American-Eurasian Journal of Sustainable Agriculture, 2(3): 300-307, 2008
ISSN 1995-0748

ORIGINAL ARTICLE

In Vitro Production of Multiple Bud Clumps (Mbcs) from Cavendish Banana Cultivar, Brasilian (AAA)

[1,2] Sreeramanan Subramaniam, [2]Xavier Rathinam, [1,2]Ranjetta Poobathy and [3]Umarani Sinniah.

[1]School of Biological Sciences, Universiti Sains Malaysia (USM), Minden Heights, 11800, Penang, Malaysia,
[2]Department of Biotechnology, AIMST University, Batu 3½, Jalan Bukit Air Nasi, Bedong, 08100, Kedah, Malaysia,
[3]Department of Crop Sciences, Faculty of Agriculture, Universiti Putra Malaysia, 43400, Seri Kembangan, Selangor, Malaysia.

Sreeramanan Subramaniam, Xavier Rathinam, and Umarani Sinniah, *In Vitro* Production of Multiple Bud Clumps (Mbcs) from Cavendish Banana Cultivar, Brasilian (AAA), *Am.-Eurasian J. Sustain. Agric., 2(3): 300-307, 2008*

ABSTRACT

Multiple bud clumps (Mbcs) are whitish compact clusters of banana proliferating shoot meristems. A single bud from Mbcs can be a good target material for genetic transformation studies and could be a potential source for the development of embryogenic cell suspension cultures in banana. In this study, Mbcs were initiated from the selected corm slices region of the cavendish banana cultivar, Brasilian (AAA) and were cultured on Murashige and Skoog medium (1962) supplemented with various concentrations of BAP hormone in both solid and liquid medium. Optimization of Mbcs production was assessed based on the number and various size of single buds obtained (< 3 mm, < 3-5> mm and >5 mm). The Mbcs production was monitored at the two weeks intervals for the period of eight weeks. Highest number of single buds of 3 different sizes was produced from multiple bud clumps at the concentration of 8 mg/L BAP in both solid and liquid medium, respectively. Single buds is the material of choice to be used as a target tissues for the genetic transformation in Cavendish banana cultivar, Brasilian (AAA) because it can be easily propagated via *in vitro* system, potentially regenerable at higher rates and could be reduced the possibility of chimeric plants since each single bud will regenerate into a single shoot within 4 weeks.

Key words: Cavendish banana Brasilian (AAA) . Multiple bud clumps. *In vitro* regeneration

Introduction

In vitro propagated of Cavendish bananas (AAA) are increasingly becoming the planting material of choice because of excellent advantages over traditional propagation and availability of disease-free material all the year around. Micropropagation of banana also plays a vital role in the distribution of germplasm, conservation, and safe exchange of internal planting material of newly selected hybrid cultivars.

Among the *in vitro* techniques used in agriculture and horticulture, meristem and shoot tip cultures of banana are the most widely used, mainly for clonal multiplication on large scale (Lee *et al.,* 1997; Navarro *et al.,* 1997; Madhulatha *et al.,* 2004). *In vitro* multiplication of banana plantlets is an excellent alternative with many advantages over field-grown material (Vuylsteke and Ortiz, 1996). According to Cote *et al.* (1993), banana is probably the most intensely micropropagated crop and also efficient the possibilities of multiplication of a valuable genotype. .

Successful generation of transgenic bananas relies on the availability of highly efficient regeneration system and type of transformation protocols. *In vitro* plant regeneration has been possible from meristematic tissues located at or adjacent of the shoot apical and adventitious meristems, as well as through somatic embryogenic

Corresponding Author: Sreeramanan Subramaniam, School of Biological Sciences, Universiti Sains Malaysia (USM), Minden Heights, 11800, Penang, Malaysia.
Tel:604-6533528 Fax:604-6565125
E-mail: sreeramanan@usm.my / sreeramanan@gmail.com

cell cultures. Both pathways have been used in genetic transformation of banana. Highly embryogenic cell suspension cultures are a better target for genetic transformation since most cells are totipotent and regeneration via somatic embryogenesis is originating from individual cells. The main difficulty with genetic transformation of meristems is that only a small quantity of cells is totipotent and regeneration from meristem makes the selection of non-chimaeric transgenic tissues difficult. Despite these limitations, transgenic banana cultivar Rastali (AAB) plants have been obtained using both *Agrobacterium* and particle-bombardment-mediated transformation (Sreeramanan *et al.,* 2006 a, b) through similar type of meristematic tissue which is known as multiple bud clumps (Mbcs).

The development of Mbcs will provide the opportunity for the establishment of regenerable from individual cells, similar to the production of protocorm-like bodies (PLBs) in orchids. Therefore, Mbcs guarantee high regeneration frequencies with low risks of chimaerism during the selection of transgenic banana and can be reduced the incidence of genetic variation known as somaclonal variation (Sreeramanan *et al.,* 2006b). In addition from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Generally, adenine-based cytokinins are the most commonly used in *Musa* spp. i*n vitro* propagation, especially N^6-benzylaminopurine (BAP). The concentration of BAP cytokinin appears to be main factor affecting multiplication rate in banana particularly to induce multiple shoots (Wong, 1986; Vuylsteke, 1989). On the other hand, there are reports on the use of diphenylurea derivates, thiadiazuron (TDZ) in *Musa* spp. which had shown significantly higher propagation rate for shoot proliferation compared to any types of other adenine-based cytokinins particularly BAP hormone (Arinaitwe *et al.,* 2000). However, in this study, we used different BAP concentrations for the optimization of induction and regeneration of the multiple bud clumps (Mbcs) of Cavendish banana cultivar Brasilian (AAA) using an optimized region of corm slice from *in vitro* rhizome culture.

Materials and methods

Plant Materials

Second generation of *in vitro* banana plantlets cultivar Cavendish Brasilian (AAA) raised from the shoot tip cultures were used for the present study.

Preparation of culture media

The basal medium employed throughout the whole study was mainly based on MS (Murashige and Skoog, 1962) medium. The culture medium consisted of a standard MS basal medium enriched with 3% sucrose with the addition of different concentrations of BAP (mg/L) hormone. The pH of the media was adjusted to 5.7 prior to the addition of gelrite at 2.75 g/L for solid media only.

Effect of 5mg/L of BAP on shoots production from five potential regions of corm tissues

Corm slices of 2mm thick and 5-8mm diameter numbered 1 to 5 were obtained from the rhizomes (number 1: the lowest region and the rest followed accordingly the numbers). Each corm slice subcultured onto MS medium supplemented with 5mg/L BAP using 25ml of solid or liquid medium in 100ml conical flasks (Duran) on a rotary shaker (liquid medium only) at 40 rpm. The cultures were incubated at 25 ± 2°C in a 16-h photoperiod under cool white fluorescent lamps (Philips TLD, 36 Watt) at 150 µmol m^{-2} s^{-1}. Experiments were carried out in a randomized design and repeated three times, and each replication containing ten corm slices per treatment from each region.

Multiple bud clumps (Mbcs) formation from corm slice (number 2) with different concentration of BAP (mg/L)

Corm slices (only from region number 3) were prepared and subsequently cultured on MS media supplemented with different BAP concentrations in solid and liquid medium (0, 2, 4, 6, 8, 10, 12, 14 and 16 mg/L). Corm slices were subcultured onto 25 ml liquid medium in 100ml conical flasks on a rotary shaker at 40 rpm. The cultures were incubated at 25 ± 2°C in a 16-h photoperiod under cool white fluorescent lamps (Philips TLD, 36 Watt) at 150 µmol m^{-2} s^{-1}. Subculture was carried out at four weeks interval on fresh medium of the same constituents. Parameters observed were the number of buds produced in three different sizes (<3mm, 3mm - 5mm and >5mm) in solid and liquid medium for eight weeks. Experiments were carried out in a randomized design and repeated three times, and each replication containing corm slices per treatment.

Frequency of single bud converting to shoot in 1mg/L of BAP

The single buds measuring 3 different sizes (<3mm, 3mm - 5mm and >5mm) were separated from the multiple bud clumps (MBCs) and then transferred on both MS solid and liquid medium supplemented with 1 mg/L of BAP, 3% sucrose 2.75 g/L Gelrite, and pH 5.7 for the regeneration of single plantlets. A total of ten replicates, with five single buds per replicate in 25 ml of solid medium in 100 ml conical flasks, were

prepared. For liquid media, single buds were subcultured onto 25ml of medium in 100ml conical flasks on a rotary shaker at 25 ± 2°C in a 16-h photoperiod under cool white fluorescent lamps (Philips TLD, 36 Watt) at 150 µmol m^{-2} s^{-1}. Observation for the first sign of shoot formation was made every five days for 30 days and each bud that showed positive sign was scored as one unit. The frequency (%) of shooting was calculated as follows: (number of shoots/ total number of single buds cultured x 100). Experiments were carried out in a randomized design and repeated three times, and each replication containing ten single buds per treatment.

Statistical data analysis

Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. All statistical analyses were performed at the level 5% using SPSS 10.0 (SPSS Inc. USA).

Results and discussion

Effect of 5mg/L of BAP on shoots production obtained from five potential regions of corm tissues

Naturally, banana has high potency to produce large number of small corms under *in vitro* condition especially in the early stage of culture (2-5th generation after obtained via surface sterilization). Thus, the *in vitro* technique for inducing cormlet initiation followed by its development to form micro sucker is prospective method for the improvement of the commercialization of banana *in vitro* technique. In this study, we used corm slices as an initial explant which can be obtained from the rhizome region for adventitious shoot formation using an optimized concentration of BAP, a cytokinin hormone. The corm tissues started swelling after one week of culture and this observation could be due to the intake of medium nutrients used for the shoots development.

Figure 1 showed that the number of shoots in the first two weeks is slightly higher in corm slice number 3 compared with the other regions. In solid media, the highest number of shoots (5.2) was attained from corm slice number 3 with 2.0 and 1.9 number shoots from corm number 2 and 4 at week 2, respectively (Fig. 1). The mean number of shoots per explant in liquid medium was higher than solid medium. The number of shoots produced at week two was statistically shows a higher significant ($p<0.05$) value in corm slice number 3 in both solid and liquid medium, compared with the other corm slice regions (Fig. 1). Meanwhile, the number of shoots was drastically increased in week four in both mediums from the five corm slice regions. Again, the mean number of shoots per explant was higher in liquid medium. In liquid media, the highest number of shoots (10.1) observed again was from corm slice number 3 followed by 5.0 on corm slice region number 1, 2 and 4 respectively (Fig. 1). Similar results pattern were obtained in solid medium but with a lower multiplication number of shoots. In week six, the numbers of shoots produced from corm slice number 3 in solid and liquid medium were again found to be highly significantly ($p<0.05$) compared to the other region of the corm slices used. The lowest number of shoots production was obtained from corm slice region five.

Inclusion of BAP hormone in the medium has been reported to form a clonal sector in cotton meristems which causes the development of multiple shoots (Hazra *et al.,* 2000). In contrary, Priyono (2001) reported that BAP has no positive effect on the cormlet development of giant cavendish banana (AAA), which all concentration of BAP tested inhibited initial development. Arinaitwe *et al.* (2000) reported that shoot proliferation from banana was cultivar dependent. They stated that increasing of BAP concentration above 16.8 uM (~ 4 mg/L) did not significantly increase shoot proliferation in *'Kibuzi'* (AAA) and *'Ndiziwemiti'* (AAB) banana cultivars. In addition, they reported in the same experiment that the cultivar *'Bwara'* (AAA) responded significantly increases in shoot proliferation rate with increasingly from 5.0 to 8.0 shoots with an increase of BAP hormone concentrations from 16.8 to 28 µM (~ 7 mg/L).

In this experiment, banana formed corm slice or known as cormlet from the apical meristem slice of naturally corm via *in vitro* system. However, other plants such as taro also formed corm from the main stem (Zhou *et al.,* 1999) and potato formed corm from axillary buds (Khuri and Moorby, 1996). Vuylsteke (1989) reported that cormlets could be obtained from apical shoot cultured on the medium contained of 2-5 mg/L BAP combined with 20% sucrose in long time subculture with the estimation of one-year old subculture for banana and two year-old subculture for plantain. In this study, the multiplication rate (average number of shoots produced per explant), were always the highest in corm slice number 3, then followed by corm slice number 2 and the lowest was in corm slice number 5. Therefore, for subsequent experiment for the optimization of multiple bud clumps (Mbcs), the corm slice from the region number 3 was used as target tissue.

Multiple bud clumps (Mbcs) formation from corm slice region (number 3) with different concentration of BAP (mg/L)

Multiple bud clumps (Mbcs) formation from the different concentrations of BAP used varied greatly throughout this experiment (Fig. 2 and 3). Multiple bud clumps (Mbcs) can be separated into three different

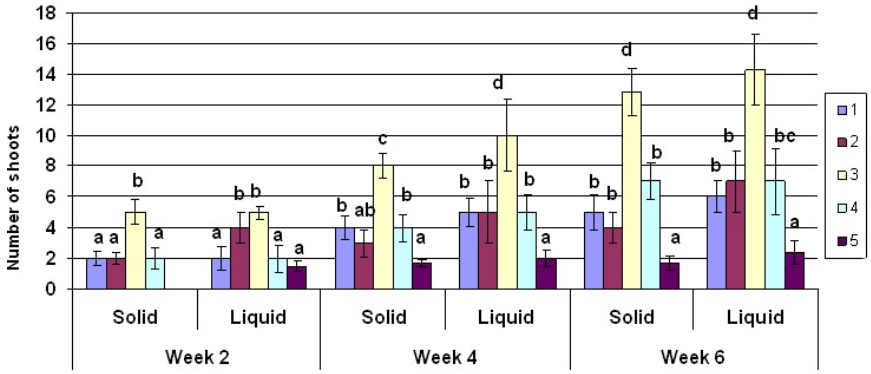

Fig. 1: Identification of target corm meristematic tissues for shoots proliferation. The data plotted were the means of 3 replicates. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).

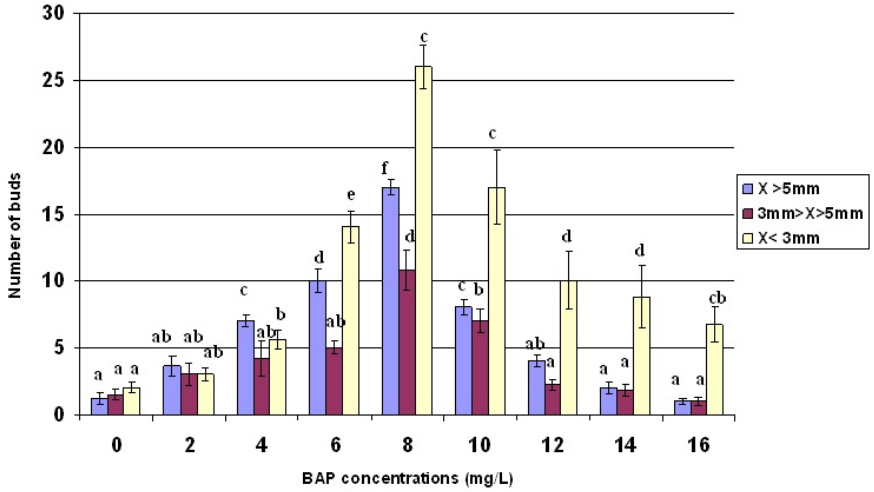

Fig. 2: Optimization of multiple bud clumps (Mbcs) in MS solid medium with different concentrations of BAP in eight weeks. The data plotted were the means of three replicates using corm slice number 3. Each replication contained ten corm slices. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).

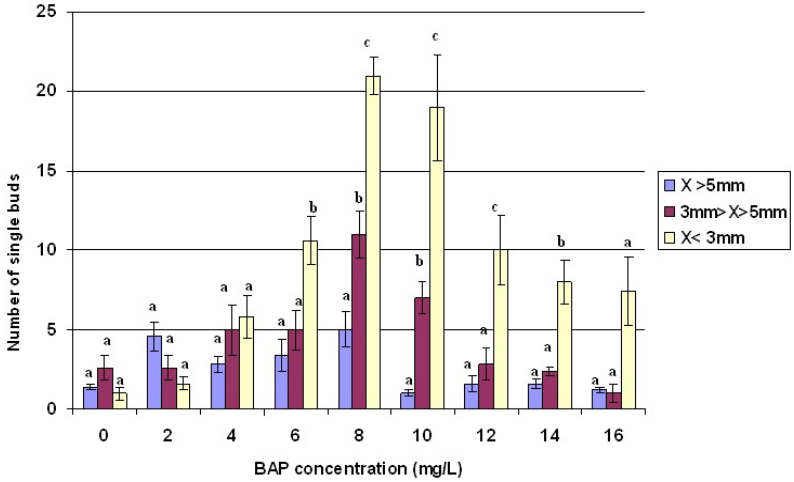

Fig. 3: Optimization of multiple bud clumps (Mbcs) in MS liquid medium with different concentrations of BAP in eight weeks. The data plotted were the means of three replicates using corm slice number 3. Each replication contained ten corm slices. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).

sizes of single buds with 3mm, between 3mm to 5mm and 5mm. A significant difference ($p<0.05$) in buds formation using BAP concentrations between 8 and 12 mg/L for eight weeks was obtained compared with other range of BAP concentrations (Fig. 2 and 3). Single buds of less than 3mm in sizes were highest in 8 mg/L of BAP, followed by sizes between 3mm and 5mm in solid medium (Fig. 2). Similar results were obtained by using liquid medium (Fig. 3).

Generally, multiple bud clumps (Mbcs) produced were less in quantity at the range of BAP concentrations which is lower than 8mg/L or more than 12mg/L of BAP in both solid and liquid medium (Fig. 2 and 3). The stunted bud production eventually formed scalps after 6 weeks using more than 12 mg/L of BAPconcentration. Scalp formation has been reported on MS medium with modified vitamins supplemented with 10µM and 100µM BAP combination with 1.0µM IAA using semi-solid media for banana cultivar, Bluggoe (ABB) (Dhed'a *et al.,* 1991). Khanam *et al.* (1996) reported that high BAP concentration along with an increase in the number of sub-cultures stimulated higher number of stunted axillary bud proliferation from shoot tips explants of different banana cultivars. Vidya and Nair (2004) reported that occurrence of somaclonal variants was observed in red banana (AAA) due to the high concentration of BAP in the culture medium. They reported that the rate of shoot multiplication depends both on the cytokinin concentration and the genotype of bananas.

Cytokinin hormone such as BAP and kinetin are generally known to reduce the dominance of apical meristems and induce axillary as well as adventitious shoot formation from meristematic explants in banana (Madhulatha *et al.,* 2004). The application of higher BAP concentrations inhibits elongation of adventitious meristems and the conversion into complete plants (Buising *et al.,* 1994; Zaffari *et al.,* 2000). However, in this study with a lower concentration of BAP produced minimum number of shoots in the cavendish banana cultivar, Brasilian (AAA) (Fig. 2). Hirimburegama and Gamage (1997) studied *in vitro* micropropagation of 12 banana cultivars. They observed that the rate was highly significant among the cultivars. Banana cultivars bearing the A genome showed higher rates of multiplication than those with B genome. Shoot regeneration from corm tissue of banana was reported by Okole and Schultz (1996) and production of multiple shoots from apical meristems of oat (Zhang *et al.,* 1996b). Thirty-four shoots per corm were obtained on MS medium supplemented with 3mg/L IBA and 1.5mg/L BAP in 90 days of culture for banana cultivar genome A. Khanam *et al.* (1996) recorded 40 shoots per explant with a mean shoot length of 1.5 cm in cultivar Amritsagar (AAA) on MS medium supplemented with 30 µM BAP. Dhed'a (1992) described those cultivars lacking a 'B' chromosome in their genome produced clumps of stunted shoots instead of scalp formation while cultivars with one or two 'B' chromosome sets in their genome generated nice and vigorous proliferation for scalp formation.

However, we have noticed that the physical appearance (quality) of the multiple bud clumps (Mbcs) and single buds changed from fresh outlook of whitish/light green colour (Fig. 4a, b and c) to slightly brownish if subculture period was done more than 4 weeks interval. This could be due to aging and the oxidation of phenolic compounds. Another attribute that make single buds of this banana cultivar an excellent target tissue for transformation studies is having a high regeneration frequency from the meristematic organ which could be obtained from liquid and solid media by using lower concentration of BAP hormone. Men *et al.* (2003) obtained vigorously growing protocorm-like bodies (PLBs) from *Dendrobium phalaenopsis* and *Dendrobium nobile* which was crucial for successful transformation event since this type of explant are actively dividing cells that could be prone to receive foreign DNA into their genome successfully (Zhao *et al.,* 2000). The ability to regenerate whole plants from adventitious shoots without an intermittent callus phase has been achieved in chrysanthemum stem thin cell layers (Teixeira da Silva and Fukai, 2003). In addition, adventitious shoot regeneration derived from an initial callus phase may result somaclonal variation compared with direct shoot regeneration (Teixeira da Silva and Fukai, 2003).

Frequency of single bud converting to shoot in 1mg/L of BAP

Information of the shoot conversion frequency is equally important in order to demonstrate that single buds from multiple bud clumps (Mbcs) are good quality meristematic regions. Usually by going through a direct regeneration system, shoot regeneration will be formed directly on an explant without an intervening callus stage. This phenomenon was preferred especially in banana cultivar, since extensive callus formation and long-term callus culture can lead to somaclonal variation incident at high percentage. The frequency of single buds converting to shoot in both solid and liquid medium is shown in Figure 5 and 6. Single isolated buds were cultured on 1mg/L of BAP on MS media and observation was carried out every 5 days for 30 days for the first sign of shoot emergence (as indicated in the Fig. 5 and 6). All the single buds cultured formed shoots within 30 days but at different time periods and frequencies. For bud size 3mm in solid medium (Fig. 4d), (Fig 4 e) the highest frequency of shooting was observed around week 3 with the highest peak at day 20 (44%) and followed by day 25 (32%).

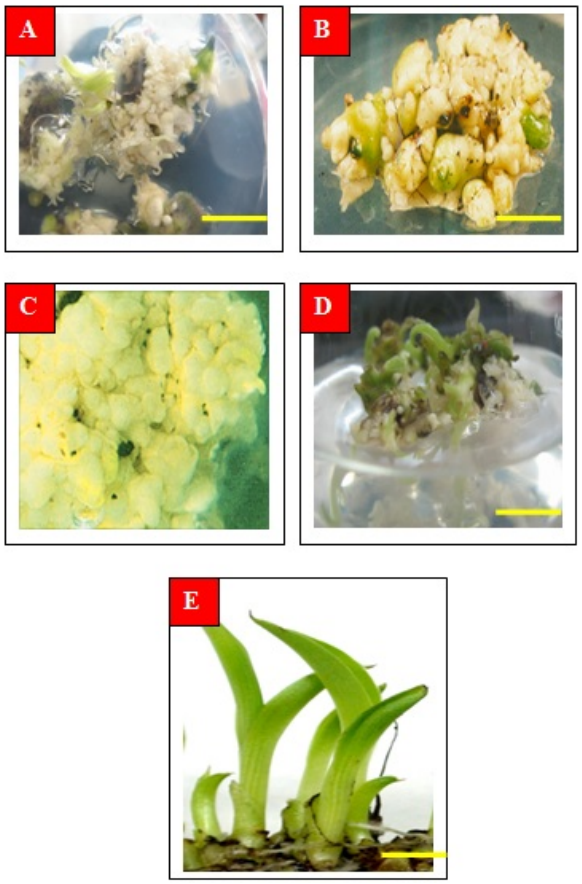

Fig. 4: Production of multiple bud clumps (Mbcs) obtained through *in vitro* culture of corm slices. (A, B) Physical appearance of Mbcs obtained from corm slice region 3; (C) Proliferation of Mbcs under microscope observation (30X); (D) Regeneration of single buds from Mbcs and (E) Putative banana plantlets suitable for hardening stage prior transfer to polybags. The bar in the bottom of each of the image represents 1.0 cm.

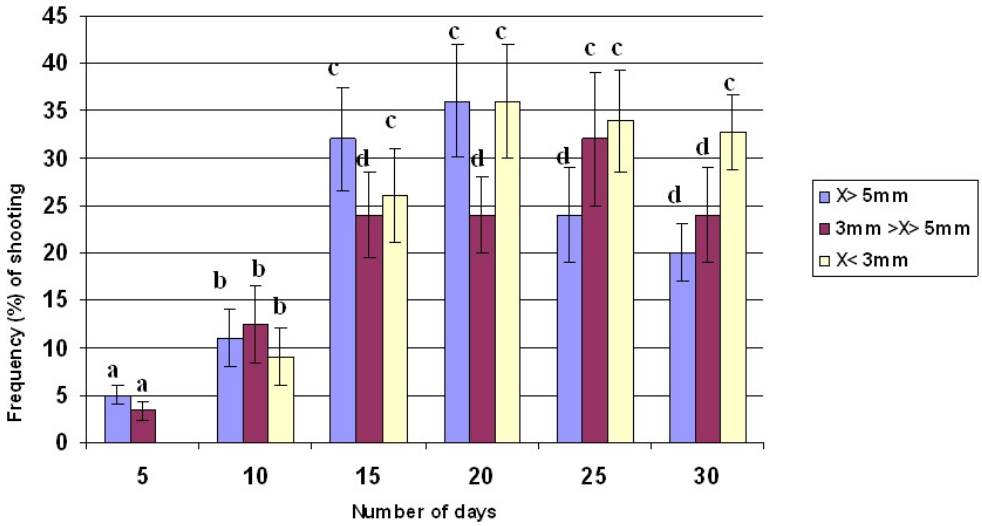

Fig. 5: Frequency of a single bud converting to shoot in MS solid medium in 1 mg/L of BAP hormone. The data plotted were the means of three replicates using with each replication contained ten single buds. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).

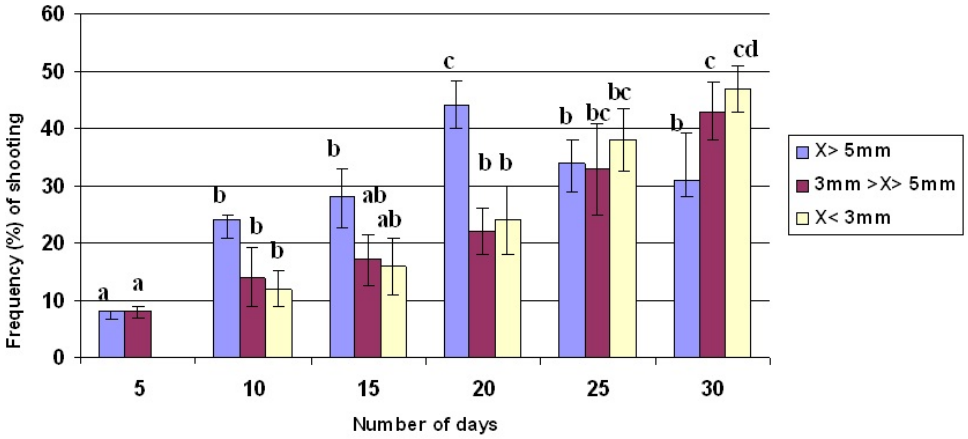

Fig. 6: Frequency of a single bud converting to shoot in MS liquid medium in 1 mg/L of BAP hormone. The data plotted were the means of three replicates using with each replication contained ten single buds. Data were analysed using one-way ANOVA and the differences contrasted using Duncan's multiple range test. Different letters indicate values are significantly different ($p<0.05$).

In liquid media, the highest frequency of shooting was observed in a similar pattern at even higher frequency of shooting (Fig.6). There was no shooting obtained from bud size 3mm for the first 5 days of cultured. However, the highest overall frequency of shooting was obtained from bud of size more than 5mm at day 20 (44.3%) in solid and liquid medium. For size between 3mm and 5mm, the highest frequency of shooting was observed at day 30 in both solid (34%) and liquid (43%) medium. However, a single bud will completely regenerate into a rooting plantlet for hardening purpose after week 10 in both solid and liquid medium.

These observations highlighted that the use of the banana cultures in liquid increases the proliferation rate. Venkatachalam *et al.* (2004) reported liquid medium supports rapid uptake and excretion of nutrients molecules from and to the cells with the establishment of specific chemical gradients appear to play a positive role towards regeneration of banana cultivar. However, the use of liquid medium in Cavendish banana cultivar Brasilian (AAA) involves the problem of vitrification as a result of immersion after a long period at *in vitro* culture condition. Vitrification known as hyperhydricity could be frequently affecting herbaceous plants such as banana and woody shoots during their *in vitro* vegetative propagation (Kevers *et al.,* 2004). Therefore, we would like to suggest the use of solid medium for the regeneration of single buds was highly preferable despite of the superiority of liquid forms of media over shoots multiplication and elongation rates in this Cavendish banana cultivar Brasilian (AAA).

Conclusions

The application of BAP hormone was effective for the stimulation of shoot bud proliferation in cavendish banana cultivar Brasilian (AAA). Corm slice number 3 produced highest number of shoots by using 5mg/L of BAP in the solid and liquid medium. Highest number of single buds produced from multiple bud clumps (MBCs) by using 8 mg/L of BAP in both solid and liquid medium in week 8. Single buds is the potential material of choice to be used as target tissues for genetic transformation in banana because since it is easily propagated via *in vitro* culture, potentially regenerable tissue and could reduced the possibility of chimeric plants since each bud regenerating into a single shoots within 4 weeks. The buds produced by this method rooted easily in MS basal medium without auxin and suitable for preservation of germplasm *in vitro* in gene bank.

Acknowledgment

This research was supported by the Malaysian Ministry of Higher Education through Fundamental Research Grant 2007 (01-01-07-095FR).

References

Arinaitwe, G., P.R. Rubaihayo and M.J.S. Magambo, 2000. Proliferation rate effects of cytokinins on banana (Musa spp.) cultivars. Scientia Horticulturae, 86: 13-21.

Buising, C.M., R.C. Shoemaker and R.M. Benbow, 1994. Early events of multiple bud formation and shoot development in soybean embryogenic axes treated with the cytokinin 6-benzylaminopurine. American Journal of Botany, 81: 1435-1448.

Blakesley, D., 1991. Uptake and metabolism of 6-benzyladenine in shoot proliferation of Musa and Rhododendron. Plant Cell, Tissue and Organ Culture, 25: 69-74.

Cote, F.X., J.A. Sanoval, P. Marie and E. Auboiron, 1993. Variations in micropropagated bananas and plantains. Fruits, 48: 11-18.

Dhed'a, D., F. Dumortier, B. Panis, D. Vuylsteke and E. De Langhe, 1991. Plant regeneration in cell suspension cultures of the cooking banana cv. 'Bluggoe' (*Musa* spp. ABB group). Fruits, 46: 125 - 135.

Hazra, S, A.V. Kulkarni A., S.M. Nalawade, A.K. Banerjee, D.C. Agrawal, K.V. Krishnamurthy, 2000. Influence of explants, genotypes and culture vessels on sprouting and proliferation of pre-existing meristems of cotton (Gossypium hirsutum L. and Gossypium arboreum L.). *In Vitro Cell. Developmental Biology Plant*, pp. 505-510.

Hirimburegama, K. and N. Gamage, 1997. Cultivar specificity with respect to *in vitro* micropropagation of *Musa* spp. (banana and plantain). Journal Horticulture Science, 72: 205-211.

Kaminck, M., 1992. Progress in cytokinin research. TIBTECH, 10: 159-162.

Kevers, C., T. Franck, R.J. Strasser, J. Dommes and T. Gasper, 2004. Hyperhydricity of micropropagated shoots: a typically stress induced change of physiological state. Plant Cell, Tissue and Organ Culture, 77: 181-191.

Khanam, D., M.A. Hoque, M.A. Khan and A. Quasem, 1996. *In vitro* propagation of banana (*Musa* spp.). Plant Cell, Tissue and Organ Culture, 6(2): 89-93.

Lee, K.S., F.J.Z. Arias, H. Brunner and R. Afza, 1997. Histology of somatic embryo initiation and organogenesis from rhizome explants of *Musa* spp. Plant Cell, Tissue and Organ Culture, 51: 1-8.

Madhulatha, P., M. Anbalagan S. Jayachandran and N. Saktivel, 2004. Influence of liquid pulse treatment with growth regulators on *in vitro* propagation of banana (*Musa* spp. AAA). Plant Cell, Tissue and Organ Culture, 76: 189-191.

Men, S., X. Ming, Y. Wang, R. Liu, C. Wei, and Y. Li, 2003. Genetic transformation of two species of orchid by biolistic bombardment. Plant Cell Reports, 21: 592- 598.

Murashige T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiology Plant, 15: 473-497.

Navarro, C., R.M. Escobedo and A. Mayo, 1997. *In vitro* plant regeneration from embryogenic cultures of a diploid and triploid, Cavendish Banana. Plant Cell, Tissue and Organ Culture, 51: 17-25.

Okole, B.N. and F.A. Schultz, 1996. Micro-sections of banana and plantains (*Musa* spp.) morphogenesis and regeneration of callus and shoot buds. Plant Science, 116: 185-195.

Priyono, 2001. Micropropagation of Banana (Musa paradisiacal) through cormlet initiation by *in vitro* culture of apical meristem slices. Jurnal ILMU DASAR, 2(1): 41-48.

Sreeramanan, S., M. Maziah, M.P. Abdullah, M. Sariah and R. Xavier, 2006a. Transient Expression of *gusA* and *gfp* Gene in *Agrobacterium*-mediated Banana Transformation Using Single Tiny Meristematic Bud. Asian Journal Plant Science, 1: 1-13.

Sreeramanan, S., M. Maziah, M. Sariah, M.P. Abdullah and R, Xavier, 2006b. Enhanced Tolerance against a Fungal Pathogen, *Fusarium oxysporum f.sp. cubense* (Race 1) in Transgenic Silk Banana. International Journal of Agriculture Research, 1(4): 342-354.

Teixeira da Silva, J.A. and S. Fukai, 2003. Four gene introduction methods affect the shoot regeneration and localization of transgene expression in greenhouse stem explants and *in vitro*-grown chrysanthemum stem thin cell layers. Journal of Biotechnology, 2(5): 114-123.

Venkatachalam, L., R. Thimmaraju, R.V. Sreedhar and N. Bhagyalakshmi, 2006. Direct shoot and cormlet regeneration from leaf explants of 'silk'banana. *In Vitro Cell. Developmental Biology Plant*, 42: 262-269.

Vidya, R. and A.S. Nair, 2002. Molecular analysis of somaclonal variation in *Musa acuminate* (AAA) cv. Red. Phytomorphology, 52: 293-300.

Vuylsteke, D. and R. Ortiz, 1996. Field performance of conventional vs. *in vitro* propagules of plantain (*Musa spp*., AAB Group). Horticulture Science, 31: 862-865.

Zaffari, G.R., G.B. Kerbauy, J.E. Kraus and E.C. Romano, 2000. Hormonal and histological studies related to *in vitro* banana bud formation. Plant Cell, Tissue and Organ Culture, 63: 187-192.

Zhang, S., M.J. Cho, T. Koprek, R. Yun, P. Bregitzer and P.G. Lemaux, 1999b. Genetic transformation of commercial cultivars of oat (*Avena sativa* L.) and barley (*Hordeum vulgare* L.) using *in vitro* shoot meristematic cultures derived from germinated seedlings. Plant Cell Reports, 18: 959-966.

Zhao, Z.Y., T.S. Cai, L. Tagliani, M. Miller, N. Wang, H. Pang, M. Rdert, S. Schroeder, D. Hondred, J. Seltzer and D. Pierce, 2000. *Agrobacterium*-mediated sorghum transformation. Plant Molecular Biology, 44: 789-798.