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COMBINATION OF BAP AND TDZ IN INDUCING THE GROWTH OF BANANA SHOOTING EXPLANT CV. BARANGAN IN VITRO

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ABSTRACT
Barangan banana (*Musa acuminata* L.) is one of the superior types of banana in the West Tanjung Jabung district, Jambi Province - Indonesia. Barangan bananas are very popular with the public and have high economic value. The problem faced in conventional cultivation and development of Barangan bananas is that it is difficult to obtain quality banana seeds in large and uniform quantities, so to overcome this we need alternative technology for propagating banana seeds through modern techniques such as plant tissue culture techniques. The advantage of this technique is that it is able to produce plant seeds en masse uniformly and in a relatively short time. In addition, the resulting progeny is also conditioned to be free from pests and diseases, especially diseases caused by bacteria and fungi.

KEY WORDS
Explant, callus, MS media, in vitro, growth regulator.

The bananas are a horticultural crop with high economic value in Indonesia and abroad. Various superior bananas are developed in Indonesia, such as kapok, raja, cavendish and barangan bananas. Barangan bananas are one of the leading types of bananas in several provinces in Indonesia, especially in North Sumatra and Jambi Provinces. This banana has a distinctive, sweet taste and fragrant aroma so it is very popular in national and international markets. Apart from its sweet and fresh taste, the nutrients contained in bananas are also high and complete, such as carbohydrates, protein, fat, cellulose, starch and tannins, vitamin A, vitamins B1, B2, B6 and B12, vitamin D, vitamin C, calcium, phosphorus, iron, sodium, potassium, magnesium and zinc (Direktorat Budidaya and Pascapanen Buah, 2012). In the ASEAN region, the value of Indonesian banana exports is in fifth place with a contribution of 0.06% to the volume of ASEAN banana exports (Kementerian Pertanian. 2014; Sonia et al., 2016). Banana production in Indonesia in 2020 tends to increase compared to previous years, but has not been able to keep up with increasing consumer demand, so efforts are still being made to extensive banana planting areas in Indonesia (Direktorat Perlindungan Hortikultura, 2019; Badan Pusat Statistik, 2021).

The banana cultivation in an extensification effort in monoculture and on a wide scale requires the availability of seeds with clear genotypes, uniform, healthy and strong and in large quantities. This of course cannot be achieved through conventional seed propagation. One alternative technology that can be applied in efforts to extensify banana plants is to use tissue culture techniques. The advantage of this technique is that it can produce plants massively, uniformly and in a relatively short time (Zulkarnain. 2009; Elyazid et al., 2021; Eliyanti et al., 2023). Apart from that, the offspring produced are also free from pests and diseases, especially those caused by bacteria and fungi (Sivakumar. 2021; Xiang. 2023). In banana plant propagation, the plant tissue culture techniques applied to date still have very varying levels of success, so ongoing testing must still be carried out (Yusnita et al., 2015; Eustache et al., 2021).

One of the factors that influence the success of plant propagation through tissue culture is the composition of the medium. The medium composition commonly used is MS medium (Murashige and Skoog, 1962). This medium is suitable for almost all plant species. Apart from the application of growth regulators (ZPT), the type and concentration of ZPT used also play an important role in plant tissue culture techniques (Hendaryono and Wijayani., 2012; Yusnita et al., 2015). The PGR that is often used in banana tissue culture is from the cytokinin group, which plays an important role in increasing cell division, callus formation, shoot proliferation, morphogenesis, organogenesis and embryogenesis (Rivai and Helmanto, 2015; Sukowardana, 2015). Cytokinins also influence shoot dominance, peripheral bud growth, and leaf senescence. There are two types of cytokinins, namely the adenine type and the phenylurea type. Adenine types include kinetine, zeatin, and benzylaminopurine (BAP), while phenylurea types include diphenylurea and tidiazuron (TDZ) (Kieber. 2002; Aina et al., 2012). TDZ and BAP are a class of synthetic cytokinins that have better abilities and are most effective in inducing shoots among other cytokinins (Wardiyati. 2018; Maninggolang et al., 2018). Something similar was also reported by Eustache et al. (2021) that BAP is an effective cytokinin for the proliferation of banana and plantain shoots in vitro, furthermore the use of TDZ also complements the effect of banana explant development in vitro. However, Budi (2020) and Nur'riyani (2021) state that whatever type of cytokinin is used, the optimal cytokinin concentration for shoot proliferation is very dependent on the plant genotype and the combination of cytokinin with other PGRs used in the in vitro media. Furthermore, various concentrations of cytokinin are used, still varies in inducing the development of plant explants in vitro. Nurmaningrum et al. (2017) reported that the combination of BAP and TDZ in MS media was effective in speeding up the initiation time and increasing the growth of alfalfa shoots. Administration of cytokinin between 0.1-10 ppm was able to induce the formation of shoots according to cultivar specifications. Previously, Suminar et al. (2016), stated that the response of banana cultivars to BAP was relatively significant compared to other types of cytokinins such as zeatin, kinetin, and 2-iP, but at low concentrations Thidiazuron (TDZ) could increase the number of shoots on banana. Another PGR combination was reported by Saini et al. (2016) that increasing the concentration of banana extract and BAP affect the emergence of shoots and roots, shoot height, number of shoots and root length and number of roots in explants of the banana cultivar "Haji".

Based on the description above, research was conducted to test the effect of the combination of BAP and TDZ on the development of Barangan banana seedling shoot explants in vitro, as well as to obtain the optimal treatment combination concentration in inducing shoot growth and multiplication in Barangan banana shoot explants.

METHODS OF RESEARCH

The research was carried out at the Plant Tissue Culture Laboratory of the Food Crops and Horticulture Service, West Tanjung Jabung Regency, Jambi Province - Indonesia. This research is collaboration between the Food Crops and Horticulture department and the Faculty of Agriculture, Jambi University, which was carried out for 6 months. The materials used were the shoots of the Barangan banana, Murashige and Skoog (MS) media (Murashige and Skoog, 1962), disinfectant (70% and 95% alcohol), distilled water, growth regulators BAP and TDZ. The tools used are culture bottles, Erlenmeyer glasses, measuring cups, pipettes, balances, pH-meters, autoclaves, magnifiers, ovens, laminar air flow, hot plates, magnetic stirrers, cameras and other tools.

The research used a Completely Randomized Design (CRD) consisting of 8 treatment combinations and 3 replications. Each experimental unit consisted of 3 bottles, so there were 72 culture bottles. Each culture bottle contains 1 explant.

The treatment combinations are:

- P1 = 2.5 ppm BAP + 0.5 ppm TDZ;
- P2 = 6.0 ppm BAP + 0.5 ppm TDZ;
- P3 = 2.5 ppm BAP + 1.0 ppm TDZ;
- P4 = 6.0 ppm BAP + 1.0 ppm TDZ;

- P5 = 2.5 ppm BAP + 1.5 ppm TDZ;
- P6 = 6.0 ppm BAP + 1.5 ppm TDZ;
- P7 = 2.5 ppm BAP + 2.0 ppm TDZ;
- P8 = 6.0 ppm BAP + 2.0 ppm TDZ.

Stages of research implementation include:

1) Sterilization of tools using 95% alcohol;

2) Sterilization and preparation of explant material;

3) Preparation of MS Media;

4) Preparation of treatment media which is added with cytokinin growth regulators (BAP and TDZ) according to the treatment concentration in Barangan banana explants;

5) Explant initiation was carried out sterilely in a laminar air flow rack. For initial initiation the explants were first cultured on MS 0 media within 1 month, which aimed to see the level of browning and level of contamination that occurred in the explant shoots of the Barangan banana before they were initiated into the treatment media;

6) Research observations were carried out on the following variables:

- Percentage of Browning Explants (%);
- Callus Observations which include time of callus appearance with HSK measurement units (days after culture), callus color observed at the end of the study, explant age 12 weeks after culture (WAC), callus structure also observed at the end of the study;
- Shoot Observation which includes time of shoot emergence, number of shoots (number of shoots per explant), shoot height were observed at the end of the study (age 12 WAC).

The observation data were analyzed statistically using ANOVA (analysis of variance), and to see the differences in influence between treatments, it was continued with the Duncan Multiple Range Test (DMRT) at α = 5% level.

RESULTS OF STUDY

The effect of giving BAP and TDZ on the percentage of browning explants is categorized into three groups as shown in Table 1.

Table 1 – Effect of various treatments on the percentage of browning explants (%)

Treatment	the percentage of browning explants (%)		
	Category 1	Category 2	Category 3
2.5 ppm BAP + 0.5 ppm TDZ	0	56	44
6.0 ppm BAP + 0.5 ppm TDZ	0	100	0
2.5 ppm BAP + 1.0 ppm TDZ	0	100	0
6.0 ppm BAP + 1.0 ppm TDZ	0	100	0
2.5 ppm BAP + 1.5 ppm TDZ	0	100	0
6.0 ppm BAP + 1.5 ppm TDZ	0	100	0
2.5 ppm BAP + 2.0 ppm TDZ	0	100	0
6.0 ppm BAP + 2.0 ppm TDZ	0	100	0

Description. Category 1: 50% (half) of the explant surface is brown or black; Category 2: almost the entire surface of the explant is brown or black, but there were still white parts; Category 3: the entire surface of the explant is brown or black.

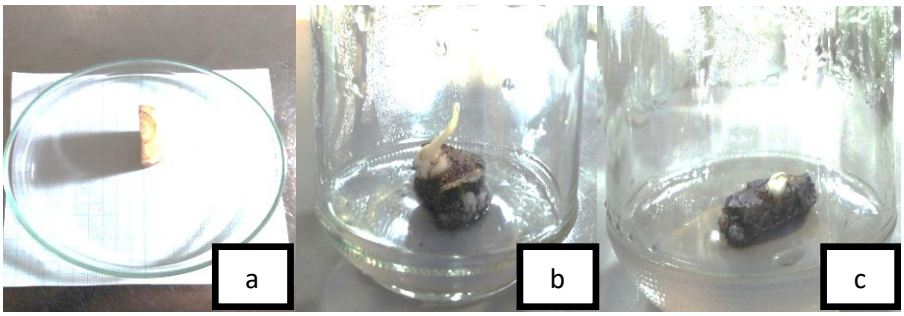

Figure 1 – Browning on the explant surface (a) initial condition, (b) almost completely/category 2, (c) completely/category 3

From the observation data on the percentage of browning explants in Table 1, it appears that in the treatment of 2.5 ppm BAP + 0.5 ppm TDZ. 56% of category 2 explants browned and 44% of category 3 explants (the entire explant surface was brown/black). Meanwhile, other treatments showed that all explants experienced category 2 browning (there were still white parts). The results of these observations can be seen in Figure 1.

Table 2 – Effect of various treatments on callus formation

Treatments	The average time of callus appearance (DAC)	Texture of Callus	Color of Callus
6.0 ppm BAP + 1.0 ppm TDZ	52 a	Compact	Clear white
2.5 ppm BAP + 2.0 ppm TDZ	50 b	Compact	Clear white
6.0 ppm BAP + 0.5 ppm TDZ	48 c	Compact	Clear white
2.5 ppm BAP + 1.5 ppm TDZ	46 d	Compact	Clear white
6.0 ppm BAP + 2.0 ppm TDZ	45 d	Compact	Clear white
6.0 ppm BAP + 1.5 ppm TDZ	44 e	Compact	Clear white
2.5 ppm BAP + 1.0 ppm TDZ	41 f	Compact	Clear white
2.5 ppm BAP + 0.5 ppm TDZ	38 g	Compact	Clear white

Note: Numbers followed by the same letter are not significantly different from DMRT results at the 5% level with respect to the time the callus appears. DAC: Day after culture.

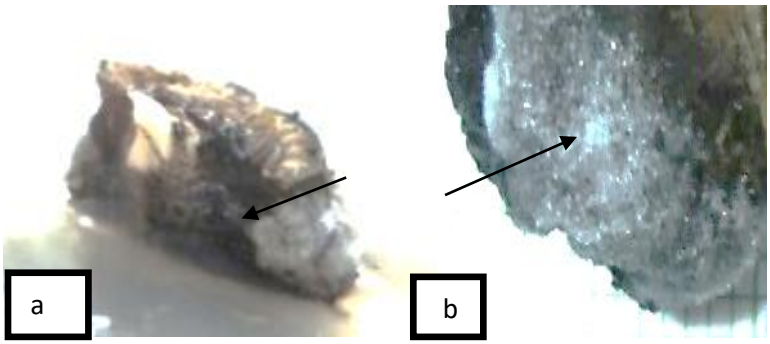

Figure 2 – (a) and (b): Callus texture (compact) and callus color (clear to white) that occurred in each treatment (age 12 DAC)

Table 3 – Effect of various treatments on shoot formation time

Treatments	Average shoot-Formation time (DAC)
2.5 ppm BAP + 0.5 ppm TDZ	51 a
2.5 ppm BAP + 1.0 ppm TDZ	48 b
2.5 ppm BAP + 1.5 ppm TDZ	46 c
2.5 ppm BAP + 2.0 ppm TDZ	46 c
6.0 ppm BAP + 0.5 ppm TDZ	46 c
6.0 ppm BAP + 1.5 ppm TDZ	43 d
6.0 ppm BAP + 2.0 ppm TDZ	39 e
6.0 ppm BAP + 1.0 ppm TDZ	37 f

Note: Numbers followed by the same letter are not significantly different according to the 5% DMRT for shoot formation time. DAC: Days after culture.

The results of the analysis of variance on the time variable for callus emergence showed that there was an influence from administering several combinations of concentrations of cytokinin growth regulators (BAP and TDZ). Based on the DMRT test results in Table 2, it appears that the time for callus to appear is different between treatments. The 6.0 ppm BAP + 1.0 ppm TDZ treatment showed the longest callus emergence time response of 52 DAC, while the 2.5 ppm BAP + 0.5 ppm TDZ treatment had the fastest callus appearance time response of 38 DAC. Furthermore, the variables callus

texture and callus color had no effect between the treatments observed, with the callus texture being compact and the callus color clear and white (Figure 2).

The results of analysis of variance on the variable time to shoot emergence (shoot formation time) showed that there was an influence from administering several combinations of cytokinin concentrations (BAP and TDZ). Based on the DMRT test results in Table 3, it appears that the 2.5 ppm BAP + 0.5 ppm TDZ treatment showed a maximum shoot emergence time of 51 DAC, while the treatment of 6.0 ppm BAP + 1.0 ppm TDZ had the fastest shoot emergence time of 37 DAC.

When administering BAP and TDZ treatments to the number of shoots, no effect was observed between the treatments, with the average number of shoots for all treatments being 1 shoot, which is found in the middle part of the Barangan banana shoot explant (Figure 3).

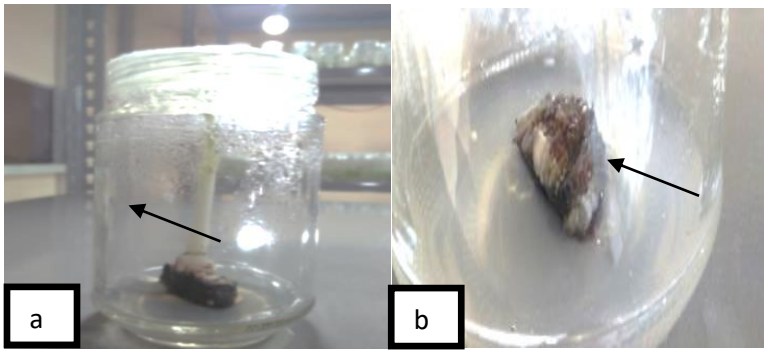

Figure 3 – (a) and (b) The number of shoots, there was 1 shoot in each treatment (explant age 12 DAC). Average shoot height per explant (cm)

The results of analysis of variance on the shoot height variable showed that there was an influence from administering several combinations of concentrations of cytokinin growth regulators (BAP and TDZ). Based on the DMRT results in Table 4, it appears that the 6.0 ppm BAP + 1.0 ppm TDZ treatment gave a different shoot height response to the 6.0 ppm BAP + 0.5 ppm TDZ treatment; 2.5 ppm BAP + 2.0 ppm TDZ; 2.5 ppm BAP + 1.5 ppm TDZ; 2.5 ppm BAP + 1.0 ppm TDZ and 2.5 ppm BAP + 0.5 ppm TDZ. However, the 6.0 ppm BAP + 1.0 ppm TDZ treatment gave the same shoot height response as the 6.0 ppm BAP + 1.5 ppm TDZ and 6.0 ppm BAP + 2.0 ppm TDZ treatments. The 6.0 ppm BAP + 1.0 ppm TDZ treatment also gave the best response for shoot height, namely 2.71 cm. Meanwhile, the treatment of 2.5 ppm BAP + 0.5 ppm TDZ gave a response to the lowest shoot height, namely 0.4 cm. The results of these observations can be seen in (Figure 4).

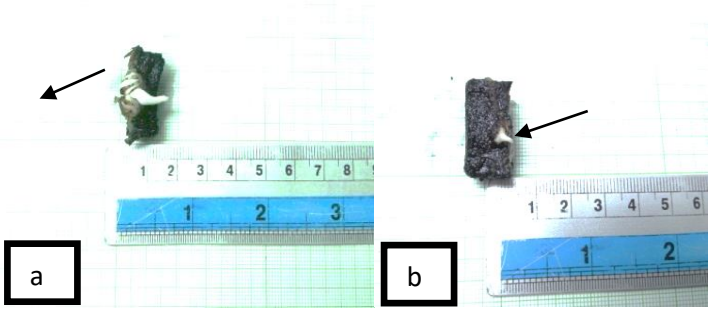

Figure 4 – Shoot height taken from the highest treatment (a) 6.0 ppm BAP + 1.0 ppm TDZ and the lowest (b) 2.5 ppm BAP + 0.5 ppm TDZ (12 weeks after culture)

DISCUSSION OF RESULTS

In this research all the shoot explants of the Barangan banana seedlings experienced browning in category 2 (brownish on almost all the explant surfaces but there were still white parts of the explants). This is due to the presence of large amounts of phenolic compounds released by the explants of Barangan banana saplings when they are cultured.

This browning problem often occurs during the culture initiation period and can cause tissue death caused by excessive oxidation of phenolic compounds due to injured explant tissue (Ko et al., 2009; Onuoha et al., 2011; Jones and Saxena. 2013; Permadi et al., 2011; Jones and Saxena. 2013; Permadi et al., al., 2023). Browning of plant tissue can be caused by enzymatic or non-enzymatic reactions depending on the involvement of enzymes in the process (Chen et al., 2012; Wang et al., 2021). These phenolic compounds are toxic and can kill explant tissue, thereby inhibiting explant growth if subculture is not carried out quickly (Amente and Chimdessa. 2021; Rodrigues et al., 2022). However, the first step to anticipate widespread explant browning in this study was to use an ascorbic acid solution to soak the explant material during preparation, carry out several subcultures and place the explants in total darkness for 1 month after culture. This is in accordance with Ngomuo et al. (2014) that soaking explants in antioxidants (polyvinylpyrrolidone and ascorbic acid) is also effective for controlling browning in various plants. Besides that, frequent subculturing and placing explants in total darkness for a certain period of time are also recommended to control browning of plant explants.

The observation of the callus formation of Barangan banana shoot explants in this study began with the appearance of swelling and small nodules on the edge of the explant which was injured due to cutting the explants, which then forms clear and white callus tissue. This is in accordance with the opinions of Puspitasari and Soegihardjo (2002) and Eustache et al. (2021) which states that from small protrusions at the edge of the explant a white callus will grow and at a certain time the callus will cover the entire surface of the explant. Based on the results of observations regarding the time the callus appears. There are different results between treatments. The fastest time for callus to appear was explants at the age of 38 DAP in the treatment of 2.5 ppm BAP + 0.5 ppm TDZ and the longest time for callus to appear was explants at the age of 52 DAP in the treatment of 6.0 ppm BAP + 1.0 ppm TDZ. Meanwhile, the callus texture and callus color looked the same between treatments, namely with a compact texture and clear white and white callus color. The formation of callus on the surface of injured tissue is caused by a stimulus for tissue recovery. This is in accordance with the opinion of Zulkarnain (2009) who states that callus develops in the tissue culture system due to a hormonal response to tissue damage. Furthermore, Lizawati (2012) stated that whether or not explants form callus depends on the part of the plant used as the explant source and the composition of the planting media used. Callus proliferation is an important step in inducing somatic embryogenesis in plant propagation through tissue culture. Embryo production through somatic embryogenesis will only be successful in callus that has embryogenic properties. According to Tefera and Wannakrairoj (2006), that explants planted in callus induction media containing BAP and TDZ can produce somatic embryogenesis callus. The combination of BAP and TDZ in callus induction media is thought to result in the binding of Cytokinin Binding Protein (CBP), which has two receptors, making it more effective in inducing cell division. Furthermore, the administration of growth regulators to modify the culture media can result in the formation of embryogenic callus which leads to the production of somatic embryos (Elyazid et al., 2021).

Based on the results of observations, the time of shoot emergence on the shoot explants of Barangan banana seedlings in various treatment combinations showed very significant differences between treatments. Providing high concentrations of cytokinins (BAP and TDZ) can induce faster shoot formation in explants. Furthermore, the shoot height of the Barangan banana seedling explants was also significantly different between treatments. Treatment of 6.0 ppm BAP + 1.0 ppm TDZ gave the best response for shoot height of 2.71 cm. Meanwhile, the 2.5 ppm BAP + 0.5 ppm TDZ treatment gave the lowest response with a shoot height of 0.4 cm. However, the number of shoots was not significantly different between the treatments that were observed (the number of shoots was 1 in each treatment). This is because when the explant is cut, a bud growth point is prepared. so that only one shoot grows in the center of the explant. According to Nurmaningrum et al. (2017) that the combination of BAP and TDZ in MS media is effective in accelerating initiation time and increasing alfalfa shoot growth. Giving cytokinin between 0.1-10 ppm is able to induce shoot formation according to cultivar specifications. Previously, Suminar et al. (2016) using a low

concentration of cytokinin TDZ was proven to induce the number of shoots in banana explants. Dewir et al. (2018) reported that TDZ is a type of cytokinin that has been proven to be effective and effective for organogenic pathways, regeneration, and development, including proliferation of axillary buds and accessory buds, somatic embryogenesis, and in vitro flowering. TDZ has facilitated the establishment of in vitro cultures for several plant species, especially woody plants and perennials, which enables genetic transformation and improvement.

Based on the results of observations that have been carried out until the end of the research, it shows that the administration of various combinations of BAP and ZDT concentrations to Barangan banana seedling explants has not been able to induce the formation of root tissue. This is thought to be because the concentration combination used was not appropriate in inducing endogenous auxin in Barangan banana seedling explants so that it was not able to stimulate the root formation process. According to Su et al. (2011), culture media without the addition of cytokinins turned out to be better at inducing roots in various plant explants. This is because cytokinins, under certain conditions, can actually inhibit endogenous auxin biosynthesis in forming roots. The same thing was reported by Roestanto et al (2018) that giving 1 ppm TDZ could increase the wet weight of orchid PLB, but has not been able to give rise to a root system, so it still needs to be combined and tested with other growth regulators. Like previous research which combined BAP with other types of growth regulators or media materials. Saini et al. (2016) reported that increasing the concentration of banana extract and BAP could increase root length and number of roots in explants of the Haji banana cultivar.

CONCLUSION

From the results of research and data analysis it was found that the combination of cytokinin growth regulators BAP and TDZ had an effect on callus formation (time of callus appearance, callus color and callus structure) and shoot formation (time of shoot emergence, number of shoots and height of shoots) in shoot explants of Barangan banana seedlings. However, various combinations of BAP and ZDT concentrations were not able to induce the root system in the shoot explants of Barangan banana saplings. Furthermore, giving a treatment combination of 6.0 ppm BAP + 1.0 ppm TDZ can stimulate the fastest shoot emergence time (age 37 DAC) and produce the highest shoots (2.71 cm).

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