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EFFICIENT REGENERATION LOCAL BANANA TISSUE CULTURE USING FLORAL APICES BY CYTOKININ COMBINATION

Efisiensi Kombinasi Sitokinin terhadap Regenerasi Kultur Jaringan Pisang Lokal menggunakan Eksplan Floral Apices

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ABSTRACT

Banana (Musa sp.*) is an important crop that is widely cultivated and consumed in Indonesia. Banana plants production can be increased through tissue culture with the use of explants and the right combination of hormones. This study was conducted to determine the use of flower apices explants on local banana varieties using a combination of cytokinin hormones containing BAP (Benzylaminopurine) and several levels of TDZ (Thidiazuron). The result of this research was analyzed using a one-way analysis of variance. The hormone combination in this study consists of MS + BAP 2 mg/L + three concentrations of TDZ (2 mg/L, 2.5 mg/L, and 3 mg/L) as a regeneration medium. Three local banana varieties (Kepok Putih, Kepok Kuning, and Pisang Susu) was cultured on the three combination medium. The results showed that the use of flower apices explants contained in banana flowers grown on MS media with a combination of cytokinin hormones could increase the efficiency of explant regeneration. Media MS + BAP 2 mg/L + TDZ 3 mg/L gave the best regeneration rate for Kepok Putih (62.50%), Kepok Kuning (62.50%) and Banana Susu (56.25%) compared to TDZ 2. mg/L and TDZ 3 mg/L. The novelty of this study can provide information in the propagation of local banana plants using flower apices explants.*

Keywords: *banana, floral apices, BAP and TDZ*

ABSTRAK

Pisang (*Musa* sp) merupakan tanaman penting yang banyak dibudidayakan dan dikonsumsi di Indonesia. Produksi tanaman pisang dapat ditingkatkan melalui kultur jaringan dengan penggunaan eksplan dan kombinasi hormon yang tepat. Penelitian ini dilakukan untuk mengetahui penggunaan eksplan *floral apices* pada varietas pisang lokal dengan menggunakan kombinasi hormon sitokinin yang mengandung BAP (*Benzylaminopurine*) dan beberapa taraf TDZ (*Thidiazuron*). Hasil penelitian ini dianalisis menggunakan RAL (Rancangan Acak Lengkap) non-faktorial dengan kombinasi hormon MS + BAP 2 mg/L + tiga konsentrasi TDZ (2 mg/L, 2,5 mg/L, dan 3 mg/L) sebagai media regenerasi tiga varietas pisang lokal (Kepok Putih, Kepok Kuning, dan Pisang Susu). Hasil penelitian menunjukkan penggunaan eksplan *floral apices* yang terdapat dalam bunga pisang yang ditanam pada media MS dengan kombinasi hormon sitokinin dapat meningkatkan efisiensi regenerasi eskplan. Media MS + BAP 2 mg/L + TDZ 3 mg/L memberikan tingkat regenerasi terbaik pada Kepok Putih (62,50%), Kepok Kuning (62,50%) dan Pisang Susu (56,25%) dibandingkan dengan penggunaan TDZ 2 mg/L dan TDZ 3 mg/L. Keterbaruan dari penelitian ini dapat memberi informasi dalam propagasi tanaman pisang lokal dengan menggunakan ekslplan floral apices.

Kata Kunci: pisang, floral apices, BAP dan TDZ

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INTRODUCTION

Banana is a fruit crop with high-level production and is widely cultivated in Indonesia. By 2021, banana production in Indonesia reached 8.741 million tons, 6.82% (558.39 thousand tons) higher than in 2020. Production of bananas in the first quarter (January to March) 2020 reached a peak of 2.32 million tonnes, with 86.82 million fruit-producing plants (Directorate of Food Crops, Horticulture and Plantation Statistic, 2022). Bananas are a fruit crop with a high demand level, but the lack of disease-free planting material due to conventional propagation methods has obstructed banana production (Mekonen et al., 2021). Tissue culture is a plant propagation technique that plays an essential role in plant genetic improvement and can be applied to plant multiplication. Some of the advantages of plant propagation using tissue culture techniques are being able to multiply banana plant seeds that are not dependent on the season, being able to produce large quantities of banana plant seeds in a short time and relatively consistent growth, having the same genetic characteristics as their parents (Aisyah, 2020).

The parts of the banana plant that can be used as tissue culture explants are pseudo-stems, tubers, and banana flowers (*floral apices* and *male floral hand*) (Vuylsteke, 1998). Banana flowers can be used as potential explants because meristem tissues can provide high opportunities for the regeneration phase and will provide controllable results (Nandariyah *et al.*, 2021). Study on the use of *floral apices* explant within the banana flowers showed promising results for tissue culture propagation. Previous research confirmed that *floral apices* explants have a high regeneration rate with low external contamination (Liu *et al.* 2017).

Besides the use of *floral apices* as an explant, exogenous hormones as a vital part of banana tissue culture has been studied earlier. The use of cytokinin hormones provides a good result for banana plant regeneration. Cytokinin hormones in the form of BAP 10 mg/L used on *male floral hand* explants gave the best effects on the formation of meristematic shoots (Kavitha *et al.*, 2020). Other studies state that the use of hormone BAP 8.9 µM (2 mg/L) combined with TDZ 9.1 µM (2 mg/L) using *floral apices* explants in several varieties of banana plants can provide the best shoot regeneration rate (Liu et al., 2017).

Floral apices can potentially be used as explants in the tissue culture propagation of banana plants. However, there has been no further study regarding the potential of floral apices explants in Indonesian local banana propagation. Because of its potential as an explanation in the previous studies, the use of floral apices as an explanation of the local banana propagation in Indonesia needs to be studied. Further observations on the propagation of Indonesian banana varieties using several methods need to be carried out by choosing the right explants and hormones. In this study, several combinations of cytokinin hormones were optimized to respond to the regeneration power of banana plants using floral apices explants.

MATERIALS AND METHODS

Time and Location

The research was conducted at the Nutraceutical and Pharmaceutical Laboratory, Integrated Laboratory Unit and Technology Innovation Center, Center of Development Advanced Science and Technology (CDAST), University of Jember. The research was carried out from January to July 2022.

Plant Material

The study used *floral apices* from three local banana varieties Kepok Putih (*Musa acuminata* x *Musa balbisiana* (ABB)), Kepok Kuning (*Musa acuminata* x *Musa balbisiana* (ABB)), and Pisang Susu (*Musa acuminata* Colla (AAAA)). Banana flowers were peeled to a size of 10 cm, then peeled again to 3 cm in the laminar airflow. Floral apices were sterilized using 10% *sodium hypochlorite* and then rinsed thrice using sterile distilled water. *Floral apices* were cut transversely 1-2 mm thick on a petri dish (one *floral apices* can be cut up to 12 slices).

Regeneration

The floral apices used as explants were planted in petri dishes containing Murashige and Skoog basal (MS) media, 2 mg/L BAP (*Benzylaminopurine*) hormone, and three different concentrations of TDZ (*Thidiazurone*) (2 mg/L, 2.5 mg/L, and 3 mg/L). The media content used was Sucrose 40 g/L, Gelrite 7 g/L, with the combination of media used: MS+BAP 2 mg/L+TDZ 2 mg/L, MS+BAP 2 mg/L+TDZ 2.5 mg/L, and MS+BAP 2 mg/L+TDZ 3 mg/L, pH adjusted to 5.8 and then autoclaved at 121OC at 17.5 pressure. Explants were stored for 4 weeks in the dark, then after 4 weeks, kept in the cabin with the photoperiod (light/dark) 16/8. Explants were sub-cultured every two weeks and observed for 6 months (24 weeks). After 6 months, observation of survive explant, regeneration and the number of initial shoots formation were counted.

Statistical Analysis

The treatment was repeated 4 times, and each experimental unit consisted of 4 explants, so there were 48 experimental units for each variety. Data results were analyzed using one-way analysis of variance (ANOVA), followed by Duncan Multiple Range Test by the least significant difference at $P = 0.05$, and data have presented a means ± standard error.

RESULTS

Survive explant

Flower explants were sterilized and then cut into 12 slices for each banana flower to determine the response of cytokinin hormones to the level of explants regeneration. The medium used is MS with BAP 2 mg/L + 3 different concentrations of TDZ hormone, and the varieties of banana flower explants used are Kepok Putih, Kepok Kuning, and Pisang Susu. The higher the hormone given will help the survive explants so that the explants can continue to the regeneration stage; the use of hormone T3 gives the result of survive explants more than 80% in each banana variety compared to the use of hormones T2 and T1 (Table 1).

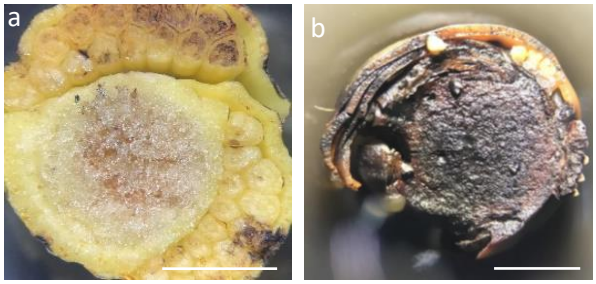

Figure 1. Difference between survive and dead explants. a. Survive explants, b. Dead explant (*scale* bar: 1 cm)

Regeneration explants

Floral apices was cultured for 6 months in 3 different media concentrations to determine the regeneration power of the explants. The analysis of the variance test on 3 banana varieties grown on 3 different types of hormone combinations resulted in explants that could regenerate well (Table 1). Explants that regenerate will grow prospective shoots, while explants that do not regenerate will not grow shoots, and explants can only grow properly (Figure 2).

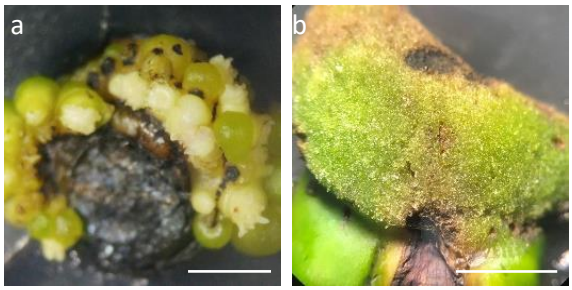

Figure 2. Regeneration of explants. a. regenerate explant, b. unregenerate explants (*scale* bar: 1 cm)

Number of shoots

The calculation of initial shoot formation in this study was carried out separately to determine the effective hormone concentration in the formation of shoots for each local banana variety. The results of the analysis of variance in Table 1 show that hormone concentration can trigger the formation of shoot initials. Hormone T3 used in this study provided the highest amount of initial shoot formation compared to the use of T1 and T2 hormones. Bud formation is characterized by the growth of potential buds on the banana flower that will enlarge and form buds (Figure 3).

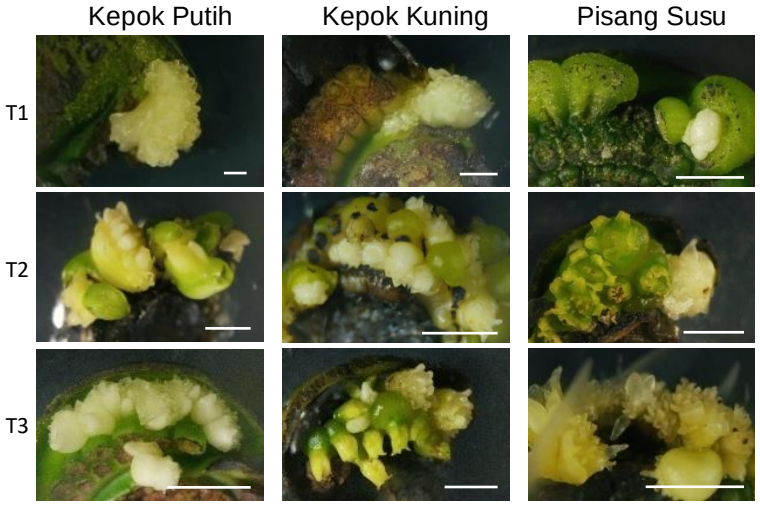

Figure 3. Explants that regenerate into shoot candidates in 3 local banana varieties grown at 3 different concentrations of cytokinin hormone (*scale* bar: 1 cm)

Table 1. Regeneration analysis of three Musa varieties using three types of media

PGR	Kepok Putih			Kepok Kuning			Pisang Susu		
	A(%)	B(%)	C	A(%)	B(%)	C	A(%)	B(%)	C
T1	56.26 ± 23.94^{b}	18.72 ± 23.94^{b}	1.75 ± 2.06^{b}	43.75 ± 23.94^{b}	18.75 ± 23.94^{b}	2.25 ± 2.63^{b}	50.00 ± 20.42^{b}	23.94 ± 23.94^{b}	2.50 ± 3.00^{b}
T2	62.50 ± 14.43^{ab}	37.50 ± 25.00^{ab}	5.00 ± 4.16^{b}	68.75 ± 31.46^{ab}	31.25 ± 57.50^{ab}	5.00 ± 7.07^{ab}	62.50 ± 32.27^{ab}	25.00 ± 28.87^{ab}	4.00 ± 5.23^{b}
T3	81.25 ± 12.50^{a}	62.50 ± 32.27^{a}	11.75 ± 5.06^{a}	87.50 ± 14.43^{a}	62.50 ± 14.43^{a}	11.50 ± 4.43^{a}	87.50 ± 14.43^{a}	56.25 ± 23.94^{a}	12.50 ± 4.80^{a}

Plant Growth Regulator three types of media T1 (MS+BAP 2 mg/L+TDZ 2 mg/L), T2 (MS+BAP 2 mg/L+TDZ 2.5 mg/L), and T3 (MS+BAP 2 mg/L+TDZ 3 mg/L). A, survive explants; B, regenerate explants; C, mean number of shoots/explants. Data are presented a means ± standard error of n=4 biological replicates. Numbers followed by the same letters are not significantly different on the Duncan Multiple Range Test $P = 0.05$.

Discussion

In vitro plant propagation is widely used to produce plants in large quantities. In traditional technique of banana propagation produces just 5 – 10 suckers per yer, resulting in a slow rate of multiplication (Subrahmanyeswari & Saikat, 2022). In vitro regeneration capabilities will open up and contribute to the genetic improvement and conversation of local babanas, which will then support banana farming by offering disease-free, genetically homogenous, and high-quality planting materials. The use of *floral apices* explants in local banana varieties, namely Kepok Putih, Kepok Kuning, and Pisang Susu, gave significantly different interactions on the percentage of survive explants, explant regeneration, and were not significantly different on the number of initial shoot formation by giving different hormone concentrations. In the same level of additional hormones, every variety has a

different ability to regenerate. Lusianto *et al*., (2021) reported that the survival rate of explants generated from male flowers of all banana species when BAP and IAA were added to the media ranged from 83,33% to 100%. Furthermore, in the initiation media the combination of both hormones induced callus growth rather than nodule or shoot production. In the study by Ahmed *et al*., (2018), it was found that the concentration of 0.5 mg/L BAP and 1.5 mg/L IBA produced the highest outcomes in shoot and root regeneration in vitro. Different types of media concentrations in the regeneration phase of banana plants is used to determine the existence of different growth power responses and to determine the concentration of the best type of media.

Research using *floral apices* explants contained in banana flowers provides results in the absence of latent contaminants from the beginning to the end of the study. *Male floral hand* explants found in banana flowers are able to provide a high level of shoot multiplication and are free from latent contaminants (Kavitha *et al*, 2021). The absence of latent contaminants in banana flowers indicates that *floral apices* as potential explants that can be used on mass plant propagation and can also provide a high level of explant regeneration ability in banana.

Explants that survive until the end of the observation (6 months) after planting are called survive explants. Survive explants are used as parameters due to its ability to regenerate from initial stage towards the explant regeneration phase. This study confirmed that the use of MS + BAP 2 mg/L + TDZ 3 mg/L gives the highest average percentage of result on the power of survive explants, on Kepok Putih 81.25%, Kepok Kuning 87.50% and Pisang Susu 87.50%. Combination of MS + BAP 2 mg/L + 3 TDZ hormone concentrations gives the best regeneration of explant. In banana plant propagation, the use of MS + BAP 2 mg/L combined with TDZ 2 mg/L hormone can have a good effect on shoot regeneration of five Chinese banana varieties (Liu *et al*, 2017), but research conducted on three Indonesian local banana varieties, by increasing the dose of the TDZ hormone to 2.5 mg/L and 3 mg/L gives a higher percentage of regeneration value compared to use the TDZ 2 mg/L hormone.

The appearance of the initial shoot is characterized by small white clumps that will grow into solid clumps and turn into shoots (Liu *et al*, 2017). Initial shoots on *floral apices* explants appear on the flower and also grow between the flowers. In contrast, the formation of shoots did not appear in the middle or stem part but only produces white spots like callus. Cytokinin hormones given as plant nutrition can affect the growth of explants. Banana *floral apices* explants with a combination of cytokinin hormones in the form of MS + BAP 2 mg/L + TDZ 2 mg/L can provide the highest number of shoots in each variety (Liu *et al*, 2017), then in this study conducted with the use of T3 hormones (MS + BAP 2 mg/L + TDZ 3 mg/L) produced in a higher percentage of initial shoot emergence compared to the use of TDZ 2 mg/L and 3 mg/L. The hormones BAP and TDZ will work together to help in bud formation. The ability to form initial shoots in each variety has a different response, proven in this study that after 6 months of research, the use of MS + BAP 2 mg/L + TDZ 3 mg/L hormones on Kepok Putih Banana gave an average initial shoots formation power of 11.75%, Kepok Kuning 11.50%, and Pisang Susu 11.50%.

CONCLUSION

The apply of three different quantities of cytokinin hormones on three local banana, especially Kepok Putih, Kepok Kuning, and Pisang Susu, can improve banana regeneration capability utilizing floral apices explants. BAP 2 mg/L + TDZ 3 mg/L medium has the best hormones for surviving explants, explant regeneration, and beginning shoot production when compared to hormones BAP 2 mg/L + TDZ 2 mg/L and BAP 2 mg/L + TDZ 2.5mg/L. This research successfully proves that floral apices explants can be employed as a suitable explant for banana plant multiplication because they are devoid of latent contamination data transmitted by the parent and provide regeneration power and the development of a large number of early shoots.

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