

INTERACTIVE EFFECTS OF ORGANIC EXTRACTS AND BAP CONCENTRATION ON IN VITRO GROWTH AND DEVELOPMENT OF BANANA EXPLANTS

Samanhudi^{1,2*}, Sri Hartati^{1,2}, Muji Rahayu¹, Iswahyudi¹, Endang Setia Muliawati¹,
Candra Lusi Novitasari¹ and Hassan Bashir³

¹Department of Agrotechnology, Faculty of Agriculture, Universitas Sebelas Maret (UNS),
Jl. Ir. Sutami 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia

²Center for Research and Development of Biotechnology and Biodiversity, Universitas Sebelas Maret (UNS),
Jl. Ir. Sutami 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia

³Department of Agricultural Science, Faculty of Agriculture, Universitas Sebelas Maret (UNS),
Jl. Ir. Sutami 36A, Kentingan, Surakarta, 57126, Central Java, Indonesia

*Corresponding author: samanhudi@staff.uns.ac.id

ABSTRACT

The efficiency of banana (*Musa acuminata* L. cv. Barangan) micropropagation depends heavily on the composition of the culture medium, particularly the type and concentration of cytokinins and supplementary organic additives. This study evaluated the interactive effects of three organic extracts—shallot (*Allium cepa* Aggregatum Group), mung bean sprout (taoge; *Vigna radiata*), and tomato (*Solanum lycopersicum*)—and four concentrations of 6-benzylaminopurine (BAP; 0.0, 1.5, 2.0, and 2.5 mg L⁻¹) on in vitro growth and development of banana explants. A completely randomized design (CRD) with a 3 × 4 factorial arrangement was employed, with three replications per treatment and one explant per culture vessel. Data were analyzed using two-way analysis of variance (ANOVA), and treatment responses were further examined through interaction and dose–response visualization, Pearson correlation analysis, and principal component analysis (PCA). BAP concentration exerted a dominant effect on root-related traits, significantly delaying root emergence ($P < 0.001$) and reducing root number and root length at higher concentrations. Shoot number and days to shoot emergence were influenced by significant extract × BAP interactions ($P < 0.05$), indicating extract-dependent modulation of cytokinin response. Leaf number and fresh weight increase exhibited high variability and were not significantly affected by treatments. Correlation and PCA revealed strong associations among shoot, root, and biomass traits, with developmental timing traits showing partial trade-offs with growth vigor. These results demonstrate that BAP concentration is the primary regulator of in vitro growth dynamics in banana, while organic extracts modulate shoot responses in an interaction-dependent manner, providing useful information for optimizing banana micropropagation protocols.

Keywords: Banana micropropagation, *Musa acuminata*, 6-benzylaminopurine, Organic extracts, In vitro culture, Multivariate analysis.

Article History: ABR-26-045 || Received: 17-Feb-2026 || Revised: 15-Aug-2026 || Accepted: 27-Sep-26 || Published Online: 20-Oct-2026

This is an open-access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. INTRODUCTION

Banana (*Musa* spp.) ranks among the most important fruit crops worldwide, serving as a major source of food, income, and nutrition across tropical and subtropical regions (Prabowo et al., 2018; da Mata et al., 2024; Tripathi et al., 2024; Ullah et al., 2025; Woeisan et al., 2026; Samanhudi et al., 2026). Large-scale production of healthy, uniform planting material is essential to sustain banana cultivation, especially under biotic and abiotic constraints (Setyawati et al., 2023). In vitro propagation has therefore become a widely adopted technique for rapidly multiplying disease-free banana plants (Chukwu et al., 2025; Rohith et al., 2025). The efficiency of micropropagation protocols, however, depends strongly on culture medium composition, especially the type and concentration of plant growth regulators (Samanhudi et al., 2021a).

Cytokinins regulate cell division, shoot initiation, and morphogenesis in tissue culture systems (Akhiriana et al., 2019; Mardhikasari et al., 2020; Samanhudi et al., 2022; Talitha et al., 2022). Among them, 6-benzylaminopurine (BAP) is one of the most commonly used cytokinins for banana micropropagation because of its effectiveness in promoting shoot proliferation (Purwanto et al., 2023; Hardarani et al., 2024; Singh et al., 2024). BAP concentration must nonetheless be carefully optimized, as excessive levels can delay organ initiation, suppress root development, and induce abnormal growth (Rachmi et al., 2020; Bhakta et al., 2022). The response to BAP also varies depending on

Citation: Samanhudi, Hartati, S., Rahayu, M., Iswahyudi, Muliawati, E.S., Novitasari, C.L., & Bashir, H. (2026). Interactive effects of organic extracts and BAP concentration on in vitro growth and development of banana explants. *Agrobiological Records*, 138-147. <https://doi.org/10.47278/journal.abr/2026.089>

explant type, genotype and the presence of additional organic components in the culture medium.

In recent years, natural organic extracts have attracted increasing attention as supplementary components in plant tissue culture media. Extracts from shallot (Rugayah et al., 2021), mung bean sprouts (taoge) (Itsaini & Anggarani, 2025) and tomato (Sohrabi et al., 2023; Setyawati et al., 2024) reportedly contain endogenous phytohormones, vitamins, amino acids, and other bioactive compounds that may improve in vitro growth. Such extracts can partially substitute or modulate synthetic growth regulators, offering a more cost-effective and biologically balanced alternative (Mardhikasari et al., 2020; Samanhudi et al., 2020). Responses to organic extracts, however, are often inconsistent and highly dependent on their interactions with the applied cytokinins.

Despite the growing use of organic additives in tissue culture, limited information exists on how different organic extracts interact with varying BAP concentrations to influence multiple developmental and growth traits simultaneously. Most previous studies focus on a narrow set of parameters, such as shoot number or rooting percentage, without considering broader trait interrelationships or multivariate growth responses. A more comprehensive evaluation integrating developmental timing, shoot and root architecture, biomass accumulation, and their interrelationships is therefore needed.

The present study aimed to evaluate the interactive effects of three organic extracts (shallot, taoge, and tomato) and different concentrations of BAP on in vitro growth and development of banana (*Musa acuminata* L. cv. Barangan) explants. Multiple traits related to shoot emergence, root initiation, leaf development, shoot and root proliferation, root length, and fresh weight increase were measured. In addition to univariate analyses, interaction patterns, dose-response trends, trait correlations, and multivariate relationships were examined to provide an integrated assessment of explant responses. The results of this study contribute to an improved understanding of how organic extracts and cytokinin concentrations jointly influence in vitro growth dynamics and may support the optimization of banana micropropagation protocols.

2. MATERIALS AND METHODS

2.1. Plant Material and Explant Preparation

Banana (*Musa acuminata* L. cv. Barangan, AAA genome group) planlets were obtained from Kebun Benih Hortikultura Salaman, Magelang, Central Java, Indonesia. Planlets had been maintained through previous subculture cycles on basal Murashige and Skoog (MS) medium without plant growth regulators (MS₀). Explants used in this experiment were three-week-old subculture-derived shoot tips excised from actively growing shoots under aseptic conditions in a laminar airflow cabinet. Prior to inoculation, explants were cleaned by removing older roots, leaves, and any blackened or necrotic tissue from the base, then trimmed to a uniform size. Each explant was weighed individually at the time of inoculation to calculate the increase in fresh weight at the end of the culture period.

2.2. Preparation of Organic Extracts

Three organic extracts were prepared from fresh plant materials: shallot (*Allium cepa* Aggregatum Group), mung bean sprouts (taoge; *Vigna radiata*), and tomato (*Solanum lycopersicum*). Fresh tissues were washed thoroughly with tap water, weighed, and homogenized with sterile distilled water at a 1:1 (w/v) ratio using a sterile blender. The resulting slurry was filtered through sterile filter cloth to remove solid residues, yielding aqueous extracts. Extracts were prepared fresh on the day of medium preparation and incorporated into the medium at a concentration of 50 mL L⁻¹ for all three extract types. The extracts were added to the medium prior to autoclaving.

2.3. Culture Medium and Treatment Combinations

Full-strength Murashige and Skoog (MS) basal medium was supplemented with 30 g L⁻¹ sucrose and solidified with 8 g L⁻¹ agar. The pH of the medium was adjusted to 5.8–6.3 using 1 N NaOH or 1 N HCl before adding agar. The medium was heated until boiling, dispensed into culture bottles (approximately 25 mL per bottle), and sterilized by autoclaving at 121°C and 1.5 kg cm⁻² pressure for 30 minutes. Organic extracts and BAP stock solutions were incorporated into the medium before autoclaving.

The experiment followed a completely randomized design (CRD) with a factorial arrangement of two factors: organic extract type (three levels: taoge, shallot, and tomato) and BAP concentration (four levels: 0.0, 1.5, 2.0, and 2.5 mg L⁻¹). This resulted in 12 treatment combinations. Each treatment was replicated three times, with one explant per culture bottle serving as the experimental unit, yielding a total of 36 experimental units.

2.4. Culture Conditions and Data Collection

Inoculated cultures were maintained in a growth room at 20–25°C under continuous illumination (24-hour photoperiod) at approximately 61 lux, with a relative humidity of approximately 51%. Culture bottles were positioned on shelving racks and periodically sprayed with ethanol to minimize external contamination. Explants were observed daily for developmental timing traits: days to shoot emergence (first visible shoot ≥3 mm from the base), days to root emergence (first visible root ≥3 mm), and days to leaf emergence (first fully opened leaf ≥5 mm). At the end of the

culture period, the following growth parameters were recorded: shoot number, root number, leaf number, root length (cm; measured using thread laid alongside the root and then measured with a ruler), and fresh weight increase (g; calculated as the difference between final planlet weight and initial explant weight). Initial explant weight was determined indirectly as the difference between the weight of the culture bottle with medium plus the explant and the weight of the bottle with medium alone.

2.5. Statistical Analysis

Data were analyzed using Python (version 3.x). Descriptive statistics (mean $\pm$ SD) were calculated for all traits across treatment combinations. Two-way analysis of variance (ANOVA) was performed to evaluate the effects of organic extract type, BAP concentration, and their interaction on each trait. Prior to ANOVA, data were assessed for normality (Shapiro–Wilk test) and homogeneity of variance (Levene’s test); where assumptions were not met, this was noted as a limitation. Treatment responses were further examined using graphical visualization of interaction and dose–response patterns, Pearson correlation analysis among all trait pairs, and principal component analysis (PCA) to identify multivariate growth patterns. Given the small sample size ($n = 3$ per treatment), statistical power was limited for some traits; this constraint is acknowledged and discussed.

3. RESULTS

3.1. Descriptive Statistics and ANOVA

Mean $\pm$ SD for all eight traits across extract and BAP treatments are presented in Table 1. The two-way ANOVA revealed that days to shoot emergence was significantly affected by the extract $\times$ BAP interaction ($p = 0.047$), whereas neither organic extract type nor BAP concentration alone had a significant main effect on this trait. Days to root emergence showed a highly significant main effect of BAP concentration ($P < 0.001$), with mean values increasing from 7.56 days at 0.0 mg L⁻¹ BAP to 27.67 days at 2.5 mg L⁻¹, but no significant interaction was detected. Days to leaf emergence were not significantly affected by any treatment factor. Shoot number showed a significant extract $\times$ BAP interaction ($p = 0.043$). Root number declined with increasing BAP concentration (main effect $P < 0.05$), falling from a grand mean of 43.22 at 0.0 mg L⁻¹ to 26.56 at 2.5 mg L⁻¹. Root length followed a similar decreasing trend. Leaf number and fresh weight increase showed no significant main effects or interaction effects, although numerical differences among treatments were evident.

Table 1: Mean $\pm$ SD of in vitro growth parameters of banana (*Musa acuminata* L. cv. Barangan) explants cultured under different organic extracts and BAP concentrations ($n = 3$ per treatment)

Extract	BAP (mg L ⁻¹)	Days to shoot	Days to root	Days to leaf	Shoot number	Root number	Leaf number	Root length (cm)	Fresh weight increase (g)
Taoge	0.0	5.33 $\pm$ 1.15	7.67 $\pm$ 2.08	11.33 $\pm$ 2.08	3.00 $\pm$ 1.73	34.67 $\pm$ 13.65	12.33 $\pm$ 5.77	18.20 $\pm$ 1.06	1.41 $\pm$ 0.89
	1.5	4.67 $\pm$ 2.08	7.00 $\pm$ 0.00	10.67 $\pm$ 4.16	3.00 $\pm$ 3.46	28.67 $\pm$ 9.87	10.00 $\pm$ 7.81	11.27 $\pm$ 9.63	1.51 $\pm$ 0.62
	2.0	6.67 $\pm$ 2.89	12.67 $\pm$ 2.89	17.00 $\pm$ 5.00	9.00 $\pm$ 9.64	25.00 $\pm$ 8.89	16.67 $\pm$ 7.37	8.77 $\pm$ 8.35	1.93 $\pm$ 0.52
	2.5	17.67 $\pm$ 11.02	23.33 $\pm$ 21.57	29.33 $\pm$ 16.50	3.00 $\pm$ 3.46	22.33 $\pm$ 15.28	12.00 $\pm$ 12.12	3.40 $\pm$ 0.95	1.57 $\pm$ 0.81
Shallot	0.0	8.67 $\pm$ 2.89	6.33 $\pm$ 1.15	14.00 $\pm$ 3.00	2.33 $\pm$ 0.58	40.00 $\pm$ 10.39	12.33 $\pm$ 2.52	25.87 $\pm$ 5.82	1.64 $\pm$ 0.43
	1.5	12.00 $\pm$ 9.17	8.67 $\pm$ 3.21	20.00 $\pm$ 9.85	2.00 $\pm$ 1.00	35.33 $\pm$ 20.65	9.67 $\pm$ 4.04	14.53 $\pm$ 7.00	1.59 $\pm$ 0.62
	2.0	6.00 $\pm$ 1.00	21.00 $\pm$ 10.54	12.33 $\pm$ 0.58	6.00 $\pm$ 1.73	35.33 $\pm$ 11.68	25.00 $\pm$ 6.56	18.63 $\pm$ 14.21	2.16 $\pm$ 0.21
	2.5	5.33 $\pm$ 0.58	35.00 $\pm$ 15.62	11.00 $\pm$ 3.61	12.67 $\pm$ 5.51	30.00 $\pm$ 4.36	29.33 $\pm$ 8.33	17.00 $\pm$ 6.02	2.72 $\pm$ 0.16
Tomato	0.0	7.33 $\pm$ 3.51	8.67 $\pm$ 3.06	16.67 $\pm$ 3.21	6.33 $\pm$ 5.13	55.00 $\pm$ 13.08	21.00 $\pm$ 10.15	28.70 $\pm$ 12.44	1.91 $\pm$ 0.59
	1.5	4.67 $\pm$ 1.15	10.00 $\pm$ 5.57	13.33 $\pm$ 6.03	5.00 $\pm$ 4.00	37.33 $\pm$ 21.22	16.67 $\pm$ 17.67	19.87 $\pm$ 12.04	1.78 $\pm$ 0.64
	2.0	8.33 $\pm$ 3.79	11.67 $\pm$ 3.21	21.67 $\pm$ 9.02	1.67 $\pm$ 0.58	41.67 $\pm$ 22.74	9.33 $\pm$ 2.89	14.80 $\pm$ 16.86	1.39 $\pm$ 0.41
	2.5	8.33 $\pm$ 5.77	24.67 $\pm$ 9.71	23.00 $\pm$ 10.15	6.00 $\pm$ 1.00	27.33 $\pm$ 33.08	18.33 $\pm$ 8.50	10.03 $\pm$ 11.26	2.27 $\pm$ 0.28

3.2. Interaction Effect Analysis (Extract $\times$ BAP)

Interaction plots for all traits are shown in Fig. 1. For days to shoot emergence, the interaction patterns differed among extracts. Under taoge extract, mean days to shoot emergence increased sharply from 6.67 days at 2.0 mg L⁻¹ BAP to 17.67 days at 2.5 mg L⁻¹, whereas shallot actually showed a decline at higher BAP levels (from 8.67 days at 0.0 mg L⁻¹ to 5.33 days at 2.5 mg L⁻¹), and tomato extract showed relatively stable values across the BAP range. Days to root emergence displayed largely parallel trends across extracts, with mean values increasing consistently at higher BAP concentrations for all three extracts, reaching 35.00, 23.33, and 24.67 days at 2.5 mg L⁻¹ BAP for shallot, taoge, and tomato, respectively. Shoot number exhibited non-parallel interaction patterns: under shallot extract, shoot number increased from 2.33 at 0.0 mg L⁻¹ to 12.67 at 2.5 mg L⁻¹ BAP, while tomato extract showed an opposite trend with shoot number declining from 6.33 at 0.0 mg L⁻¹ to 1.67 at 2.0 mg L⁻¹. Root number and root length decreased with increasing BAP across all extracts. Interaction patterns for days to leaf emergence, leaf number, and fresh weight increase were less structured, with overlapping trends among extract-BAP combinations.

3.3. Dose–response Patterns of BAP Concentration

Across traits, mean responses showed clear dose-dependent trends with increasing BAP concentration (Fig. 2). Root-related traits exhibited strong monotonic responses: mean days to root emergence increased from 7.56 days at 0.0 mg L⁻¹ BAP to 27.67 days at 2.5 mg L⁻¹, while mean root length declined from 24.26 cm to 10.14 cm across the same concentration range. Root number similarly decreased from 43.22 to 26.56 per explant with increasing BAP. Shoot-related traits showed more variable, non-linear responses. Mean shoot number increased from 3.89 at 0.0 mg L⁻¹ to 7.22 at 2.5 mg L⁻¹, with greater variability at higher concentrations. Fresh weight increase showed a gradual numerical increase from 1.66 g at 0.0 mg L⁻¹ to 2.19 g at 2.5 mg L⁻¹, although variability among treatments was high.

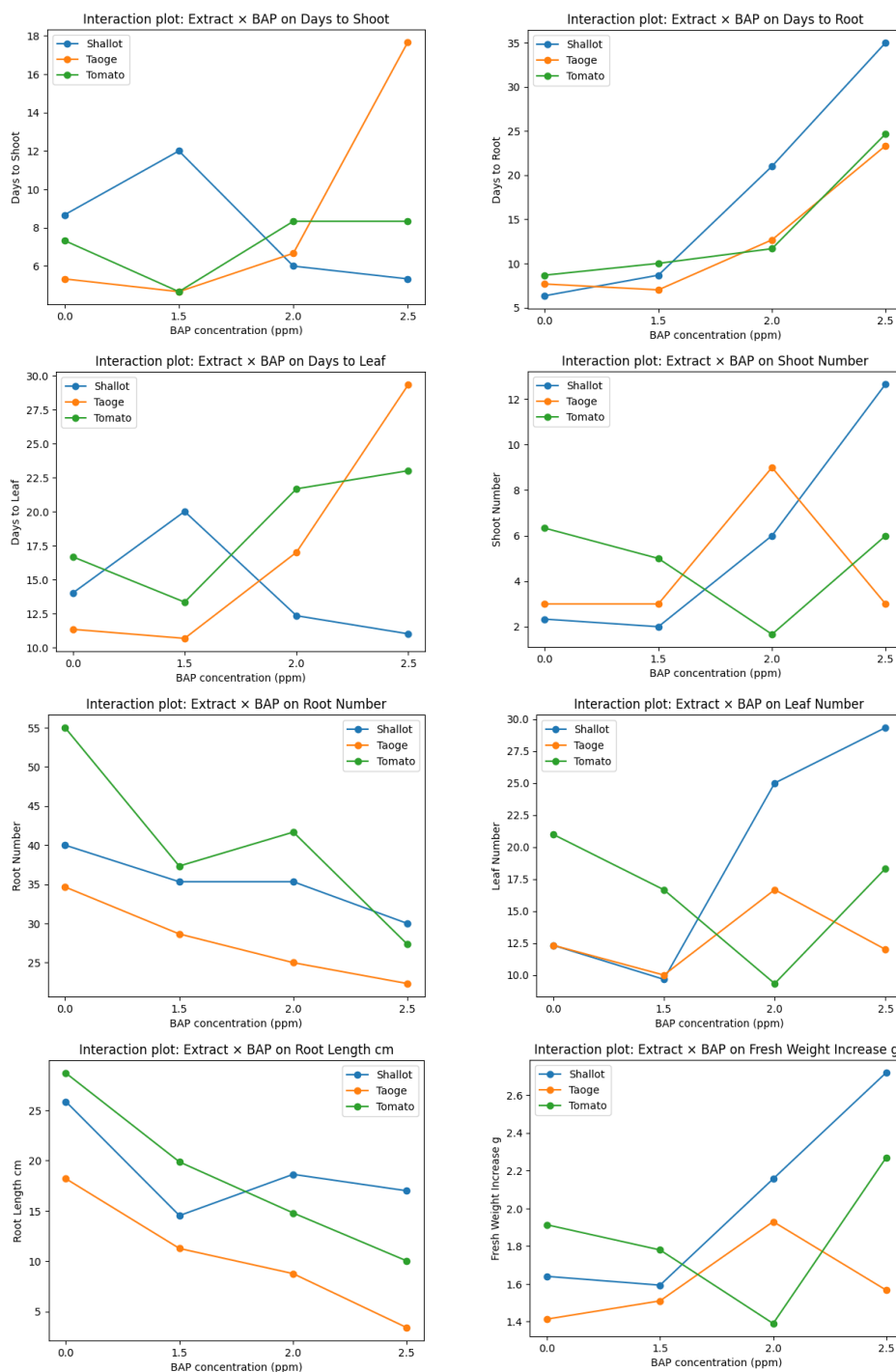


Fig. 1: Interaction of organic extracts and BAP concentration on in vitro growth traits of banana. Panels show extract × BAP interaction plots (left) and BAP main-effect plots (right) for each trait.

3.4. Correlation and Principal Component Analysis

Correlation analysis revealed several strong associations among traits (Fig. 3). Days to shoot emergence was strongly correlated with days to leaf emergence ($r = 0.82$). Shoot number showed strong positive correlations with leaf

number ($r = 0.75$) and fresh weight increase ($r = 0.73$). Root number was positively correlated with root length ($r = 0.71$). Negative correlations were observed between developmental timing traits and growth traits; for example, days to shoot emergence showed negative correlations with shoot number ($r = -0.24$) and fresh weight increase ($r = -0.23$).

Principal component analysis explained 68.56% of the total variation in the first two components (PC1 = 40.03%, PC2 = 28.53%; Fig. 4). PC1 was primarily associated with shoot number, leaf number, root number, root length, and fresh weight increase, while PC2 was associated mainly with developmental timing traits. Individual explant scores overlapped across extract types with no distinct clustering by organic extract, whereas dispersion along PC1 and PC2 reflected variation associated with BAP concentration and overall growth responses.

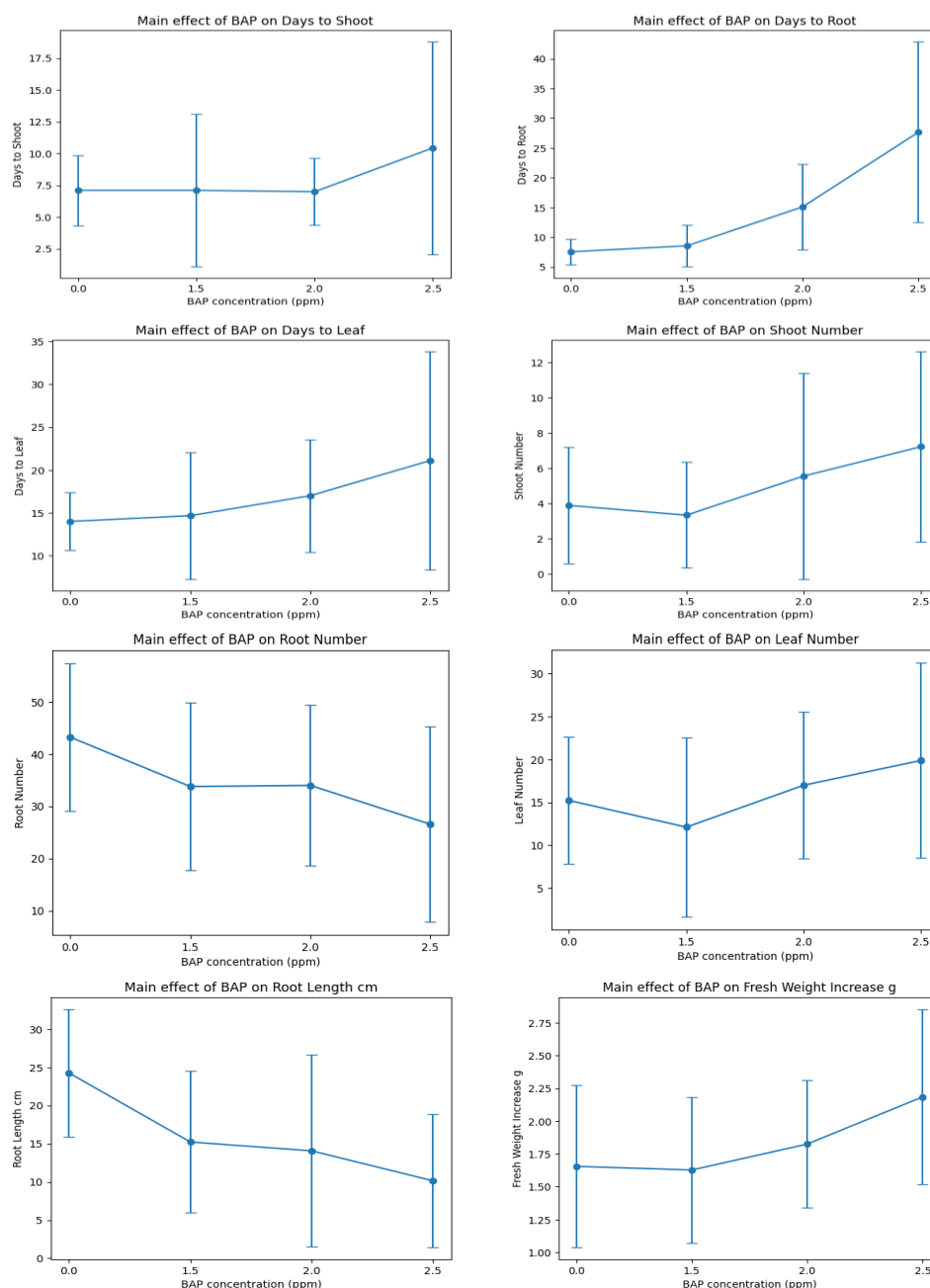


Fig. 2: Main effects of BAP concentration on in vitro growth traits of banana. Points represent treatment Error bars represent mean $\pm$ SD ($n = 3$).

4. DISCUSSION

4.1. Dominant Role of BAP in Regulating Root Development

Across all analytical approaches, BAP concentration emerged as the primary determinant of root-related traits. Increasing BAP levels delayed root emergence, reduced root number, and shortened root length (Plihal et al., 2013;

Nazir et al., 2022). This pattern is well documented in banana and other monocot tissue culture systems, where elevated cytokinins antagonize auxin-mediated root primordia formation, thereby suppressing root system development (Cárdenas-Aquino et al., 2023; Chai et al., 2024). The parallel dose-response trends observed across all three organic extracts further indicate that the endogenous bioactive compounds present in shallot, taoge, and tomato extracts were insufficient to counteract the inhibitory influence of high BAP on rooting. A comparable dominance of cytokinin dosage over organic additives has been reported in banana micropropagation systems supplemented with coconut water and various plant extracts (Mardhikasari et al., 2020; Dzhumanazarova et al., 2024; Mekawy et al., 2025).

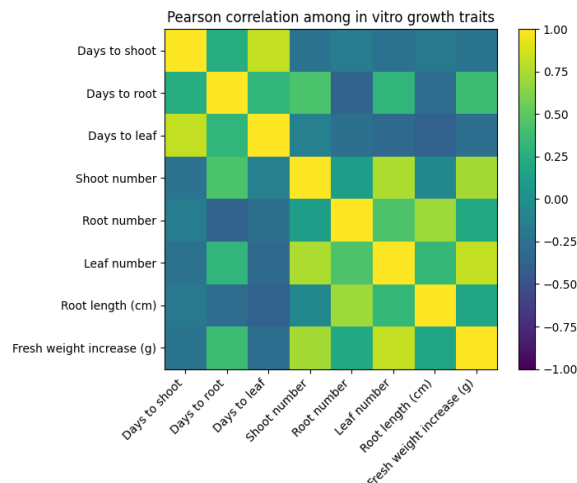


Fig. 3: Pearson correlation matrix showing relationships among eight in vitro growth and developmental traits of banana.

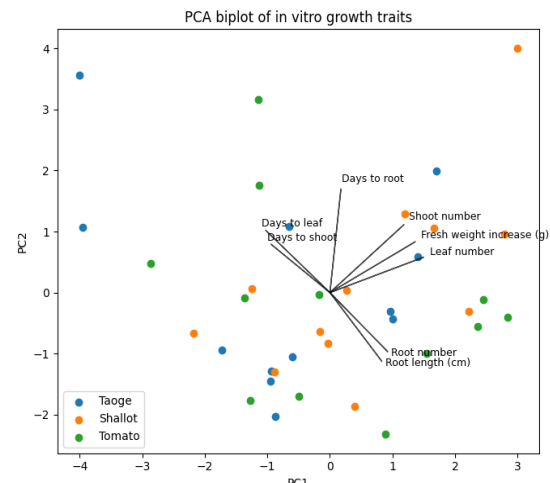


Fig. 4: Principal component analysis (PCA) biplot summarizing multivariate variation among banana explants based on eight in vitro growth and developmental traits.

4.2. Extract-dependent Modulation of Shoot Proliferation

In contrast to root traits, shoot-related parameters were more strongly influenced by extract $\times$ BAP interactions. Shoot number increased under shallot extract at higher BAP concentrations, while responses under taoge and tomato extracts were either variable or showed the opposite trend (decreasing shoot number with increasing BAP under tomato). Such interaction-dependent responses indicate that organic extracts modulate cytokinin effectiveness rather than acting as independent growth stimulants (Grzegorzczak-Karolak et al., 2015; Al Ibrahim et al., 2025). Plant-derived organic supplements contain endogenous cytokinins, vitamins, sugars, and amino acids that may either enhance or buffer hormonal signaling depending on concentration and tissue sensitivity (Pramesthi et al., 2025; Smýkalová et al., 2025). Several earlier studies have reported enhanced shoot multiplication in banana when BAP is combined with organic supplements such as banana homogenate, tomato extract, or onion extract, although the magnitude of response is typically extract-specific and genotype-dependent (Oumar et al., 2017; Bhakta et al., 2022; Narwade et al., 2024). The non-parallel interaction patterns observed here are consistent with those reports and highlight the importance of evaluating organic additives within specific cytokinin regimes rather than in isolation.

4.3. Developmental Timing and Growth Trade-offs

Developmental timing traits, including days to shoot and leaf emergence, showed weaker and more variable responses than structural growth parameters (Hairuddin & Mawarini, 2021; Arief et al., 2025). The strong positive correlation between shoot and leaf emergence times indicates coordinated developmental progression (Yuniastuti et al., 2018; Samanhudi et al., 2021b; Muthi'ah et al., 2023), while negative correlations between developmental timing and traits such as shoot number and fresh weight increase suggest a trade-off between early establishment and subsequent growth vigor. Similar trade-offs have been reported in banana tissue culture, where rapid shoot initiation tends to be associated with higher multiplication rates and biomass accumulation (Hairuddin & Mawarini, 2021; Arief et al., 2025). The delayed emergence observed at higher BAP concentrations, particularly under taoge extract at 2.5 mg L^{-1} , may reflect hormonal imbalance caused by excessive cytokinin disrupting meristem organization and delaying organ differentiation.

4.4. Leaf Development and Biomass Accumulation

Leaf number and fresh weight increase did not show significant main or interaction effects, despite strong correlations with shoot number (Mia et al., 2025). This finding indicates that leaf production and biomass

accumulation were primarily driven by shoot proliferation rather than by direct regulation of BAP concentration or extract type. Comparable results have been reported in banana micropropagation studies, in which biomass accumulation tracked shoot density rather than hormone dosage per se (Kumari et al., 2015; Basiri et al., 2022; Al-Ali et al., 2023; Mia et al., 2025). The high variability observed for these traits likely reflects internal physiological status and explant vigor once shoot initiation has occurred. Such variability is common in tissue culture systems with small replication ($n = 3$), and this limitation should be considered when interpreting non-significant effects.

4.5. Multivariate Integration of Growth Responses

PCA provided an integrated view of treatment effects by condensing complex trait relationships into two principal axes. The first component represented overall growth vigor, with heavy loadings on shoot number, root traits, and increases in fresh weight, whereas the second component captured variation in developmental timing. Similar multivariate structuring has been reported in tissue culture studies where PCA was used to disentangle hormonal and nutritional effects on growth (AlGhamdi et al., 2015; Samanhudi et al., 2020). The substantial overlap among treatments in PCA space confirms that organic extract type alone did not produce distinct growth syndromes; instead, BAP concentration was the dominant driver of overall explant performance, consistent with the univariate ANOVA results. These observations reinforce the value of multivariate approaches for validating and contextualizing univariate findings in tissue culture research.

4.6. Implications for Banana Micropropagation

From a practical standpoint, the results highlight the need to carefully optimize BAP concentration when incorporating organic extracts into banana micropropagation protocols. Although organic supplements can boost shoot proliferation under certain conditions, excessive cytokinin levels consistently impair root development, which is critical for subsequent acclimatization and field establishment (Samanhudi et al., 2021a; Samanhudi et al., 2024). The interaction-dependent nature of shoot responses further suggests that organic additives should be selected and tested in combination with specific hormone regimes rather than treated as universal growth enhancers.

4.7. Limitations

Several limitations of this study should be acknowledged. The replication level ($n = 3$ per treatment) limited statistical power, particularly for traits with high variability. The sterilization of organic extracts by autoclaving may have degraded heat-sensitive bioactive compounds, potentially attenuating extract effects. Additionally, the use of a 24-hour continuous photoperiod differs from the more common 16/8 h light/dark cycle used in many banana tissue culture protocols, and this may have influenced developmental responses. Future studies should consider higher replication, filter-sterilization of organic extracts, and evaluation under standard photoperiod regimes.

5. CONCLUSION

In vitro growth and development of banana (*Musa acuminata* L. cv. Barangan) explants were primarily governed by BAP concentration, with root-related traits showing strong dose-dependent inhibition at higher cytokinin levels. Organic extracts alone did not exert dominant effects but significantly modulated shoot responses through interaction with BAP, particularly influencing shoot proliferation and shoot emergence timing. Developmental timing, shoot growth, root architecture, and biomass accumulation were closely interrelated, as revealed by correlation and principal component analyses. Excessive BAP consistently suppressed root development regardless of extract type, underscoring the need for careful cytokinin optimization when organic supplements are incorporated into culture media. The integrated analytical approach adopted in this study provides a more comprehensive understanding of explant responses than conventional single-trait analyses and supports the rational optimization of banana micropropagation protocols through balanced use of synthetic growth regulators and organic additives.

Declarations

Funding: The Directorate of Research and Community Service, Ministry of Higher Education, Science, and Technology, supported this research through the Applied Research (Penelitian Terapan) scheme (Contract No.: 1186.1/UN27.22/PT.01.03/2025), fiscal year 2025.

Acknowledgement: The authors thank the staff of the Laboratory of Plant Physiology and Biotechnology, Faculty of Agriculture, Universitas Sebelas Maret, for technical assistance during the experimental work.

Conflicts of Interest: All authors declare that they do not have any potential conflict of interest.

Data Availability: All the data is available in the article.

Citation: Samanhudi, Hartat, S., Rahayu, M., Iswahyudi, Muliawati, E.S., Novitasari, C.L., & Bashir, H. (2026). Interactive effects of organic extracts and BAP concentration on in vitro growth and development of banana explants. *Agrobiological Records*, 138-147. <https://doi.org/10.47278/journal.abr/2026.089>

Ethics Statement: This study involved no experiments with humans or animals; therefore, ethical approval was not required.

Author's Contribution: Samanhudi: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing; Sri Hartati: Supervision, Resources, Writing – review & editing; Muji Rahayu: Supervision, Methodology, Resources; Iswahyudi: Supervision, Resources; Endang Setia Muliawati: Supervision, Methodology, Resources; Candra Lusi Novitasari: Investigation, Methodology, Data curation, Writing – original draft; Hassan Bashir: Formal analysis, Visualization, Writing – original draft, Writing – review & editing.

Generative AI Statement: The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

Publisher's Note: All claims stated in this article are exclusively those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, and the reviewers. Any product that may be evaluated/assessed in this article or claimed by its manufacturer is not guaranteed or endorsed by the publisher/editors.

REFERENCES

- Akhiriana, E., Samanhudi, & Yunus, A. (2019). Coconut water and IAA effect on the in vitro growth of *Tribulus terrestris* L. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(1). <https://doi.org/10.1118/actaun201967010009>
- Al Ibrahim, T. B., Qomariah, U. K. N., & Nasirudin, M. (2025). The Effect of Concentration and Soaking Time on The Shallots Extract against the Growth of Stem Cuttings Dersono Guava (*Syzygium malaccense*). *AGRICUS: Advances Agriculture Science & Farming*, 5(2), 115-120. <https://doi.org/10.32764/agarius.v5i2.6199>
- Al-Ali, A. M., Dewir, Y. H., & Al-Obeed, R. S. (2023). Influence of cytokinins, dark incubation and air-Lift bioreactor culture on axillary shoot proliferation of Al-Taif rose (*Rosa damascena* tringintipetala (Diek) R. Keller). *Horticulturae*, 9(10), 1109. <https://doi.org/10.3390/horticulturae9101109>
- AlGhamdi, S., Leoncik, V., Plant, K. E., & Plant, N. J. (2015). Synergistic interaction between lipid-loading and doxorubicin exposure in Huh7 hepatoma cells results in enhanced cytotoxicity and cellular oxidative stress: implications for acute and chronic care of obese cancer patients. *Toxicology Research*, 4(6), 1479-1487. <https://doi.org/10.1039/C5TX00173K>
- Arief, N. H. A. Z., Rahayu, E. S., Pa'ee, F., & Habibah, N. A. (2025). Effect of BAP and Picloram on Shoot Induction (*Musa acuminata* Colla var. Mulu Bebek). *Biosaintifika: Journal of Biology & Biology Education*, 17(1), 107-117. <https://doi.org/10.15294/biosaintifika.v17i1.19605>
- Basiri, Y., Etemadi, N., Alizadeh, M., & Alizargar, J. (2022). In vitro culture of *Eremurus spectabilis* (Liliaceae), a rare ornamental and medicinal plant, through root explants. *Horticulturae*, 8(3), 202. <https://doi.org/10.3390/horticulturae8030202>
- Bhakta, S., Negi, S., Tak, H., Singh, S., & Ganapathi, T. R. (2022). MusaATAF2-like protein regulates shoot development and multiplication by inducing cytokinin hypersensitivity and flavonoid accumulation in banana plants. *Plant Cell Reports*, 41(5), 1197-1208. <https://doi.org/10.1007/s00299-022-02849-y>
- Cárdenas-Aquino, M. D. R., Camas-Reyes, A., Valencia-Lozano, E., López-Sánchez, L., Martínez-Antonio, A., & Cabrera-Ponce, J. L. (2023). The cytokinins BAP and 2-iP modulate different molecular mechanisms on shoot proliferation and root development in lemongrass (*Cymbopogon citratus*). *Plants*, 12(20), 3637. <https://doi.org/10.3390/plants12203637>
- Chai, L. C., Alderson, P. G., & Chin, C. F. (2024). Exogenous Cytokinin Induces Callus and Protocorm-Like-Bodies Formation in In Vitro Root Tips of *Vanilla planifolia* Andrews. *Tropical Life Sciences Research*, 35(1), 235. <https://doi.org/10.21315/tlsr2024.35.1.13>
- Chukwu, S.C., Awala, S.K., Angombe, S., Valombola, J.S., Nanhapo, P.I., Mberama, C., Rafii, M.Y., Oladosu, Y., Thomas, B., Okporie, E.O. and Musa, I., (2025). Recent progress in tissue culture techniques and biotechnological innovations for banana production (*Musa* spp.): a review. *Discover Plants*, 2(1), 13. <https://doi.org/10.1007/s44372-025-00099-2>
- da Mata, D. A., da Mata, D. A., de Souza Oliveira, V., Sousa, I. T., Porcino, M. M., da Silva, J. R. S., ... (all author are required) & da Silva, E. P. M. (2024). Agronomic perspective: exploring the diversity and potentialities of banana cultivation (*Musa* spp.). *Scientific Electronic Archives*, 17(4). <https://doi.org/10.36560/17420241952>
- Dzhumanazarova, A., Stozhko, N., Dzhorupbekova, D., Gutsalyuk, N., & Bukharinova, M. (2024). Biological preparations from crop waste for organic farming. In *E3S Web of Conferences* (Vol. 537, p. 10010). EDP Sciences. <https://doi.org/10.1051/e3sconf/202453710010>
- Grzegorzczak-Karolak, I., Kuźma, Ł., & Wysokińska, H. (2015). The effect of cytokinins on shoot proliferation, secondary metabolite production and antioxidant potential in shoot cultures of *Scutellaria alpina*. *Plant Cell, Tissue and Organ Culture* (PCTOC), 122(3), 699-708. <https://doi.org/10.1007/s11240-015-0804-5>
- Hairuddin, R., & Mawariani, M. (2021). Organogenesis of Cavendish Banana (*Musa acuminata* L.) Plant in Various Concentration of ZPT IAA (Indole Acid Acid) and BAP (Benzyl Amino Purine) in Invitro Culture. *Argotech Journal* Vol 6, No 1 June (2021), 6(1). <https://doi.org/10.31327/atj.v6i1.1563>

Citation: Samanhudi, Hartat, S., Rahayu, M., Iswahyudi, Muliawati, E.S., Novitasari, C.L., & Bashir, H. (2026). Interactive effects of organic extracts and BAP concentration on in vitro growth and development of banana explants. *Agrobiological Records*, 138-147. <https://doi.org/10.47278/journal.abr/2026.089>

- Hardarani, N., Karina, J., & Susanti, H. (2024). Pengaruh Konsentrasi BAP (Benzil Amino Purin) terhadap Multiplikasi Subkultur Tunas Pisang Kepok Timbatu. Daun: Jurnal Ilmiah Pertanian dan Kehutanan, 11(2), 125-140. <https://doi.org/10.33084/daun.v11i2.7857>
- Itsnaini, P. N., & Anggarani, M. A. (2025). Phenolic and Antioxidant Activity of Moringa-Bean Sprouts Extract as PGRs for Soybeans. Jurnal Pijar Mipa, 20(6), 1092-1097. <https://doi.org/10.29303/jpm.v20i6.9941>
- Kumari, M., Khalid, H., & Nasim, M. (2015). Growth parameter study in Camelina sativa, a potential biofuel crop, under in-vitro culture conditions. The Indian Journal of Agricultural Sciences, 85(8), 1055-1058. <https://doi.org/10.56093/ijas.v85i8.50844>
- Mardhikasari, S., Yunus, A., & Samanhudi, S. (2020). Modification of media for banana in vitro propagation with foliar fertilizer and coconut water in cv. Rajabulu. Caraka Tani: Journal of Sustainable Agriculture, 35(1), 23-32. <https://doi.org/10.20961/carakatani.v35i1.27756>
- Mekawy, A. A. I., Alqhtani, A. H., Ali, A., & Yosri, M. (2025). In vitro detection of antimicrobial impact, organic acids production and phytochemical screening of Musa acuminata wastes after treatment with fungi. Brazilian Journal of Biology, 85, e288774. <https://doi.org/10.1590/1519-6984.288774>
- Mia, M. A. S., Al Mamun, S. A., Masror, T. Z., Hossain, M. J., & Islam, M. M. (2025). Optimization of BAP concentration for in vitro mass multiplication of G9 and Agnishwar banana (Musa spp.) varieties. Plant Tissue Culture and Biotechnology, 35(1), 51-65. <https://doi.org/10.3329/ptcb.v35i1.81768>
- Muthi'ah, A., Sakya, A. T., Setyawati, A., Samanhudi, & Rahayu, M. (2023). Callus induction of Calotropis gigantea using BAP and 2, 4-D in vitro. In IOP Conference Series: Earth and Environmental Science (Vol. 1177, No. 1, p. 012021). IOP Publishing. <https://doi.org/10.1088/1755-1315/1177/1/012021>
- Narwade, A. V., Singh, M., Bisnae, K. D., & Modi, P. K. (2024). In vitro micropropagation of ten different Banana genotypes by using single in vitro culture media. International Journal of Agricultural Invention, 9(2), 172-177. <https://doi.org/10.46492/IJAI/2024.9.2.26>
- Nazir, U., Gul, Z., Shah, G. M., & Khan, N. I. (2022). Interaction effect of auxin and cytokinin on in vitro shoot regeneration and rooting of endangered medicinal plant Valeriana jatamansi Jones through tissue culture. American Journal of Plant Sciences, 13(2), 223-240. <https://doi.org/10.4236/ajps.2022.132014>
- Oumar, S., Kouassi, K. M., Koffi, K. E., Kouakou, K. E. P., & Aké, S. (2017). Effect of adenine sulphate, casein hydrolysate and spermidine on in vitro shoot multiplication of two banana varieties (FHIA-21 and PITA-3). African Journal of Biotechnology, 16(46), 2152-2159. <https://doi.org/10.5897/AJB2017.16247>
- Plíhal, O., Szüčová, L., & Galuszka, P. (2013). N9-substituted aromatic cytokinins with negligible side effects on root development are an emerging tool for in vitro culturing. Plant Signaling & Behavior, 8(6), e24392. <https://doi.org/10.4161/psb.24392>
- Prabowo, H., Samanhudi, Yuniastuti, E., & Yunus, A. (2018). Effects of media combination with concentration of AB-Mix nutrient on growth of banana shoots on in vitro. Bulgarian Journal of Agricultural Science, 24(3).
- Pramesthi, A. M., Az-Zahra, S., Tamidiyono, M. B., Salamah, U., Mafiroh, W. U., & Tunjung, W. A. (2025). In vitro seed germination and phytochemical content of kaffir lime (Citrus hystrix DC.) in response to auxin and cytokinin treatments. ScienceAsia, 51(1). <https://doi.org/10.2306/scienceasia1513-1874.2025.016>
- Purwanto, E., Arniputri, R.B., Handoyo, G.C., Yunus, A., Samanhudi, Purnomo, D., Sakya, A.T., Rahayu, M., Setyawati, A. and Brimantara, F., (2023). In vitro growth of Dendrobium stratiotes on various medium and growth regulator. In IOP Conference Series: Earth and Environmental Science (Vol. 1165, No. 1, p. 012019). IOP Publishing. <https://doi.org/10.1088/1755-1315/1165/1/012019>
- Rachmi, D., Samanhudi, & Purnomo, D. (2020). The Effectiveness of Fruit Extract and Temperature for In Vitro Culture of Kepok Banana (Musa balbisiana). In IOP Conference Series: Earth and Environmental Science (Vol. 466, No. 1, p. 012008). IOP Publishing. <https://doi.org/10.1088/1755-1315/466/1/012008>
- Rohith, E., Prakasha, D. P., Kavitha, R., Nagaraju, V., & Dasari, M. (2025). Influence of different plant growth regulators on in-vitro propagation and innovative technique for enhancing quality mass production in banana cultivation. SKUAST Journal of Research, 27(3), 438-444. <https://doi.org/10.5958/2349-297X.2025.00051.9>
- Rugayah, Karyanto, A., Ermawati & Suselawati, D., (2021). Pengaruh Pemberian Ekstrak Bawang Merah dan Kecambah dengan Pemberian Pupuk Cair Hayati terhadap Pertumbuhan Seedling Manggis (Garcinia mangostana L.). Jurnal Agrotropika, 20(2), 139-149. <https://doi.org/10.23960/ja.v20i2.5373>
- Samanhudi, Sakya, A. T., Setyawati, A., & Muawanah, M. (2022). Agarwood (Aquilaria malaccensis) diversity conservation by in vitro culture with IAA and yeast extract. Biodiversitas: Journal of Biological Diversity, 23(5). <https://doi.org/10.13057/biodiv/d230525>
- Samanhudi, Widijanto, H., Muliawati, E.S., Favreta, V.F., & Hidayanto, M., (2021a). The growth response of banana cv. Barangan (Musa acuminata L.) from tissue culture with organic manure and arbuscular mycorrhizal fungi. Indian Journal of Agricultural Research, 55(5), 597-602. <https://doi.org/10.18805/IJAR.E-633>
- Samanhudi, Widijanto, H., Tetrani, A.S., & Salsabill, S., (2024). Application of organic fertilizers and arbuscular mycorrhiza dosage to increase the growth of Kepok banana seedlings (Musa paradisiaca L.) derived from tissue culture. Australian Journal of Crop Science, 18(5), 253-258.
- Samanhudi, Yunus, A., & Rahmanto, R., (2021b). In vitro propagation of Tribulus terrestris with IAA and BAP concentrations. Journal of Biodiversity and Biotechnology 1(1): 17. <https://doi.org/10.20961/jbb.v1i1.50245>
- Samanhudi, H.S., Rahayu, M., Setyawati, A., Arniputri, R.B., Iswahyudi, W.I., Wulandari, I.R., & Bashir, H. (2026). Coconut water as a cost-effective alternative to synthetic cytokinins in banana micropropagation and optimization of auxin-cytokinin ratios. Agrobiological Records, 24, 60-73. <https://doi.org/10.47278/journal.abr/2026.025>

Citation: Samanhudi, Hartat, S., Rahayu, M., Iswahyudi, Muliawati, E.S., Novitasari, C.L., & Bashir, H. (2026). Interactive effects of organic extracts and BAP concentration on in vitro growth and development of banana explants. Agrobiological Records, 138-147. <https://doi.org/10.47278/journal.abr/2026.089>

- Setyawati, A., Samanhudi, Hartati, S., Gusniar, N., Fathin, T.S., & Prihanto, J., (2024). In vitro application of kinetin and tomato extract on orchid growth of *Dendrobium stratiotes*. *Annals of Biology*, 40(2), 211–218.
- Setyawati, A., Samanhudi, Prameswari, W., Syukri, D., Ramadhani, D.F., & Talitha, O., (2023). In vitro propagation and secondary metabolite production of medicinal plant of *Euchresta horsfieldii* (Lesch) Benn. *Plant Breeding and Biotechnology*, 11(1), 34–48. <https://doi.org/10.9787/PBB.2023.11.1.34>
- Singh, J., Sengar, R. S., Prasad, M., & Kumar, A. (2024). Effect of different plant growth regulators on in-vitro micropropagation of banana cultivar Grand Naine (*Musa* spp.). *Journal of Advances in Biology & Biotechnology*, 27(3), 90-98. <https://doi.org/10.9734/jabb/2024/v27i3724>
- Smykalová, I., Doležal, K., Plačková, L., Plíhalová, L., Zatloukal, M., & Griga, M. (2025). The effect of supplementing in vitro flax (*Linum usitatissimum* L.) culture media with a synthetic cytokinin derivative (BAP9 THP) and a cytokinin metabolism modulator (INCYDE). *Plant Growth Regulation*, 105(4), 1125–1138. <https://doi.org/10.1007/s10725-025-01326-2>
- Sohrabi, O., Hatamzadeh, A., Ghasemnezhad, A., Samizadeh, H., & Erfani-moghadam, V. (2023). Development of a novel protocol for enhanced tomato callus formation and growth through the use of medicinal plant extracts. PREPRINT (Version 1) available at Research Square. <https://doi.org/10.21203/rs.3.rs-3054989/v1>
- Talitha, O., Samanhudi, S., Setyawati, A., Rahayu, M., & Sakya, A. T. (2022). The effect of growth concentration on in vitro shoot multiplication of crown flower (*Calotropis gigantea*). *Plant breeding and biotechnology*, 10(4), 244-256. <https://doi.org/10.9787/PBB.2022.10.4.244>
- Tripathi, L., Ntui, V. O., & Tripathi, J. N. (2024). Application of CRISPR/Cas-based gene-editing for developing better banana. *Frontiers in bioengineering and biotechnology*, 12, 1395772. <https://doi.org/10.3389/fbioe.2024.1395772>
- Ullah, M.H., Azhar, A., Maqbool, T., Hamza, A., Khan, U.U., Aslam, P., Malik, B., Mubarak, E., Ghafoor, A., Shaukat, M., & Rasool, G. (2025). Eco-friendly synthesis of silicon nanoparticles from banana peel extract and their in vitro and in vivo potential in glioblastoma treatment. *Agrobiological Records*, 22, 150-162. <https://doi.org/10.47278/journal.abr/2025.057>
- Yuniastuti, E., Widodo, C. E., Samanhudi, & Delfianti, M. N. I. (2018). Effect of benzyl amino purine and indole-3-acetic acid on propagation of *Sterculia foetida* in vitro. In *IOP Conference Series: Earth and Environmental Science* (Vol. 142, No. 1, p. 012011). IOP Publishing. <https://doi.org/10.1088/1755-1315/142/1/012011>
- Woeisan, S., Promptong, N., Tubtimhin, S., & Limpiteeprakan, P. (2026). Comparative performance of mulching board materials made of banana stalks and sugarcane bagasse on Chinese kale growth. *International Journal of Agriculture and Biosciences*, 15(5), 2114-2122. <https://doi.org/10.47278/journal.ijab/2026.131>