EFFECTS OF BENZYLADENINE ON IN VITRO SHOOT MULTIPLICATION OF BANANA (*Musa paradisiaca* Linn) cv. AMBON KUNING AND TANDUK

Dwi Hapsoro, Mochamad Ivan Alisan, Titiek Ismaryati, and Yusnita

Department of Agronomy, Faculty of Agriculture, University of Lampung
Jl. Sumantri Brojonegoro 1 Bandar Lampung, Indonesia.
E-mail: dwhapsoro@gmail.co.id, dwihapsoro@unila.ac.id

ABSTRACT

Objective of this research was to investigate effects of benzyladenine (BA) concentrations on in vitro shoot multiplication of banana (*Musa paradisiacal* Linn) cv. Ambon Kuning and Tanduk. Shoot buds (0.5 x 0.5 x 0.5 cm^3) as explants were surface sterilized by shaking them in Na-hypochlorite solution (1.05%) added with 1-2 drops of Tween 20 for 15 minutes and shaking them again in the same solution (0.5%) for 5 minutes. The explants were then rinsed in sterile distilled water at least three times. The explants were aseptically cultured on semi-solid medium consisting of MS salts, 30 g/l sucrose, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glycine, 100 mg/l myo-inositol,2 g/l gelrite as solidifying agent, and different concentrations of BA (0, 1, 2, and 5 mg/l) as treatment. One explant was cultured on one 250 ml-culture bottle containing 20 ml medium. The pH of medium was adjusted to 5.8 prior to being autoclaved at 1.2 kgf/cm2 for 15 minutes. Each treatment consisted of at least 10 culture bottles. Data was subjected to analysis of variance and differences in treatment means was detected using least significant difference (LSD). Results of the experiment showed that BA was effective to induce shoot multiplication of both cultivars of bananas. Increased BA concentrations led to increased number of shoots. Best shoot multiplication was achieved using 2 mg/l BA, resulting in 6.8 shoots per explants for cv. Ambon Kuning and 7.3 shoots per explants for cv. Tanduk. All shoots were successfully rooted and acclimatized.

Keywords: banana, *Musa paradisiacal* Linn, benzyladenine, in vitro, shoot multiplication.

INTRODUCTION

Bananas (*Musa paradisiaca* Linn) are one of the most important fruits in the world as indicated by being the biggest imported fruits by volume and the second biggest after citrus fruits by value (UNCTAD, 2010). Indonesia is one of the main actors in banana production, sharing 7% of the total production of banana in the world. Other important producers of banana are India (21%), Brazil (9%), China (9%), The Philippines (9%), Ecuador (8%), and the rest of the world (37%) (UNCTAD, 2010).

Even though Indonesia is recorded as one of important producers of banana, most of its production is not for export. The main exporters of banana are Ecuador, Costa Rica, The Philippines, Colombia, and Guatemala. To become one of the main exporters of banana Indonesia should support commercial production of banana. This requires best practices on farm level, in which availability of high quality planting materials is a must. Most banana farmers in Indonesia use suckers as planting materials, which are not uniform and can hardly be produced in a large number.

Tissue culture-derived planting materials could be an alternative to suckers. Propagation by tissue culture can be used to produce high quality planting materials in a large number in a relatively short time. In vitro propagation of banana has been reported by several researchers (Cronauer and Krikorian, 1984; Mateille and Foncelle, 1988; Gupta, 1986; Yusnita et al., 1996; 1997). Routine procedure of in vitro propagation of banana, in fact, has been established. An agricultural company in Lampung, for example, is currently using tissue culture to produce planting materials for its large area of banana plantation.

Even though routine protocol of in vitro propagation of banana has been established, there are many other banana cultivars that need to be studied to find out the protocol of propagating them in vitro. Since genotype of banana plants has been reported to influence their regerability in vitro

(Hirimburegama and Gamage, 1997), research still need to be done to propagate genotypes that have not been propagated in vitro yet. In vitro propagation of banana cv.Ambon Kuning (AAA) has been reported (Yusnita et al., 1996; Murad, 2008, Anegra, 2008) but in vitro propagation of banana cv.Tanduk (AAB) has not been reported yet. This research was conducted with aims of investigating effects of benzyladenine concentrations on in vitro propagation of banana cv. Ambon Kuning and Tanduk.

MATERIALS AND METHODS

Explants

Banana plants as a source of explants were those of cv. Ambon Kuning (AAA) and Tanduk (AAB) derived from the Stock of Banana Plants in Padang Cermin, Lampung, Indonesia. Explants used in this experiment were shoot buds (0.5 x 0.5 x 0.5 cm) excised from rhizome of banana plants. The explants were surface sterilized by shaking them in Na-hypochlorite solution (1.05%) added with 1-2 drops of Tween 20 for 15 minutes and shaking them again in the same solution (0.5%) for 5 minutes. The explants were then rinsed in sterile distilled water at least three times.

Medium

The sterile explants were cultured on semi solid pre-condition medium for one week before being subjected to experimental treatments. The pre-condition medium consisted of MS salts (Murashige and Skoog, 1962), 30 g/l sucrose, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glycine, 100 mg/l myo-inositol, and 2 g/l gelrite as solidifying agent. The medium as treatments were the same as the pre-condition medium except that it was added with different concentrations of benzyladenine (BA) (0, 1, 2, and 5 mg/l). The pH of all of the media was adjusted to 5.8 before being autoclaved at 1.2 kgf/cm2 for 15 minutes. The media were dispensed into 250 ml-culture bottle, 20 ml each.

Experimental Design

Explants of banana plants cv Ambon Kuning and Tanduk were cultured on pre-condition media for one week. Uncontaminated explants were then subcultured on treatment media containing different concentrations of BA) (0, 1, 2, and 5 mg/l) for 12 week. Prior to being cultured on treatment media, each explants was sliced longitudinally on meristem to inhibit apical dominance. Subculture was done every 4 weeks. Experiment was factorial and arranged in a completely randomized design with 4 replicates, 3 culture bottles with one explants each being an experimental unit. One factor was cultivars (Ambon Kuning and Tanduk) and another factor was concentrations of BA (0, 1, 2, and 5 mg/l). After 12 weeks in culture number of shoots, number of shoot buds and shoot length were recorded. Data were subjected to analysis of variance and difference between treatment means was detected using least significant difference (LSD).

RESULTS AND DISCUSSION

Results

Results of the experiment showed that benzyladenine (BA) concentrations significantly affected number of shoot buds, number of shoots, and shoot length, while cultivars only significantly influenced number of shoot buds but had no significant effect on number of shoots and shoot length. Interaction of BA concentrations and cultivars significantly affected number of shoot, but had no significant effects on number of shoot buds and shoot length (Table 1). Explants were put on pre-condition media for one week, transferred to treatment media for 12 weeks then data were recorded. Representation of appearance of the culture up to 12 weeks was depicted in Figure 1.

Table 1. Results of analysis of variance of effects of benzyladenine (BA) concentrations and cultivars on number of shoot buds, number of shoots and shoot length in in vitro culture of banana (*Musa paradisiaca* Linn) 12 weeks in culture

Variables	BA concentrations (A)	Cultivar (B)	A x B Interaction
Number of shoot buds per explant	*	*	ns
Number of shoots per explant	*	ns	*
Shoot length (cm)	*	ns	ns

* : significant at α = 0.05

ns : not sigificant at α = 0.05

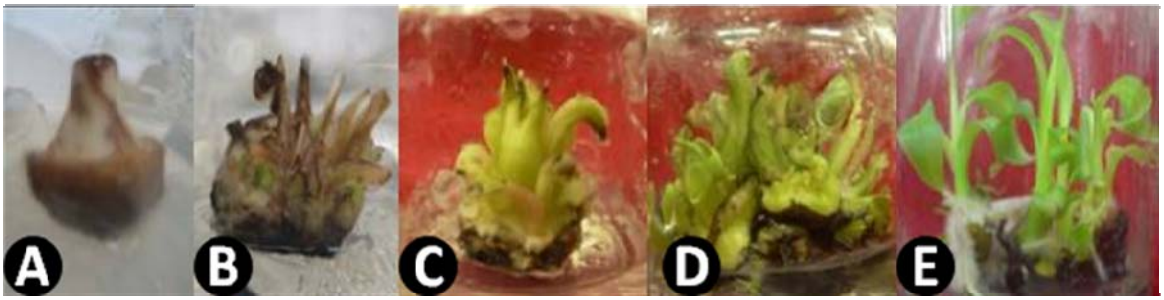

Figure 1. In vitro culture of banana plants (*Musa paradisiaca* Linn). Explant was put on pre-condition media for 1 week (A), then transferred to treatment media (B to E). Subcultures onto the treatment was done every 4 weeks. B, C, D, and E were culture of 1, 4, 8, 12 weeks of age, respectively.

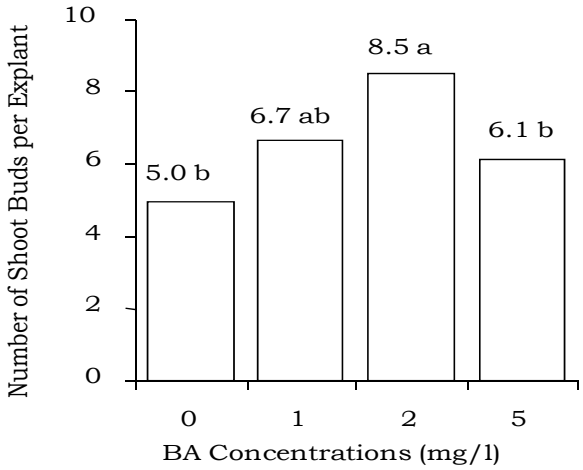

Figure 2. Number of shoot buds produced by explants of banana plants (*Musa paradisiaca* Linn) cv.Ambon Kuning and Tanduk cultured in vitro as affected by benzyladenine (BA) concentrations after 12 weeks in culture. Data for the two cultivars were pooled since there was no effects of interaction between cultivars and BA concentrations on number of shoot buds per explant. Numbers followed by the same letters were not significantly different based on LSD (P<0.05).

Figure 2 showed that addition of BA resulted in an increase in number of shoot buds of both cultivars of banana plants. Number of shoot buds increased significantly as the concentrations of BA increased up to 2 mg/l; more BA added led to a significant decrease in number of shoot buds. Highest number of shoot buds (8.5 shoot buds per explants) was obtained at 2 mg/l BA. Cultivar

Tanduk produced more shoot buds than Ambon Kuning, averaging 9.4 shoot buds per explant compared to 3.7 shoot buds per explant (Figure 3).

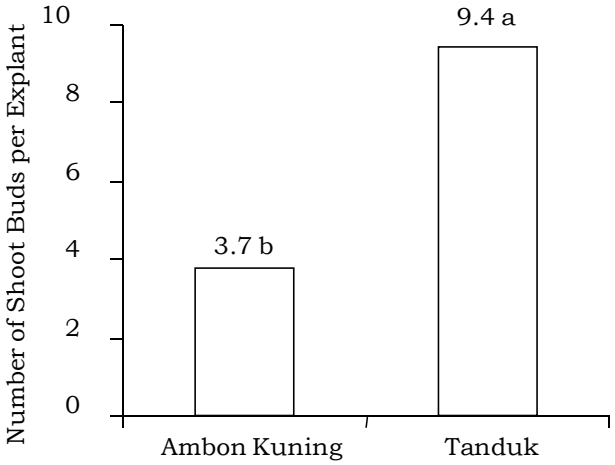

Figure 3. Number of shoot buds produced by explants of banana (*Musa paradisiaca* Linn) cv. Ambon Kuning and Tanduk cultured in vitro for 12 weeks on media containing different concentrations of benzyladenine (BA)(0, 1, 2, and 5 mg/l). Data for the effects of BA concentrations were pooled since there was no effects of interaction between cultivars and BA concentrations on number of shoot buds per explant. Numbers followed by the same letters were not significantly different based on LSD (P<0.05).

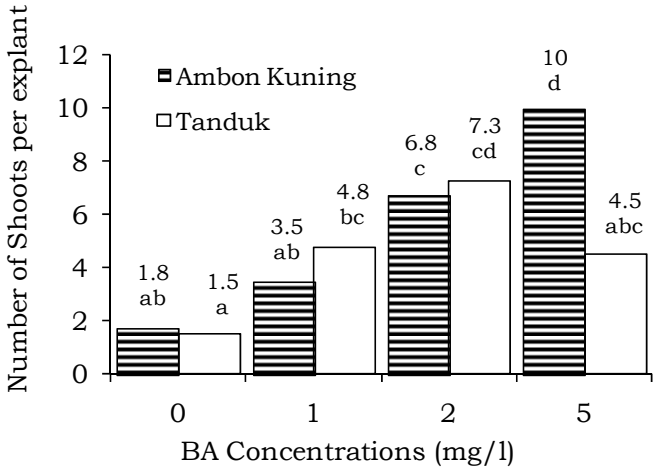

Figure 4. Effects of benzyladenine (BA) concentrations and cultivars on number of shoots produced by explants of banana (*Musa paradisiaca* Linn) cultured in vitro for 12 weeks. Numbers followed by the same letter were not significantly different based on LSD (P<0.05).

Addition of BA also resulted in an increase in number of shoots (Figure 4). An increase in BA concentrations up to 2 mg/l led to an increase in number of shoots of both cultivars. Further increase in BA concentrations, however, resulted in a significant increase in number of shoots of cv.Ambon Kuning but a significant decrease in number of shoots of cv. Tanduk. Highest number of shoots of cv Tanduk (7.3 shoots per explants) was attained at 2 mg/l BA, but highest number of shoots of cv Ambon Kuning (10 shoots per explants) was obtained at 5 mg/l BA. Shoot length decreased as the concentrations of BA increased (Figure 5). Appearance of in vitro culture of banana cv Ambon Kuning and Tanduk was shown in Figure 6 and 7. Shoots have been rooted and the plantlets have been successfully acclimatized (Figure 8).

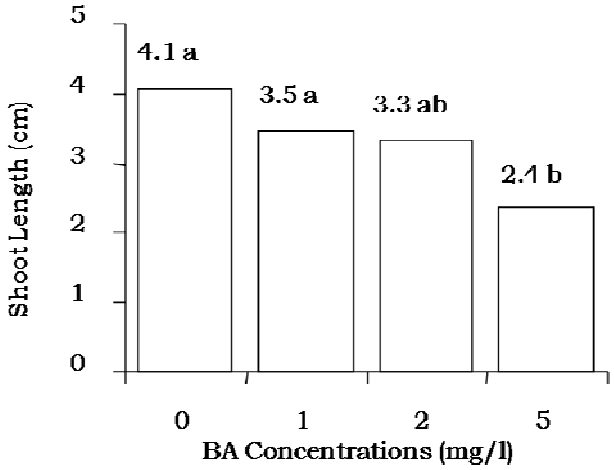

Figure 5. Length of shoot produced by explants of banana (*Musa paradisiaca* Linn) cv.Ambon Kuning and Tanduk cultured in vitro for 12 weeks on media containing different concentrations of benzyladenine (BA). Data for the effects of the two cultivars were pooled since there was no significant effects of cultivars and interaction between cultivars and BA concentrations on shoot length. Numbers followed by the same letters were not significantly different based on LSD (P<0.05).

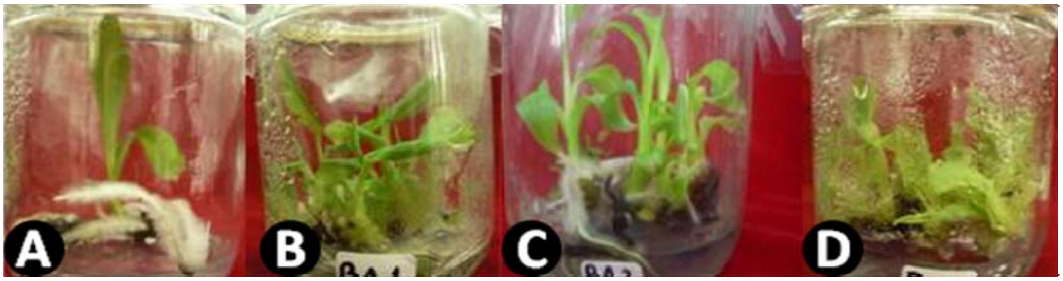

Figure 6. In vitro culture of banana cv. Ambon Kuning as affected by concentrations of benzyladenine (BA). A= 0 mg/l BA, B= 1 mg/l BA, C= 2 mg/l BA, and D= 5 mg/l BA. The cultures were 12 weeks old.

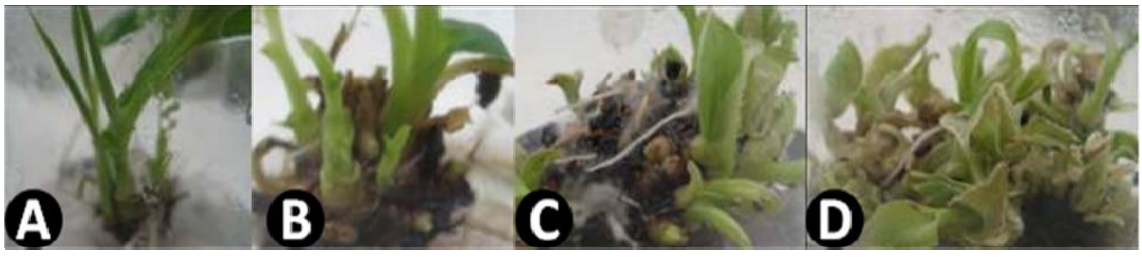

Figure 7. In vitro culture of banana cv. Tanduk as affected by concentrations of benzyladenine (BA). A= 0 mg/l BA, B= 1 mg/l BA, C= 2 mg/l BA, and D= 5 mg/l BA. The cultures were 12 weeks old.

Discussion

Plant propagation by use of tissue culture is commonly conducted by inducing shoot multiplication, rooting the shoots, and acclimatizing the plantlets. Murashige (1974) devided in vitro propagation into three stages. The first stage is called culture establishment, in which explants does

not show microbial contamination and exhibit sign of initial growth. The second stage is shoot multiplication, in which shoot multiplication occurs. The third stage is rooting the shoots and acclimatizing the plantlets. In this present study, all the three stages have been conducted. Culture establishment of both cultivar Ambon Kuning and Tanduk has been acchieved using the protocol reported in this study. Shoot multiplication has also been attained by addition of benzyladenine (BA). Highest number of shoots of cv.Ambon Kuning (10 shoots per explant) was obtained at 5 mg/l BA, whereas highest number of shoots of cv. Tanduk (7.3 shoots per explant) was acchieved at 2 mg/l BA. Rooting the shoots has also been conducted and the plantlets have been successfully acclimatized (Figure 8).

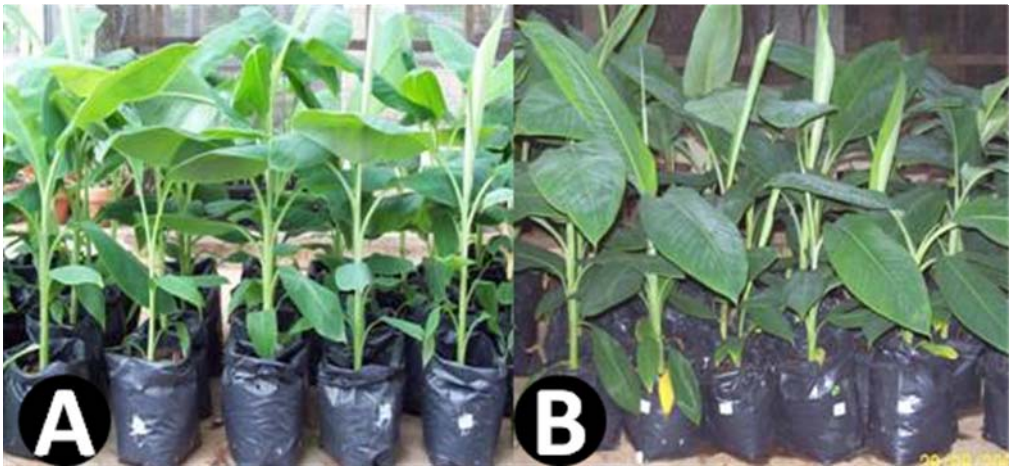

Figure 8. Tissue culture-derived planting materials of banana cv.Tanduk (A) and cv.Ambon Kuning (B). Plants were derived from acclimatized plantlets 6-8 cm in length grown for 3 months in media contained in polybags.

Plant tissue culturists have been applying principles of regeneration published in classical paper written by Skoog and Miller (1957). They reported that high auxin to cytokinin ratio induced root formation. Low auxin to cytokinin ratio promoted shoot formation. Balanced concentrations of auxin and cytokinin resulted in callus formation. In this experiment, addition of BA was intended to make auxin to cytokinin ratio low so as to induce shoot formation. This study showed that addition of BA to the culture media was effective to induce formation of shoots of banana cv. Ambon Kuning and Tanduk. However, it was noted that without addition of BA multiple shoots were induced (Figure 4), suggesting the presence of endogenous cytokinin. Use of BA as shoot inducer has also been reported by other researchers (Yusnita et al., 1996; 1997; Mateille and Foncelle, 1988; and Gupta, 1986).

Even though regenerability are usually influenced by genotypes, in this present study cv. Ambon Kuning (AAA) and Tanduk (AAB) showed no difference in regenerability as indicated by number of shoots produced by the two cultivars in response to BA (Table1). However, it is worth noting that the two genotypes produced significantly different number of shoot buds, Tanduk producing more buds than Ambon Kuning (Figure 3). Since shoots were resulted from the elongation of shoot buds, cv.Tanduk has higher potential to produce more shoots than cv. Ambon Kuning, suggesting that the media used in this study was more favorable for elongation of shoots of cv.Ambon Kuning than for cv. Tanduk. Therefore, furher research to find out more favorable media for elongation of shoots of cv. Tanduk need to be done.

CONCLUSIONS

In vitro propagation of banana plants cv. Ambon Kuning and Tanduk has been established using MS medium supplemented with 30 g/l sucrose, 0.1 mg/l thiamine-HCl, 0.5 mg/l pyridoxine-HCl, 0.5 mg/l nicotinic acid, 2 mg/l glycine, 100 mg/l myo-inositol,2 g/l gelrite as solidifying agent, and 2 mg/l benzyladenine (BA). BA was proven to be effective to induce shoot multiplication. Substantial number of shoots of the two cultivars were produced using the media. Even though cultivar Ambon Kuning produced more shoots than Tanduk, the latter produced more shoot buds that potentially can become shoots when subjected to favorable culture media.

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