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Full Length Research Paper

Micropropagation of some Malaysian banana and plantain (*Musa* sp.) cultivars using male flowers

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Male inflorescences have potential to be used as explants for rapid micropropagation of *Musa* sp. The male flowers of four banana cultivars, namely 'Berangan', 'Rastali', 'Nangka' and 'Abu' belonging to three genome types in *Musa* (AAA, AAB, and ABB), were cultured onto Murashige and Skoog (MS) medium which was supplemented with 1 mg/L of TDZ, BAP, Kin, 2-ip and Zea. The number of shoots was found to significantly increase in both TDZ and BAP treatments, as compared to other cytokinins. TDZ at 0.4, 0.6 and 0.8 mg/L, in particular, appeared to be optimum for shoot induction in 'Berangan-AAA', 'Rastali-AAB' and 'Nangka-AAB' and 'Abu-ABB', respectively. However, all the cultivars showed their highest response to regeneration at 8 mg/L of BAP. After the initiation of the explants onto the MS media for all the cultivars, the highest number of cauliflower-like bodies' (CLBs) clusters was observed at two months of culture. The number of induced 'CLBs' cluster is dependent on the size of male buds. Male inflorescences with the size of 20 mm were found to induce more 'CLBs' clusters. Meanwhile, the number of shoots produced is dependent on both the cytokinins and cultivars used.

Key words: Proliferation rate, *Musa*, cytokinin, male flower, male inflorescence, micropropagation.

INTRODUCTION

Bananas and plantains are perennial herbaceous monocots which belong to the *Musa* genus of the *Musaceae* family. They are cultivated in the tropical and sub-tropical areas all over the world, with a yearly production of approximately 70 million tonnes. They are ranked as the fourth important fruit crops which provide diet to millions of people, especially in South East Asia (FAOstat, 2005). As one of the origins of bananas, Malaysia has a great variety of them, that is, with around 50 types with capacity to be exported to other countries. Some of the banana varieties have already been cultivated for export purposes, but the quality of the fruit still need to be further enhanced for more exportation. The high sterility of most cultivated bananas has historically prevented conventional breeding programs, as it has similarly affected plant propagation. Moreover, the longer time required by bananas to generate makes it even more difficult to breed them (Sasson, 1997). Meanwhile, micropropagation has played a key role in banana and plantain breeding programs worldwide (Rowe and Rosales, 1996; Vuylsteke et al., 1997). Among others, *in vitro* culture is of great advantage for mass propagation of various vegetative propagated crops. Plantlets produced through micropropagation method have been found to establish faster, healthier, stronger, shorter production cycle and higher yields than those produced through conventional methods (Ortiz and Vuylsteke, 1996). Banana is probably the most intensely micropropagated crop. However, a large number of banana genotypes need to be screened for commercial micropropagation and genetic improvement. Different explants have been used for banana and plantain propagations. Although, *in vitro* propagation of bananas using shoot tips has been reported for many commercial cultivars (Kulkarni et al., 2004, 2006), male inflorescence can also be applied as a potential regenerable explant. Specifically, male inflorescence

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Abbreviations: TDZ, Thidiazuron; **BAP,** 6-benzylaminopurine; **Kin,** kinetin; **Zea,** zeatin; **2-ip,** 2-isopentenyl adenine; **CLBs,** cauliflower-like bodies; **MS,** Murashige and Skoog medium.

reduces contamination rate during micropropagation as compared to soil grown suckers. Moreover, *in vitro* culture of inflorescence apices offers an opportunity to select a male bud with desirable characteristics such as a greater number of hands and fruits per bunch *in situ* (Resmi and Nair, 2007). Therefore, the male inflorescence culture can help increase the efficiency of micro-propagation, as well as produce plantlets from the parts which could be lost during harvesting. Male inflorescence direct regeneration of some Indian banana cultivars (Swamy and Sahijram, 1989; Resmi and Nair, 2007). This study explored the capacity of the male inflo-rescences of some Malaysian banana and plantain key cultivars for plantlet production.

MATERIALS AND METHODS

Plant materials

After emergence at 10 weeks old, male flower bunches were collected from *Musa* sp. cv. 'Berangan' AAA-group, 'Rastali' and 'Nangka' AAB-group and 'Abu' ABB-group of field-grown plants. The sizes of these male inflorescences were reduced to 4 cm in non-sterile conditions. They were surface sterilized by immersing in 70% ethyl alcohol for 5 min under a laminar flow. The tiny bracts which protect the male flowers (hands) were removed with caution to avoid damages to the apical dome and this was done until the first one was reached to be used as explants. The male inflorescences were grouped to three different sizes of 15, 20 and 25 mm length, while hands were used as the explants for micropropagation.

Media preparation and culture conditions

The explants were cultured onto the Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) which was supplemented with 1 mg/L of thidiazuron (TDZ), 6-benzylaminopurine (BAP), kinetin (Kin), zeatin (Zea) and 2-isopentenyl adenine (2-ip) 30 g/L sucrose and 3 g/L Gelrite®. The pH of the culture medium was adjusted to 5.7 before autoclaving at 121°C for 17 min. Zea and 2-ip were added to the MS media after autoclaving by filtration. The cultures were maintained at a temperature of 25 ± 2°C, with a photoperiod of 16 h/day. The sub-culture was carried out every 30 days. The experiment was repeated using different concentrations of TDZ (0.2 - 1 mg/l) and BAP (2 - 10 mg/L) to determine the optimum concentration for plant regeneration. The cultures were maintained according to the procedure explained in the earlier section.

Data collection and analysis

The data were collected weekly based on the number of induced CLBs cluster. Both the mean values and standard errors are shown based on the average of four replicates. Meanwhile, Duncan's Range Test (Duncan, 1995) was used for dissociation of means.

RESULTS

All explants were found to expand and they became green around 10 to 15 days after their initiation. They responded to cytokinin through induction of CLBs clusters which became visible after four weeks (Figure 1). After 60 days, the MS medium, which was supplemented with 1 mg/L of TDZ and BAP, produced average of 4.5 and 3.9 'CLBs' clusters (Figure 2). Meanwhile, the average number of the 'CLBs' clusters produced by 1 mg/L of Kin, 2-ip and Zea were 3.1, 1.75 and 1.3, respectively. The explants which were cultured onto the MS medium without any cytokinin did not respond to proliferation and their colour just turned green. Among the different cytokinins used, both TDZ and BAP showed the highest effects in inducing the 'CLBs' clusters in all the cultivars. Different concentrations of TDZ and BAP were used to compare the proliferation and determine the optimum concentration for a high 'CLBs' induction (Figures 3 and 4). When the concentrations of the BAP and TDZ were increased, the total number of the 'CLBs' clusters induced were decreased. Apparently, the 'CLBs' clusters produced were found to vary among the cultivars in the different concentrations of TDZ. In particular, 'Berangan' showed the highest 'CLBs' cluster (7.75) induction at 0.4 mg/L of TDZ. However, the highest 'CLBs' cluster inductions (with 6.5, 6.25 and 7.25 respectively) were obtained when TDZ was used for 'Rastali' and 'Nangka' (0.6 mg/L) and 'Abu' (0.8 mg/L). The MS medium supplemented with 8 mg/L of BAP induced the highest 'CLBs' cluster in all the cultivars. The number of 'CLBs' cluster significantly varied with different concentrations of BAP. Nevertheless, the 'CLBs' clusters produced were less in quantity at BAP concentration lower than 6 mg/L or more than 12 mg/L of BAP in all the cultivars. Specifically, the cultivar 'Berangan' induced the highest 'CLBs' cluster (8.5) as compared to other cultivars (7.75, 8 and 7 'CLBs' cluster in 'Rastali', 'Nangka' and 'Abu', respectively) in 8 mg/L of BAP. Based on the cor-responding monthly multiplication rates of the male flowers shown in Figure 5, high values were obtained for all the cultivars after the first cycle of sub-cultures in 8 mg/L of BAP as an optimum concentration. The average number of 'CLBs' cluster produced by 'Berangan' was 7.25 per explant after two months of culture. However, 'Rastali', 'Nangka' and 'Abu' induced an average of 6, 6.25 and 4.5 'CLBs' clusters, respectively. The number of 'CLBs' clusters increased when the male inflorescence with the size of 20 mm was used for micropropagation (Figures 6 and 7). After the second sub-culture, the 'CLB' clusters converted to shoots (Figure 8). The average number of shoots were observed in 'Berangan' with 13.5 and 11.5 shoots per explant at 0.4 and 8 (mg/L) of TDZ and BAP, respectively. These were 12.5 and 11 for 'Rastali' and 'Nangka' at 0.6 and 8 (mg/L) of TDZ and BAP. Meanwhile, 'Abu' was found to induce 10.5, and 9.5 shoots per explant at 0.8 and 8 (mg/L) of TDZ and BAP, respectively.

DISCUSSION

Immature male flowers of bananas and plantains have

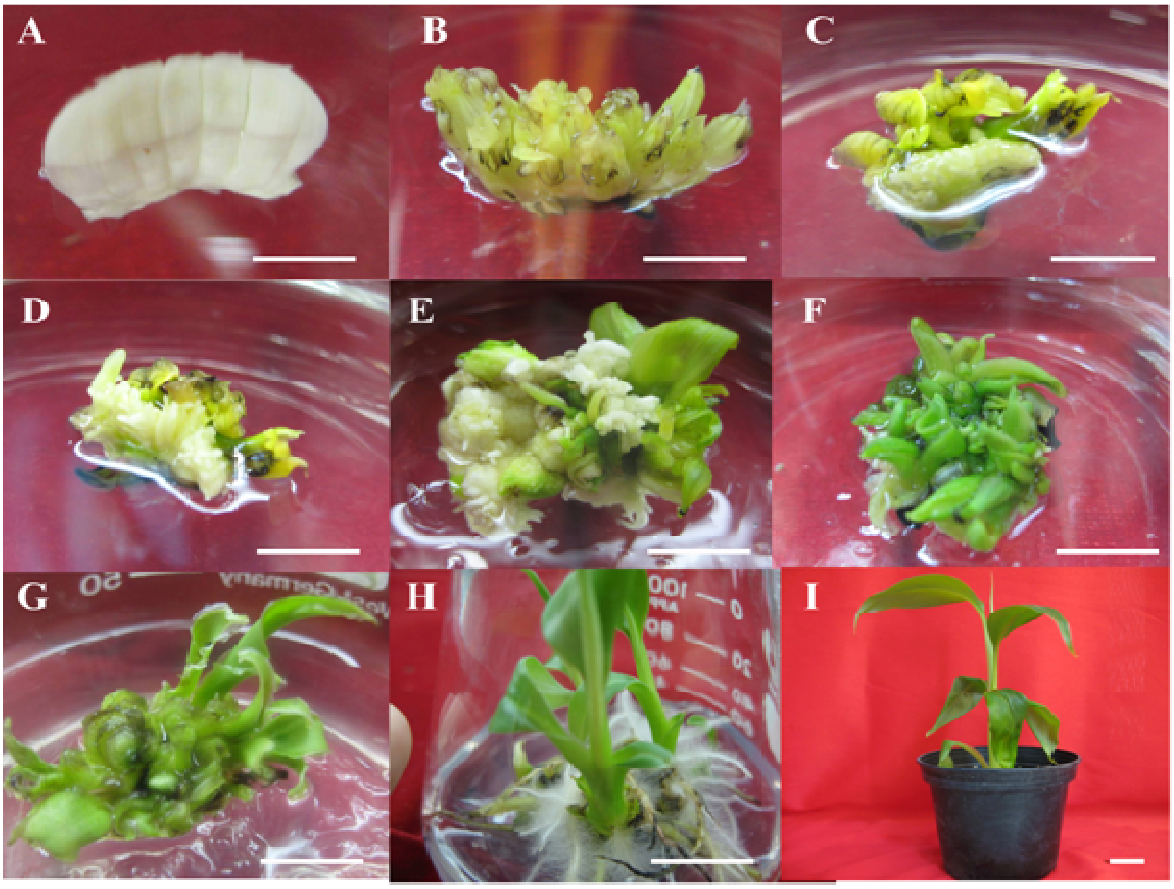

Figure 1. Direct regeneration of male flowers of Pisang Nangka in MS media supplemented with 0.6 mg/L of TDZ. (**A**) A group of male flower (hands) used for proliferation. (**B**) The hands becoming green after 15 days. (**C** and **D**) Cauliflower-like bodies' clusters produced by (A) male flower in left picture. (**E**, **F** and **G**) Cauliflower-like bodies' converting to shoot. (**H**) Plantlet produced by male flowers; Bar = 3 mm. (**I**) Plantlet transferred to pot; Bar = 10 mm.

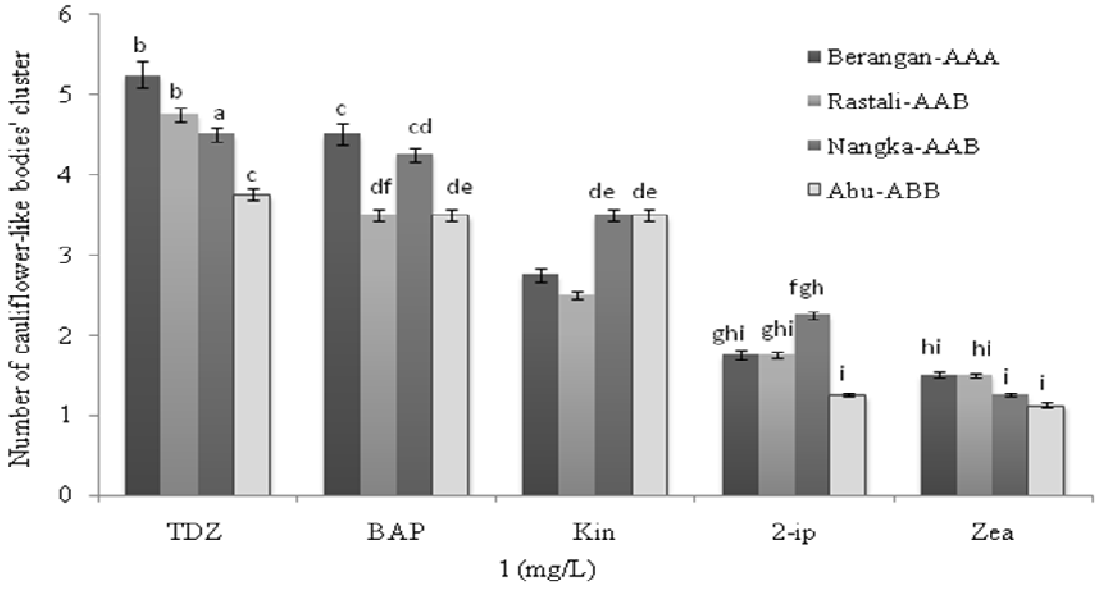

Figure 2. Effect of 1 mg/L of various cytokinins on proliferation induction of male flowers after two successive subcultures from primary found explants. The total numbers of cauliflower-like bodies' cluster are shown. Different letters indicate values are significantly different ($p < 0.05$).

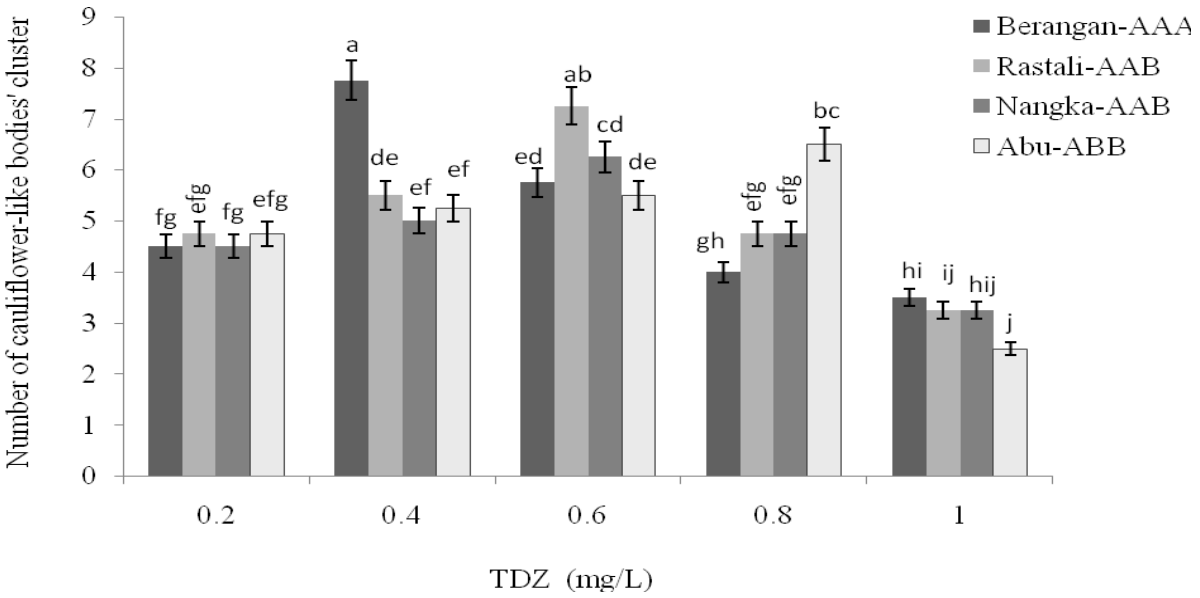

Figure 3. Optimization of CLBs cluster in MS media with different concentrations of TDZ after two continuously subcultures cycle from initially established explants. The total numbers of induced CLBs clusters are shown. Different letters indicate values are significantly different ($p < 0.05$).

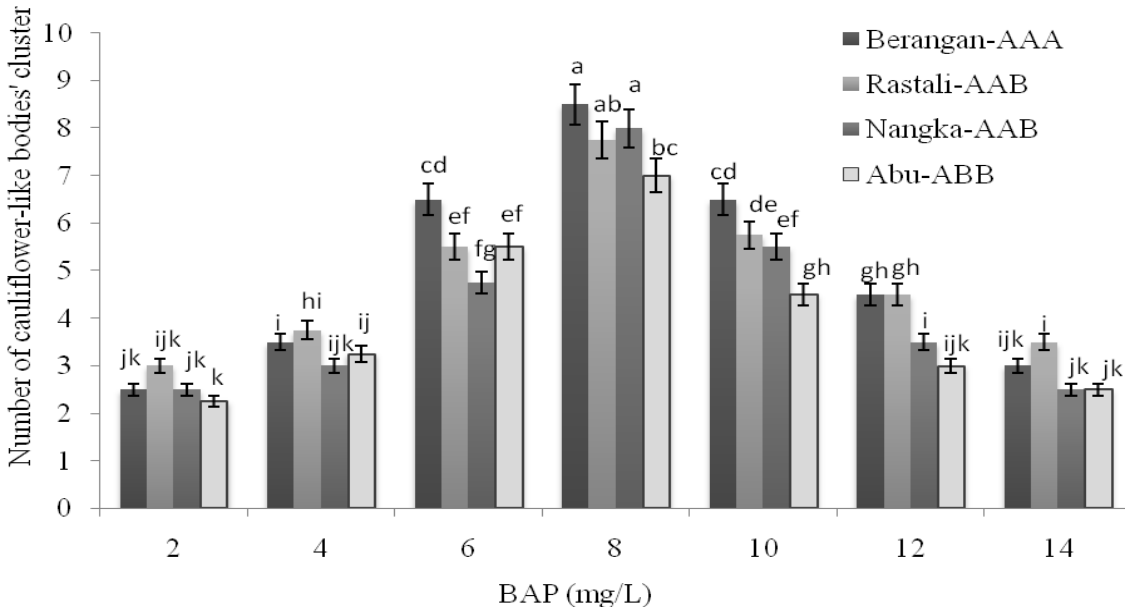

Figure 4. Optimization of CLBs cluster in MS media with different concentrations of BAP after two continuously subcultures cycle from initially established explants. The total numbers of induced CLBs clusters are shown. Different letters indicate values are significantly different ($p < 0.05$).

shown their potentials as suitable explants for direct regeneration, as suggested by Krikorian et al. (1993). Apparently, the male flowers responded to regeneration through induction of CLBs clusters. A similar regeneration response was also observed in an *in vitro* culture of floral apices of bananas and other monocots (Cronauer and Krikorian, 1985a, b; Swamy and Sahijram, 1989; Verron et al., 1995; Resmi and Nair, 2007). In this study, the multiplication rate of the male flowers was found to be significantly dependent on the type of cytokinins. Thus, cytokinins are necessary as a pre-requisite of male flower regeneration. Comparable results have been reported for *Musa* cv. Cavendish, bamboo and ginseng inflorescences which have not shown any organogenesis responses or

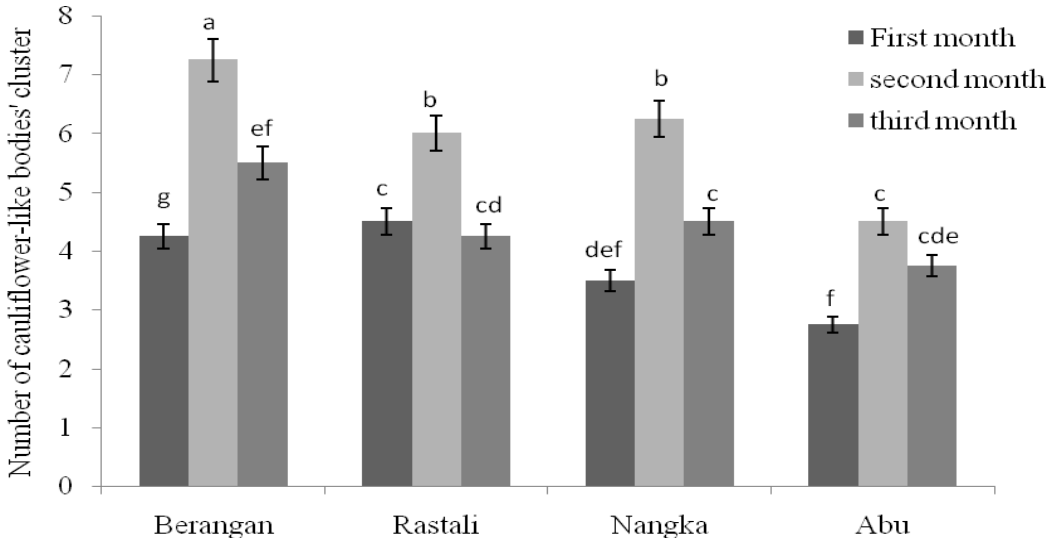

Figure 5. Optimization of Cauliflower like bodies (CLBs) clusters induction from initially established male flower in 8 mg/L of BAP during three successive subcultures. The total numbers of induced CLBs clusters are shown. Different letters indicate values are significantly different ($p < 0.05$).

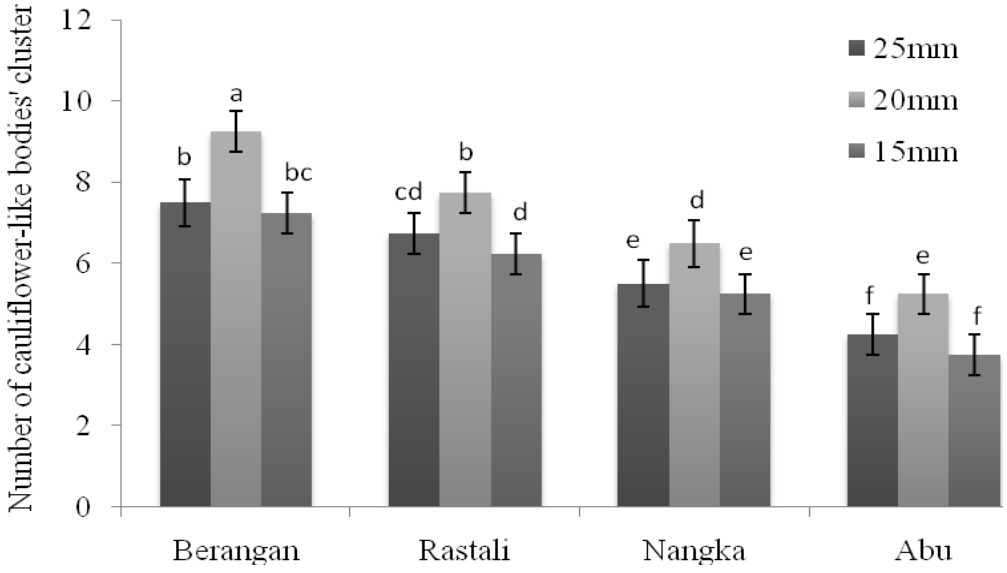

Figure 6. Effect of different initiate explant size on multiplication of male flowers after 2 successive subcultures. The explants were cultured onto 8 mg/L of BAP as the best concentration for all cultivars. The total numbers of induced CLBs clusters are shown. Different letters indicate values are significantly different ($p < 0.05$).

proliferation in MS basal medium lacking any cytokinins (Lin et al., 2003; Lin et al., 2004; Bernardo and Purificacio´n, 2008). Strosse et al. (2008) studied the effects of different cytokinins on banana proliferation. They observed that TDZ and BAP stimulated multiplication to a higher extent compared to Kin, 2-ip and Zea. In particular, cytokinins have been found to reduce the dominance of apical meristems and induce axillary shoots, as well as formation of adventitious shoot from meristematic explants (Madhulatha et al., 2004).

The results showed that the concentration of cytokinins affected the multiplication rate of male flowers. Bernardo and Purificacio´n (2008) obtained the highest multiplication rate (2.89) of the male inflorescence of 'Cavendish' (AAA) on the medium containing 1 mg/L (5 µM) of TDZ. In this study, 0.4 mg/L was found to be the optimum concentration with a high proliferation rate (0.97) for 'Berangan' with AAA genotype as compared to Cavendish. This

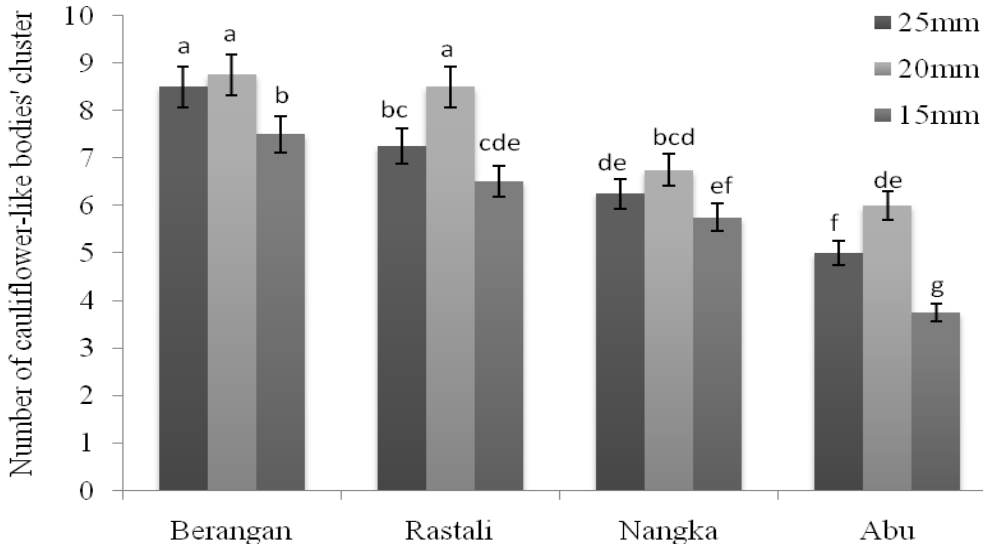

Figure 7. Effect of Different sizes on multiplication of male flowers after 2 successive subcultures. The explants were cultured onto 0.4, 0.6 AND 0.8 mg/L of TDZ for Berangan-AAA, Rastali and Nangka and Abu-ABB respectively as the optimum concentrations for regeneration. The total numbers of induced CLBs clusters are shown. Different letters indicate values are significantly different ($p < 0.05$).

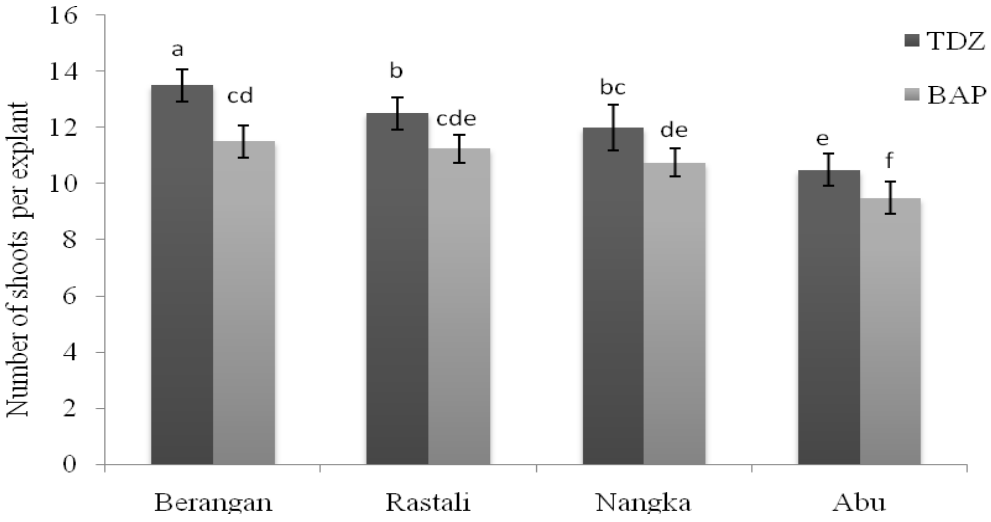

Figure 8. The total number of shoots per explant converted from flower clusters after 120 days of initiation. The explants were cultured onto 8 mg/L of BAP as the best concentration for all cultivars and 0.4 (mg/L) for Berangan-AAA, 0.6 (mg/L) for Rastali and Nangka and 0.8 (mg/L) for Abu-ABB of TDZ as the optimum concentrations for regeneration. Different letters indicate values are significantly different ($p < 0.05$).

could be due to the physiological differences between the cultivars. In the case of TDZ, the induction of 'CLBs' has been found to be genetic-dependent. Similar results have been reported by Strosse et al. (2004) whereby they stated that the rate of shoot multiplication is dependent both on the concentration and genotype of cytokinins. It was observed that the cultivars bearing the A genome showed higher rates of multiplication than those with B genome (Arinaitwe et al., 2000). Although the optimum concentration of TDZ varied among the genotypes, it was the same in BAP. The high performance of BAP over other cytokinins in the multiplication of shoot tips has been reported in different cultivars of banana (Wong, 1986; Gilmar et al., 2000). In addition BAP also has an important role in stimulating and proliferating lateral bud growth in other plants such as *Centella asiatica*,

Kaempheria galanga and *Bacopa monerria* (Shirin et al., 2000; Tiwari et al., 2000; Tiwari et al., 2001). The role of BAP in multiple shoot clusters induction from the terminal floral apices of *Musa acuminate* cv. 'Dwarf Cavendish' (AAA), inoculated on the modified MS medium supplemented with 5 mg/L (22.2 µM) of BAP, has previously been reported (Cronauer-Mitra and Krikorian,1984; Jarret et al., 1985; Resmi and Niar, 2007). In a comparable study, Asnita and Norzulaani, (2006) reported shoot-like structures on the MS medium, supplemented with 7 mg/L of (31.0 µM) BAP, which gave a large number of shoot formation from the male inflorescence of *M. acuminate* cv. 'Berangan'. Each banana cultivar has an optimum concentration for maximum response to proliferation (Vuylsteke, 1989). In this study, all the cultivars showed their highest 'CLBs' inductions to BAP at 8 mg/L. However, their proliferation capacity was found to be significantly dependent on the cultivars. Specifically, the size of the male inflorescences tip influenced the regeneration response because of the disposition of male flowers in male bud. Bernardo and Purificacio´n (2008) showed that the highest clusters (62.5-93.8%) were induced in bananas male flowers of 'small' class size. Similarly, the high organogenesis responses were obtained from the male inflorescence of 'Cavendish' after 45 days of culture. In addition to normal shoots, abnormal shoots were also observed during the micropropagation of the male flowers, and this was due to the high concentrations of BAP and TDZ. Vidhya and Nair (2002) also reported the occurrence of green somaclonal variants during the micropropagation of 'Red' banana which resulted from the high concentration of BAP in the medium.

In summary, micropropagation of inflorescences can provide disease-free plantlet. Therefore, this strategy can also be applied for mass micropropagation of bananas and plantains to produce disease-free plantlet. Moreover, it can be helpful in any cases where suckers of elite cultivars are unavailable.

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