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Effect of The Application of ZPT IAA (*Indole Acetic Acid*) and Kinetin on Multiplication of Granola Potato Shoots (*Solanum Tuberosum* L.) In Vitro

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Abstract: Seedlings produced through tissue culture with an imbalanced ratio of growth regulators often exhibit poor quality and uneven growth. The success of shoot multiplication in granola potato can be improved by optimizing the ratio of growth regulators, particularly auxins and cytokinins. This study aimed to determine the optimal concentrations of Indole-3-acetic acid (IAA) and kinetin for shoot multiplication of granola potato. The experiment was conducted from June to October 2024 at the Tissue Culture Laboratory of Jember State Polytechnic. A Completely Randomized Factorial Design (RALF) was used, consisting of two factors and three replications. The first factor was IAA concentration, comprising 0.4 mg L^{-1}, 0.7 mg L^{-1}, and 1.0 mg L^{-1}. The second factor was kinetin concentration, consisting of 0.5 mg L^{-1}, 1.0 mg L^{-1}, and 1.5 mg L^{-1}. The results showed a significant interaction between 0.4 mg L^{-1} IAA and 0.5 mg L^{-1} kinetin in accelerating callus emergence, with the earliest appearance observed at 6.33 days. This indicates that a balanced ratio of auxin and cytokinin can promote faster callus development. The combination of 0.7 mg L^{-1} IAA and 1.0 mg L^{-1} kinetin resulted in the earliest shoot emergence at 5.67 days. This is likely because IAA promotes cell elongation, while kinetin stimulates cell division. Treatment with 1.0 mg L^{-1} IAA showed a highly significant effect on the number of segments (24.89) and the number of roots (29.22). Meanwhile, treatment with 0.7 mg L^{-1} IAA significantly increased root length (5.91 cm). These results suggest that IAA plays a vital role in promoting cell elongation and enhancing cell wall plasticity, enabling cells to expand and elongate more effectively.

Keywords: Granola Potato; Tissue Culture; Plant Growth Regulators, Number Of Shoots, Callus

1. Introduction

Potatoes play a significant role in various aspects of life, both as a food source and for industrial purposes. In Indonesia, potato consumption continues to increase each year in line with population growth. In 2022, national potato production reached 1.5 million tons; however, in 2023, it declined by approximately 18% to 1.2 million tons (BPS, 2024). One of the main factors contributing to this decline is the limited availability and use of high-quality potato seed. A key challenge in providing quality seed is the difficulty in producing large quantities of potatoes in a short period. Generally, potatoes are propagated vegetatively through tubers. However, this method has several drawbacks, including limited seed production, susceptibility to pests and diseases, and dependence on seasonal conditions (Putri et al., 2021). To address these challenges, an alternative method that can be applied is in vitro propagation using tissue culture techniques.

In vitro propagation is a method used to grow plant cells, tissues, or organs into complete plants by utilizing artificial media under sterile conditions (Lintong et al., 2022). One technique applied in in vitro propagation is shoot multiplication. Shoot multiplication is a stage in tissue culture aimed at producing a large number of plantlets. The success of plant tissue culture is influenced by severa/l factors, such as sterilization, explant selection, and environmental conditions, including pH, lighting, temperature, and the concentration of plant growth regulators (PGRs) in the culture medium (Pangestika et al., 2015). Among these factors, PGRs play a crucial role in stimulating the growth and development of explants. One commonly used auxin is Indole-3-acetic acid (IAA), which functions in various plant growth processes, including cell division, shoot development, cambial activity, and root formation (Pangestika et al., 2015). A study by Riyanto (2022) on the use of IAA for shoot multiplication of the Atlantic potato variety showed that a concentration of 0.5 mg L^{-1} resulted in the highest survival rate of plantlets. In addition, research by Omer et al. (2021) on in vitro propagation and minituber production in potatoes revealed that an IAA concentration of 0.4 mg L^{-1} significantly increased both the number and length of roots in two tested varieties.

The application of cytokinin and auxin hormones must be adjusted to appropriate concentrations. A higher cytokinin-to-auxin ratio generally promotes shoot formation. One widely used cytokinin is kinetin, which stimulates shoot growth, influences cellular metabolism, activates dormant cells, and plays a key role in cell division (Buchory, 2008). Riyanto (2022) reported that the application of 2.0 mg L^{-1} kinetin in the shoot multiplication of Atlantic potatoes resulted in the highest plantlet survival rate and also increased the number of nodes, leaves, and lateral shoots. Meanwhile, Omer et al. (2021) found that a kinetin concentration of 0.4 mg L^{-1} produced the best results in enhancing the number of plantlets, shoots, and leaves during in vitro potato propagation and minituber production.

Due to their significant role in supporting shoot regeneration, IAA and kinetin are widely utilized in in vitro culture. Therefore, further research is needed to evaluate the application of these plant growth regulators—IAA and kinetin—in the shoot multiplication of granola potatoes (*Solanum tuberosum* L.) through in vitro culture techniques.

2. Materials and Methods

This study was conducted from June to October 2024 at the Plant Tissue Culture Laboratory, Jember State Polytechnic, East Java. The objective of this study was to investigate the effect of different concentrations of the growth regulators IAA and kinetin on the in vitro growth of granola potato (Solanum tuberosum L.) explants. The explants used were apical buds of the granola potato variety, obtained from local farmers in Sariwani Village, Sukapura Subdistrict, Probolinggo Regency.

The equipment used in this study included an autoclave, oven, laminar airflow cabinet (LAFC), refrigerator, culture bottles, analytical balance, Petri dishes, hot plate, magnetic stirrer, scalpel, forceps, blades, graduated pipettes, Erlenmeyer flasks, micropipettes, measuring cylinders, beakers, Bunsen burner, lighter, pan, pH meter, hand sprayer, cutter, scissors, ruler, stationery, and a camera. The chemical materials used included Murashige and Skoog (MS) medium, sucrose, agar, distilled water, 70% and 96% ethanol, Tween-20, 10% and 5% Clorox solution, fungicide, bactericide, NaOH, HCl, and the plant growth regulators IAA and kinetin. All materials were prepared under sterile conditions, and the culture media were adjusted according to the treatment design.

The experiment was arranged in a factorial completely randomized design (CRD), consisting of two factors: IAA concentration (0.4 mg L^{-1}, 0.7 mg L^{-1}, and 1.0 mg L^{-1}) and kinetin concentration (0.5 mg L^{-1}, 1.0 mg L^{-1}, and 1.5 mg L^{-1}). These two factors resulted in nine treatment combinations (I1K1, I1K2, I1K3, I2K1, I2K2, I2K3, I3K1, I3K2, and I3K3),

each replicated three times, producing a total of 27 experimental units. Each unit consisted of one culture bottle containing one explant.

The initial step involved sterilizing all equipment. All tools were first washed with detergent and running water, followed by autoclaving at 121°C for 20 minutes. MS media were prepared by mixing macro and micronutrient stock solutions, 30 g/L sucrose, and the designated concentrations of growth regulators. The pH was adjusted to 5.7–5.8 using either NaOH or HCl. Agar (8 g/L) was then added, and the mixture was heated until fully dissolved. The media were poured into culture bottles (25 mL per bottle), labeled, and autoclaved again at 121°C for 60 minutes.

Explants in the form of potato bud eyes were selected and thoroughly cleaned before sterilization. They were soaked in a solution of bactericide and fungicide (2 g/L each) with Tween-20 for 20 minutes, followed by immersion in 70% ethanol for 30 seconds, 10% Clorox for 10 minutes, and 5% Clorox for 7 minutes, with sterile distilled water used to rinse the explants after each step. Sterilized explants were transferred aseptically under the LAFC. Each explant was cut to a length of 0.5–1 cm and planted into the culture bottles using sterile forceps. The bottles were then tightly sealed, wrapped with plastic film, labeled, and placed in the culture room.

During the incubation period, the explants were maintained in a culture room at a temperature of 20–25°C with a light intensity of approximately 1000–2000 lux. The culture environment was kept clean by regularly spraying 70% ethanol. Contaminated explants were immediately removed to prevent the spread of contamination.

The observed variables included: the time of callus emergence (days after planting), callus quality (color and texture), time of shoot emergence, number of shoots, shoot height, number of leaves, number of stem nodes, root length, and number of roots. Observations were conducted periodically up to the 8th week after planting. The data obtained were analyzed using analysis of variance (ANOVA). If significant differences were found among the treatment combinations, further analysis was conducted using Duncan's Multiple Range Test (DMRT) at 5% and 1% significance levels to determine statistically significant differences.

3. Results and Discussion

Time of Callus Emergence in Granola Potato

Table 1.1. Time of Callus Emergence in Granola Potato at Different Concentrations of IAA and Kinetin (Days After Planting)

Concentracion of	**Concentration of Kinetin (mg L^{-1})**		
IAA (mg L^{-1})	**0,5**	**1,0**	**1,5**
0,4	6,33 A a	6,33 A a	7,00 A a
0,7	6,33 A a	6,67 A a	7,00 A a
1,0	6,67 A a	9,00 B a	7,00 A b

Note: *Numbers followed by the same uppercase letters in the same column and numbers followed by the same lowercase letters in the same row indicate no significant difference based on the 5% DMRT (Duncan's Multiple Range Test).*

Callus formation in Granola potato explants was characterized by the appearance of translucent white tissue resembling water droplets or mucus at the cut surfaces and wound sites of the explant. This tissue gradually developed into small nodules, eventually forming callus aggregates (Waryastuti et al., 2017). This process is a physiological response of the plant to mechanical injury inflicted on the explant tissue. According to Mastuti et al. (2018), the cells surrounding the wounded area undergo rapid division to cover the damaged parts, forming a meristematic callus layer.

Based on the observations in this study, a significant interaction was found between IAA and kinetin concentrations, where the combination of 0.4 mg L^{-1} IAA and 0.5 mg L^{-1}

kinetin resulted in the earliest callus emergence, occurring at 6.33 days after planting (DAP). Conversely, the combination of 1.0 mg L^{-1} IAA and 1.0 mg L^{-1} kinetin showed the slowest callus emergence, at 9.00 DAP. This suggests that a balanced ratio of auxin and cytokinin at lower concentrations tends to accelerate callus formation.

Setyorini (2019) stated that an initial balance between auxin and cytokinin hormones can promote faster callus induction. An imbalance or dominance of one hormone over the other may direct the differentiation process toward specific organs and inhibit callus cell division. The addition of auxin, such as IAA—being a natural hormone readily recognized by plant tissues—provides an effective stimulus for cell division when combined with an appropriate cytokinin concentration (Sudrajad et al., 2015).

In addition to callus initiation time, callus quality was also evaluated to assess the treatment effects. Based on visual observations at 40 DAP, all treatment combinations produced callus with a translucent white color and friable texture. This color and texture indicate that the callus cells were still in an active division phase. Rasud et al. (2020) explained that white callus reflects actively dividing cell masses, while yellowish-white callus indicates a near-end stage of cell division, and brownish callus shows signs of aging. The translucent white callus formed in this study did not yet contain chlorophyll, and thus could not be classified as embryogenic callus. Embryogenic callus is typically green, compact in texture, and contains nodules indicating the initial stage of somatic embryo formation (Asmono et al., 2016). Therefore, the callus observed in this study was classified as non-embryogenic.

In terms of texture, all callus produced exhibited a friable structure. According to Idris et al. (2019), friable callus has good regenerative potential due to its loosely arranged cells, which are easily separated and suitable for subculture. Millenia et al. (2022) further noted that friable texture is associated with the effects of auxin, which increases cell wall elasticity, facilitates water absorption, and causes cell swelling.

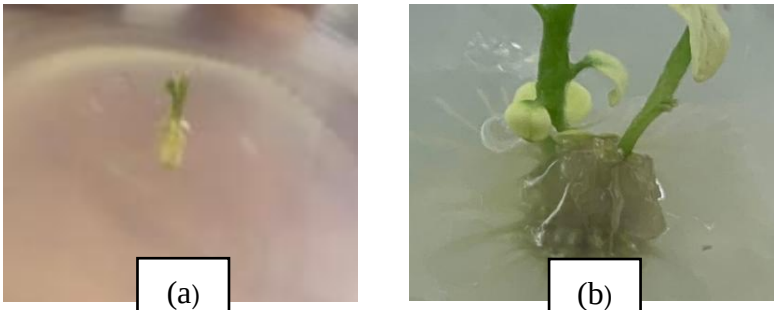


Figure 1. Initial formation of Granola potato callus (a); Granola potato callus with white color and friable texture (b).

Time of Shoot Emergence in Granola Potato

Table 1.2. Time of Shoot Emergence in Granola Potato at Different Concentrations of IAA and Kinetin Growth Regulators

Concentracion of	**Concentration of Kinetin (mg L^{-1})**		
IAA (mg L^{-1})	**0,5**	**1,0**	**1,5**
0,4	8,67 B b	6,67 AB a	6,33 A a
0,7	6,33 A a	5,67 A a	6,33 A a
1,0	6,67 A a	8,00 B a	8,00 B a

Note: *Numbers followed by the same uppercase letters in the same column and numbers followed by the same lowercase letters in the same row indicate no significant difference based on the 5% DMRT (Duncan's Multiple Range Test).*

The results of the study revealed a significant interaction between the concentrations of IAA and kinetin on the time of shoot emergence in Granola potato explants. The most

effective treatment combination for accelerating shoot formation was 0.7 mg L^{-1} IAA and 1.0 mg L^{-1} kinetin, which resulted in the earliest shoot emergence at 5.67 days after planting (DAP).

The interaction between auxin (IAA) and cytokinin (kinetin) plays a crucial role in stimulating shoot growth. Kinetin is known to have a primary function in promoting cell division, and is generally more dominant in initiating shoot formation. When both hormones are applied in the appropriate concentrations, they can create a hormonal balance that supports optimal growth without suppressing the activity of the other (Munggarani et al., 2018).

While higher concentrations of kinetin can enhance shoot multiplication frequency, excessive or sole application of cytokinin may lead to morphological abnormalities in the resulting shoots. Additionally, IAA contributes to cell elongation and growth, supporting the regeneration of shoots from callus tissue (Rohmah, 2021).

Figure 2. First Emergence of Granola Potato Shoots

Number of Shoots in Granola Potato

The results of the study showed that the combination of IAA and kinetin treatments had no significant effect on the number of shoots in Granola potato explants. The average number of shoots formed in each treatment combination ranged from 1 to 2 shoots, suggesting that the applied plant growth regulator (PGR) treatments were not sufficiently effective in enhancing shoot multiplication. This may be attributed to the presence of endogenous hormones—naturally produced within the plant and translocated to specific parts in response to growth requirements (Asra et al., 2020).

Although the application of exogenous hormones such as IAA and kinetin can influence the plant's morphological and physiological structures, their effects may not always be significant due to possible disruptions in the balance of endogenous hormones. Therefore, careful selection of exogenous hormone concentrations is essential to avoid interfering with the plant's natural hormonal mechanisms.

Shoot Height in Granola Potato

The study also indicated that the combination of IAA and kinetin treatments did not significantly affect the shoot height of Granola potato explants. The average shoot height across all treatments was relatively uniform, which was consistent with the number of shoots formed, as those values were also relatively similar. Physiologically, IAA—an auxin hormone—functions to promote cell elongation, while kinetin—a cytokinin—supports cell division and morphogenesis. However, the effectiveness of these exogenous hormones largely depends on the balance of endogenous hormones present in plant tissues. The concentrations used in this study were likely insufficient to trigger a significant physiological response (Handoko et al., 2020).

Number of Leaves in Granola Potato

The results also revealed that the combination of IAA and kinetin treatments had no significant effect on the number of leaves in Granola potato explants. Each treatment produced a relatively similar number of leaves, which was consistent with the results observed for shoot height. This indicates a positive correlation between leaf growth and shoot height, although both were not significantly influenced by the different hormone concentrations applied. The number of leaves formed is regulated by the activity of the apical meristem, which initiates leaf primordium development through active cell division. Auxin and gibberellins are known to play a role in stimulating this process, particularly in the early and subsequent stages of leaf development (Hindriana et al., 2023). However, in this study, the concentrations of exogenous hormones used were presumably not sufficient to elicit an optimal morphogenic response, resulting in no significant differences in leaf number among treatments.

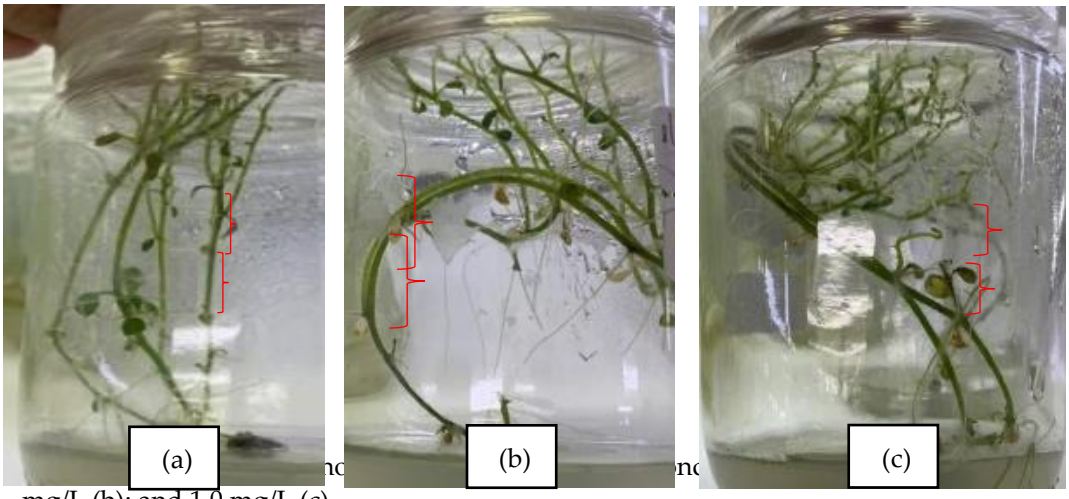
Number of Nodes in Granola Potato

Table 1.3. Number of Nodes in Granola Potato at Different IAA Growth Regulator Concentrations

IAA Concentration (mg L^{-1})	Number of Nodes (Units)	DMRT Value at 1% Level
1,0	24,8 a	-
0,7	21,1 ab	4,844
0,4	17,7 b	5,052

Note: Numbers followed by the same lowercase letter indicate no significant difference based on the 1% DMRT test.

The results of the study showed that treatment with IAA at a concentration of 1.0 mg L^{-1} produced the highest number of internodes in Granola potato explants, with an average of 24.8 internodes, followed by concentrations of 0.7 mg L^{-1} (21.1 internodes) and 0.4 mg L^{-1} (17.7 internodes). Auxin plays a crucial role in stimulating cell division and elongation, as well as regulating cellular metabolism and development through its polar transport mechanism to target tissues. A higher concentration of IAA can deliver a stronger signal to the target tissues, thereby promoting more robust internode growth (Aiman et al., 2022). However, the uneven distribution of auxin within plant tissues leads to varying growth responses depending on the sensitivity of each tissue to the hormone (Adamowski, 2015).



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mg/L (b); and 1.0 mg/L (c).

Number of Roots in Granola Potatoes

Table 1.4. Number of Roots in Granola Potatoes at Different IAA Growth Regulator Concentrations

IAA Concentration (mg L^{-1})	Number of Roots (units)	DMRT Value at 1% Level
1,0	29,2 a	-
0,7	26,2 ab	5,138
0,4	20,0 b	5,358

Note: Numbers followed by the same lowercase letter indicate no significant difference based on the 1% DMRT test.

The results of the study showed that the application of IAA at a concentration of 1.0 mg L^{-1} produced the highest number of roots in Granola potato explants, with an average of 29.2 roots, followed by 0.7 mg L^{-1} (26.2 roots), and 0.4 mg L^{-1} (20 roots). This indicates that increasing IAA concentration positively affects the number of roots formed. IAA, a type of auxin hormone, plays a critical role in root initiation by stimulating cell division in the lower stem tissues. Auxins trigger metabolic activities that generate energy, thereby supporting the formation and differentiation of primary roots (Harahap et al., 2023).

Although higher concentrations of IAA tend to increase root number, its effectiveness still depends on the balance with other hormones and the physiological condition of the explant. IAA concentrations that exceed the optimal threshold may, in fact, inhibit root growth (Louw et al., 2018). Therefore, selecting the appropriate concentration is essential to optimize root formation in in vitro culture of Granola potatoes.

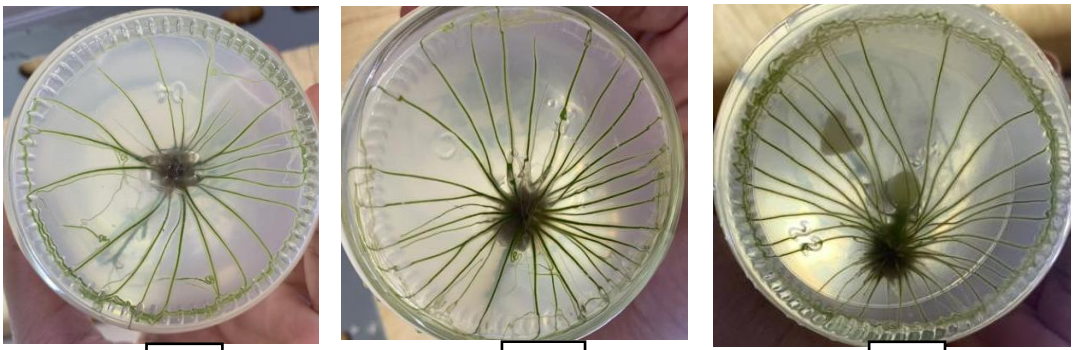

Figure 1.4. Root formation of Granola potato in in vitro culture using various concentrations of IAA: 0.4 mg/L (a); 0.7 mg/L (b); 1.0 mg/L (c).

Root Length of Granola Potato

Table 1.5. Root Length of Granola Potato at Different IAA Growth Regulator Concentrations

IAA Concentration (mg L^{-1})	Root Length (cm)	DMRT Value at 5% Level
0,7	5,9 a	-
0,4	5,7 ab	0,736
1,0	5,1 c	0,772

Note: Numbers followed by the same lowercase letter indicate no significant difference based on the 5% DMRT (Duncan's Multiple Range Test).

The application of IAA at a concentration of 0.7 mg L^{-1} resulted in the longest root length in Granola potato explants, measuring 5.9 cm, followed by 0.4 mg L^{-1} (5.7 cm), while the shortest root length was observed at 1.0 mg L^{-1} (5.1 cm). These results indicate

that a moderate concentration is more effective in stimulating root elongation compared to higher concentrations. The plant growth regulator IAA, which belongs to the auxin group, plays a crucial role in promoting cell division and elongation in the root apical meristem, thereby contributing to increased root length. IAA enhances the plant's ability to absorb water and nutrients through its longer root system (Nurhanis et al., 2019). Therefore, applying IAA at an optimal dose is essential for achieving maximum root growth.

4. Conclusions

This study demonstrated that the combination of 0.4 mg L^{-1} IAA and 0.5 mg L^{-1} kinetin had a significant effect on callus initiation time (6.33 days), while the combination of 0.7 mg L^{-1} IAA and 1.0 mg L^{-1} kinetin significantly affected the time of shoot emergence (5.67 days). The application of IAA in MS medium at a concentration of 1.0 mg L^{-1} produced the best results in terms of the number of nodes (24.9) and number of roots (29.2), while a concentration of 0.7 mg L^{-1} resulted in the longest root length (5.9 cm). Meanwhile, kinetin application had no significant effect on the in vitro shoot multiplication of Granola potatoes.

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