RESEARCH ARTICLE

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IN VITRO REGENERATION OF BANANA GENOTYPES POSSESSING DISTINCT GENOMES BY USING MALE FLOWER EXPLANTS

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SUMMARY

The use of male flowers as explants offers an efficient protocol for overcoming the contamination problems encountered in plant propagation via tissue culture. This study aimed to evaluate the *in vitro* regeneration efficiency of commercial and local banana cultivars possessing different genomes by using male flower and flower base explants. Five commercial and local banana cultivars, i.e., 'Ambon' (AAA), 'Rutai' (AAB), 'Talas' (AAB), 'Kepok' (ABB), and 'Klutuk' (BB), were studied during 2020 at the Laboratory of Biotechnology, Department of Agroecotechnology, Mulawarman University, Samarinda, Indonesia. The nodule initiation stage was conducted in Murashige and Skoog medium containing indole acetic acid (IAA) (0.15, 0.30, and 1.00 mg L^{-1}) combined with benzyl amino purine (BAP). After 8 weeks, the explants were transferred into multiplication media to induce shoot formation. The multiplication medium was the same as used at the initiation stage. However, the BAP concentration was increased 10-fold. Results revealed that in all the banana genotypes, the callus was dominantly induced at the initiation stage and failed to form shoots at the multiplication stage when male flower explants were used. However, by using male flower base explants, nodules and shoots were successfully induced in all genotypes with specific medium composition. High nodule formation was observed in the genotypes 'Talas' (on medium with 1 mg L^{-1} IAA combined with 2.00 or 4.00 mg L^{-1} BA) and 'Klutuk' (on medium with 0.30 mg L^{-1} IAA and 2.0 mg L^{-1} BAP). In these genotypes, 100% of the explants were able to form nodules. Nodules were successfully induced in the banana cultivars 'Talas' and 'Kepok' with 12–20, 19, and 2–5 shoots per explant at the multiplication stage. A high concentration of cytokinin (BAP) ranging from 20 mg L^{-1} to 40 mg L^{-1} in combination with low auxin (IAA) was found to be highly effective in inducing shoot formation in the genotypes 'Rutai', 'Talas', and 'Kepok'. The banana genotype 'Ambon' showed low shoot formation at the initiation stage. In conclusion, *in vitro* regeneration was successfully used and performed well in the local banana cultivars, 'Talas', 'Rutai', and 'Kepok' by using flower base explants.

Keywords: Local banana cultivars, *in vitro* regeneration, genomes, male flower, flower base

Key findings: This study demonstrated that successful banana *in vitro* regeneration could be achieved by using flower base explants instead of male flowers. In addition, the *in vitro* regeneration capability of local banana cultivars from Indonesia was genotype-dependent, and the genotypes containing A and B genomes were more effective than the banana

genotypes possessing only the A or B genome. The present findings will be of great interest and contribution to local banana propagation, conservation, cultivation, and genetic improvement.

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INTRODUCTION

After tomatoes, banana is the most important fruit consumed by people worldwide (World-Atlas, 2020). It serves as a staple food that supports and complements the nutrient needs of the human body (De *et al.,* 2009). It plays an important role in supporting global food security, especially in supplementing micronutrients, i.e., vitamins A and C and potassium (Ashokkumar *et al.,* 2018).

The variability of genome origin determines banana characteristics and applications. Modern bananas are mostly derived from the interspecific triploid hybrids of wild *Musa* species, i.e., *Musa acuminata* (2n = 2x = 22), which contains the A genome and is referred to as the dessert banana origin, and *Musa balbisiana* (2n = 2x = 22), which contains the B genome and is referred as the cooking banana origin (Espino *et al.,* 1992). Chromosome restitution at meiosis has produced higher-order combinations of distinct groupings at the triploid or occasionally tetraploid level (De-Langhe *et al.,* 2010). Triploids, such as AAA, AAB, ABB, and BBB types, arose as the results of multiple hybridizations among parental species (AA and BB bananas) with their AB progenitor. These crosses support unequal chromosomal allocation at meiosis, producing triploid species (D'Hont *et al.,* 2000). Given that diploid species (AA and BB) are seeded and produce small, starchy fruit with a small amount of fleshy parts, triploids with seedless and parthenocarpic fruit and optimal vigor and plant growth are preferred by consumers. The A genome contributes sweet flesh that can be eaten uncooked. The B genome accounts for starchy bananas that have to be cooked before being eaten and are referred to as plantains or cooking bananas. Most cultivated and domesticated bananas are derived from triploids (3n = 33). Given that AAA triploids produce sweet and uncooked bananas, these genotypes are often referred to as dessert bananas. AAB and ABB bananas are mainly but not exclusively starchy bananas. Most diploid cultivars exhibit weak plants with small bunches, and the occasionally occurring tetraploids are physically large but have small bunches (De-Langhe, 1986).

Indonesia is the center of the biodiversity and origin of many species, including bananas. Approximately 300 local banana cultivars exist in Indonesia and are disseminated throughout the Indonesian islands as endemic/local or introduced cultivars. They vary by genome origin from AA, BB, AAB, AAA, AAB, ABB, BB, and AB (Sunaryo *et al.,* 2019). Some bananas that are cultivated in East Africa have been suggested to originate from Southeast Asia, including Indonesia (Perrier *et al.,* 2019). Several local and commercial bananas from Indonesia have been identified by using morphological analysis, and their genomic composition has been revealed (Sunaryo *et al.,* 2017; Sunaryo *et al.,* 2019). The results of genetic diversity analysis with ISSR markers also supported the previously reported genomic composition (Sunaryo *et al.,* 2020). Identification showed that the local and commercial bananas in Indonesia belong to the AA ('Liar/Monyet' banana), AAA ('Ambon' and 'Kapas' bananas), AAB ('Rutai', 'Talas', 'Raja', and 'Susu' bananas), ABB ('Kepok' and 'Awak' bananas), AB ('Tanduk' banana), and BB ('Klutuk' banana) groups (Sunaryo *et al.,* 2019; 2020).

Tissue culture technology promises many opportunities in banana propagation, conservation, cultivation, and genetic improvement. The exploration of *in vitro* regeneration capability will open and contribute to the genetic improvement and conservation of local bananas and subsequently support banana cultivation by supplying disease-free, genetically homogenous, and high-quality planting materials (Pillay *et al.,* 2011; Ali *et al.,* 2011; Hussein, 2012). The tissue culture of bananas is usually conducted by regenerating explants from microbuds isolated from the suckers, peepers, and lateral buds (Vuylsteke, 1989). Such an approach faces the problems of the limited number of suckers and the high rate of bacterial or fungal contamination (Blomme *et al.,* 2011; Dubois and Coyne, 2011).

Banana tissue culture is facing some potential problems. The main problem is to avoid explant contamination because most explants are derived from plant parts that contain shoot meristems, such as parental pseudostem, small suckers, peepers, or lateral buds, that are sensitive to soil microorganism contamination. Contaminant organisms, such as bacteria, viruses, and fungi, can be deposited in the plants and are often difficult to handle. Serial explant sterilization has to perform to eradicate potential contaminants. Another constraint is explant browning caused by the polyphenolic compounds that are abundantly released by banana explants. Browning will inhibit explant growth, mostly considerably reducing explant viability and finally increasing the mortality rate of the explants. Regular explant subcultures and/or some chemical compounds, such as ascorbic or citric acid, activated carbon, and polyvinylpyrrolidone (PVP), are usually applied to combat browning. Different banana genotypes/genomes have different responses to plant growth regulators (Strosse *et al.,* 2004). The appropriate application of exogenous hormones in terms of type and concentration to the tissue culture medium triggers the *in vitro* regeneration of a specific banana genotype via shoot and/or root development. Therefore, studying tissue culture media, especially the application of plant growth regulators, and exploring other types of explants are crucial for the establishment of banana tissue culture.

The use of banana male buds as an explant source offers a good alternative for banana *in vitro* propagation. This approach is easy to handle, has low contamination rates, and provides numerous explants. Given that banana flowers are naturally covered by bracts, they are infrequently contaminated by viruses, bacteria, or fungi. The abundant flowers present in one male bud of banana also supply sufficient numbers of explants. However, studies on banana *in vitro* regeneration by using male flowers remain very limited. In this manuscript, we report the success of the *in vitro* regeneration of local bananas cultivated in East Kalimantan, Indonesia, with different genomes by using male flower explants.

MATERIALS AND METHODS

Plant material

Five commercial and local banana cultivars i.e., 'Ambon' (AAA genome), 'Rutai' and 'Talas' (AAB genome), 'Kepok' (ABB genome), and 'Klutuk' (BB genome) were used in this study, which was performed during 2020 at the Laboratory of Biotechnology, Department of Agroecotechnology, Mulawarman University, Samarinda, Indonesia. 'Ambon', 'Kepok', and 'Klutuk' are commercial banana cultivars in Indonesia, whereas 'Rutai' and 'Talas' are local bananas that originated from East Kalimantan. The male buds were taken just after the last hand of the banana bunch emerged (after approximately 6–11 banana hands in total). All male buds were collected from healthy plants cultivated in the Samarinda, Penajam Pasir Utara, and Kutai Kertanegara districts of East Kalimantan Province, Indonesia.

Explant preparation and sterilization

Two types of explants were isolated from the male bud, i.e., male flower explants containing the male flower ornaments (compound tepal, free tepal, filament, style, stigma, and anthers if any) and the male flower base explants (Figure 1).

Banana male buds were washed by using running water, treated with 0.2 mg L^{-1} fungicide (Dithane M-45) for an hour, and then rinsed and washed by using distilled water. The subsequent sterilization steps were conducted in a laminar air flow cabinet. The bract was removed, and young, fresh, and healthy male flowers were taken (Figure 1D). The male flower base explants were obtained by removing the base from male flowers. Subsequently, the flower base was sliced into small sizes with thicknesses of approximately 1 cm (Figure 1E). Explants (male flowers and flower bases) then were exposed to a series of sterilization processes: immersion in 70% alcohol for 2 s; immersion in 30% of NaClO and two drops of Tween-20 solution for 10 min; and rinsing for three times in sterile water. The sterilization process was repeated by using the same procedure with the NaClO concentrations reduced to 10% and 1%.

Shoot initiation and multiplication

Both explants were inoculated into the initiation media to trigger nodule initiation. Four levels of media composition, i.e., (1) Murashige and Skoog (MS) + 0.15 mg L^{-1} indole acetic acid (IAA) + 2 mg L^{-1} benzyl amino purine (BAP), (2) MS + 0.30 mg L^{-1} IAA + 2 mg L^{-1} BAP, (3) MS + 0.15 mg L^{-1} IAA + 4 mg L^{-1} BAP , and (4) MS + 0.30 mg L^{-1} IAA + 4 mg L^{-1} BAP, were applied for male flower and flower base explants. Other initiation media for flower base explants were prepared by using BA as a cytokinin source, i.e., (5) MS + 1 mg L^{-1} IAA + 2 mg L^{-1} BA, (6) MS + 1 mg L^{-1} IAA + 4 mg L^{-1} BA, (7) MS + 1 mg L^{-1} IAA + 6 mg L^{-1} BA, and (8) MS + 1 mg L^{-1} IAA + 8 mg L^{-1} BA. All experimental units were replicated three times.

After 8 weeks in the initiation stage, the explants were then transferred into the multiplication media, i.e., (1) MS + 0.15 mg L^{-1} IAA + 20 mg L^{-1} BAP, (2) MS + 0.30 mg L^{-1} IAA + 20 mg L^{-1} BAP, (3) MS + 0.15 mg L^{-1} IAA + 40 mg L^{-1} BAP, and (4) MS + 0,30 mg L^{-1} IAA + 40 mg L^{-1} BAP. A reference multiplication medium from Strosse *et al.* (2004), MS + 0.175 mg L^{-1} IAA + 22.5 mg L^{-1} BAP, was also included in this experiment. All experimental units in the multiplication stage were replicated three times. The explants were subcultured every 2 weeks by using the same media to mitigate the explant browning problem.

Growth observation

Explant growth was observed at the initiation stage by using the following parameters: percentages of surviving explants (4 weeks after inoculation), explants forming callus (8 weeks after inoculation), and explants forming nodules (8 weeks after inoculation). At the multiplication stage, growth observation was conducted on the basis of the percentages of explants that formed shoots (8 weeks after inoculation in multiplication media) and the number of shoots per explant (8 weeks after inoculation in multiplication media).

RESULTS

Male flower explants

The survival rates of the explants derived from the male flowers of all local bananas were high and ranged from 83.33% to 100% (Table 1). This result indicated the successful initiation of *in vitro* regeneration by using male flower explants. Almost all surviving explants (58.33% to 100%) formed calli after 6 weeks in the initiation medium (Table 1, Figure 2). This result indicated that when using male flower explants, the plant

growth regulator combination in the initiation media (BAP and IAA) triggered callus formation rather than nodule or shoot formation. Shoot initiation failed in all banana cultivars at the multiplication stage in all medium combinations (Table 1). This result demonstrated that the given multiplication media was inappropriate for callus regeneration. The 'Kepok' cultivar showed exceptional results. Specifically, 8.33% of the explants formed shoots that produced eight shoot numbers per explant (Table 1).

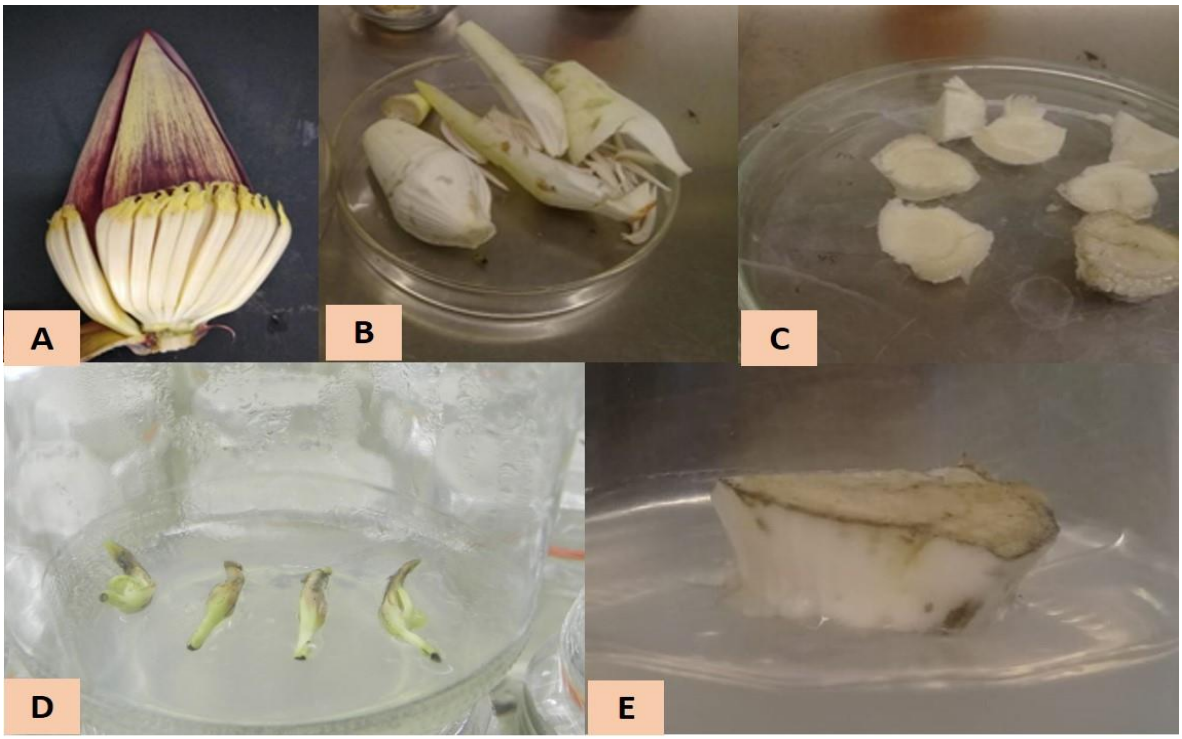

Figure 1. Preparation of explants from banana male buds: A. Intact male bud containing a series of male flowers. B. Male flowers taken and separated from the male bud. C. Flower basal slices. D. Male flower explants. E. Flower basal explant.

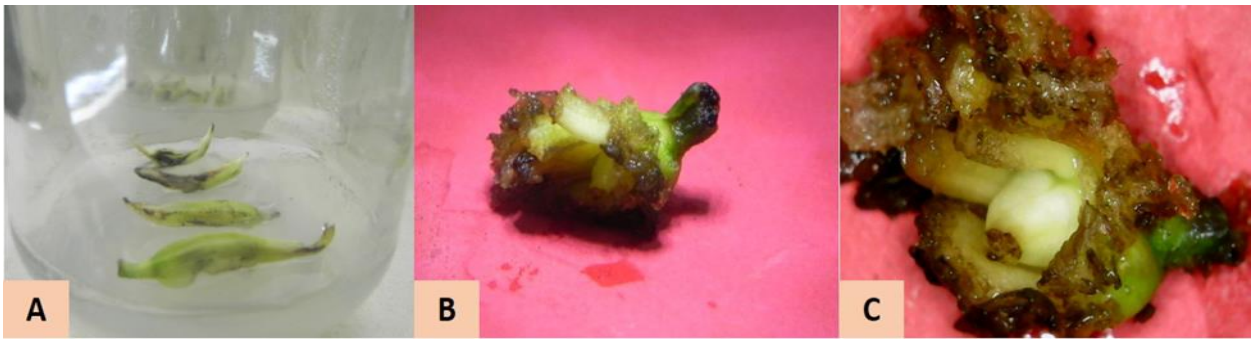

Figure 2. *In vitro* regeneration of local banana by using male flower explants: A. Male flower explants in initiation medium. B. Callus development at tepal scars 4 weeks after inoculation. C. Explant development after 8 weeks in the initiation medium.

Table 1. Shoot initiation and multiplication of local banana cultivars from Indonesia by using male flower explants.

Media composition	Banana cultivars				
	'Ambon' (AAA)	'Rutai' (AAB)	'Talas' (AAB)	'Kepok' (ABB)	'Klutuk' (BB)
Intiation Stage					
Surviving explants (%) at 4 weeks after inoculation					
MS (no PGR supplement)	100.00	91.67	100.00	83.33	100.00
MS + 0.15 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	100.00	91.67	83.33	83.33	91.67
MS + 0.15 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	91.67	83.33	100.00	100.00	100.00
MS + 0.30 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	100.00	100.00	100.00	100.00	100.00
MS + 0.30 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	100.00	100.00	100.00	100.00	91.67
Callus-forming explants (%) at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	91.67	91.67	83.33	83.33	83.33
MS + 0.15 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	91.67	83.33	100.00	100.00	50.00
MS + 0.30 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	100.00	91.67	100.00	100.00	58.33
MS + 0.30 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	100.00	83.33	91.67	100.00	75.00
Multiplication stage					
Shoot-forming explants (%) at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	8.33	0.00
MS + 0.30 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
Number of shoots per explant (%) at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	8.00	0.00
MS + 0.30 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00

Male flower bases as explants

The use of male flower base as explants in the banana *in vitro* regeneration experiments showed much better results than the use of male flower explants (Table 2, Figure 3). Although the rates of surviving explants were almost similar (66.67% to 100%), significant differences were observed in the emergence of nodules, instead of calli, among certain cultivars and medium compositions (Table 2, Figure 3A and B). For the 'Ambon' cultivar, nodule-forming explants nodules were obtained only in media that contained BA as the cytokinin source (MS + 1.0 mg L^{-1} IAA + 4.0 mg L^{-1} BA) instead of BAP. In 'Rutai' and 'Klutuk', nodules were formed only in media containing BAP but not in the media containing BA. Interestingly, in 'Talas' and 'Kepok', although nodules could be initiated in the medium containing BAP or BA, the intensity of nodule formation was higher in 'Talas' banana rather than in 'Kepok'. The highest percentage of nodule-forming explants (100%) was observed in 'Talas' banana inoculated into the media supplemented with 2.0 or 4.0 mg L^{-1} BA in combination with high auxin concentration (IAA 1 mg L^{-1}) and 'Klutuk' banana in MS media containing 0.30 mg L^{-1} IAA + 2.0 mg L^{-1} BAP (Table 2).

After the initiation stage, the explants were transferred into multiplication media to initiate shoot formation and shoot multiplication. Shoots were successfully initiated and developed from the nodules of 'Rutai', 'Talas', and 'Kepok' in different media compositions (Table 2, Figure 3). However, although the explants of 'Ambon' and 'Klutuk' successfully developed nodules, they did not form shoots. The highest percentage of shoot-forming nodules was observed in 'Rutai' banana in MS media containing 0.15 mg L^{-1} IAA + 20.0 or 40.0 mg L^{-1} BAP. Consistent results were observed in the multiplication medium containing 0.175 mg L^{-1} IAA + 22.5 mg L^{-1} BAP, a

reference multiplication medium from Strosse *et al* (2004). In this medium, shoots could be successfully initiated and multiplied from the nodules in 'Rutai', 'Talas', and 'Kepok' but not from those in 'Ambon' or 'Klutuk' (Table 2). The reference medium appeared to be appropriate for the shoot multiplication of bananas containing A and B genomes ('Rutai', 'Talas', and 'Kepok').

DISCUSSION

In this study, we compared the effectiveness of using male flowers and male flower bases as explants for the *in vitro* propagation of bananas with different genomic backgrounds. The results showed that the two kinds of banana male bud-derived explants showed high survival rates: approximately 83% to 100% for male flowers and 66% to 100% for male flower base (Tables 1 and 2). Explant survival in tissue culture is closely related to the browning conditions of phenotypes with high phenolic content, including banana. In this study, the explants were subcultured every 2 weeks to avoid browning. However, some explants still experienced browning in this experiment, degrading physiological conditions and influencing the survival rates of explants especially during the initiation stage.

Browning is manifested by the appearance of a brown or black color, inhibiting growth and development or even causing explants to die (Hutami, 2008). It occurs due to the influence of the accumulation of oxidized phenolic compounds, mostly polyphenol oxidase and tyrosinase enzymes, due to mechanical stress or injury to the explants. These enzymes are naturally synthesized by plants as self-defense mechanisms under oxidative conditions caused by mechanical stress injury (Titov *et al.,* 2006). Polyphenol oxidase functions as a phytoalexin (Ozyigit *et al.,* 2007), which is very important in plant defense against fungal, viral, and bacterial infections (Onuoha *et al.,* 2011). The phenolic compounds present in *Musa* spp. include dopamine, catechins, chlorogenic acid, cinnamic acid, hydroxybenzoic acid, resorcinol, pyrogallic acid, salicylic acid, ferulic acid, vanillin, coumarin, and P-coumaric acid (Khalil *et al.,* 2007).

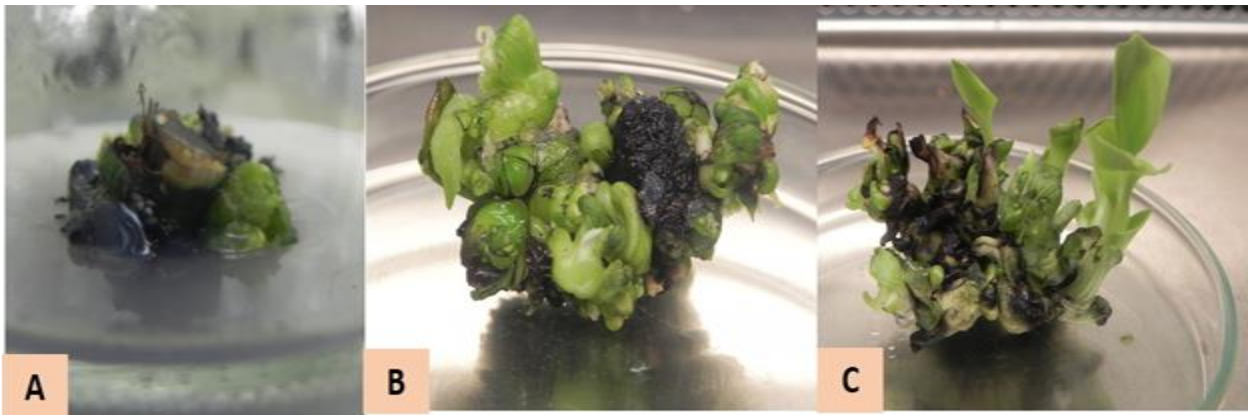

Figure 3. *In vitro* regeneration of local banana by using flower base explants: A. Initial nodule development in the initiation stage medium (2 weeks). B. Nodule development 8 weeks after inoculation. C. Shoot formation from the nodule after 4 weeks in the multiplication media.

Table 2. Shoot initiation and multiplication of local bananas from Indonesia by using flower base explants.

Media composition	Banana cultivars				
	'Ambon' (AAA)	'Rutai' (AAB)	'Talas' (AAB)	'Kepok' (ABB)	'Klutuk' (BB)
Initiation stage					
Surviving explants (%) at 4 weeks after inoculation					
MS (no PGR supplement)	83.33	100.00	91.67	91.67	100.00
MS + 0.15 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	66.67	100.00	100.00	66.67	100.00
MS + 0.15 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	100.00	100.00	100.00	66.67	100.00
MS + 0.30 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	100.00	100.00	100.00	100.00	100.00
MS + 0.30 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	100.00	100.00	100.00	100.00	66.67
MS + 1.0 mg L^{-1} IAA + 2.0 mg L^{-1} BA	66.67	66.67	100.00	66.67	100.00
MS + 1.0 mg L^{-1} IAA + 4.0 mg L^{-1}. BA	66.67	100.00	100.00	100.00	66.67
MS + 1.0 mg L^{-1} IAA + 6.0 mg L^{-1} BA	100.00	100.00	100.00	66.67	100.00
MS + 1.0 mg L^{-1} IAA + 8.0 mg L^{-1} BA	100.00	100.00	100.00	100.00	100.00
Nodule-forming explants (%) at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	0.00	66.67	0.00	0.00	33.33
MS + 0.15 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	0.00	66.67	0.00	0.00	66.67
MS + 0.30 mg L^{-1} IAA + 2.0 mg L^{-1} BAP	0.00	33.33	33.33	0.00	100.00
MS + 0.30 mg L^{-1} IAA + 4.0 mg L^{-1} BAP	0.00	0.00	33.33	33.33	0.00
MS + 1.0 mg L^{-1} IAA + 2.0 mg L^{-1} BA	0.00	0.00	100.00	0.00	0.00
MS + 1.0 mg L^{-1} IAA + 4.0 mg L^{-1} BA	33.33	0.00	100.00	0.00	0.00
MS + 1.0 mg L^{-1} IAA + 6.0 mg L^{-1} BA	0.00	0.00	33.33	33.33	0.00
MS + 1.0 mg L^{-1} IAA + 8.0 mg L^{-1} BA	0.00	0.00	33.33	0.00	0.00
Multiplication stage					
Shoot-forming nodules (%) at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	66.67	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	66.67	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	33.33	0.00
MS + 0.175 mg L^{-1} IAA + 22.5 mg L^{-1} BAP	0.00	66.67	66.67	33.33	0.00
Number shoots per explants at 8 weeks after inoculation					
MS (no PGR supplement)	0.00	0.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	12.00	0.00	0.00	0.00
MS + 0.15 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	20.00	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 20.0 mg L^{-1} BAP	0.00	0.00	0.00	0.00	0.00
MS + 0.30 mg L^{-1} IAA + 40.0 mg L^{-1} BAP	0.00	0.00	0.00	3.00	0.00
MS + 0.175 mg L^{-1} IAA + 22.5 mg L^{-1} BAP	0.00	16.00	19.00	5.00	0.00

The surviving explants of the male flowers remained fresh, and their color changed from white to green. After 2 weeks, the explants started to form white calli (Figure 2). Calli were formed after the explant swelling at the part of the compound tepal scarred after it fell from the ovary (Figure 2). The swelling is a sign of rapid cell division due to callus development. The application of auxin (IAA) in the initiation medium, although at low concentrations, significantly contributed to callus formation. Auxin at low concentrations combined with cytokinin at high concentrations is commonly applied to trigger callus formation (Tan *et al.,* 2010; Abdul Rahman *et al.,* 2019; Irene *et al.,* 2019). When using male flower explants, callus emergence was observed in all medium combinations and for all banana cultivars, with the percentage varying between 50% to 100% (Table 1). This result indicated that the use of 0.15 and 0.30 mg L^{-1} IAA combined with 2 or 4 mg L^{-1} BAP could initiate callus formation with different effectiveness depending on the banana genotype.

The failure of shoot formation in the multiplication stage when using

callusing explants was observed in male flower explants (Table 1). In this study, no medium combination triggered shoot initiation in all banana genotypes. The combination of low IAA concentrations with very high BAP concentrations (10-fold) is appropriate for multiplying existing shoots/buds in *in vitro* banana regeneration (Strosse *et al.,* 2004) and inappropriate for differentiating calli into shoots. In many species, the shoot induction of calli in many species is usually induced by the higher concentration of cytokinin relative to that of auxin (Philips *et al.,* 1995; Islam and Alam, 2018). Subsequent investigation is required to reveal the combination of auxin and cytokinin concentrations that is appropriate for regenerating shoots from calli. The exceptional appearance of shoot growth at 8.33% observed in 'Kepok' might be due to the retention of a small part of the floral base in the male flower explant (Table 1). Therefore, this condition resulted in a significant shoot formation as observed in an experiment using flower base explants. This result was in line with the results of banana *in vitro* regeneration by using flower base explants (Table 2).

The regeneration pattern observed when flower bases were used was different from that observed when male flower explants were used (Figures 2 and 3). The emergence of nodules from the flower base (Figure 3A) did not occur in male flower explants. The nodules subsequently developed (Figure 3B), and shoots were successfully initiated from nodules. This result suggested that nodules emerged from the meristematic cells existing in the flower base. Nodules are a group of cells in a particular part of the callus that resembles a cambium and are often called meristemoids (Wattimena, 1991). In cauliflower, lumps or swelling explants, which appear similar to white flower buds, are called nodules or cauliflower-like bodies (Darvari *et al.,* 2010). Nodules or swelling explants occur due to the presence of endogenous auxin activity, which is present in meristematic cells (Rainiyati *et al.,* 2009). In banana, nodules are initiated from the side adjacent to the bract attachment (flower base) that is meristematic (Darvari *et al.,* 2010). The nodules regenerate into compact nodules, and some of them turn into shoots (Figure 3 B). The application of cytokinins is reported to be capable of reducing the apical dominance of meristems and inducing the formation of axillary shoots or adventitious shoots from meristematic tissues (Madhulatha *et al.,* 2004).

Nodules were successfully initiated in all banana genotypes when using flower base explants, although the success of the initiation medium for each genotype was different (Table 2). Compared with other cultivars ('Rutai', 'Talas', 'Kepok', and 'Klutuk'), 'Ambon' had the lowest nodule formation response. This genotype formed nodules only in a medium containing 1.0 mg L^{-1} IAA and 4.0 mg L^{-1} BA (Table 2). The weaker response of 'Ambon' than that of other banana cultivars has also been reported in a previous study involving male flower explants (Swamy and Sahijram, 1989). On the other hand, 'Talas' (AAB genome), a local banana from East Kalimantan, showed high nodule formation in a medium containing either BAP or BA at the concentrations of 2.0, 4.0, 6.0, and 8.0 mg L^{-1}. These results indicated that different banana genotypes showed different responses to the initiation medium. The suitability between the genotype and the concentration of plant growth regulator supplemented in the initiation media is highly influenced by the genetic base of the plants (Wirakarnain, 2008).

At the multiplication stage, nodules were successfully regenerated to form shoots in male flower base explants. Culture growth in the shoot multiplication stage starts with the formation of white meristematic nodules (scalp), which then turn green. The green nodules subsequently grow into young shoots and leaves (Fitramala *et al.,* 2016). However, shoots also grow directly from the explant network without the formation of a meristematic nodule (scalp) first. Shoots originating from meristematic nodules are

numerous but require a longer time for elongation, growth, and leaf formation. Each shoot or bud produced at the multiplication stage can be subsequently subcultured to produce additional shoots.

Shoot-forming nodules were observed in 'Rutai' (AAB genome), 'Talas' (AAB genome), and 'Kepok' (ABB genome) banana (Table 2). 'Rutai' and 'Talas' showed higher shoot number per explant (approximately 12–20 shoots per explant) than 'Kepok' banana (2–5 shoots per explant). The high response of the three banana cultivars suggested that the multiplication medium containing a high concentration of cytokinin (20–40 mg L^{-1} BAP) in combination with a low concentration of auxin (less than 0.30 mg L^{-1} IAA) is suitable for culturing bananas with A and B genomes, in which the response of bananas containing the AAB genome was higher than that of the bananas containing the ABB genome. The multiplication rate of bananas via *in vitro* regeneration highly depends on genotype and cytokinin concentration. In media with high cytokinin concentrations, cultivars possessing only the A genome produce only 2–4 new shoots, whereas those containing one or two B genomes can initiate many shoots (cluster of shoots and buds) (Strosse *et al.,* 2004). In the current study, media with a high concentration of cytokinin, including the multiplication medium of Strosse *et al* (2004) (MS + 0.175 mg L^{-1} IAA + 22.5 mg L^{-1} BAP), could not induce shoot formation in cultivars possessing only the A genome, such as 'Ambon', or in those possessing only the 'B genome', such as 'Klutuk'. The high nodule formation percentage of 'Klutuk' (BB genome) at the initiation stage did not translate into shoot formation at the multiplication stage. Although the nodules that appear at the initiation stage can become microshoots at the multiplication stage, not all nodules can form shoots (Ernawati, 2005). Subsequent investigation is required to optimize the medium composition to initiate and increase shoot formation in 'Ambon' or 'Klutuk'. The reference media by Strosse *et al.* (2004) can efficiently induce shoot formation in explants containing meristematic cells as also shown in this study (Table 2). However, this was highly dependent on the genotype. The different responses of banana cultivars to exogenous plant growth regulators is dependent on genotype (Arinatwe *et al.,* 2000). This genotype-dependent trait causes differences in the growth patterns of each cultivar even though they are treated by using the same combination of plant growth regulators.

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