



In Vitro Effects of Yeast Extract and Indole-3-Acetic Acid on Shoot Emergence and Height in Mas Kirana Banana

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Abstract

Mas Kirana banana (*Musa sp.*) is a superior local variety from Lumajang, East Java, valued for its favorable fruit size and consumer preference. Conventional propagation of this variety is limited due to its low sapling production (2–3 per clump), making large-scale cultivation inefficient. Tissue culture techniques offer an alternative approach to accelerate propagation. This study aimed to evaluate the effects of yeast extract and indole-3-acetic acid (IAA) on the in vitro growth of Mas Kirana banana explants. The experiment was arranged in a factorial completely randomized design (CRD) with two factors: yeast extract at four concentrations (0, 400, 800, and 1200 mg L⁻¹) and IAA at four concentrations (0, 0.75, 1.5, and 2.25 ppm). Growth responses were assessed based on shoot emergence, shoot height, root development, and leaf formation. Yeast extract significantly affected shoot emergence time; however, its effect was inhibitory rather than promotive. The control (0 mg L⁻¹) produced the fastest emergence (11 DAP), whereas higher yeast concentrations (400–1200 mg L⁻¹) delayed emergence to 13–22 DAP. IAA significantly influenced shoot height, with the best performance observed at 1.5 ppm, while other growth parameters remained unaffected. These findings indicate that the effectiveness of yeast extract and IAA is highly concentration-dependent, and their roles in improving micropropagation of Mas Kirana banana require further optimization. This study provides baseline information to support the development of more efficient tissue culture protocols for large-scale propagation of this essential local variety.

Keywords: Mas Kirana banana; tissue culture; yeast extract; indole-3-acetic acid (IAA); micropropagation

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Introduction

Bananas (*Musa spp.*) remain a crucial global fruit crop, particularly in Indonesia, where they significantly contribute to food security and rural livelihoods. In 2022, Indonesia produced approximately 9.3 million metric tons of bananas, ranking among the world's top producers (1). Among Indonesia's esteemed local cultivars, the Mas Kirana banana from Lumajang Regency in East Java is particularly valued for its ideal fruit size, which is convenient for single consumption yet satisfying in quality (2). However, conventional propagation via suckers remains inefficient, as

Mas Kirana plants produce a limited number of tillers (2–3 per clump), constraining both multiplication speed and dissemination (2).

To overcome these limitations, tissue culture offers a compelling alternative. Micropropagation enables the rapid production of high-quality, disease-free planting material, thereby enhancing crop uniformity and performance (3). Recent studies have reinforced its benefits: tissue-cultured banana plantlets show more consistent growth patterns, achieve earlier fruiting, and yield improvements compared to conventionally propagated

counterparts, all while reducing the need for chemical fertilizers and supporting sustainable agricultural practices (4).

Central to thriving tissue culture is the culture medium, which must supply macro- and micronutrients, vitamins, carbon sources, and plant growth regulators (PGRs) in balanced proportions. Among PGRs, auxins, notably indole-3-acetic acid (IAA), are essential for inducing callus, promoting root formation, and guiding organogenesis. Simultaneously, organic growth additives, such as yeast extract, can potentiate *in vitro* growth by supplying amino acids, peptides, vitamins, and growth-promoting compounds. Incorporating such additives has been shown to accelerate shoot proliferation and enhance overall plant vigor. Furthermore, recent reviews suggest that these bio-organic supplements may help maintain genetic fidelity in regenerated plants, as assessed by molecular markers such as RAPD, ISSR, SSR, and SCoT.

Although several studies have examined the independent effects of auxins (such as Indole-3-acetic acid, IAA) or organic additives (such as yeast extract) on *in vitro* plant growth, no prior research has investigated their combined effects on the micropropagation of Mas Kirana banana, a valuable yet under-propagated local cultivar. This study is the first to explore the interaction between yeast extract and IAA in influencing shoot emergence, shoot height, and other growth parameters in this cultivar.

We hypothesized that combining yeast extract and IAA would synergistically enhance the early growth responses of Mas Kirana banana explants *in vitro*. Clarifying their combined effects could provide a basis for developing more efficient, cost-effective, and sustainable tissue culture protocols for large-scale propagation.

Therefore, this study aimed to evaluate the interaction between yeast extract and IAA on the *in vitro* growth of Mas Kirana banana. By integrating organic and hormonal media enhancements, we strive to develop a more effective tissue culture protocol tailored to this cultivar, supporting both commercial propagation and the conservation of valuable local germplasm.

Material and Method

Shoot tips of *Musa acuminata* cv. Mas Kirana was used as an explant and cultured on Murashige and Skoog (MS) basal medium supplemented with yeast extract and indole-3-

acetic acid (IAA). The medium also contained benzylaminopurine (BAP) at a concentration of 1 ppm, and the pH was adjusted to 5.8 before sterilization by autoclaving at 121 °C for 15 minutes. Sterile distilled water was used to prepare the media, and explants were surface-sterilized with 70% ethanol before culture. All procedures were conducted under aseptic conditions using standard tissue culture equipment, including laminar airflow cabinets, autoclaves, hot plate stirrers, magnetic stirrers, pH meters, scalpels, and culture racks.

The experiment was arranged in a factorial completely randomized design (CRD) with two factors: yeast extract at four concentrations (0, 400, 800, and 1200 mg L⁻¹) and IAA at four concentrations (0, 0.75, 1.5, and 2.25 ppm). This produced sixteen treatment combinations, coded as R0A0 through R3A3, representing all possible combinations of yeast extract and IAA levels. Each of the 16 treatment combinations was replicated three times, with one explant per replicate, resulting in a total of 48 experimental units.

Several growth parameters were observed to evaluate treatment effects. These included the time to the emergence of the first shoot, the number of shoots produced, and the height of the shoots. Root development was assessed by recording the time of root initiation, the number of roots per explant, and the root length. Leaf formation was also monitored by recording the time to the emergence of the first leaf and the total number of leaves produced per explant.

Data obtained from the observations were subjected to analysis of variance (ANOVA) at a 5% significance level. When significant differences were detected, Duncan's Multiple Range Test (DMRT) at the 5% level was used to separate treatment means. In addition, correlation and regression analyses were performed to examine the relationship between variables, and results were presented in both tabular and graphical form to provide a comprehensive interpretation of the findings.

Results and Discussion

Time of Shoots Emergence

The interaction between yeast extract and Indole-3-acetic acid (IAA) did not significantly affect the time of shoot emergence, and IAA alone also showed no effect. However, yeast extract had a significant but inhibitory effect on this parameter.

The control treatment (0 mg L⁻¹) produced the fastest shoot emergence at 11 days after planting (DAP), while higher yeast concentrations (400, 800, and 1200 mg L⁻¹) delayed emergence to 13–22 DAP (Table 1). This pattern indicates that increasing yeast concentrations beyond a minimal level can slow down the initiation of shoots in Mas Kirana banana explants. Similar observations have been reported in other studies where inappropriate concentrations of organic supplements caused osmotic or metabolic stress, thereby reducing early morphogenesis. Although yeast extract can serve as a source of amino acids, vitamins, and growth-promoting compounds (5,6), its optimal dose varies by species, and excessive levels may disrupt the hormonal balance required for shoot initiation. While yeast has been reported to enhance shoot proliferation in other species (10,11), its effect here was opposite, suggesting a species-specific and concentration-dependent response.

Table 1. Yeast effect of yeast on the emergence time of Mas Kirana banana shoots

Yeast Concentration (mg L ⁻¹)	Shoot emergence time (DAP)
0	11.00±0.55 a
400	21.08±0.89 b
800	13.17±0.75 ab
1200	21.58±0.53 b

Explanation: DAP = days after planting, the numbers followed by the same letters in the same column were not significantly different from the DMRT test at the 5% level.

Time of Root Emergence

The interaction between the two treatment factors showed no significant effect on root emergence time; neither treatment factor had a substantial impact on its own. The appearance of a root occurs between 13.67 DAP and 39.33 DAP, with an average of 23.94 DAP. Yeast concentrations of 0 mgL⁻¹ and IAA 1.5 ppm showed the fastest root appearance at 13.67 DAP (Figure 1). Based on this, it can be said that the addition of auxin can stimulate the appearance of roots in banana explants, such as those of the Mas Kirana variety. Pamungkas (7) explains that the addition of auxin to the media can increase root primordia that grow, so that more roots are produced. The higher the concentration of auxin given, the more permeable the cells will be, which will encourage the emergence of primordial roots in the explants. Akhariana et al. (8) added that the emergence of roots in plantlets is significant because the roots function to absorb water, minerals, and other materials needed for plant growth.

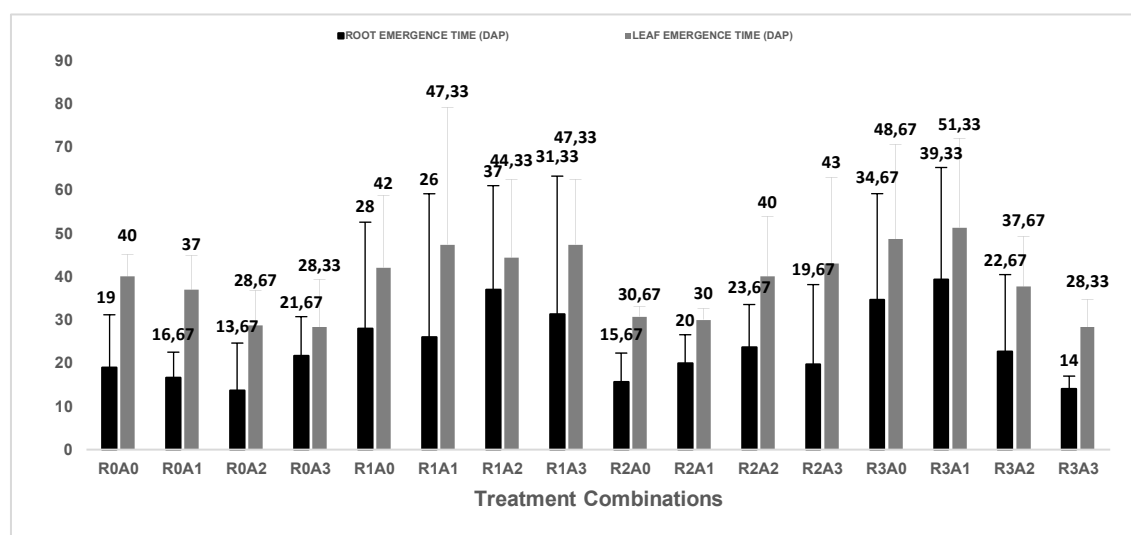


Figure 1. Histogram of the yeast and IAA addition effect on the average of root emergence time and leaf emergence time of Mas Kirana banana explants

Time of Leaf Emergence

The R factor (yeast administration) and factor A (IAA administration), as well as the interaction of the two, did not have a significant or non-significant effect on leaf emergence. This is not in accordance with the opinion of

Triharyanto et al. (9), which states that administration of 0.5 ppm IAA along with 4 ppm BAP accelerates the time of root and leaf emergence on multiplication of Raja Bulu banana. The appearance of leaves ranged from 28.33 to 51.33 DAP with an average of 39.04

DAP. Figure 7 shows that the appearance of leaves in all treatments is different. This is presumably because the content of endogenous hormones in each explant is different, so that when the leaves appear, they also vary depending on the hormonal balance in the explants.

Number of Shoots

The interaction between the two treatment factors did not show a significant effect on the number of shoots. This also occurs in the analysis of variance per treatment. This

does not correspond to the opinion of Damiska et al. (10), which states that yeast addition affects the number of shoots of mangosteen explants. Observation of the optimal yeast concentration, which involves an increase in the number of shoots, is treatment R3 (9% yeast), with an average of 0.387 shoots. This is because the content of amino acids and proteins in yeast can accelerate the process of cell differentiation, resulting in the formation of more new shoots and an increase in their number.

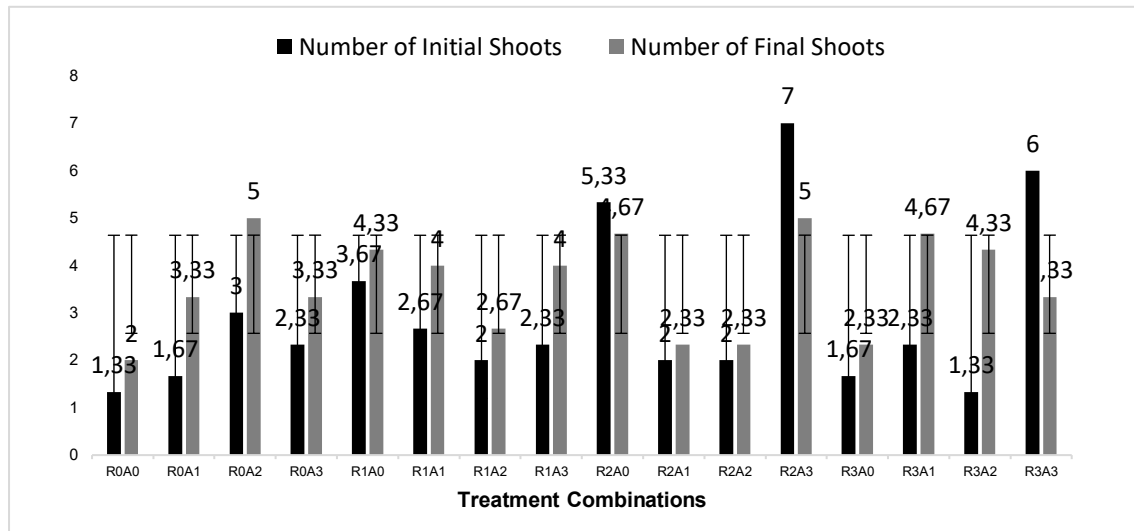


Figure 2. Histogram of yeast and IAA addition effect on the average number of shoots of Mas Kirana banana explant

The final number of shoots was calculated to determine the number of shoots from the initial number of explant shoots. Figure 2 shows that the combination of 1200 mg L⁻¹ yeast treatment and IAA at 1.5 ppm is the most effective treatment for the variable number of shoots of Mas Kirana banana explants, specifically three shoots. In combination with yeast treatment 800 mg L⁻¹ + IAA 0 ppm, yeast 800 mg L⁻¹ + IAA 2.25 ppm, and yeast 1200 mg L⁻¹ + IAA 2.25 ppm showed a decrease in the average number of shoots compared to the initial shoots. Based on this, it can be said that the higher the concentration of yeast given, the more it tends to inhibit the formation of shoots of banana explants, Mas Kirana. However, this differs from the research by Zunafika et al. (11), which states that the higher the concentration of yeast given, the greater the formation of shoots in yellow Kepok banana explants. The final number of shoots formed by the end of the observations differs. And it is thought to be influenced by the size of different explants. Additionally, the number of initial shoots that are not the same

also affects the final number of shoots that form.

Shoots Height

The interaction between yeast and IAA did not yield significant results in terms of shoot height, nor did the yeast treatment alone. This is not in line with the opinion of Marlina et al. (12), which states that varying concentrations of yeast affect the length/height of mangosteen explant shoots. The best observation of shoot length was in the R2 treatment (yeast at a concentration of 9%), with an average shoot length of 2.78 cm. This is because yeast contains nitrogen, vitamins, and carbon compounds that play a crucial role in plant physiological processes, such as protein, nucleic acid, and coenzyme formation. However, the IAA treatment factor showed significant results for shoot height. The treatment of IAA concentration of 1.5 ppm was significantly different from the treatment of IAA concentration of 2.25 ppm, but not substantially different from the treatment of IAA concentrations of 0 ppm and 0.75 ppm. Based on further testing, it is evident that the

addition of IAA at a concentration of 1.5 ppm yields the best average shoot height of 18.90 cm (Table 2). The shortest shoot height is observed in the IAA treatment with a concentration of 2.25 ppm, which averages 13.64 cm. Hartati et al. (13) explained that some explants produce sufficient quantities of auxin. Still, to support tissue culture growth, they require additional auxin from the outside, specifically through the addition of growth regulators and organic compounds. Farida and Muslihatin (14) added that auxin stimulates cell elongation by adding plasticity to the cell wall, making it loose, so that water can enter the cell wall.

Table 2. The average shoot height of Mas Kirana banana plants at any IAA concentration

IAA Concentration (ppm)	Shoots height (cm)
0.00	14.98±0.55 ab
0.75	17.19±0.37 ab
1.50	18.90±0.11 b
2.25	13.64±0.74 a

Explanations: The numbers followed by the same letters in the same column were not significantly different from each other using the DMRT test at the 5% level.

Root Length

The interaction between the two treatment factors had no significant effect on root length. That yeast treatment had no significant effect on root length. This is not in accordance with the opinion of Jainol and Jualang (15) that the multiplication of orchids with the addition of 2 gL⁻¹ yeast extract has the highest root length of 15.18 mm; however, according to the opinion of Putri et al. (16), yeast inhibits the growth of garlic explants as indicated by the number and length of roots. Yeast extract contains several types of vitamins, including thiamine, riboflavin, pyridoxine, niacin, and pantothenic acid (17). Abrahamian and Kantharajah (18) stated that riboflavin, thiamine, calcium, and vitamin E play a role in stimulating the process of root formation. However, in this study, the vitamins did not have a significant effect on the growth of root numbers because the concentration had not reached the optimal point.

Giving IAA at various concentrations also does not have a significant effect on root length. This is presumably because endogenous auxin in shoots is used as a source for explants, and exogenous auxin is given to accumulate explants. This accumulation of auxin will inhibit root lengthening. This is in

accordance with the opinion of Putri et al. (2019), which states that high endogenous auxin concentrations can cause cell shortening. The root lengths ranged from 12.8 to 51.77 cm, with an average of 29.45 cm across all treatments (Figure 3).

Root Number

The interaction between the two treatment factors did not show a significant effect on the number of roots. The yeast and IAA concentrations also did not affect the number of roots. This is presumably because the content of auxin, which is too high, inhibits root growth. According to Bharati et al. (20), Auxin concentration (both IAA and IBA) can increase root response, but only to a certain threshold level. If fewer or more rooting responses are obtained, the correct concentration is needed for optimal response. The percentage of the best root response in this study is for ½ MS + IAA 2.5 mg L⁻¹ media, while the optimal root number is achieved with ½ MS + IAA 1.5 mg L⁻¹. Sutriana et al. (21) noted that auxin plays a role in cell extension and tissue enlargement, cell division, and adventitious root formation, while also inhibiting the formation of axillary and adventitious buds. The number of banana roots formed ranged from 8.67 to 23.67 roots, with an average of 16.83 roots across all treatments (Figure 3).

Leaves Number

The interaction between yeast treatment and IAA did not have a significant effect on the number of leaves, nor did each yeast and IAA treatment. Yeast does not have a substantial impact on the number of leaves, nor does IAA significantly influence the number of leaves. This differs from the study by Zulwanis et al. (22), which showed that an optimal concentration of yeast extract, as high as 1.3 g/L, was sufficient to produce growth in the number of leaves, with an average of 6 strands, for 60 days after planting. Yeast extract is an additional supplement that contains nitrogen compounds. Nitrogen is a macronutrient, making up amino acids, chlorophyll, and other compounds that are needed by plantlets during their growth. The high concentration of nitrogen (N) in the media can stimulate plants to synthesize more protein for leaf growth.

The number of leaves varied across all treatments. This is presumably because the content of endogenous hormones in each explant is not the same, so the number of leaves also varies.

Yuniastuti et al. (23) explained that variations in the number of leaves produced were possible because endogenous hormone levels were not the same for each explant, so the response to the addition of growth regulators also varied. The

number of leaves formed ranged from 5.67 to 15.67 leaves with an average of 9.58 leaves (Figure 3). The more leaves a plant has, the more photosynthates it produces for food reserves (9).

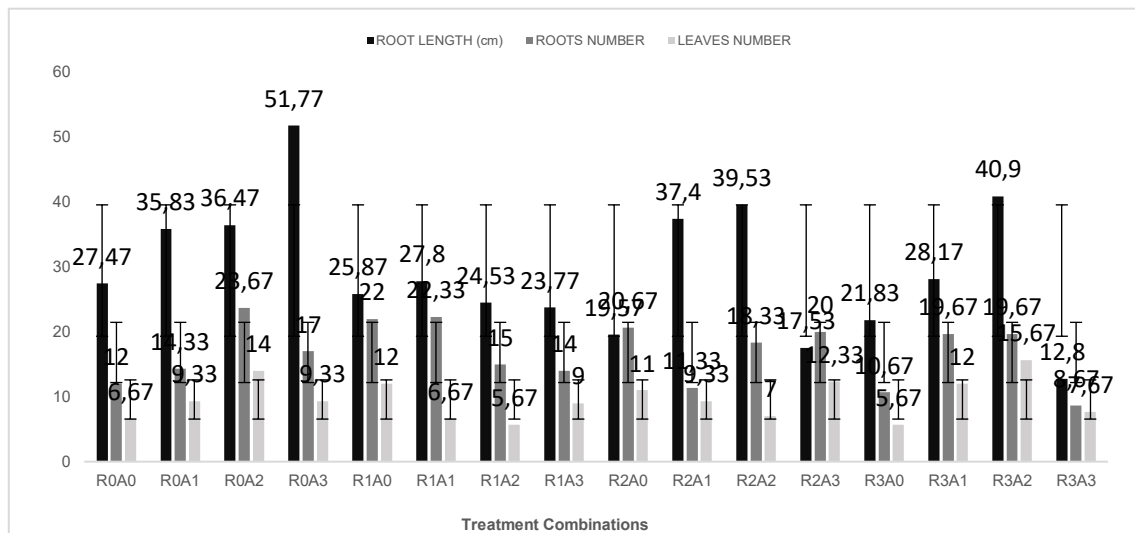


Figure 3. Histogram of yeast and IAA addition effect on the average of root length, roots, and number of leaves of Mas Kirana banana explant

Conclusion

This study demonstrated that the addition of yeast extract and Indole-3-acetic acid (IAA) had limited interactive effects on the in vitro growth of Mas Kirana banana explants. Yeast extract significantly influenced shoot emergence time; however, higher concentrations delayed emergence, with the fastest response occurring in the control (0 mg L⁻¹). This indicates that yeast acted as an inhibitory factor rather than a promotive one for shoot initiation in this study. In contrast, the IAA application, particularly at 1.5 ppm, enhanced shoot height, indicating its role in promoting cell elongation. Other growth parameters, including root emergence, root length, number of roots, and number of leaves, were not significantly affected by either yeast or IAA treatments. These findings suggest that the response of Mas Kirana banana explants to yeast extract and IAA is highly concentration-dependent and species-specific. Future studies should explore lower yeast concentrations (<400 mg L⁻¹), broader auxin ranges, and combinations with Benzylaminopurine (BAP) or other cytokinins to identify media formulations that can synergistically improve shoot multiplication and rooting. Optimizing these factors will support the development of efficient, large-scale micropropagation protocols for this essential local cultivar.

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