. Similar findings have been reported, showing high survival rates of sugarcane explants cultured on media supplemented with cytokinin and auxin at moderate concentrations [17,18]. Therefore, the uniform survival observed in this study confirms that the culture conditions were appropriate for subsequent evaluation of growth parameters.

b. Shoot Emergence Time

Values in Table 2 followed by the same letter within the same column are not significantly different according to Duncan's Multiple Range Test (DMRT) at the 5% significance level. The interaction between BAP and NAA significantly affected shoot emergence time. The earliest shoot emergence occurred in P2 (0.5 mg/L BAP without NAA) at 1.75 weeks after culture (WAC), whereas P0 and P1 (without BAP) produced no shoots throughout the observation period. Higher BAP concentrations

combined with NAA, particularly P5 and P9, delayed shoot emergence to 5.25 WAC (Figure 2).



(A) P0 (BAP 0 mg/L + NAA 0 mg/L), **(B)** P1 (BAP 0 mg/L + NAA 0.1 mg/L), **(C)** P3 (BAP 0.5 mg/L + NAA 0.1 mg/L), **(D)** P7 (BAP 1.5 mg/L + NAA 0.1 mg/L), **(E)** P5 (BAP 1.0 mg/L + NAA 0.1 mg/L), **(F)** P4 (BAP 1.0 mg/L + NAA 0 mg/L), **(G)** P8 (BAP 2.0 mg/L + NAA 0 mg/L), **(H)** P9 (BAP 2.0 mg/L + NAA 0.1 mg/L), **(I)** P2 (BAP 0.5 mg/L + NAA 0 mg/L), dan **(J)** P6 (BAP 1.5 mg/L + NAA 0 mg/L

Figure 2. Documentation of Sugarcane Explants at 8 WAC. A, B: Treatment that did not produce shoot. C, D: Shoots emerged rapidly but failed to develop. E, G, H: Treatments that delayed leaf shoot emergence but supported good shoot growth until the end of the observation period. F: Treatments that required a relatively long time for shoot emergence but produced the highest number of shoots. I: The fastest shoot initiation. J: Treatments that promoted relatively rapid shoot emergence and produced the tallest shoots.

The earlier shoot emergence at low BAP concentration highlights the role of cytokinin in stimulating cell division and activating axillary meristems [19]. The effective shoot induction under low BAP without NAA further suggests that cytokinin dominance favored shoot initiation in 'AAS Agribun' [15]. Conversely, excessive auxin may interfere with shoot induction through hormonal antagonism between auxin and cytokinin [8]. The mean shoot emergence time for each treatment is presented in Figure 3.

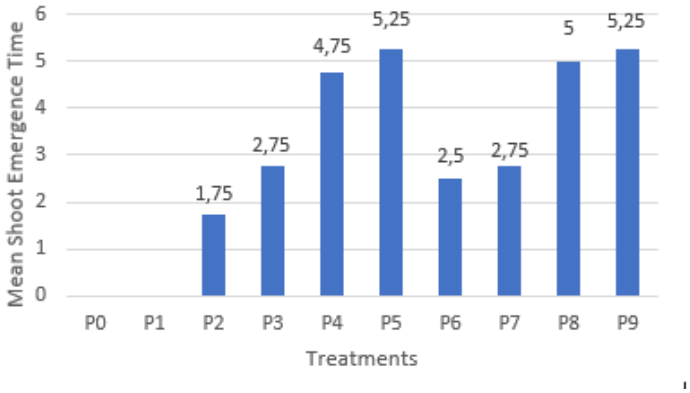


Figure 3. Mean Time to Shoot Emergence Each Treatment

The results indicate that 0.5 mg/L BAP was the most favorable concentration for shoot emergence in 'AAS Agribun'. This concentration may effectively stimulate axillary bud development without causing excessive hormonal effects. Higher BAP levels may disrupt the hormonal balance required for shoot initiation. These findings support the concept that plant morphogenesis depends not only on individual hormone concentrations but also on the balance between exogenous regulators and endogenous hormones.

The delayed shoot emergence in treatments containing 0.5 mg/L NAA may indicate that additional auxin was less favorable for shoot initiation in 'AAS Agribun'. Exogenous NAA may have altered the hormonal balance toward processes other than shoot initiation, thereby delaying bud emergence. Thus, low BAP without NAA appeared more favorable for accelerating shoot initiation during the initiation stage of this cultivar.

c. Number of Shoots

The interaction between BAP and NAA significantly affected the number of shoots (Table 2), with P4 (1.0 mg/L BAP without NAA) producing the highest mean of 0.21 shoots per explant (Figure 2). In contrast, P0 and P1, which contained no BAP, produced no shoots during the initiation phase. These results indicate treatment means presented in Figure 4.

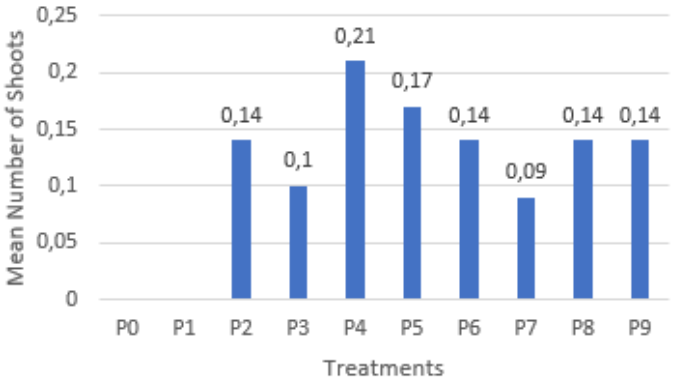


Figure 4. Mean Number of Shoots

BAP promotes mitotic activity and reduces apical dominance, thereby stimulating axillary bud proliferation [4]. However, NAA did not consistently increase shoot formation, and its addition often resulted in fewer shoots than BAP alone. This suggests that higher auxin levels may shift morphogenic responses toward other developmental pathways [5,20]. Similarly, previous studies found that cytokinin dominant media produced more shoots than media with balanced or auxin dominant compositions [9].

DMRT at the 5% significance level showed that P4 (1.0 mg/L BAP) produced the highest number of shoots, indicating that this concentration was more favorable for axillary bud multiplication in 'AAS Agribun'. Although P2 (0.5 mg/L BAP) induced earlier shoot emergence, it produced fewer shoots (0.14) than P4. This suggests that a slightly higher BAP concentration may better support subsequent axillary bud proliferation after shoot initiation.

The suppressive effect of NAA observed in treatments like P3, P5, and P7 where shoot numbers were lower than their NAA free counterparts highlights the sensitivity of 'AAS Agribun' to the auxin and cytokinin ratio (Figure 2). The data suggests that at the initiation stage, the presence of exogenous auxin may interfere with the "breaking" of apical dominance provided by BAP. Therefore, to maximize the multiplication rate of sugarcane cultivar 'AAS Agribun', the use of BAP as a single plant growth regulator at a moderate concentration is recommended over combined hormone formulations.

d. Shoot Height

The interaction between BAP and NAA significantly affected shoot height at the 5% significance level (Table 2). P6 (1.5 mg/L BAP without NAA) produced the

highest shoot height (0.80 cm), whereas treatments containing 0.5 mg/L NAA, particularly P1 and those with lower BAP concentrations, showed reduced or no shoot elongation (Figure 2). These results treatment means presented in Figure 5.

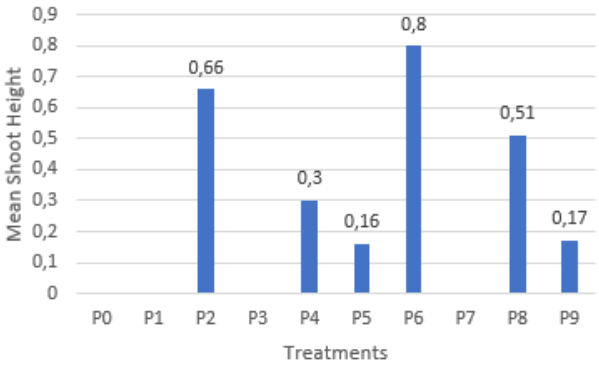


Figure 5. Mean Shoot Height

Cytokinin promotes cell division, while shoot elongation depends on balanced hormonal interactions, with moderate BAP concentrations supporting active meristematic growth [21][22]. Although 0.5 mg/L BAP accelerated shoot emergence, 1.5 mg/L BAP produced greater shoot height, indicating that the higher concentration was more favorable for shoot elongation under the conditions of this study. P3 (0.5 mg/L BAP + 0.5 mg/L NAA) and P7 (1.5 mg/L BAP + 0.5 mg/L NAA) showed shoot emergence at 2.75 weeks after culture but no measurable shoot elongation or leaf formation, with low shoot numbers of 0.10 and 0.09 shoots per explant, respectively. In contrast, the NAA-free treatments P2 and P6 produced shoot heights of 0.66 and 0.80 cm, respectively, whereas NAA-containing treatments generally showed lower shoot elongation [23,25]. These results suggest that 0.5 mg/L NAA did not enhance early axillary shoot development and may have created a hormonal balance less favorable for shoot elongation in 'AAS Agribun'.

This finding is consistent with recent studies showing that sugarcane responses to plant growth regulators vary with genotype and hormone combination. Lutfiani et al. (2022) reported that BAP and NAA combinations affected shoot multiplication in sugarcane, while Gupta et al. (2024) found that in vitro responses varied with plant growth regulator composition [15][26]. Thus, the poor elongation observed in P3 and P7 may reflect the specific hormonal

requirements of 'AAS Agribun' rather than a general inhibitory effect of NAA on sugarcane shoot growth. Among the treatments, P6 (1.5 mg/L BAP without NAA) produced more vigorous and elongated shoots, with the absence of NAA potentially favoring cell expansion during the initiation stage. Therefore, 1.5 mg/L BAP without NAA appeared more favorable for shoot elongation in 'AAS Agribun' under the conditions of this study [27].

e. Number of Leaves

The interaction between BAP and NAA significantly affected leaf number at the 5% significance level (Table 2), with P2 (0.5 mg/L BAP without NAA) producing the highest mean of 0.96 leaves per explant, followed by P6 (1.5 mg/L BAP) at 0.75 leaves. Treatments containing NAA generally produced fewer leaves, while P0 and P1 produced none due to the absence of shoot emergence. These results indicate that leaf formation in 'AAS Agribun' was more favorable under low to moderate BAP without NAA during the initiation stage, as shown in Figure 6.

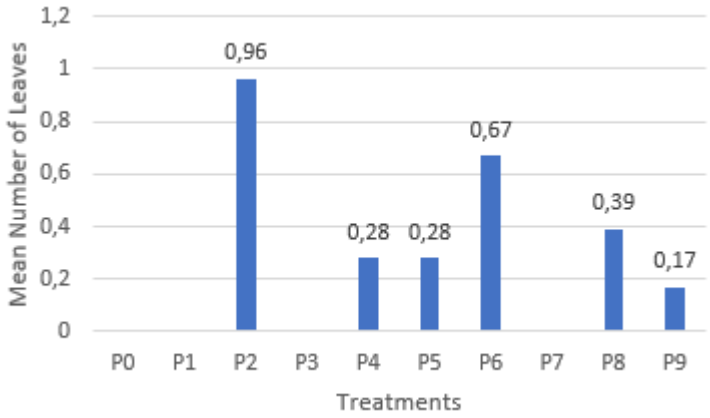


Figure 6. Mean Number of Leaves.

Leaf development is closely associated with successful shoot induction and elongation. Adequate cytokinin concentration promotes differentiation of leaf primordia and supports organogenesis [24][26]. Conversely, the addition of auxin at higher proportions may suppress leaf formation due to hormonal imbalance [17].

Conversely, the addition of 0.5 mg/L NAA may suppress leaf formation due to hormonal imbalance. Treatments P3, P5, and P7 produced significantly fewer leaves than treatments containing BAP alone. This may occur because NAA shifts hormonal signals toward cell elongation or callus formation rather than leaf

differentiation. Thus, 0.5 mg/L BAP without NAA appeared more favorable for leaf production in 'AAS Agribun'.

Furthermore, Leaves in cytokinin dominant media, particularly P2 and P6, showed vibrant green color and healthy morphology. Gebreyohannes et al. (2026) reported genotype specific leaf responses to BAP and NAA, indicating that leaf development depends on genotype and PGR composition. Similarly, the higher leaf production in P2 (0.5 mg/L BAP) and P6 (1.5 mg/L BAP), both without NAA, suggests that cytokinin dominant media favored leaf development in 'AAS Agribun' [28].

4. Conclusion

The interaction between Benzyl Amino Purine (BAP) and Naphthalene Acetic Acid (NAA) significantly affected all growth parameters of axillary shoots of sugarcane cultivar 'AAS Agribun' under *in vitro* conditions, with a 100% explant survival rate observed across all treatments, indicating that the culture conditions and plant growth regulator concentrations were within the physiological tolerance range of the explants. The optimal treatment varied among growth parameters: 0.5 mg/L BAP without NAA produced the fastest shoot emergence (1.75 WAC) and highest leaf number (0.96 leaves), 1.0 mg/L BAP without NAA produced the highest shoot number (0.21 shoots), and 1.5 mg/L BAP without NAA produced the greatest shoot height (0.80 cm). Overall, BAP at 0.5–1.5 mg/L without NAA was more favorable for axillary shoot initiation and early growth of sugarcane cultivar 'AAS Agribun' under the conditions of this study, although its effectiveness may vary among cultivars.

5. Recommendations

It is recommended to use BAP at concentrations ranging from 0.5 mg/L to 1.5 mg/L as the optimal dosage for inducing early explant growth. For future research development, further studies should be conducted by combining these recommended BAP doses with other types of auxins. This would allow for an

evaluation of their effectiveness in promoting shoot and root growth, as well as enhancing overall plantlet quality compared to the use of NAA.

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