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Growth of Banana cv. Kayu (*Musa paradisiaca* cv. Kayu) Meristemoids on Various Type of Media In Vitro

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ABSTRACT

Banana is an important fruit commodity in Bali that has high demand from the tourism sector and religious ceremonies. However, its availability has not been fulfilled continuously because local production is limited and still relies on supplies from outside the region. One of the efforts to provide quality seedlings and preserve local banana genetic resources is through tissue culture techniques. This study aimed to obtain the best media composition for the growth of Timber banana meristemoids in vitro. The research was conducted at the Tissue Culture Laboratory of the Faculty of Agriculture, Udayana University from February to November 2023, using a two-factor factorial design with five replications. The first factor was media type (MS, BMM, and AGS), while the second factor was growth regulator (Z0 = no ZPT, Z1 = BAP 5 ppm, Z2 = TDZ 2 ppm, Z3 = BAP 5 ppm + TDZ 2 ppm). Variables observed included shoot emergence, number of shoots, number of leaves, number of roots, and root length. The results showed no interaction between treatments, but MS media gave the best results in almost all variables observed. ZPT treatment significantly affected the number of leaves, with 2 ppm TDZ producing the best growth in the number of leaves, number of roots, and root length. Thus, MS media is the optimal media for in vitro culture of Timber banana meristemoids, while the use of 2 ppm TDZ is effective in increasing the growth of vegetative organs.

Keywords: Wood banana, Meristemoid, Media Type

INTRODUCTION

Banana is a fruit commodity that is much needed in Bali by hotels and restaurants to support Bali as a tourist destination and also needed during Hindus religious holidays such as Galungan and Kuningan. Until now, the fulfilment of these fruit needs is still imported from Java (Bali.bisnis.com., 2016). Suparyana et al. (2017) mentioned that the demand for fruit in Bali is very high and exceeds the production capacity of Balinese farmers. This shows that the commodity is not continuously available and needs a solution.

One of the factors suspected to be the cause is the lack of intensive banana cultivation in Bali. Intensive cultivation requires a sufficient number of good quality seedlings. Conventional propagation by farmers cannot fulfil the needs of intensive cultivation, as the number of seedlings produced is very limited. Tissue culture technology offers a solution for the availability of healthy seedlings, in large quantities and identical to their parents. Dwiyani (2015) states that tissue culture is one of the methods used for plant propagation.

Seedlings produced from this technology are not only identical to their parents because it is classified as vegetative propagation, but also clean, healthy and in relatively large quantities.

Banana Kayu is one of the local Balinese genetic resources that has unique characteristics, especially its long shelf life and flavour that is highly preferred by many people. The uniqueness of Indonesian bananas, including Balinese, was pointed out by Valmayor *et al* (2000). In addition, the existence of wooden banana species is very rare in the market, so efforts to conserve this genetic resource need to be made. One of these efforts can be done through *ex situ* conservation by in vitro propagation. In vitro propagation will produce plants that are genetically similar to their parents (Dwiyani, 2015), so that the genetic conservation of Wood banana can be done.

Previous research for banana has successfully been conducted through direct organogenesis, on Raja and Kayu banana cultivars but only using one type of media, MS (Dwiyani et al., 2022). The study resulted in an inadequate number of plantlets as per the principle of in vitro propagation. In this study, different types of media will be tried in combination with various cytokinin growth regulators to stimulate shoots.

This study will evaluate and analyse the use of various types of in vitro media using meristemoid planting material. Meristemoid is a new formation (propagules) formed from explants grown in vitro, consisting of a collection of meristematic cells, resembling callus but containing bud primordia. In this study, meristemoids were generated from banana stem explants with media containing cytokinin. There were three types of media tried in this study, namely MS (Murashige & Skoog) media, a medium commonly used in in vitro culture; BMM (Banana Multiplication Medium) media; Banana AGS Basal Medium. The latter two are specially formulated media for banana plants. This type of media will be combined with cytokinin growth regulators that function to stimulate shoot growth. The aim of the study is to obtain the best media composition for the growth of wood banana meristemoids in vitro.

MATERIALS AND METHODS

The research was conducted from February to November 2025 at the Tissue Culture Laboratory, Faculty of Agriculture, Udauyana University. The plant material used was the meristemoid of Banana Wood plant, which was grown from banana stem explants in December 2024. The chemicals required were MS, BMM, and AGS media, sugar, BAP, Thidiazuron, bioagar. Alcohol for sterilisation and distilled water are also required. The equipment used were laminar, autoclave, petri dish, spatula, bunsen lamp, magnetic steerer, measuring cup, digital scale, pH meter.

The study used factorial design, two factors, with 5 replicates. The first factor is the type of media, namely MS (Murashige & Skoog Media), BMM (Banana Multiplication Medium); AGS (Banana AGS Basal Medium). The second factor is the growth regulator, namely: Z0= without ZPT, Z1= Benzyl amini purine (BAP) 5 ppm, Z2= Tidiazuron (TDZ) 2 ppm, Z3= BAP 5 ppm + TDZ 2 ppm. Thus there were 12 treatments, repeated 5 times, so there were 60 experimental units. Each unit was represented by one Observations were made on: time of shoot growth, number of shoots, number of leaves, number of roots and root length.

RESULTS AND DISCUSSION

The observation results (Table 1) showed that the type of planting media gave a significant effect on most of the growth variables of wood banana plantlets in vitro. MS media proved to give the best results with

an average number of shoots of 2.41, number of leaves of 2.21, number of roots of 2.11, and root length of 2.45 cm. These values were statistically significantly different compared to BMM and AGS media, which only produced lower vegetative growth in almost all parameters. The superiority of MS media in supporting the growth of wood banana meristemoids is most likely due to the more balanced composition of macro and micro nutrients, especially the availability of nitrogen in the form of nitrate and ammonium, as well as sufficient vitamin and sugar content to support cell metabolism, division, and tissue differentiation (Joju *et al*., 2024). Similar research was also reported by Jafari et al. (2023) which stated that MS media is the most versatile and effective media in supporting the growth of various Musa species compared to banana-specific media. Meanwhile, for the parameter of shoot emergence, no significant differences were found between media, with a range of 3.09-3.53 days. This indicates that the initial speed of shoot emergence is more influenced by the physiological condition of the explants, while the growing medium is more decisive in the next phase of growth. In other words, although the initial initiation is relatively the same in all media, shoot development, leaf formation and root induction are highly dependent on the nutrient composition of the media.

The growth regulator (ZPT) factor also had a different effect on plantlet growth. The treatment without ZPT (Z0) produced fewer leaves (1.27 leaves), indicating that the presence of exogenous hormones is absolutely necessary to stimulate the vegetative growth of banana wood in vitro. The application of 2 ppm TDZ (Z2) showed the most optimal results with an average number of leaves of 2.29, number of roots of 2.15, and root length of 2.39 cm. The effectiveness of TDZ is related to its nature as a very strong synthetic cytokinin, capable of triggering cell division, inducing shoot multiplication, and increasing the differentiation of vegetative organs. These results are in line with the research of Das *et al*. (2025) who reported that TDZ is effective in increasing the number of shoots and vigour of Musa spp. plantlets, and in line with the findings of Molla *et al*. (2020) which showed that TDZ can work better than BAP in spurring shoot multiplication and root development in local banana varieties. In contrast, the treatment of BAP 5 ppm (Z1) and the combination of BAP 5 ppm + TDZ 2 ppm (Z3) gave lower results than TDZ alone. This may be due to the competition or antagonistic effect between BAP and TDZ, resulting in reduced growth induction effectiveness. Research by Memon *et al*. (2020) also reported that the use of BAP in high concentrations tends to produce lower shoot multiplication and can cause abnormalities in banana plantlets, while single TDZ produces more vigorous and balanced growth between shoots and roots.

Furthermore, the analysis showed that there was no interaction between the treatment factors (media × ZPT) on all observation variables. This indicates that the combination of the type of growing media and the application of ZPT does not have a synergistic or antagonistic effect, so that the influence of both stands independently. In other words, the type of planting media plays a role according to the composition of nutrients and the balance of nutrients it contains, while ZPT works through physiological mechanisms regulating cell division and differentiation without any interdependence. The media factor proved to make a more dominant contribution in determining the success of meristemoid growth, especially in the parameters of the number of shoots, number of leaves, number of roots, and root length, while in the parameter of shoot emergence no significant differences were found between media. This condition confirms that the speed of shoot initiation is more determined by the

physiological potential of the explants, while the media plays more of a role in supporting the development of the next growth phase. In contrast, the ZPT factor did not have a significant effect on most of the observed variables, except for the number of leaves that showed different responses between treatments. This finding shows that ZPT, especially TDZ, is more functional in spurring the growth of certain vegetative organs, while the overall success of plantlet growth is more determined by the quality of the growing medium used.

The results of this study indicate that Murashige and Skoog (MS) media is the most suitable media for the growth of wood banana meristemoids in vitro compared to BMM and AGS media. MS media has the advantage of containing more balanced macro and micro nutrients so that it can support cell metabolism, division, and tissue differentiation in various plant species. This explains why MS is not only used for banana culture, but has also been widely applied to various types of horticultural plants (Das *et al*., 2025). Research on the banana cultivar 'Amrit Sagar' also confirmed that MS media enriched with TDZ produced optimal shoot regeneration and maintained genetic stability, making it superior to other media (Das *et al*., 2025).

In addition, the role of growth regulators (ZPT) is very important in promoting the growth of in vitro cultures. This study proved that the application of ZPT produced a better response than the treatment without ZPT, indicating that wooden banana culture requires exogenous hormones to support organogenesis. Among the various types of ZPT, tidiazuron (TDZ) at a concentration of 2 ppm gave the best results on the number of leaves, number of roots, and root length. TDZ is known as a synthetic cytokinin that is very potent in inducing shoot formation and vegetative multiplication. In fact, TDZ has a dual mechanism because at certain concentrations it can stimulate root formation, something that rarely happens with conventional cytokinins (Niazian *et al*., 2019).

Table 1. Effect of treatments on observed variables

Treatment	Time to Emergence Shoots (days)	Number of Buds (fruit)	Number of Leaves (strands)	Number of Roots (pieces)	Root Length (cm)
Media Type Factor:					
BMM	3,26 a	1,36 b	1,30 b	1,27 b	1,37 b
AGS	3,09 a	1,28 b	1,87 a	1,56 a	1,52 b
MS	3,53 a	2,41 a	2,21 a	2,11 a	2,45 a
BNT 5%	1,28	0,46	0,74	0,78	0,89
Growth regulator factor:					
Z0	2,87 a	1,43 a	1,27 b	1,34 a	1,51 a
Z1	3,32 a	1,86 a	1,97 a	1,66 a	1,64 a
Z2	3,38 a	1,59 a	2,29 a	2,15 a	2,39 a
Z3	3,60 a	1,86 a	1,65 a	1,43 a	1,58 a
BNT 5%	-	-	0,86	-	-

Notes: Numbers followed by the same letters show no significant difference based on BNT test at 5% level. BMM=Banana Multiplication Medium; AGS= Banana AGS Basal Medium; Z0= control/no ZPT; Z1= Benzyl amini purine (BAP) 5 ppm, Z2= Tidiazuron (TDZ) 2 ppm, Z3= BAP 5 ppm+ TDZ 2ppm

Recent research has also shown similar results. For example, in banana cv. Neypoovan, MS media treatment with TDZ combination resulted in higher shoot multiplication than the control treatment (Joju *et al*., 2024). Similarly, Ngomuo et al. (2022) reported that TDZ was able to increase root length and shoot number simultaneously, signalling the flexibility of this hormone in regulating morphogenesis pathways. Singh *et al*. (2021) also found that the combination of TDZ with auxin produced a synergistic effect that significantly improved shoot growth in banana cultures. These findings reinforce the results of this study that the right concentration of TDZ (2 ppm) is a key factor in the success of in vitro propagation of wood banana.

Figure 1 shows the differences in phenotypes of plantlets grown on three types of media (BMM, AGS, and MS) with four variations of ZPT treatments (control, BAP 5 ppm, TDZ 2 ppm, and a combination of BAP 5 ppm + TDZ 2 ppm). Visually, it can be seen that MS media produces plantlets with healthier growth, vigour, and uniformity compared to BMM and AGS media. On MS media with 2 ppm TDZ treatment, plantlets showed more leaves, longer root growth, and fresh green leaf colour, indicating optimal nutrient availability and hormonal response. This supports the quantitative results in Table 1, where the MS + TDZ 2 ppm treatment gave the highest values in number of leaves, number of roots, and root length.

In contrast, plantlets on BMM media appeared smaller with a limited number of leaves and roots, and even the control treatment without ZPT showed a dwarf phenotype with pale leaves and minimal root growth. AGS media showed an intermediate response, where the addition of ZPT could improve growth, but the results were still lower than MS media. The phenotypes that emerged in the 5 ppm BAP treatment and the combination of BAP + TDZ showed less optimal growth compared to TDZ alone, both on MS and other media. This indicates that BAP in high concentrations can be less effective or even cause an antagonistic effect on TDZ activity, so that growth is not as good as the treatment with TDZ alone. From a visual aspect, it can be seen that the use of 2 ppm TDZ on MS media produces plantlets with the most ideal morphology for successful in vitro propagation, namely a greater number of shoots, wider leaves, longer roots, and green leaf colour indicating healthy plant physiology. Meanwhile, the no ZPT control on all media produced less developed phenotypes, confirming the important role of hormonal regulation in plantain tissue culture. These findings are consistent with the study of Das et al. (2025) who reported that TDZ is highly effective in inducing shoot multiplication in Musa spp. and Joju et al. (2024) who showed that MS media is able to support the morphological growth of banana plantlets better than banana-specific media (such as BMM and AGS). Therefore, the combination of MS media with 2 ppm TDZ can be considered as the most optimal treatment in producing vigorous banana wood plantlets in vitro.

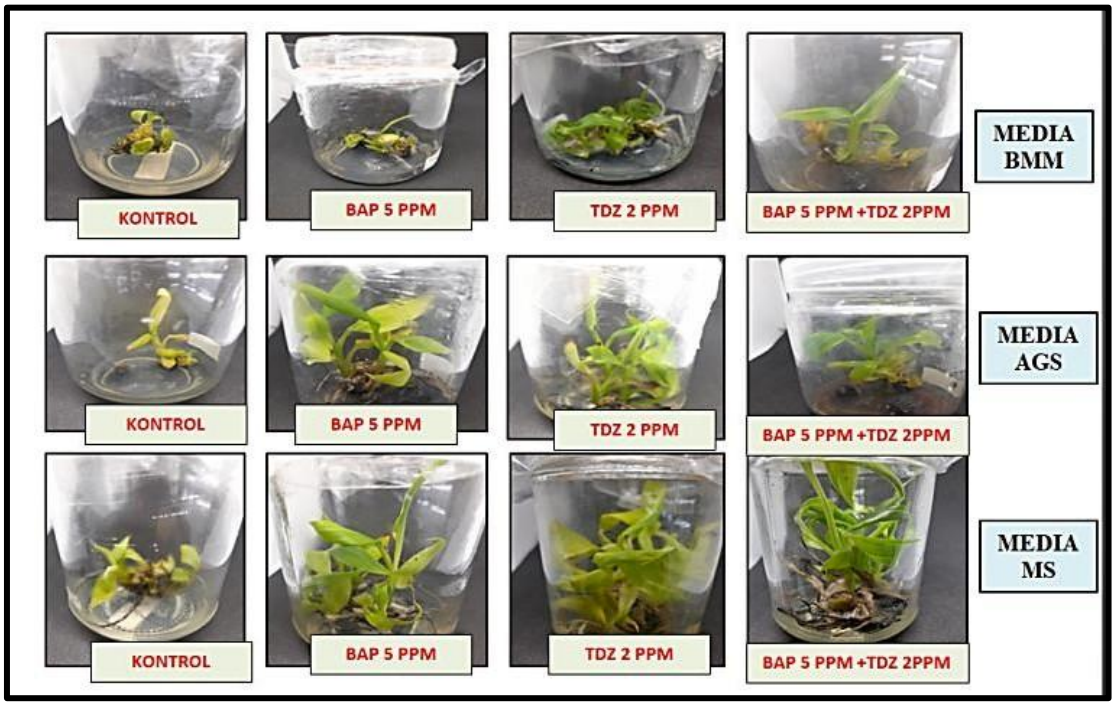

Figure 1. Phenotypes of Plantlets of Plantain Wood on Various Treatments

CONCLUSIONS

This study shows that the type of culture media significantly affects the growth of wood banana meristemoids in vitro. Murashige & Skoog (MS) media proved to give the best results compared to BMM and AGS media, as indicated by higher average number of shoots, number of leaves, number of roots, and root length. Growth regulator (ZPT) factors also had an effect, especially on the number of leaves, where the administration of tidiazuron (TDZ) at a concentration of 2 ppm produced the most optimal response compared to other treatments. Further analysis showed that there was no interaction between the media and ZPT factors, indicating that they work independently. In this case, the media plays a more dominant role in supporting growth, while TDZ serves an important function in stimulating the formation of certain vegetative organs. Overall, the combination of MS media with 2 ppm TDZ can be considered as the best treatment because it can produce vigorous plantlets with healthy phenotypes, more leaves, longer roots, and fresh green leaf colour.

SUGGESTION

Based on the results of this study, the use of MS media with the addition of 2 ppm TDZ is recommended as the most suitable treatment for in vitro propagation of banana wood on a mass scale. However, further research still needs to be done by exploring variations in TDZ concentration or its combination with auxin to increase the effectiveness of shoot multiplication and root formation. In addition, it is also important to test the genetic stability of the tissue-cultured plantlets to ensure genetic uniformity and purity, especially in the context of conservation of local Balinese genetic resources. In the next stage, in vitro-cultured

plantlets need to be further tested in the acclimatisation phase in the field to determine their adaptability and growth success rate before being developed in wide-scale cultivation.

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