PLANT SCIENCE TODAY
Vol 13(1): 1-9
https://doi.org/10.14719/pst.9494

eISSN 2348-1900

RESEARCH ARTICLE

Combination of NAA and BAP significantly enhances plantlet regeneration of *Saccharum officinarum* L. cv. PSJK 922

Muhammad Hamzah Solim & Ita Dwimahyani*

National Research and Innovation Agency, Research Organization for Agriculture and Food, Bogor 16915, West Java, Indonesia

*Correspondence email - itad001@brin.go.id

Received: 18 May 2025; Accepted: 24 October 2025; Available online: Version 1.0: 09 January 2026; Version 2.0: 26 January 2026

Cite this article: Solim MH, Dwimahyani I. Combination of NAA and BAP significantly enhances plantlet regeneration of *Saccharum officinarum* L. cv. PSJK 922. Plant Science Today. 2026; 13(1): 1-9. https://doi.org/10.14719/pst.9494

Abstract

This study aimed to optimize callus induction and plantlet regeneration protocols for the commercial sugarcane cultivar PSJK 922. Callus induction from spindle leaf roll segments was performed using Murashige and Skoog (MS) medium supplemented with 2,4-D at concentrations of 0, 1, 2, 3 and 4 mg/L. Data were recorded on Callus Induction Frequency (CIF) and Callus Fresh Weight (CFW). The best callus with a friable texture and creamy white colour, was subcultured and transferred to regeneration media containing 25 combinations of NAA and BAP, ranging from 0 to 2 mg/L. Plantlet regeneration was evaluated over 14 weeks under controlled conditions. The parameters included plantlet regeneration, number of shoots, number of roots, shoot length, root length and Plantlet Vigour Index (PVI). The results showed that 2,4-D at concentrations of 3 and 4 mg/L were most effective for generating morphogenic callus with high CIF (83.33 %) and CFW (0.3834-0.4541 g). The statistical analysis revealed a significant effect of NAA and BAP combinations on plantlet regeneration (77.33 %, $p < 0.047$) observed from callus induced with 3 mg/L. In particular, the optimal regeneration medium was found to be 0.5 mg/L NAA and 1.5 mg/L BAP, when using callus induced by 3 mg/L 2,4-D, leading to higher shoot elongation (4.74 cm ± 0.32) compared to most other treatments. The combination exhibited the highest PVI (around 456), which indicates healthy plantlet development and overall quality. Thus, these findings can help optimize tissue culture protocols for elite sugarcane as well as large-scale propagation for accelerating germplasm multiplication and serving as a foundation for genetic transformation of elite lines.

Keywords: BAP; callus; *in vitro* technique; NAA; somatic embryogenesis; sugarcane

Introduction

Sugarcane is the world's primary source of sugar, supplying around 70 % of global sugar production (1). Enhancing sugarcane's productivity, crop performance and resilience in various conditions are key focuses of modern agricultural biotechnology (2–4). A key challenge in Indonesia is that the commercial sugarcane cultivar PSJK 922, despite its strong agronomic potential, still lacks an optimized *in vitro* regeneration protocol to support breeding and large-scale propagation. *In vitro* culture systems offer valuable solutions, as established protocols can enhance genetic diversity, enable rapid propagation and improve germplasm conservation, thereby strengthening the sugarcane industry (5). In Indonesia, the sugarcane cultivar PSJK 922 is a commercial sugarcane, which has stem lengths of approximately 278–283 cm and a productivity of 12.89 t/ha (6). Its high yield performance makes it an important variety for supporting the rising demand in the national sugar industry. However, the lack of an optimized *in vitro* regeneration protocol limits its application in advanced biotechnological approaches for genetic improvement and large-scale propagation. Among the regeneration strategies, somatic embryogenesis has emerged as an effective technique for sugarcane improvement.

Somatic embryogenesis is a plant tissue culture techniques that enables efficient clonal propagation, facilitates genetic transformation and supports mutation breeding by allowing the regeneration of complete plants from single somatic cells (4,7–9). To initiate somatic embryogenesis and successful plant regeneration, a critical early step is callus induction, which provides the undifferentiated tissue required for further development into somatic embryos and plantlets (8,10). Thus, understanding the factors influencing callus induction becomes crucial for improving the efficiency of somatic embryogenesis.

The efficiency of callus formation is largely influenced by the type and concentration of Plant Growth Regulators (PGRs), especially 2,4-D, which is known to induce dedifferentiation and promote cell proliferation (5,8,11,12). Establishing optimal conditions for callus induction is essential, as the quality and responsiveness of the callus with a friable texture, white to light green colour and embryogenic morphology directly affect the success of subsequent regeneration (11). For plantlet development, auxin and cytokinin combinations, such as BAP and NAA are commonly applied and the relative ratios determine shoot and root differentiation (11,13). However, the ratios of PGRs must be carefully adjusted to maximize the production of healthy plantlets (14,15).

Despite the extensive application of tissue culture in sugarcane, regeneration efficiency can vary significantly across cultivars, requiring cultivar-specific protocol optimization (4,5,16).

PSJK 922 is widely cultivated due to its high yield potential, yet it has shown limited efficiency in tissue culture responses, highlighting the need for a protocol to accelerate germplasm multiplication. Therefore, this study aimed to develop and optimize a reliable and efficient *in vitro* callus induction and plantlet regeneration protocol specifically for the sugarcane cultivar PSJK 922 from Indonesia.

Materials and Methods

Plant material

Spindle leaf roll segments as explants were taken from 4-month-old sugarcane (*Saccharum officinarum* L. cv. PSJK 922) (Fig. 1A) in the field in Pasar Jumat, Lebak Bulus, South Jakarta, Indonesia. This variety is a commercial sugarcane from Indonesia that has a stem length of around 278–283 cm with a productivity of almost 13 t ha^{-1} (6). In this study, there were two experiments, including callus induction and plantlet regeneration, which were carried out from January to November 2024.

Medium preparation

MS medium supplemented with plant growth regulator, which is 2,4 -dichlorophenoxyacetic acid (2,4-D), is used for stage 1 and benzyl amino purin (BAP) and naphthalene acetic acid (NAA) for stage 2 (17). The MS formulation was prepared by dissolving stock solutions of macro and micro-nutrients. A 10 × concentrated stock solution of macro-nutrients (containing NH_4NO_3, KNO_3, $CaCl_2{\cdot}2H_2O$, $MgSO_4{\cdot}7H_2O$ and KH_2PO_4) and a 100 × stock of micronutrients (including $MnSO_4{\cdot}H_2O$, $ZnSO_4{\cdot}7H_2O$, H_3BO_3, KI, $Na_2MoO_4{\cdot}2H_2O$, $CuSO_4{\cdot}5H_2O$ and $CoCl_2{\cdot}6H_2O$) were added to distilled water. Additionally, a separate 100 × vitamin stock was also added, which contained glycine (2 mg/L), pyridoxine-HCl (0.5 mg/L), nicotinic acid (0.5 mg/L) and thiamine-HCl (0.1 mg/L). The medium was further supplemented with 30 g/L sucrose as a carbon source, myo-inositol (100 mg/L) and solidified with 6 g/L agar.

For callus induction, 2,4-D was added after autoclaving to achieve final concentrations of 0, 1, 2, 3 and 4 mg/L (Fig. 1B). The pH of the medium was adjusted to 5.8 using pH meter (EZ-9909SP model) before autoclaving at 121 °C for 15 min. After that, the media was aseptically transferred into sterile bottles. For the regeneration stage, the medium was supplemented with various combinations of BAP and NAA, each filter-sterilized and added aseptically after autoclaving (cooled to ~50 °C), since these PGRs are relatively heat-labile and may degrade at high temperatures. The sterilized medium was poured into sterile culture bottles.

Callus induction

In the first stage of the experiment, the explants were washed under running tap water with liquid detergent (1 % SteriOne, v/v) for 10 min and then washed thrice, followed by surface sterilization with 20 % commercial Clorox solution "Bayclin" (containing 5.25 % sodium hypochlorite) for 15 min. After sterilization, the explants were rinsed thrice with distilled water in a Laminar Air Flow Cabinet (LAFC) (Esco, LHG-4AG-F8).

The explants (0.5 cm) were prepared as cross-sections of spindle leaf roll segments and were aseptically cultured on MS medium supplemented with varying concentrations of plant growth hormone 2,4-D (0, 1, 2, 3 and 4 mg/L). Each treatment consisted of three replicates, with two explants placed per bottle. The cultures were incubated in the dark condition at 27 ± 2 °C for 3 weeks (Fig. 1C).

The parameters were CIF (%) and was calculated as well as and CFW (g) formula with modifications, as follows (18):

$$\text{CIF (\%)} = \frac{\text{Number of explants that formed callus}}{\text{Total number of explants cultured}} \text{ X } 100 \quad \text{(Eqn. 1)}$$

$$\text{CFW (g)} = \text{final weight - initial weight} \quad \text{(Eqn. 2)}$$

CFW was the fresh callus biomass calculated by subtracting the initial weight (Petri dish and medium) from the final weight (Petri dish, medium and callus). The CFW is measured using an analytical balance (0.0001 g). The CFW was transferred onto a new MS medium without hormones for three weeks with single subculture, to increase the biomass as a source for regeneration treatments.

Plantlet regeneration

The best callus which showing friable texture and white-creamy color obtained from the first stage was transferred to fresh MS medium supplemented with BAP (0, 0.5, 1, 1.5 and 2 mg/L) and NAA (0, 0.5, 1, 1.5 and 2 mg/L), resulting in a total 25 treatment combinations (Table 1). Each treatment consisted of three replicates, with three callus pieces cultured per bottle. In this experiment, the same NAA and BAP combinations were tested to evaluate both shoot and root induction simultaneously. As preliminary trials indicated that these combinations produced acceptable responses and were used for both shoot and root induction in this study for further optimization. The plantlet regeneration treatments were cultured in the controlled room (16 hr light and 8 hr dark) at 27 ± 2 °C for 14 weeks.

The parameters were Plantlet Regeneration (PR), Number of Shoots (NS) per callus and Number of Roots (NR) per callus, average of Shoot Length (SL) and Root Length (RL). PVI, a measure used to evaluate the growth potential and overall quality, indicating optimal regeneration with shoot and root development, which was calculated (19,20).

$$\text{PVI} = \text{PR} \times (\text{SL} + \text{RL}) \quad \text{(Eqn. 3)}$$

Statistical analysis

All parameters in experiment 1 and 2 were tested using one-way Analysis of Variance (ANOVA) and using an independent t-test to compare the performance of two callus sources. MS Excel and Jamovi version 2.6.26 were used to analyse data and ImageJ (scale bar) https://imagej.net/ij/ and https://sankeymatic.com/build/ used for visualization (21,22). Post-hoc test used Tukey's honest significant difference (Tukey's HSD) procedure was used to compare means at the 95 % probability level.

Results and Discussion

Callus induction

The callus induction response of sugarcane (cv. PSJK 922) was evaluated under different concentrations of 2,4-D (0, 1, 2, 3 and 4 mg/L). The results indicated that explants cultured on MS medium without 2,4-D showed 0 % callus induction, showing that auxin is necessary to trigger dedifferentiation in this sugarcane variety (11). Callus induced with 2,4-D at 3 and 4 mg/L regenerated separately into roots and plantlets in different concentration of NAA-BAP media

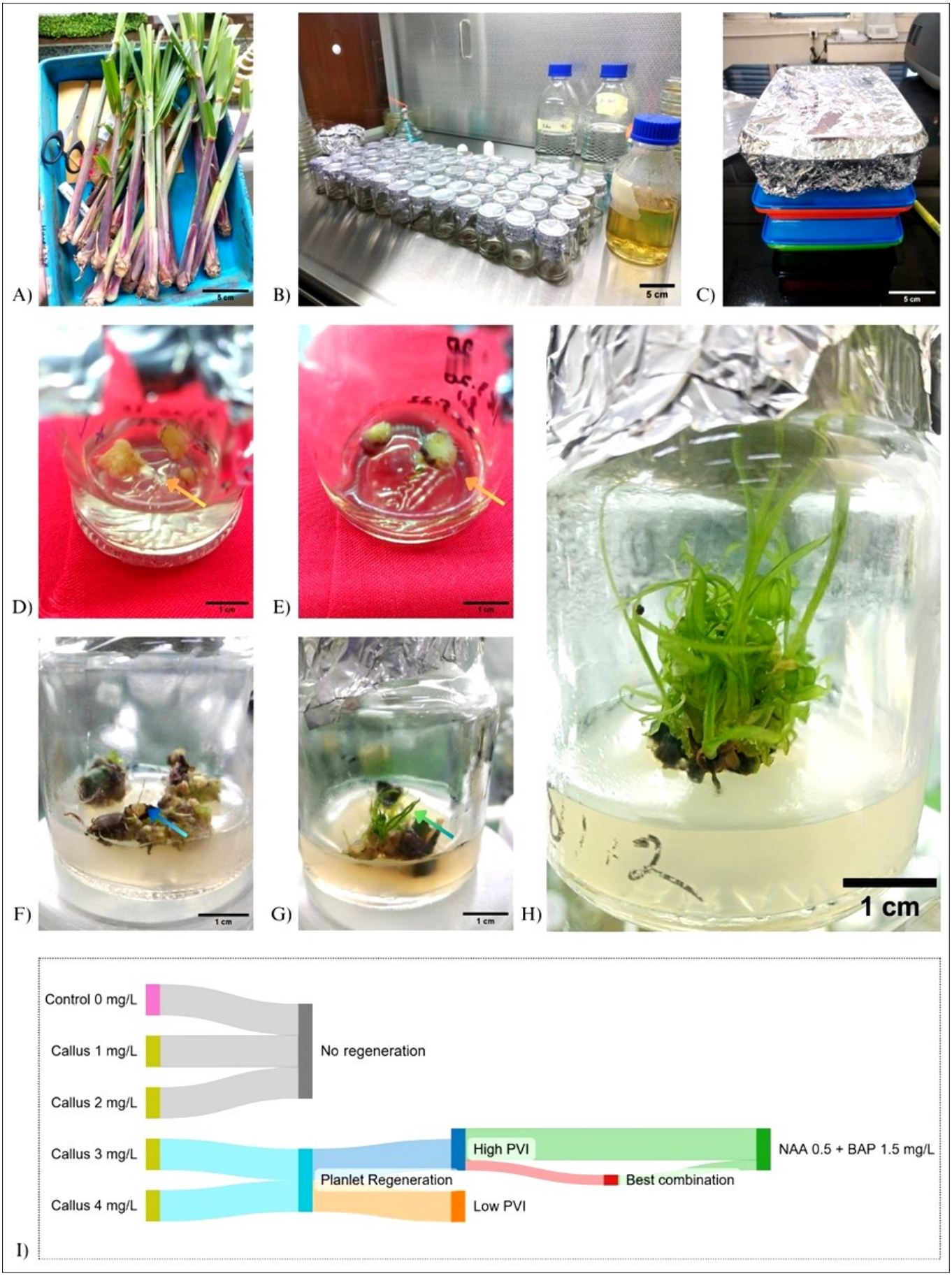

Fig. 1. *In vitro* technique for sugarcane cv. PSJK 922. (A) Explant source from 4-month-old sugarcane; (B) Media preparation for callus induction using 2,4-D (1 to 4 mg/L); (C) The culture store in the dark condition at 27±2 °C for 3 weeks; (D-E) Formed callus from 2,4-D 3 and 4 mg/L treatments, respectively; (F) Root formation in regeneration medium (NAA_2 + $BAP_{0.5}$); (G) Plantlet emergence in regeneration medium ($NAA_{0.5}$+ $BAP_{1.5}$); (H) Plantlet regeneration showing shoot and leaf development in regeneration medium $NAA_{0.5}$+ $BAP_{1.5}$; (I) Sankey chart demonstrates experiment pathway from callus induction to the best combination media for sugarcane PSJK 922. Arrows: callus (orange), roots (blue), shoots (green). The scale bar of each figure was measured by ImageJ

combinations (Fig. 1D-I). CIF increased in a dose-dependent response, reaching the highest frequency (83.33 %) at both 3 mg/L and 4 mg/L (Fig. 2A). However, lower concentrations (2 mg/L and 1 mg/L) resulted in reduced CIF of 66.67 % and 33.33 %, respectively. In similar, CFW peaked at 3 mg/L (0.4541 g), with a slightly lower value at 4 mg/L (0.3834 g) (Fig. 2B). Meanwhile, calli induced at 1 mg/L and 2 mg/L produced CFW at 0.2029 g and 0.2773 g, respectively.

The results showed callus formation depends on genotype, culture media, type of explant and their interaction. The elite cultivars should be used as explants with good embryogenic potential, which is consistent with our observation that genotype influenced strongly callus induction efficiency (5). For culture media, observed that the maximum callus induction occurred on MS media supplemented with 2 mg/L 2,4-D, whereas optimal induction at 2.5 mg/L 2,4-D (10,11). However, our study demonstrated higher efficiency at 3-4 mg/L, indicating that cultivar-specific responses can change the optimal hormone concentration. Similarly, callus induction declined at 4 and 5 mg/L, but 3.5 mg/L as the ideal concentration (16). Besides, we observed that injured cells of the immature explant enhanced nutrient uptake capacity and promoted callus proliferation, aligning with other reports recommending immature tissues for embryogenic callus formation (5,11). However, hormone level must be balanced carefully, as inappropriate concentrations of PGRs can alter the endogenous hormones in the explant and trigger callus growth. Therefore, it is crucial to optimize the concentration to get maximum callus growth in the shortest possible time (16,23).

PR based on two callus sources

The best callus with CIF > 80 % and CFW > 0.3 g from 2,4-D 3 mg/L and 2,4-D 4 mg/L were selected and subcultured for three weeks to increase biomass and subsequently transferred to regeneration medium containing a combination of NAA and BAP (Supplementary Table 1 & 2). The focus was directed toward optimizing and evaluating these hormones, which have also been reported to induce high regeneration capacities (11). Plant tissues use PGRs to develop their organogenic potential during *in vitro* regeneration. An optimal level of auxins and cytokinins is necessary for this stage, as these hormones interact during developmental processes, including the formation of the whole plant body through the generation of shoot and root meristems (11,24).

Table 1. Combination of BAP and NAA for plantlet regeneration

BAP (mg/L)	NAA (mg/L)				
	0	0.5	1	1.5	2
0	B_0N_0	$B_0N_{0.5}$	B_0N_1	$B_0N_{1.5}$	B_0N_2
0.5	$B_{0.5}N_0$	$B_{0.5}N_{0.5}$	$B_{0.5}N_1$	$B_{0.5}N_{1.5}$	$B_{0.5}N_2$
1	B_1N_0	$B_1N_{0.5}$	B_1N_1	$B_1N_{1.5}$	B_1N_2
1.5	$B_{1.5}N_0$	$B_{1.5}N_{0.5}$	$B_{1.5}N_1$	$B_{1.5}N_{1.5}$	$B_{1.5}N_2$
2	B_2N_0	$B_2N_{0.5}$	B_2N_1	$B_2N_{1.5}$	B_2N_2

Based on the results of the independent t-test, we observed that calli induced from 2,4-D, 3 mg/L exhibited significantly higher PR (77.33 %) in regeneration media (NAA + BAP) compared to 2,4-D 4 mg/L (PR at 64 %) (Fig. 3A). However, the number of shoots per callus did not differ significantly between treatments (1.64 ± 0.10 for 2,4-D 3 mg/L and 1.48 ± 0.11 2,4-D 4 mg/L; $p = 0.270$), showing that shoot induction may be more influenced by the regeneration media components rather than the initial auxin concentration during callus induction (Fig. 3B). The next findings indicate a clear pattern which the higher auxin concentration during callus induction (2,4-D 4 mg/L) led to root development, producing significantly more roots (2.58 ± 0.30) and longer root length (2.86 ± 0.11), showing significantly higher than 3 mg/L treatment (NR at 1.84 ± 0.16 and RL at 2.06 ± 0.12) (Fig. 3C, E). Meanwhile, the lower auxin (2,4-D 3 mg/L) during callus induction promoted shoot elongation (3.15 cm ± 0.17), which was significantly greater than 4 mg/L treatment (SL at 2.62 cm ± 0.17) (Fig. 3D).

The regeneration response between calli from 3 mg/L and 4 mg/L treatments on MS medium supplemented with NAA and BAP, the callus of 4 mg/L showed a higher NR and longer RL than callus of 3 mg/L treatment, although both calli showed comparable shoot numbers (not significant) but SL significantly greater in callus from 3 mg/L treatment. The roots were established on MS medium containing 1.5 mg/L NAA and the highest root frequency and

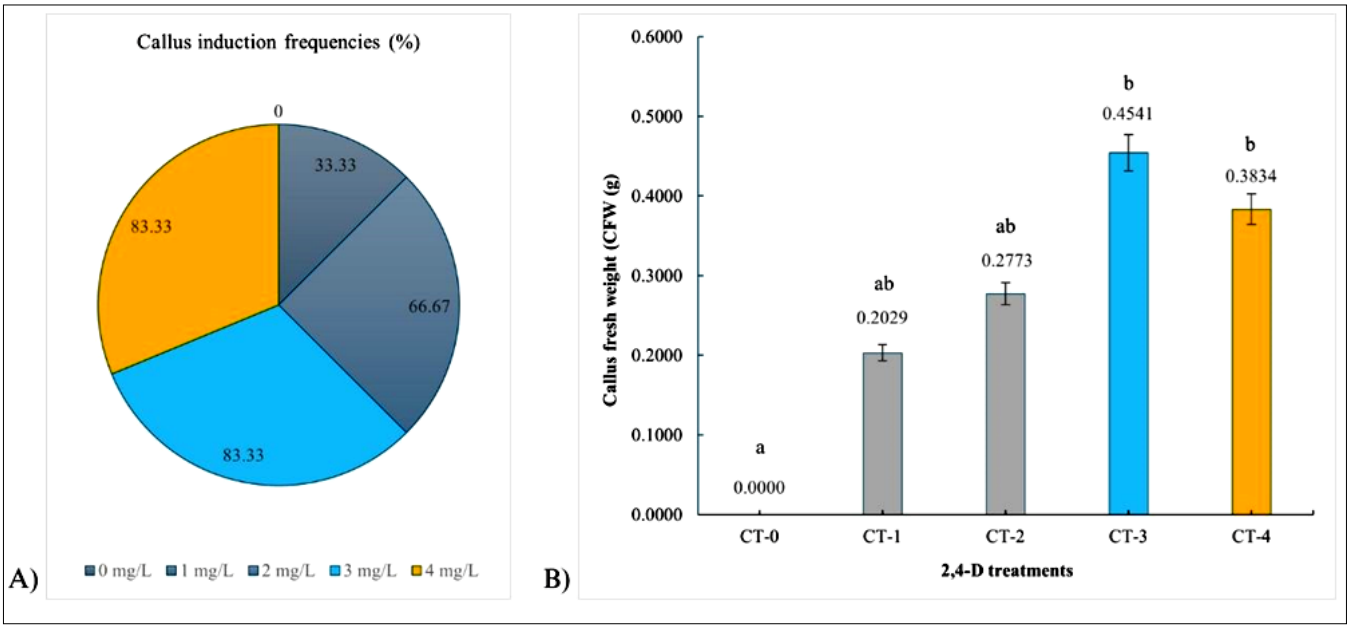

Fig. 2. Effect of different concentrations of 2,4-D on the percentage of callus induction from explants. (A) Callus induction frequencies (%); (B) fresh weight (g) obtained from explants cultured on different concentrations of 2,4-D. Means in the same bar followed by the same lowercase letter are not significantly different based on Tukey's HSD test at the 95 % probability level. CT (Callus Treatment): 2,4-D at 0, 1, 3, 4 mg/L

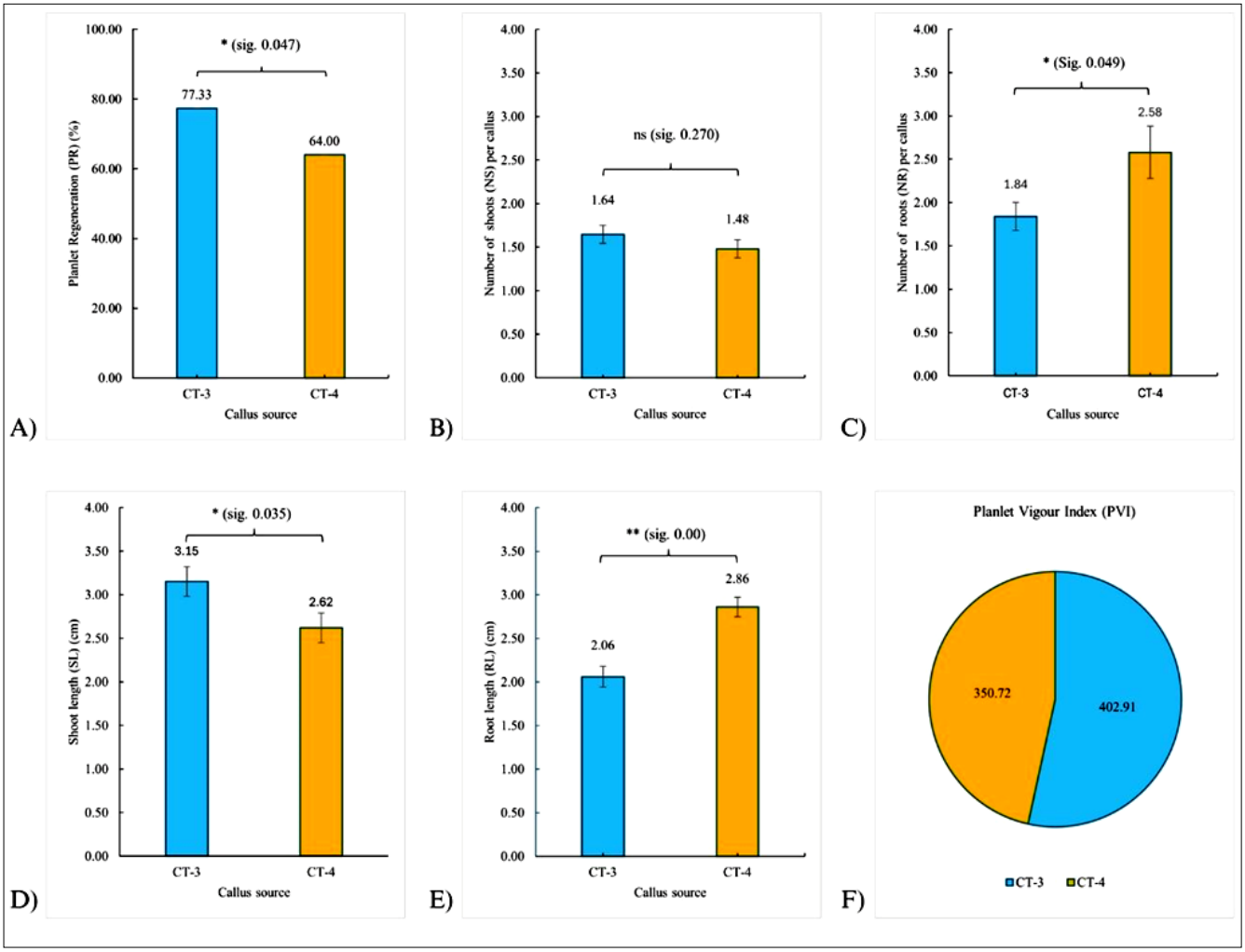

Fig. 3. Effect of MS media supplemented with NAA and BAP combinations on callus from 2,4-D 3 and 4 mg/L treatments for five parameters. (A) Plantlet regeneration (%); (B) Number of Shoots (NS) per callus; (C) Number of Roots (NR) per callus; (D) Shoot Length; (E) Root Length; (F) Plant Vigour Index (PVI) for two callus sources. Data (A-E) were analyse using independent t-test to test two callus sources (3 and 4 mg/L) in regeneration media. CT (Callus Treatment) – 2,4-D at 0, 1, 3, 4 mg/L. *significant at $p \leq 0.05$; **significant at $p \leq 0.01$; ns = nonsignificant

maximum RL of sugarcane was obtained with 2 mg/L NAA (11,16). Besides, the interaction between auxins and cytokinins influenced callus induction and plant regeneration. In the study, the effective balance of NAA and BAP demonstrated how auxin-cytokinin crosstalk regulate developmental process, with higher auxin favoring root-related responses and elevated cytokinin promoting shoot formation from callus, consistent with previous reports (24–26).

The PVI which integrates regeneration percentage, SL and RL into a single composite value reflecting overall regeneration quality, was calculated PVI for two callus sources (2,4-D 3 mg/L and 2,4-D 4 mg/L) (19). The PVI of 3 mg/L (402.91) was higher than 4 mg/L (350.72), indicating that calli from 3 mg/L exhibited better overall regeneration performance under NAA and BAP combination (Fig. 1I). Additionally, Pearson correlation analysis among parameters revealed strong positive correlations between PR and NS (r = 0.718) and between PR and SL (r = 0.649), indicating that efficient regeneration protocols not only enhance the proportion of regenerated plantlets but also promote the development of multiple shoots with greater elongation (Fig. 4). In addition, RL had a negative correlation with SL (r = -0.418), implying a balance in resource allocation between the organs. However, the strong positive correlation between RL and NR (r = 0.912). This indicate that once root initiation occurs, plants tend to allocate resources toward

	PR	*NS*	*NR*	*SL*	*RL*
PR	1				
NS	0.71876568	1			
NR	-0.0138695	-0.0691618	1		
SL	0.64934409	0.66568754	-0.4030703	1	
RL	0.01673571	-0.0363981	0.91238139	-0.4182003	1

Fig. 4. Pearson correlation analysis among parameters. Blue and red indicate strong positive and negative correlations, respectively. PR: Plantlet Regeneration; NS: Number of Shoots per callus; NR: Number of Roots per callus; SL: Shoot Length; RL: Root Length

extensive root system which may improve nutrient uptake and survival in later growth stages.

Effect of regeneration media on PR, SL, RL of 2,4-D 3 mg/L

The response of callus (2,4-D 3 mg/L) to various concentrations of NAA and BAP indicates that these hormones are essential for successful PR, with the highest PR percentages. The maximum PR (> 80 %) was achieved when moderate concentrations of both hormones were present, while regeneration declined when either NAA or BAP was absent or at low concentration, as depicted in Fig. 5. Shooting and rooting were achieved simultaneously under the NAA and BAP combinations, with the response depending on the concentration used. Both high and moderate concentrations of NAA

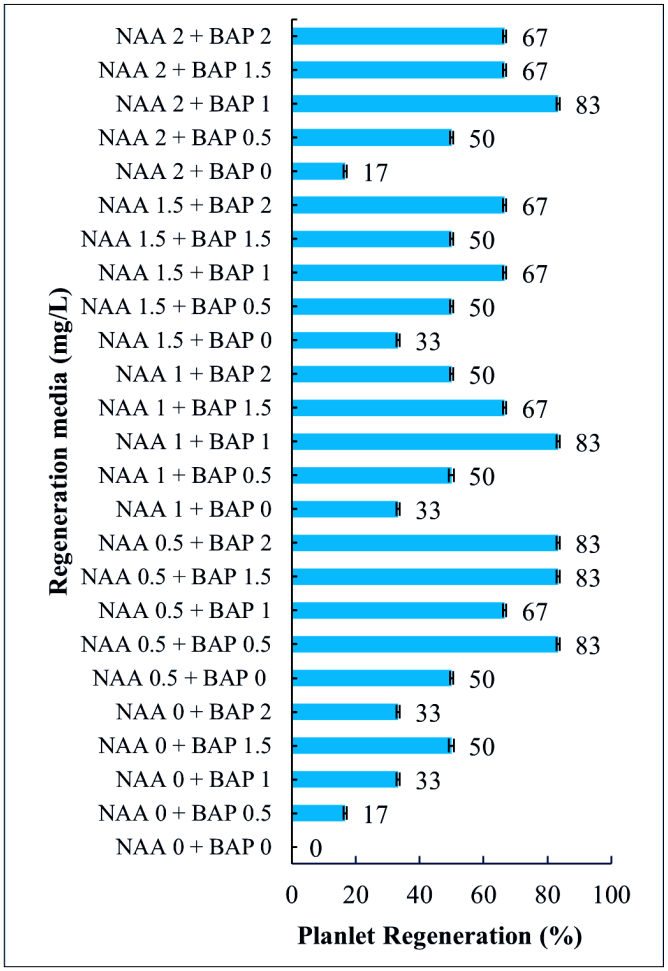

Fig. 5. Percentage of plantlet regeneration in response to various NAA and BAP combinations.

(0.5-2 mg/L) combined with a moderate amount of BAP (1-2 mg/L) that support optimal shoot and root development, leading to complete plantlet formation.

In contrast, regeneration percentages dropped significantly in media where either NAA or BAP was absent or present at very low concentrations, such as 2 mg/L NAA and 1.5 mg/L BAP, both yielded only 17 % regeneration, while without NAA and BAP failed to induce any regeneration (0 %). This confirms the synergistic effect of auxin and cytokinin, where auxin levels are sufficient to initiate rooting and cytokinin is present to stimulate shoot formation (11,16,24).

Shoot development was particularly responsive to cytokines supply. This hormone regulates meristem function with studies showing that excess cytokinin promotes shoot formation from callus. The positive role of cytokinin in regulating shoot meristem activity is further demonstrated by recent studies that demonstrate that cytokinin deficiency decreases shoot meristem size and activity (24,26). It is evident that media containing higher concentrations of BAP with lower or moderate NAA levels, promoted greater shoot elongation, as illustrated in Fig. 6. It was observed that lower NAA (0.25 mg/L) concentration in combination with BAP (2 mg/L), highly induced shoot regeneration (11). Similarly, we also found all treatments with BAP and lower NAA 0.5 mg/L (three combinations), showed highest shoot regeneration (above 80 %), as shown in Fig. 5. However, we observed the highest SL was observed in the medium containing 0.5 mg/L NAA + 1.5 mg/L BAP, which reached approximately 5.0 cm and was significantly different from other treatments (Fig. 6). Other high-performing combinations included 0.5 mg/L NAA + 0.5 mg/L BAP, 0.5 mg/L NAA + 1 mg/L BAP and 0.5 mg/L NAA + 2 mg/L BAP, both yielding SL above 4.5 cm. Previous studies reported that although BAP works in a specific sugarcane variety, this hormone is efficient for shoot induction in sugarcane (16).

On the other hand, root formation was enhanced under auxin-rich (NAA) conditions, especially when combined with low to moderate concentrations of cytokinin (BAP), significantly enhanced root elongation. The longest roots were observed in the treatment 2 mg/L NAA + 0.5 mg/L BAP, reaching nearly 3.5 cm. This supports the notion that auxin dominance is crucial for promoting root development in tissue culture. It is a slight difference where, roots were easily induced on ½ MS augmented only with 1.5 mg/L NAA (11). Low NAA concentration on rooting showed a positive impact (27). On the contrary, MS media supplemented with higher auxins like NAA (2 mg/L) induced the highest root frequency and maximum RL in a specific sugarcane variety (16).

A general trend shows that SL increased with rising BAP concentrations up to 1.5 mg/L but tended to plateau or slightly decline at 2 mg/L BAP, especially when combined with higher NAA concentrations (Fig. 6). In contrast, media without BAP or with high NAA and low BAP concentrations generally resulted in shorter shoots, with the lowest SL recorded in 2 mg/L NAA_2 at around 0.5 cm. The dashed curve (trend line) supports this trend, indicating an optimal peak in SL around mid-range cytokinin levels, showing that cytokinin dominance in the regeneration media is crucial for enhanced shoot elongation in this crop. Cytokinins are essential for shoot regeneration, regulating meristem function. Excess cytokinin promotes shoot formation from callus, while deficiency reduces shoot meristem size and activity (16,24,26). The trend line reflects an increase in RL with increasing auxin concentration, peaking when BAP is balanced or reduced (Fig. 7). Collectively, these results underscore that auxin and cytokinin can also act antagonistically which high auxin promotes root growth but suppresses shoot elongation, while high cytokinin enhances shoot development (24,25).

Based on the PVI analysis, the data shows significant variation across different combinations of NAA and BAP. The combination of 0.5 mg/L NAA + 1.5 mg/L BAP emerged as the most effective treatment. This medium produced the highest SL of approximately 4.9 cm, a respectable RL of around 0.6 cm and achieved more than 80 % of PR. This combination showed the highest PVI of 456, which is the highest among all tested treatments. The high PVI value indicates plant with optimal regeneration with shoot and root development, thereby reflecting not only the quantity but also the robustness and overall quality of the regenerated plantlets. Meanwhile, 0.5 mg/L NAA + 2 mg/L BAP, 2 mg/L NAA + 1 mg/L BAP and 0.5 mg/L NAA + 0.5 mg/L BAP also demonstrated strong performance with high regeneration rates and various length for SL and RL. However, their estimated PVI values (ranging from approximately 365 to 390) were still lower than that of 0.5 mg/L NAA + 1.5 mg/L BAP. A high index indicates plant health and robustness and a strong seedling or plant development, efficient nutrient uptake and potential for survival under field conditions (19,28). Furthermore, It was observed that the vigour improvement is better for the selection of progenies (29). In this study, the PVI based on the treatments (0.5 mg/L NAA + 1.5 mg/L BAP) with a high vigour index promotes plantlet growth, making them suitable for large-scale propagation and stress resilience studies, especially to guide and promote callus and PR in somatic embryogenesis.

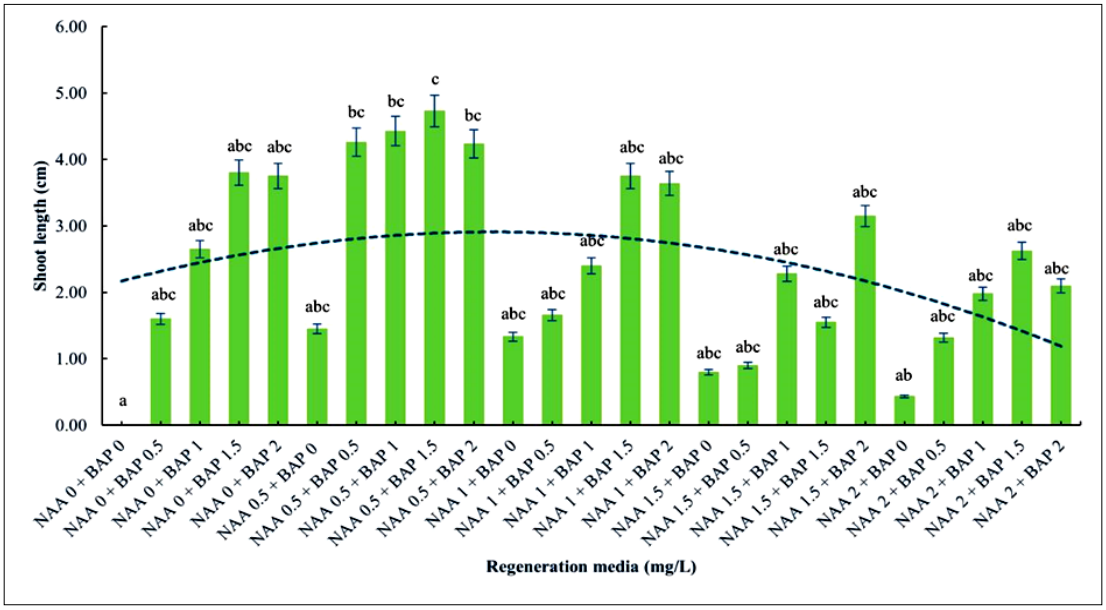

Fig. 6. Effect of NAA and BAP combinations on shoot length (cm) of regenerated plantlets. Means in the same column followed by the same lowercase letter are not significantly different based on Tukey's HSD test at the 95 % probability level

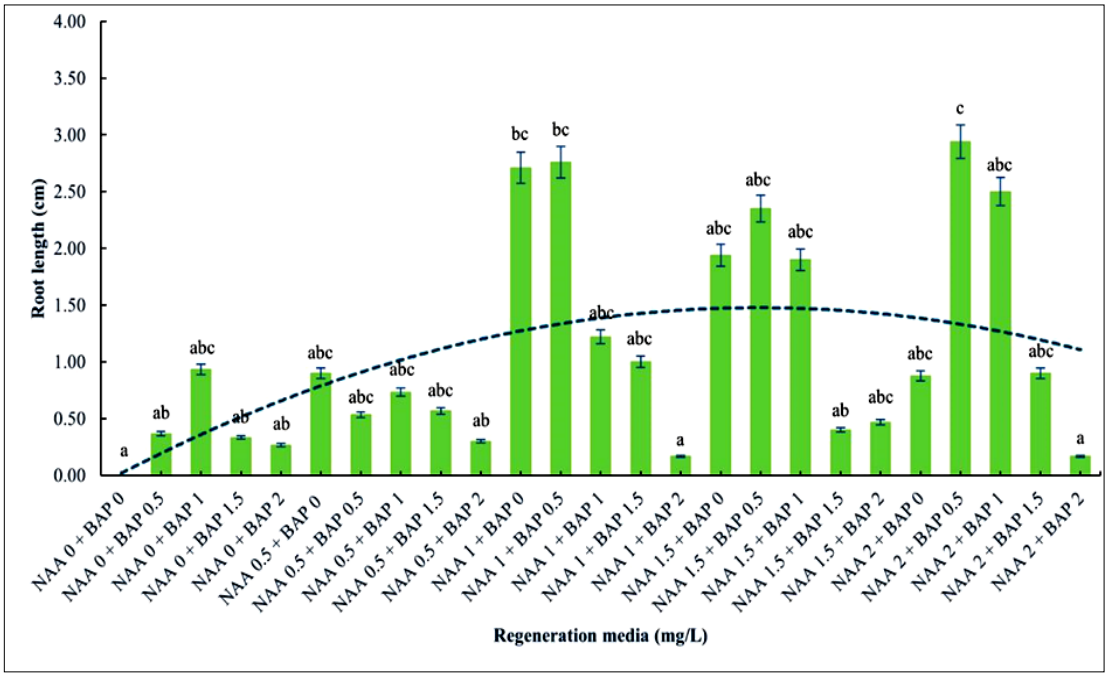

Fig. 7. Root length (cm) of plantlets regenerated under different NAA and BAP combinations. Means in the same column followed by the same lowercase letter are not significantly different based on Tukey's HSD test at the 95 % probability level

Conclusion

In conclusion, morphogenic callus formation was more successfully induced by 2,4-D at concentrations of 3 mg/L and 4 mg/L, producing friable and white-creamy calli. Callus induced with 3 mg/L showed the highest regeneration percentage (77.33 %), when cultured on a regeneration medium (NAA + BAP). Specifically, combination of 0.5 mg/L NAA + 1.5 mg/L BAP is highly recommended for PR. This combination led to the highest PVI (around 456), including regeneration frequency (83 %), shoot elongation (4.74 cm ± 0.32) and RL (0.57 cm ± 0.32). These results can be used to improve tissue culture procedures, especially for large-scale propagation and further developmental research.

Acknowledgements

The authors are grateful to Yulidar, technician who assisted the authors and all scientists involved in the Radiation Technology for Agricultural Productivity and Quality Enhancements (RADIANT) program, for their advice and support. This research was partially funded by the Research Organization for Agriculture and Food, National Research and Innovation Agency (BRIN).

Authors' contributions

MHS carried out the experiments, analysis and visualization. ID was responsible for conceptualization, methodology and interpretation. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used PDFgear to help find appropriate journals and to chat for inquiries or information and used Grammarly to clarify and correct grammar. After using these tools/services, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

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