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Optimization of in Vitro Culture of Taro Banana (*Musa paradisiaca* var. *sapientum* L.) Based on Thidiazuron: A Foundation for Developing a Biotechnology Booklet to Enhance Decision-Making Skills

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Abstract: This study aims to optimize the concentration of Thidiazuron (TDZ) in the in vitro culture of taro banana (*Musa paradisiaca* var. *sapientum* L.) and to integrate the results as a foundation for developing a biotechnology booklet to enhance decision-making skills. The research was conducted using an experimental method with various TDZ concentration treatments, observing parameters such as the number of shoots and leaves produced. To bridge the gap between biotechnological research and its application in education, this study investigates the optimization of Thidiazuron (TDZ) concentrations for the in vitro multiplication of taro banana (*Musa paradisiaca* var. *sapientum* L.). The results showed that the use of TDZ effectively induced shoot formation, with the optimal concentration obtained at 0.01 mg/L TDZ, producing an average of 22.25 shoots and 24.25 leaves. In addition to generating biological data, each stage of this study reflected the implementation of decision-making skills, including problem identification, information gathering, criteria determination, solution selection, and result evaluation. These findings serve as the foundation for developing a biotechnology booklet as a contextual learning medium that not only facilitates the understanding of biotechnology concepts but also trains students to make scientific and reflective decisions.

Keywords: Biotechnology booklet; Decision-making skills; In vitro culture; Taro banana; Thidiazuron

Introduction

Banana (*Musa paradisiaca* L.) is one of the most important tropical plants with high economic and social value in Indonesia (Due et al., 2019; Ekayanti et al., 2023). Almost all parts of the banana plant can be utilized, including its fruit, stem, leaves, and corm. Ideally, banana production should be carried out sustainably and efficiently to meet both food and economic needs. One local variety with great potential is the taro banana (*Musa paradisiaca* var. *sapientum* L.), which is widely favored for its taste and high market value (Budi et al., 2022; Sirappa, 2021).

However, the production of taro bananas in the field remains limited. Conventional propagation through suckers takes a long time, produces non-uniform seedlings, and is susceptible to disease infection. This condition becomes a major obstacle in efforts to increase the productivity of taro bananas. One effective biotechnological approach to overcome this limitation is the in vitro culture technique, which allows for rapid, uniform, and pathogen-free plant propagation

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under sterile conditions (Budi et al., 2022; Wulansari et al., 2017). In this technique, the use of plant growth regulators (PGRs) plays a crucial role, particularly the cytokinin group, which promotes shoot formation and multiplication. One of the widely used cytokinins is Thidiazuron (TDZ) (Hafizh et al., 2018; Wang et al., 2025). Different concentrations of TDZ can influence the number, size, and quality of shoots produced.

Although numerous studies have demonstrated the effectiveness of TDZ in other plant tissue cultures, specific data regarding the effect of TDZ concentration on the multiplication of taro banana shoots are still limited. Moreover, biotechnological research of this kind is rarely utilized directly as a learning resource to foster students' decision-making skills, even though the scientific process of determining the optimum TDZ concentration strongly reflects such abilities. This condition highlights a gap between biotechnological research findings and their application in contextual 21st-century learning, which emphasizes critical thinking and evidence-based decision-making.

In the context of science learning, particularly biotechnology, decision-making skills represent a crucial ability that reflects the scientific thinking process of students. This skill involves the ability to identify problems, collect and analyze information, determine alternative solutions, select the most rational decision, and evaluate the outcomes of the chosen decision (Farmer et al., 2005; Ghazal et al., 2018; Mettas, 2011).

To bridge the gap between biotechnological research and its application in education, this study investigates the optimization of Thidiazuron (TDZ) concentrations for the in vitro multiplication of taro banana (*Musa paradisiaca* var. *sapientum* L.). The findings are expected not only to enhance the effectiveness of plant propagation but also to serve as the foundation for developing biotechnology learning media based on research results.

Through such learning media, students can be trained in decision-making processes for example, by interpreting experimental data from various TDZ concentrations and determining the most optimal treatment based on empirical evidence. Thus, this research holds dual strategic value, supporting the advancement of agricultural biotechnology while simultaneously strengthening critical thinking and decision-making skills as part of 21st-century competencies in biology education.

Method

This study employed a laboratory experimental design with a contextual analytical approach. The focus of the research was to examine the effect of various concentrations of Thidiazuron (TDZ) on the in vitro shoot multiplication of taro banana (*Musa paradisiaca* var. *sapientum* L.). In addition, the experimental results were analyzed to identify their potential application in biotechnology learning, particularly as the basis for developing a biotechnology learning booklet aimed at strengthening data-driven decision-making skills.

The research was conducted from June to September 2025 at the Plant Tissue Culture Laboratory, UPT Seed Center for Food Crops and Horticulture (BPTH), Banjarbaru, South Kalimantan. The material used consisted of in vitro plantlet explants of taro banana. The culture medium used was Murashige and Skoog (MS) supplemented with TDZ at various concentrations (0.00–0.06 mg/L). Other components included sucrose, peptone, agar, NaOH, and HCl. The main equipment used included a laminar air flow cabinet, autoclave, analytical balance, and various glassware under aseptic conditions.

The experiment was arranged in a Completely Randomized Design (CRD) with a single factor, namely TDZ concentration, consisting of seven treatments and four replications, resulting in 28 experimental units. Each explant (±2 cm) was planted on sterile MS medium and incubated under controlled light and temperature conditions. Observations were made on shoot initiation time, number of shoots, number of leaves, and shoot morphology at four weeks after planting.

Data Collection and Analysis

Quantitative data (number of shoots and leaves) were analyzed using one-way ANOVA, followed by the Least Significant Difference (LSD) test. Qualitative data (morphology and induction time) were analyzed descriptively.

Subsequently, the experimental results were interpreted pedagogically to identify instances of decision-making that could serve as learning materials. For example, students can compare the results across different TDZ concentrations and determine the most effective treatment based on empirical evidence.

Although the study primarily focused on optimizing tissue culture conditions, the results were also evaluated from an educational relevance perspective, particularly in designing research-based learning activities that train students to make decisions critically, rationally, and based on scientific data.

Result and Discussion

The research data included shoot induction time, number of shoots, number of leaves, and shoot morphology of taro banana (*Musa paradisiaca* var. *sapientum* L.) explants cultured on media with various concentrations of Thidiazuron (TDZ): P0 (0.00 mg/L), P1 (0.01 mg/L), P2 (0.02 mg/L), P3 (0.03 mg/L), P4 (0.04

mg/L), P5 (0.05 mg/L), and P6 (0.06 mg/L). Quantitative observations were carried out at four weeks after planting (4 WAP), while the initial observation for shoot initiation was conducted daily from the time of planting.

All explants across treatments (P0–P6) began to show the formation of shoot primordia during the first week after planting (1 WAP). These protrusions then developed into visible shoot buds, followed by elongation and leaf formation. Thus, in general, TDZ at the tested concentration range did not inhibit early shoot induction; all treatments exhibited similar initiation responses at 1 WAP.

Analysis of variance (ANOVA) of the 4 WAP data showed that TDZ application had a significant effect on the parameters of shoot number and leaf number at the 5% significance level. For shoot number, Fcount = 3.87 > Ftable = 2.51 ($\alpha = 0.05$), indicating a significant difference among treatments. Similarly, leaf number showed Fcount = 7.74 > Ftable = 2.57 ($\alpha = 0.05$), confirming significant differences among treatments.

The application of TDZ to the culture medium significantly affected the average number of shoots and leaves of taro banana at 4 WAP. It was observed that increasing TDZ concentration did not always correspond to an increase in shoot or leaf production.

Treatment P1 (0.01 mg/L TDZ) produced the highest average number of shoots (22.25 shoots), which was significantly different from other treatments. This indicates that a low concentration of TDZ is sufficient to effectively induce and stimulate optimal shoot multiplication. In contrast, at higher concentrations such as P6 (0.06 mg/L TDZ), the average shoot number drastically decreased to 7.5 shoots, suggesting an inhibitory effect at elevated doses.

A similar pattern was observed in leaf formation. Treatment P1 again produced the highest average number of leaves (24.25 leaves), significantly higher than other treatments. Conversely, increasing TDZ concentration led to a marked reduction in leaf number; treatments P4 to P6 produced only around 4–5 leaves per explant.

These findings emphasize the importance of determining the appropriate growth regulator dosage in in vitro culture. The results can also serve as contextual learning material in the development of a biotechnology booklet, where students can analyze experimental data and make scientific decisions regarding the most effective TDZ concentration based on empirical evidence.

Differences in responses among treatments indicate that the effectiveness of TDZ in stimulating shoot and leaf formation strongly depends on its concentration. At low concentrations (0.01 mg/L), TDZ actively promotes cell division and meristematic differentiation, accelerating the formation of adventitious shoots. TDZ belongs to the phenylurea-type cytokinin group, known for its high activity and efficiency in stimulating shoot proliferation compared to conventional cytokinins such as BAP (N6-Benzylaminopurine) (Dimas et al., 2023; Nofitria et al., 2024).

However, at higher concentrations (≥0.03 mg/L), a significant decrease in shoot and leaf number was observed. This may be attributed to toxic effects or hormonal imbalance induced by excess TDZ, leading to tissue thickening, excessive callus formation, or shoot growth inhibition. Similar phenomena have been reported in previous studies, showing that TDZ concentrations beyond the optimum threshold reduce explant regeneration due to the accumulation of growth regulators that suppress shoot-inducing gene expression (Dimas et al., 2023; Ratnasari. et al., 2016; Rodinah et al., 2018).

Furthermore, the reduced leaf number at higher concentrations indicates a disruption in morphogenetic processes due to physiological stress caused by TDZ overload. Under such conditions, vegetative growth becomes suboptimal as more metabolic energy is directed toward cellular defense mechanisms against hormonal stress.

The morphology of shoots produced on each treatment medium is presented in Figure 1.

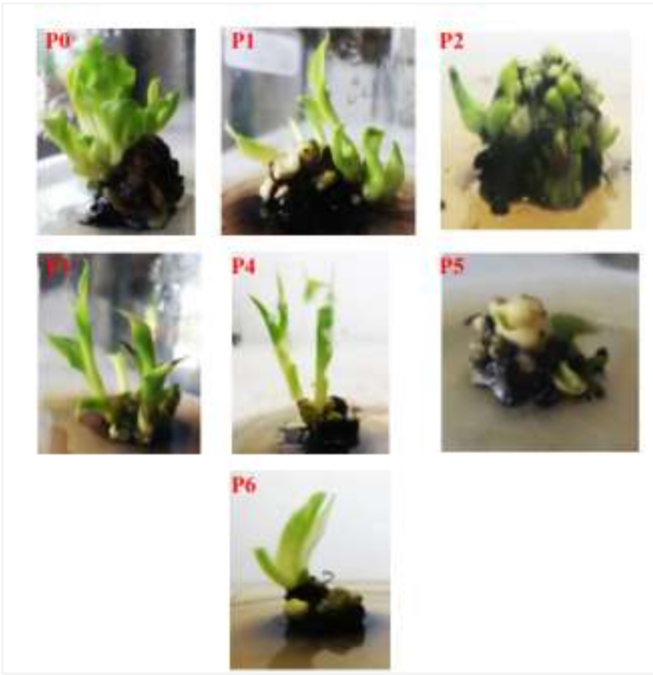

Figure 1. Explants produced on the medium at 4 weeks after planting (4 WAP)

Thus, the results of this study confirm that a TDZ concentration of 0.01 mg/L represents the optimum level for achieving the best shoot multiplication and leaf growth in the in vitro culture of Musa paradisiaca var. sapientum. These findings align with the theory that the effectiveness of cytokinins depends on the balance of their concentration and the type of plant tissue being cultured.

Potential of Research Procedures to Enhance Decision-Making Skills

The potential of the research procedures to support decision-making skills is illustrated in Figure 2.

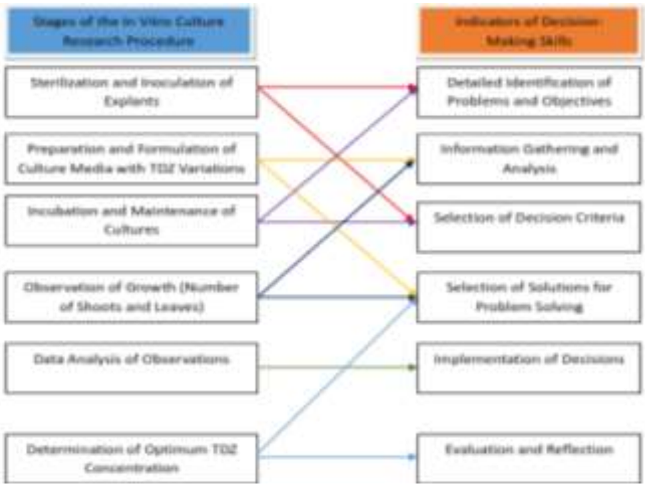

Figure 2. Potential of research procedures to enhance decision-making skills

Sterilization and Inoculation of Explants

At the initial stage, researchers must identify key problems such as potential sources of contamination and the need for proper aseptic conditions. In addition, it is necessary to collect and analyze information from previous studies or experiments to determine the most effective sterilization method and optimal chemical immersion duration. These decision-making skills ensure that the initial stage proceeds without contamination and aligns with the research objectives (Muktamar et al., 2024).

Preparation and Formulation of Culture Media with TDZ Variations

At this stage, researchers gather data and information on the effects of various TDZ concentrations on culture growth from literature or previous studies. Subsequently, they establish decision criteria for example, determining the range of TDZ concentrations to be tested based on practicality, effectiveness, and suitability for the specific plant species used (Kusakli et al., 2024).

Incubation and Maintenance of Cultures

During incubation, researchers must identify emerging issues such as abnormal growth or media contamination. This stage requires the ability to revisit and realign with the research objectives to ensure that culture maintenance remains on target (Samancı et al., 2023). At the same time, researchers select decision criteria such as determining the temperature, lighting, and incubation duration that best support optimal culture growth.

Observation of Growth (Number of Shoots and Leaves)

The observation stage involves collecting empirical data on culture growth through measurable parameters (Samancı et al., 2023; Siagian et al., 2023). From these results, researchers then select the best solutions to address identified issues. For instance, if growth performance is suboptimal at certain concentrations, the media composition or incubation conditions are adjusted accordingly.

Data Analysis of Observations

In the data analysis stage, researchers evaluate growth results from various treatments and select the most effective solutions based on statistically significant findings (Fernández-Morante et al., 2022). The resulting decisions are then implemented as recommendations for the most efficient TDZ concentration and culture procedures for subsequent stages.

Determination of Optimum TDZ Concentration

The final stage reflects the culmination of all prior decision-making processes. Researchers implement the determined optimal TDZ concentration that yields the best growth outcomes (Fischhoff, 2013). Afterward, evaluation and reflection are conducted to assess the success of the applied methods and identify aspects that require improvement for future research.

Based on the analysis of the relationship between the stages of the in vitro culture procedure of taro banana and the indicators of decision-making skills, it is evident that each laboratory activity represents a complex process of scientific reasoning.

Each stage requires the application of decision-making skills, including problem identification, information analysis, criteria setting, solution selection, decision implementation, and evaluation. This demonstrates that in vitro culture activities are not merely technical tasks but also serve as an avenue for cultivating critical and systematic thinking among students (Mettas, 2011).

This relationship forms the foundation for developing a biotechnology booklet that functions not only as a practical guide but also as a learning medium to foster decision-making skills. By integrating experimental processes with reflective decision-making, students can gain a more contextual and meaningful understanding of biotechnology concepts.

Conclusion

This study concludes that Thidiazuron effectively stimulates in vitro shoot formation in taro banana, with 0.01 mg/L identified as the optimum concentration, producing on average 22.25 shoots and 24.25 leaves per explant, and thus providing the most effective growth response. Each stage of the tissue culture procedure is closely aligned with key decision-making indicators, including problem identification, information collection and analysis, criteria setting, solution selection, and evaluating outcomes. By integrating these technical and reasoning components, the research provides a basis for developing a biotechnology booklet that functions both as a practical protocol and a contextual learning medium to strengthen students' data-based decision-making, thereby advancing both in vitro culture optimization and innovative biotechnology education.

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The authors declare no conflict of interest.

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