

Propagation of Shafira Taro (*Colocasia Esculenta* Var. Antiquorum) Applied TDZ, BAP, TDZ AND NAA, BAP and NAA In Vitro

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Abstract

Shafira taro (*Colocasia esculenta* var. antiquorum) is a type of small-tubered taro, also known as Japanese taro, which is traded internationally. This study aims to examine the effect of various concentrations of Thidiazuron (TDZ) and Benzyl Amino Purine (BAP), as well as the combination of Thidiazuron with Naphtalene Acetic Acid (NAA) and BAP with NAA, on the multiplication of Shafira taro shoots through in vitro culture techniques. The method used in this study uses in vitro culture techniques, which are modern plant propagation science known in the world of biotechnology and allow controlled propagation and improvement of plant species. An essential component of this technique is using plant growth regulators (PGRs), critical for modulating various physiological processes in plant cells and tissues. PGRs promote growth and differentiation, optimize secondary metabolite production, and increase plant resistance. This study used a combination of main PGR treatments (TDZ, BAP, and NAA) with a total of 13 (thirteen) treatment combinations using a randomized block design, namely: k0: Control, t1: TDZ 1 ppm, t2: TDZ 2 ppm, t3: TDZ 3 ppm, b1: BAP 1 ppm, b2: BAP 2 ppm, b3: BAP 3 ppm, t1n1: TDZ 1 ppm + NAA 0.5 ppm, t2n1: TDZ 2 ppm + NAA 0.5 ppm, t3n1: TDZ 3 ppm + NAA 0.5 ppm, b1n1: BAP 1 ppm + NAA 0.5 ppm, b2n1: BAP 2 ppm + NAA 0.5 ppm, b3n1: BAP 3 ppm + NAA 0.5 ppm. Each treatment was repeated thrice, with three tissue culture bottles per repetition, resulting in 117 tubes containing one explant. The results showed that the highest number of roots, leaves, and shoots was achieved with 1 ppm BAP, while the optimal fresh weight and shoot height were obtained with 2 ppm BAP. Treatments involving combinations of TDZ, TDZ + NAA, and BAP + NAA did not produce significant results for shoot multiplication.

Keywords— Benzyl Amino Purine; In Vitro; Naphthalene Acetic Acid; Shafira Taro; Thidiazuron

Introduction

The taro plant (*Colocasia esculenta* var. Antiquorum) is a species that has been widely cultivated in Indonesia, especially in areas such as Cisarua, Bantaeng, Malang, and Buleleng. Wudali et al. (2023) stated that this plant is not only cultivated for its edible tubers but also has a wealth of bioactive compounds, including phenols, flavonoids, and their various derivatives, which

contribute to its pharmacological activities to enhance immunity such as antioxidant, antimicrobial, antidiabetic, anti-inflammatory, and anticancer effects. In Japan, taro demand is met mainly through imports from China, with annual imports ranging from 55,000 to 60,000 tons, causing a supply deficit of around 40,000 to 45,000 tons (Zulianti et al., 2022).

Indonesia has the potential to address the shortage of taro supply in Japan, given its status as an agricultural country marked by two distinct seasons that can support sustainable agricultural efforts throughout the year. Currently, Indonesia wants to become the leading taro exporting country to Japan, competing with China; this can be realized due to Indonesia's diverse agricultural landscape and strategic land use planning (Siskha et al., 2021).

Indonesia's focus on identifying suitable land contours for taro cultivation is crucial given the crop's importance in food security and agricultural diversification. Taro is categorized as a tuber crop after cassava and sweet potato in Indonesia. Among the various types of taro, a variety known as satoimo taro, or Japanese taro, is characterized by its smaller tuber size and has the potential for stockpiling and economic development. This focus on land suitability and crop diversification is crucial to optimize agricultural productivity and ensure food security in Indonesia in the face of climate change (Syafiuddin et al., 2021)

Increasing taro production requires a sustainable availability of adequate crop seeds, unhindered by constraints related to seasonal variations, land accessibility, and prolonged planting duration. Consequently, increasing food productivity and ensuring the availability of large seedlings can be realized by adopting increasingly advanced technologies. This initiative is important, as it can potentially increase the productivity of increasingly scarce food crops. One such technological approach involves in vitro shoot propagation techniques, which aim to meet market demand (Lone et al., 2020).

In vitro culture techniques are essential in modern plant biotechnology, enabling controlled propagation and improvement of plant species. Yunita (2023) stated that an important component of this technique is using plant growth regulators (PGRs), which are important for modulating various physiological processes in plant cells and tissues. PGRs are important for promoting growth and differentiation, optimizing the production of secondary metabolites, and increasing plant resistance. ZPT induces organogenesis, such as shoot and root formation, which is crucial for successful plant regeneration (Bidabadi & Jain, 2020).

Plant growth regulators' importance in in vitro culture is underlined by their ability to influence outcomes such as biomass accumulation, flavonoid production, and successful plant regeneration. Different combinations of plant growth regulators are required to optimize both parameters, highlighting the specific approaches required for different plant species and desired outcomes (Kaban et al., 2024).

Literature Review

The success of in vitro culture is highly dependent on the composition of the growth medium, which includes the right balance of nutrients and PGRs. Murashige Skoog (MS) medium, often supplemented with various PGRs, is the standard choice for many plant species. Adjusting the concentration of these regulators can significantly impact the efficiency of micropropagation and the quality of the resulting plantlets (Garg et al., 2024; Sidik et al., 2024).

In vitro culture techniques are essential in plant biotechnology, the role of growth regulators (PGRs) in modulating physiological processes, thereby increasing the propagation and resilience of plant species through controlled conditions and nutrient management (Pasternak &

Steinmacher, 2024).

The media used in tissue culture is important in the propagation process. The efficacy of plant propagation and development through tissue culture methods largely depends on the type of specific media used. The media used in tissue culture significantly impact the growth and development of explants and the seedlings produced. Various basic media, including MS (Murashige Skoog) media, are used. This preference is due to the increased salt concentration and high nitrate content in MS media. In the context of in vitro culture, growth regulators, commonly called regulators, are very important in influencing growth and morphogenesis in cell, tissue, and organ cultures. Growth regulators that can be used for in vitro taro culture include Thidiazuron (TDZ), Benzyl Amino Purine (BAP), Naphthalene Acetic Acid (NAA), and Indole Acetic Acid (IAA). The use of these regulators is intended to enhance plant resistance to biotic and abiotic stresses by modulating stress-related pathways, thereby improving overall plant health and productivity (Gujar et al., 2024; Kaya & Özkaya, 2024)

Thidiazuron is a type of cytokinin hormone that can be used effectively in in vitro plant culture. Incorporating cytokinins into in vitro culture media is essential for the induction of growth and development processes in explants. NAA is classified as an auxin hormone used for cell division and root differentiation in tissue culture. Applying A can also suppress the adverse effects caused by BAP, improving the quality during in vitro rooting. NAA is mainly used for rooting, while BAP is a classification of synthetic cytokinins widely used in plant tissue culture and agricultural practices to enhance growth and yield (Abouelsaad & Brengi, 2022).

The novelty in this research is the integration or combination of Naphthalene Acetic Acid (NAA) with Thidiazuron (TDZ) and Benzyl Amino Purine (BAP), which represents a significant advance in plant growth regulation, as previous studies have mainly focused on the effects of TDZ and BAP alone or NAA and BAP. This combination has the potential to be developed to improve plant propagation and growth in a variety of species. Martins et al. (2020) reported in their study on *Alcantarea imperialis* that NAA can reduce the adverse physiological effects caused by prolonged exposure to BAP, improving overall plant quality. The interaction of NAA with other growth regulators such as gibberellic acid (GA3) has also been shown to improve flower traits and cross-pollination rates in rice, further emphasizing the versatility of NAA in plant growth regulation (Elshamey et al., 2022).

While the combination of NAA with TDZ and BAP has potential, it is important to consider that the effectiveness of these growth regulators may vary based on plant species and environmental conditions. Further research is needed to optimize the application of the combination of these three growth regulators in various contexts.

Research Method

The research was conducted at the Tissue Culture Laboratory, Faculty of Agriculture, Hasanuddin University, Makassar, from December 2023 to February 2024. The materials used were Shafari taro explants, MS media stock solution, ZPT (TDZ, BAP, and NAA), distilled water, 96% alcohol, granulated sugar, and agar powder. The tools used were analytical scales, measuring flasks, Erlenmeyer flasks, beakers, measuring cups, petri dishes, droppers, measuring pipettes, plastic funnels, pH meters, ovens, and autoclaves, while the tools for preparing and planting explants were: tweezers, scissors, scalpels, blades, petri dishes, hand sprayers, and laminar air flow.

This study used a Randomized Block Design (RBD) with 13 treatments, namely: k0: Control, t1: TDZ 1 ppm, t2: TDZ 2 ppm, t3: TDZ 3 ppm, b1: BAP 1 ppm, b2: BAP 2 ppm, b3: BAP 3 ppm,

t1n1: TDZ 1 ppm + NAA 0.5 ppm, t2n1: TDZ 2 ppm + NAA 0.5 ppm, t3n1: TDZ 3 ppm + NAA 0.5 ppm, b1n1: BAP 1 ppm + NAA 0.5 ppm, b2n1: BAP 2 ppm + NAA 0.5 ppm, b3n1: BAP 3 ppm + NAA 0.5 ppm. The combination of treatments was 13, where each treatment consisted of three groups, each treatment in each group consisted of three culture bottles as experimental units. Hence, the total number of treatments was 117 bottles/treatment units. Each culture bottle was planted with 1 explant.

Preparation of Stock Solutions and Media: Formulation of stock solutions can simplify the processes involved in media preparation. MS media stock solutions are preserved in vials. These stock solutions are prepared into groups: macronutrient stock solutions (A, B, C, D) and micronutrient stock solutions (E, F).

MS media is formulated by measuring each solution carefully according to the treatment concentration required for the experiment. Then, this solution is put into a volumetric flask filled with distilled water (aquades), corresponding to half of the total amount of media intended to be produced. Next, add 30 grams/L of sugar to the mixture, then add the TDZ concentration according to the predetermined treatment level, namely 1 ppm, 2 ppm, and 3 ppm, then stir vigorously to ensure that the components are thoroughly homogenized so that they achieve a uniform consistency. After all components are carefully combined, distilled water (aquades) is added to the mixture until it reaches the desired volume. The pH measurement should ideally be in the range of 5.6 to 5.8. If the measured pH is lower than the threshold of 5.6, then a few drops of sodium hydroxide (NaOH) must be added wisely to the solution until the pH level reaches the desired range. Next, the addition of agar as much as 7 grams/L / L, which is then heated while stirring continuously until the agar powder is completely dissolved and produces a clear and homogeneous solution, which is then carefully filled into culture bottles, where each bottle is filled with about 20 to 30 milliliters. After filling, the culture bottle is securely closed with aluminum foil and placed into the autoclave for critical sterilization. Sterilization of the media is carried out under controlled conditions, especially at a pressure of 1.23 kg cm⁻², and maintained for about 15 minutes at a temperature of 121 degrees Celsius to ensure that all microbial contaminants are effectively eliminated.

Results and Discussion

Number of Leaves

The observation results of the number of leaves of Shafira Taro show that the treatment has no genuine, significant effect on the number of leaves. The BAP treatment produced a very real difference from TDZ, where the number of leaves was 21.04 and 10.63, respectively. This is because BAP added to the MS media will further stimulate the growth and development of the canopy of the taro plant. The addition of BAP is considered more stable and efficient than other cytokinins; BAP can also induce the formation of shoots and leaves. In addition, BAP can stimulate the formation of plant organs (Shallal, 2024).

Likewise, the number of leaves produced in the BAP + NAA treatment was 14.89 strands, which was significantly different from the TDZ + NAA treatment, which produced several leaves of 8.26 strands, but lower than the single BAP treatment. It is suspected that the addition of NAA causes upregulation of genes related to potassium uptake and transport, as well as antioxidant-related genes critical for root development under stress conditions (Zhou et al., 2024).

Table 1. Orthogonal Contrast Test of Number of Leaves

Implementation	Average		Symbol	
k0 vs k1-k12 (Control vs All Treatments)	12.29	vs	13.70	tn
k1-k6 vs k7-k12 (TDZ and BAP vs TDZ and BAP + NAA)	15.83	vs	11.58	**
k1-k3 vs k4-k6 (TDZ vs BAP)	10.63	vs	21.04	**
k1 vs k2k3 (TDZ 21ppm vs TDZ 2 ppm, TDZ 3 ppm)	12.67	vs	9.61	tn
k2 vs k3 (TDZ 2 2 ppm vs TDZ 3 ppm)	11.66	vs	7.56	tn
k4 vs k5k6 (BAP 1 ppm vs BAP 2 ppm, BAP 3 ppm)	23.00	vs	20.06	tn
k5 vs k6 (BAP 2 ppm vs BAP 3 ppm)	19.33	vs	20.78	tn
k7-k9 vs k10-k12 (TDZ+NAA vs BAP+NAA)	8.26	vs	14.89	**
k7 vs k8k9 (TDZ 1 ppm + NAA vs TDZ 2 ppm and TDZ 3 ppm + NAA)	11.89	vs	6.45	**
k8 vs k9 (TDZ 2 ppm + NAA vs TDZ 3 ppm + NAA)	7.44	vs	5.45	tn
k10 vs k11k12 BAP 1 ppm + NAA vs BAP 2 ppm and BAP 3 ppm + NAA)	10.67	vs	17.00	**
k11 vs k12 (BAP 2 ppm + NAA vs BAP 3 ppm + NAA)	10.78	vs	23.22	**

Description: ** marks are the same in the same column, very different

Number of Shoots

The observations of the number of shoots of Shafira Taroshow show that the treatment has an insignificant, significant, and very significant effect on the number of shoots. The number of shoots in the BAP treatment was greater (5.56 shoots) and was significantly different compared to the TDZ treatment (2.89 shoots). These results explain that the BAP treatment also stimulates optimal shoot growth in addition to the number of leaves. Treatment with the addition of BAP in MS media has a good effect on shoot formation and produces many shoots (Turang et al., 2023). According to Septasari & Mercuriani (2023), BAP can induce axillary shoots for maximum in vitro differentiation, where the application of BAP addition to MS media in vitro can accelerate protocorms and facilitate the early stages of organogenesis, so that BAP effectively stimulates shoot multiplication (Hany et al., 2023).

Meanwhile, the BAP + NAA treatment results produced more shoots, namely 3.81 shoots, and were significantly different from the TDZ + NAA treatment's 2.19 shoots. This shows that growth regulators such as BAP (6-benzyl aminopurine) and NAA (naphthalene acetic acid) have increased shoot proliferation and taro rooting. Applying MS media supplemented with BAP and NAA effectively encourages shoot proliferation without callus formation (Kepue et al., 2021).

Table 2. Orthogonal Contrast Test of Shoot Count

Implementation	Average		Symbol	
k0 vs k1-k12 (Control vs All Treatments)	1.45	vs	3.54	tn
k1-k6 vs k7-k12 (TDZ and BAP vs TDZ and	4.07	vs	3.00	**



BAP + NAA)

k1-k3 vs k4-k6 (TDZ vs BAP)	2.59	vs	5.56	**
k1 vs k2k3 (TDZ 21ppm vs TDZ 2 ppm, TDZ 3 ppm)	2.89	vs	2.44	tn
k2 vs k3 (TDZ 2 2 ppm vs TDZ 3 ppm)	3.11	vs	1.78	tn
k4 vs k5k6 (BAP 1 ppm vs BAP 2 ppm, BAP 3 ppm)	6.00	vs	5.33	tn
k5 vs k6 (BAP 2 ppm vs BAP 3 ppm)	5.33	vs	5.33	tn
k7-k9 vs k10-k12 (TDZ+NAA vs BAP+NAA)	2.19	vs	3.18	**
k7 vs k8k9 (TDZ 1 ppm + NAA vs TDZ 2 ppm and TDZ 3 ppm + NAA)	2.78	vs	1.89	tn
k8 vs k9 (TDZ 2 ppm + NAA vs TDZ 3 ppm + NAA)	2.11	vs	1.67	tn
k10 vs k11k12 BAP 1 ppm + NAA vs BAP 2 ppm and BAP 3 ppm + NAA)	3.11	vs	4.17	tn
k11 vs k12 (BAP 2 ppm + NAA vs BAP 3 ppm + NAA)	3.11	vs	5.22	**

Description: ** marks are the same in the same column, very different

Number of Roots

BAP treatment produced the most significant number of roots and significantly differed from TDZ treatment. BAP treatment produced more roots and significantly differed from TDZ treatment (Table 3). This shows that BAP can also stimulate the initiation of root formation. Shallal (2024) stated that micro propagation can indirectly support root development by providing healthy shoots for further rooting processes.. However, adding BAP in higher concentrations to the media causes the explants to focus more on shoot development, thus inhibiting the explants from forming roots (Navarro & Canales, 2021).

The number of roots produced in the BAP + NAA treatment was not significantly different from that produced in the TDZ + NAA treatment. This is likely because the NAA added was still low, so the cytokinins in BAP still defeated its effect. Higher Cytokinin use can suppress the auxin signaling pathway, inhibiting root growth. This suppression is part of a complex regulatory network involving other hormones such as ethylene, which also regulate root growth (Zhang et al., 2024).

The BAP treatment produced more roots (11.78 strands) and significantly differed from the number of roots in the TDZ treatment (3.00 strands). It is suspected that the BAP concentration of 2 ppm shows the appropriate concentration to stimulate the initiation of leaf formation. This is in line with the research of Rimy et al (2024), which stated that the combination of BAP and kinetin was optimal for shoot initiation and regeneration, while IBA and NAA were effective for root induction. This illustrates the importance of choosing the right PGR for various stages of plant development. Likewise, in Dendrobium Red Emperor 'Prince': The study found that BAP 2 ppm was optimal for inducing axillary buds, affecting the time of shoot emergence and the growth of the crown and roots (Septasari & Mercuriani, 2023).

Table 3. Orthogonal Contrast Test of Number of Roots



Implementation	Average			Symbol
k0 vs k1-k12 (Control vs All Treatments)	16.33	vs	5.86	**
k1-k6 vs k7-k12 (TDZ and BAP vs TDZ and BAP + NAA)	7.39	vs	4.33	*
k1-k3 vs k4-k6 (TDZ vs BAP)	3.00	vs	11.78	**
k1 vs k2k3 (TDZ 21ppm vs TDZ 2 ppm, TDZ 3 ppm)	4.67	vs	2.17	tn
k2 vs k3 (TDZ 2 2 ppm vs TDZ 3 ppm)	2.00	vs	2.33	tn
k4 vs k5k6 (BAP 1 ppm vs BAP 2 ppm, BAP 3 ppm)	16.33	vs	9.50	*
k5 vs k6 (BAP 2 ppm vs BAP 3 ppm)	11.67	vs	7.33	tn
k7-k9 vs k10-k12 (TDZ+NAA vs BAP+NAA)	4.11	vs	4.56	tn
k7 vs k8k9 (TDZ 1 ppm + NAA vs TDZ 2 ppm and TDZ 3 ppm + NAA)	7.33	vs	2.50	tn
k8 vs k9 (TDZ 2 ppm + NAA vs TDZ 3 ppm + NAA)	3.67	vs	1.33	tn
k10 vs k11k12 BAP 1 ppm + NAA vs BAP 2 ppm and BAP 3 ppm + NAA)	4.67	vs	4.50	tn
k11 vs k12 (BAP 2 ppm + NAA vs BAP 3 ppm + NAA)	2.67	vs	6.33	tn

Description: ** marks are the same in the same column, very different

Conclusion

The conclusion of this study shows that the number of leaves, shoots, and roots produced in the BAP treatment is better than in the TDZ treatment. Likewise, the number of leaves and shoots produced in the BAP + NAA combination treatment is better than that of the TDZ + NAA combination treatment. Therefore, further research is needed on combining various concentrations of BAP and NAA in the propagation of shafira taro shoots. The combination of hormones has been proven effective in inducing the formation of shoots and leaves simultaneously, thereby accelerating the process of plant regeneration from explants. With this approach, the community can produce high-quality taro seeds in a short time, controlled and free from disease, which is very important to support the needs of agribusiness and conservation of local taro germplasm. The results of this study also have the potential to be the basis for developing tissue culture protocols for other tuber plants with similar physiological characteristics.

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