Tropical Life Sciences Research, 23(2), 67–80, 2012

Effects of N^6-benzylaminopurine and Indole Acetic Acid on In Vitro Shoot Multiplication, Nodule-like Meristem Proliferation and Plant Regeneration of Malaysian Bananas (*Musa* spp.)

Philip Sipen and Michael R Davey*

Plant and Crop Sciences Division, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough LE12 5RD, United Kingdom

Abstrak: Kepekatan berbeza N^6-benzylaminopurine (BAP) dan asid indole asetik (IAA) dalam medium Murashige dan Skoog telah dikaji kesannya terhadap penggandaan pucuk, proliferasi bintil meristem dan penjanaan semula pokok pisang Malaysia, kultivar Pisang Mas, Pisang Nangka, Pisang Berangan dan Pisang Awak. BAP pada 1–14 mg L^{-1} dengan atau tanpa 0.2 mg L^{-1} IAA, atau BAP pada 7–14 mg L^{-1} dengan kepekatan IAA yang sama telah dikaji untuk penggandaan pucuk dan proliferasi bintil meristem masing-masing. Penjanaan semula dari *scalp* telah dinilai menggunakan 1 mg L^{-1} BAP dan 0.2 mg L^{-1} IAA berasingan ataupun dalam kombinasi kedua-dua pengawal atur pertumbuhan tersebut. Data penggandaan pucuk, proliferasi bintil meristem dengan penjanaan semula pokok telah direkodkan selepas pengkulturan selama 30 hari. Maksimum 5 pucuk untuk setiap pucuk asal telah dicapai dalam medium yang dibekalkan dengan BAP pada 5 mg L^{-1} (Pisang Nangka), 6 mg L^{-1} (Pisang Mas and Pisang Berangan), atau 7 mg L^{-1} (Pisang Awak), dengan 0.2 mg L^{-1} IAA. BAP pada 11 mg L^{-1} dengan 0.2 mg L^{-1} IAA telah menghasilkan bintil meristem yang proliferasi paling banyak dalam empat kultivar pisang. Penjanaan semula dari *scalp* optimum dalam semua kes dengan medium yang mengandungi 1 mg L^{-1} BAP dan 0.2 mg L^{-1} IAA. Ini merupakan laporan pertama tentang induksi berjaya bintil meristem dan penjanaan semula pokok dari *scalp* kultivar pokok pisang Malaysia iaitu Pisang Mas, Pisang Nangka, Pisang Berangan dan Pisang Awak.

Kata kunci: Pisang, Meristem, Penjanaan Semula Pokok, Scalp, Pucuk Pokok

Abstract: Different concentrations of N^6-benzylaminopurine (BAP) and indole acetic acid (IAA) in Murashige and Skoog based medium were assessed for their effects on shoot multiplication, nodule-like meristem proliferation and plant regeneration of the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. BAP at 1–14 mg L^{-1} with or without 0.2 mg L^{-1} IAA, or BAP at 7–14 mg L^{-1} with the same concentration of IAA, was evaluated for shoot multiplication from shoot tips and the proliferation of nodule-like meristems from scalps, respectively. Plant regeneration from scalps was assessed using 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA separately, or a combination of these two growth regulators. Data on shoot multiplication, the proliferation of nodule-like meristems with associated plant regeneration were recorded after 30 days of culture. A maximum of 5 shoots per original shoot tip was achieved on medium supplemented with BAP at 5 mg L^{-1} (Pisang Nangka), 6 mg L^{-1} (Pisang Mas and Pisang Berangan), or 7 mg L^{-1} (Pisang Awak), with 0.2 mg L^{-1} IAA. BAP at 11 mg L^{-1} with 0.2 mg L^{-1} IAA induced the most highly proliferating nodule-like meristems in the four banana cultivars. Plant regeneration from scalps was optimum in all cases on medium containing 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. This is the first report on the successful induction of highly proliferating nodule-like meristems and plant regeneration from scalps of the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak.

*Corresponding author: mike.davey@nottingham.ac.uk

Keywords: Bananas, Meristems, Plant Regeneration, Scalps, Shoot Tips

INTRODUCTION

Bananas (*Musa* spp.) are important fruit crops cultivated in more than 120 countries throughout the tropics and subtropics, where they make a significant contribution to food security and income (Ghosh *et al.* 2009; Shapira *et al.* 2009). According to The Food and Agriculture Organization Corporate Statistical Databases of the United Nations (FAOSTAT 2009), the world production of bananas in 2007 was about 86 million tonnes, which were harvested from an area of 5 million hectares, the top five producing countries being India, China, the Philippines, Brazil and Ecuador. In 2007, about 80% of world production was consumed locally in the producing countries, with the remaining being exported and amounting to 18 million tonnes, worth US $7.2 billion. The leading exporters were Ecuador, Costa Rica, the Philippines, Colombia and Guatemala (FAOSTAT 2009). Bananas ranked fifth in the world trade for agricultural crops in terms of their economic value (Aurore *et al.* 2009).

Worldwide, banana production is affected by various biotic and abiotic stresses (Heslop-Harrison & Schwarzacher 2007). Biotechnological approaches have been exploited extensively for the reliable propagation, genetic improvement, conservation and distribution of banana germplasms (Arvanitoyannis *et al.* 2008). For example, cultured materials such as shoot tips, anthers, highly proliferating cauliflower (nodule)-like meristems, embryogenic cell suspensions (ECS) and isolated protoplasts, have been exploited in a range of applications, including micropropagation (Strosse *et al.* 2008), genetic transformation (Kulkarni *et al.* 2007; Ghosh *et al.* 2009; Xiao *et al.* 2009), cryopreservation (Assani *et al.* 2003; Nahamya 2000; Sipen *et al.* 2011a & 2011b; Smith *et al.* 2005; Strosse *et al.* 2006; Sadik *et al.* 2007) and conservation (Panis & Thinh 2001).

Highly proliferating nodule-like meristems, induced by the culture of shoot tips on medium with BAP or thidiazuron (Nahamya 2000; Sadik *et al.* 2007), were established primarily to provide meristem clumps (scalps), the latter being the uppermost parts of these highly proliferating structures (Panis *et al.* 1990). The induction of highly proliferating nodule-like meristems and their response to BAP and thidiazuron were cultivar-dependent (Sadik *et al.* 2007). Scalps have been used as an alternative to zygotic embryos and immature male flowers for the establishment of ECS in some cultivars (Nahamya 2000; Sadik *et al.* 2007). Attempts have been made to genetically transform scalps, although chimeric plants were regenerated (Acereto-Escoffie *et al.* 2005). Scalps of several banana cultivars have been cryopreserved, emphasising the importance of these structures for germplasm conservation of bananas (Panis & Thinh 2001; Agrawal *et al.* 2004; Strosse *et al.* 2006).

In Malaysia, there has been a decline in banana cultivation and production due to pests, diseases, high labour costs and marketing issues (Chai *et al.* 2004). The quality of Malaysian bananas must be enhanced to meet market and export standards (Jalil *et al.* 2003). Efforts are being made to improve

production, genetic and conservation aspects of Malaysian bananas. Currently, there are no reports on the establishment of highly proliferating nodule-like meristems of Malaysian banana cultivars. The present investigation evaluated the effects of different concentrations of BAP and IAA to develop efficient protocols to regenerate multiple shoots from shoot tips, and to establish highly proliferating nodule-like meristems with associated plant regeneration from scalps in the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak.

MATERIALS AND METHODS

Plant Materials

Multiple shoots (30–40-day old) of the Malaysian banana cultivars Pisang Mas AA (Accession code: ITC.1403), Pisang Nangka AAA (ITC.0004), Pisang Berangan AAA (ITC.1287) and Pisang Awak ABB (ITC.0213), were obtained from the *Musa* Germplasm Transit Center of Bioversity International, Katholieke Universitiet Leuven, Belgium [Fig. 1(a)]. Shoots were supplied in 50 ml Universal tubes (5 tubes per cultivar) each tube containing 10 ml of semi-solid P5 medium. The P5 medium was based on the formulation of Murashige and Skoog (MS; 1962), with 30 g L^{-1} sucrose, 2.2 mg L^{-1} BAP, 0.2 mg L^{-1} IAA, 10 mg L^{-1} ascorbic acid, and semi-solidified with 3 g L^{-1} Gelrite® (Sigma-Aldrich, Poole, UK) at pH 6.2. Shoots were incubated in a controlled environment room at 25±2°C with a 16 h photoperiod (180 µMol m^{-2} s^{-1} daylight tubes, TLD/58W 35V; Phillips, Croydon, UK), designated as incubation condition 1 (IC1).

Multiplication and Maintenance of Shoots

Axenic shoots of all banana cultivars were multiplied by separating shoot clusters into individual shoots. Shoot tips, each approximately 1 cm in length, were excised from each shoot by cutting the upper pseudostem 0.5 cm above the apical meristem and trimming the rhizome to 0.5 cm in length, with attached leaves. Outer leaf sheaths, roots, any necrotic tissue and traces of Gelrite® were removed from the explants. Excised shoot tips were cultured on 50 ml aliquots of semi-solid medium (designated as PM4 medium) in 175 ml capacity screw-capped Powder Round glass jars (Beatson Clark Co. Ltd., Rotherham, UK; 4 shoot tips per jar). The composition of PM4 medium was similar to that of semi-solid P5 medium, except that PM4 medium was semi-solidified with 8 g L^{-1} agar (Sigma, Poole, UK) at pH 5.8. The rhizomes were fully or partially submerged in the medium with their pseudostems erect. Cultures were maintained under IC1 with subculture every 30–60 days.

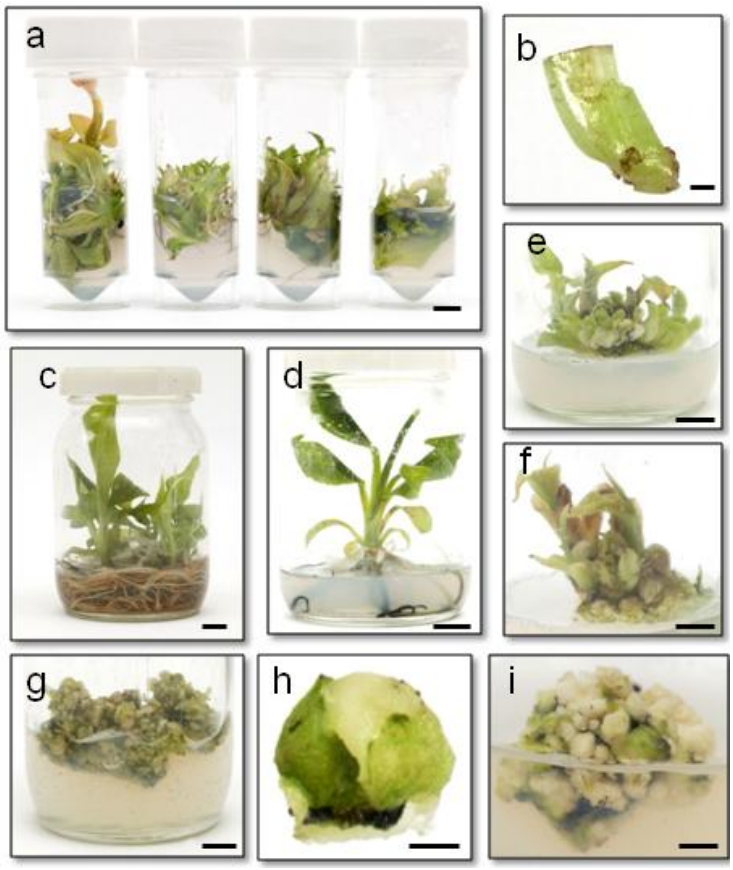

Figure 1: Shoot multiplication of Malaysian banana cultivars: (a) multiple shoots (30–40-day-old) of Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak (left to right) on semi-solid P5 medium; (b) a shoot tip excised from a 30-day-old plant of Pisang Nangka; (c) multiple shoots of Pisang Nangka after 30 days of culture of an excised shoot tip on semi-solid PM4 medium; (d) a single rooted shoot; (e) multiple shoots 1 cm or more than 1 cm in height; (f) a mixture of shoots 1 cm or more than 1 cm and shoots less than 1 cm in height; (g) multiple shoots less than 1 cm in height (highly proliferating nodule-like meristems); (h) a scalp excised from highly proliferating nodule-like meristem; (i) highly proliferating nodule-like meristems induced from a scalp. Bars = 1.5 cm (a, c), 1.25 mm (b), 1 cm (d, e, g), 1 mm (h), 3 mm (f, i).

Evaluation of the Effect of BAP and IAA on Shoot Multiplication from Shoot Tips

Experiments were conducted to evaluate the effect of different concentrations of BAP, in combination with or without 0.2 mg L^{-1} IAA, on shoot multiplication from shoot tips of the banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. Tips (1 cm or more than 1 cm in height) were excised from shoots 30 days after subculture; these shoots having at least two open leaves,

with or without roots. Excised shoot tips were cultured on semi-solid medium supplemented with different concentrations and combinations of BAP and IAA; (a) 0, 1, 2, 3, 4, 5 or 6 mg L^{-1} BAP with 0 mg L^{-1} IAA, and (b) 1–14 mg L^{-1} BAP with 0.2 mg L^{-1} IAA. The other components and pH of all culture media were similar to those of PM4 medium. Shoot tips were cultured individually on 50 ml aliquots of medium in 175 ml Powder Round glass jars (10 shoot tips per treatment), under IC1. The number of shoots 1 cm or more than 1 cm in height was recorded after 30 days of culture; data are presented as the mean of 3 replicates per subculture cycle over 3 subculture cycles. All data were expressed as a percentage of the number of shoot tips initially cultured.

Evaluation of the Effect of BAP with IAA on the Induction of Highly Proliferating Nodule-like Meristems from Scalps

Shoots less than 1 cm in height that developed from shoot tips cultured in 7–14 mg L^{-1} BAP with 0.2 mg L^{-1} IAA were designated as scalps (Panis *et al.* 1990). The effect was evaluated of the same treatments on the induction of highly proliferating nodule-like meristems from scalps of banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. Scalps 0.5 cm in height were excised from 30–40 days old materials and cultured on semi-solid medium with 7–14 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. The other components and pH of all culture media were similar to those of PM4 medium. Scalps were cultured individually on 50 ml aliquots of medium in 175 ml glass jars (10 scalps per treatment), under IC1. Assessment of the number of scalps that induced highly proliferating nodule-like meristems (consisting of many shoots less than 1 cm in height) were made after 30 days of culture and expressed as a percentage of the number of scalps initially cultured. Data are presented as the mean of 3 replicates per subculture over 3 subcultures.

Evaluation of the Effect of BAP and IAA on Plant Regeneration from Scalps

The effect was evaluated of 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA separately, or in combination, on plant regeneration from scalps of Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. The other components and pH of all culture media were similar to those of PM4 medium. Scalps, each 0.5 cm in height, were excised from 30–40-day-old materials and cultured individually on 50 ml aliquots of medium in 175 ml glass jars (10 scalps per treatment), under IC1. Growth parameters of shoots such as (a) stem length and diameter, (b) number, length and width of leaves, and (c) number and length of roots, were assessed by counting and measuring, whichever was applicable, after 30 days of culture. Data were presented as the mean of 3 replicates.

Experimental Design and Statistical Analyses

Experiments involved a completely randomised design and were repeated 3 times. Statistical analyses were performed, where applicable, using MINITAB version 15 (Minitab Inc. PA, USA). Means and standard error of means (SEM) were used throughout. The statistical significance between mean values was assessed using a conventional one-way analysis of variance (Snedecor & Cochran 1989) and the post-Hoc Tukey-Honestly Significant Difference test. A

probability of $p<0.05$ was considered significant. Prior to statistical analysis, the original percentage data were subjected to arcsin transformation using MINITAB version 15.

RESULTS

Effect of BAP and IAA on Shoot Multiplication from Shoot Tips

After 30 days of culture on medium with different concentrations of BAP, and with or without 0.2 mg L^{-1} IAA, 5 developmental patterns of shoot tips were observed; (a) necrotic, (b) single shoots, (c) multiple shoots 1 cm or more than 1 cm in height, (d) a mixture of shoots less than 1 cm, 1 cm, or more than 1 cm in height, and (e) multiple shoots less than 1 cm in height. Figures 1(b)–(d) shows the developmental patterns of shoot tips of the cultivar Pisang Nangka after 30 days of culture on medium containing different concentrations of BAP and IAA.

In treatments with 0–6 mg L^{-1} BAP alone, the number of shoots per tip increased with increasing BAP concentration in all cultivars [Fig. 2(a)]. Most shoots were obtained for Pisang Mas (4 shoots) with 5 or 6 mg L^{-1} BAP, and with 6 mg L^{-1} BAP for Pisang Nangka and Pisang Berangan. Four to 6 mg L^{-1} BAP was optimal for the cultivar Pisang Awak. Figure 2(b) shows the effect of 1–6 mg L^{-1} BAP with 0.2 mg L^{-1} IAA on the mean number of shoots per shoot tip, with most shoots in Pisang Mas and Pisang Berangan being induced with 6/0.2 mg L^{-1} BAP/IAA. The number of shoots in Pisang Nangka and Pisang Awak obtained with 5/0.2 and 6/0.2 mg L^{-1} BAP/IAA was significantly different ($p<0.05$), compared to the responses of the other cultivars. Figure 2(c) shows the effect of 7–14 mg L^{-1} BAP with 0.2 mg L^{-1} IAA. The response to 7 mg L^{-1} BAP was similar to 6 mg L^{-1} BAP, with Pisang Awak being better than at 6 mg L^{-1} BAP. Shoot multiplication decreased progressively in 8–14 mg L^{-1} BAP with 0.2 mg L^{-1} IAA.

Effect of BAP with IAA on the Induction of Highly Proliferating Nodule-like Meristems from Scalps

After 30 days of culture, 4 developmental patterns of scalps were observed for all cultivars; (a) necrotic material, (b) multiple shoots 1 cm or more than 1 cm in height, (c) a mixture of shoots 1 cm, more than 1 cm, and less than 1 cm in height, and (d) compact multiple shoots less than 1 cm in height (designated as proliferating nodule-like meristems). Figure 1(e)–(i) shows the developmental pattern of scalps for the cultivar Pisang Nangka. Induction of highly proliferating nodule-like meristems from scalps is shown in Figure 3; induction increased with increasing BAP concentration. The greatest mean induction of 100% was significantly higher ($p<0.05$) with BAP/IAA of 11–14/0.2 mg L^{-1} for all cultivars.

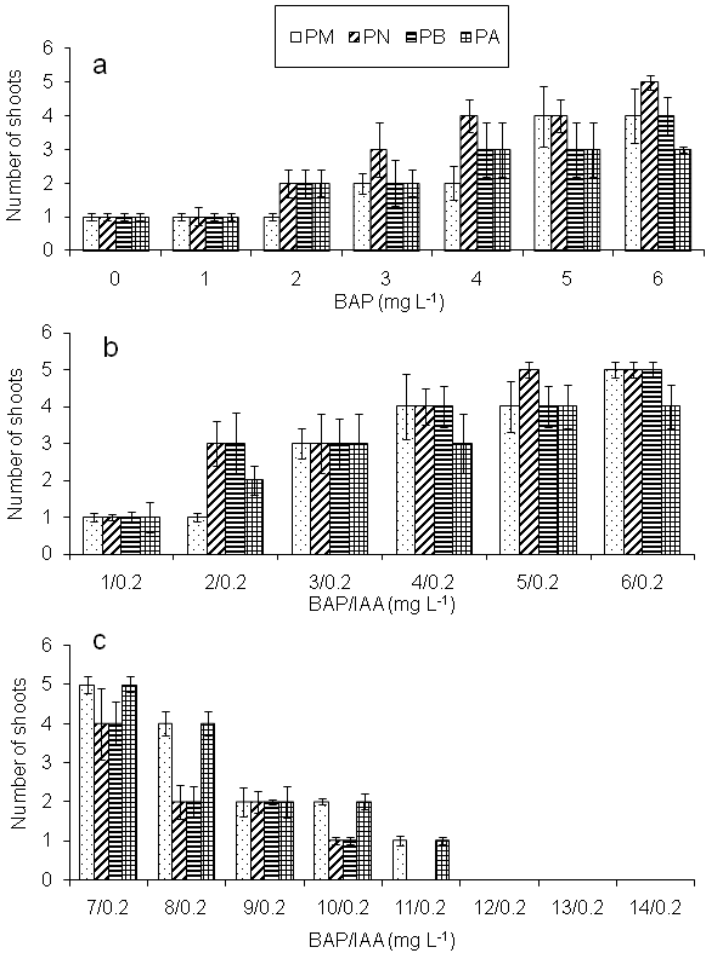

Figure 2: Mean number of shoots that proliferated from shoot tips of the banana cultivars Pisang Mas (PM), Pisang Nangka (PN), Pisang Berangan (PB) and Pisang Awak (PA) after 30 days of culture on medium containing (a) 0–6 mg L^{-1} BAP, (b) 1–6 mg L^{-1} BAP with 0.2 mg L^{-1} IAA, and (c) 7–14 mg L^{-1} BAP with 0.2 mg L^{-1} IAA. Bars represent SEM, n = 90 shoot tips.

Effects of BAP and IAA on Plant Regeneration from Scalps

The effects are shown in Figures 4–6 of different concentrations and combinations of BAP and IAA on (a) stem length and diameter, (b) number, length and width of leaves, and (c) number and length of roots, of plants regenerated from scalps. The greatest mean stem length and diameter of plants regenerated from scalps of all cultivars were significantly higher (p<0.05) with 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, compared to other treatments (Fig. 4). The mean stem length was 4.1, 3.6, 3.6 and 3.5 cm, with a stem diameter of 1.4, 1.6, 1.6 and 1.3 cm for Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak, respectively.

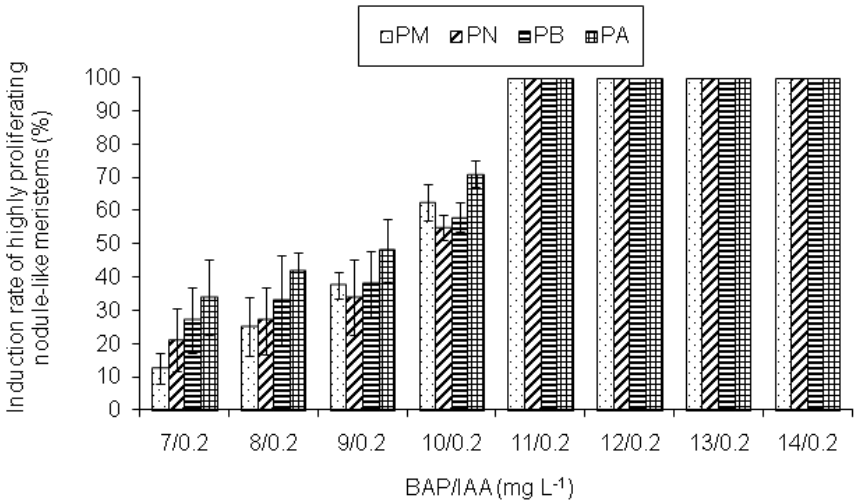

Figure 3: Induction of highly proliferating nodule-like meristems from scalps of banana cultivars Pisang Mas (PM), Pisang Nangka (PN), Pisang Berangan (PB) and Pisang Awak (PA) after 30 days of culture on medium containing different BAP concentrations with 0.2 mg L^{-1} IAA. Bars represent SEM, n = 90 scalps.

The mean number of leaves (4 leaves) on plants regenerated from scalps of the four banana cultivars was similar in all treatments (data not shown). The greatest mean leaf length and width in plants regenerated from scalps were significantly higher (p<0.05) at 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA, compared to other treatments (Fig. 5). Mean leaf length was 4.9, 5.4, 5.2 and 4.3 cm for Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak, with leaf widths of 1.4, 1.7, 1.5 and 1.4 cm for these cultivars, respectively.

The mean number and length of roots on plants regenerated from scalps were maximum with 0.2 mg L^{-1} IAA, irrespective of cultivar (Fig. 6), with a maximum of 7 roots for Pisang Nangka and Pisang Berangan. Mean root length was also maximal (4.5 cm) in the latter two cultivars.

DISCUSSION

The current study showed that BAP was effective when combined with IAA, for optimum shoot multiplication from shoot tips of the banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak, although the most effective BAP concentration and shoot multiplication rate differed amongst all the banana cultivars. The synergistic action of BAP and IAA for shoot multiplication has been reported earlier for other banana cultivars, and the findings of the current investigation agreed with those reported by other workers who induced multiple shoots from tips of other banana cultivars. Abeyaratne and Lathiff (2002) reported that 10 mg L^{-1} BAP with 0.75 mg L^{-1} IAA stimulated a maximum of 10 shoots per meristem in the banana cultivar Rathambala AAA, while 4 mg L^{-1} BAP

with 0.2 mg L^{-1} IAA induced 4–5 new shoots per apical explant in several AAA banana cultivars such as Alanya 5, Anamur 10 and Bozyazi 14 (Gubbuk & Pekmezci 2004), Dwarf Cavendish, Gazipasa 5 and Anamur 4 (Gubbuk *et al.* 2005). Strosse *et al.* (2008) also reported that 2.2 mg L^{-1} BAP with 0.2 mg L^{-1} IAA was most effective for shoot multiplication in several banana cultivars.

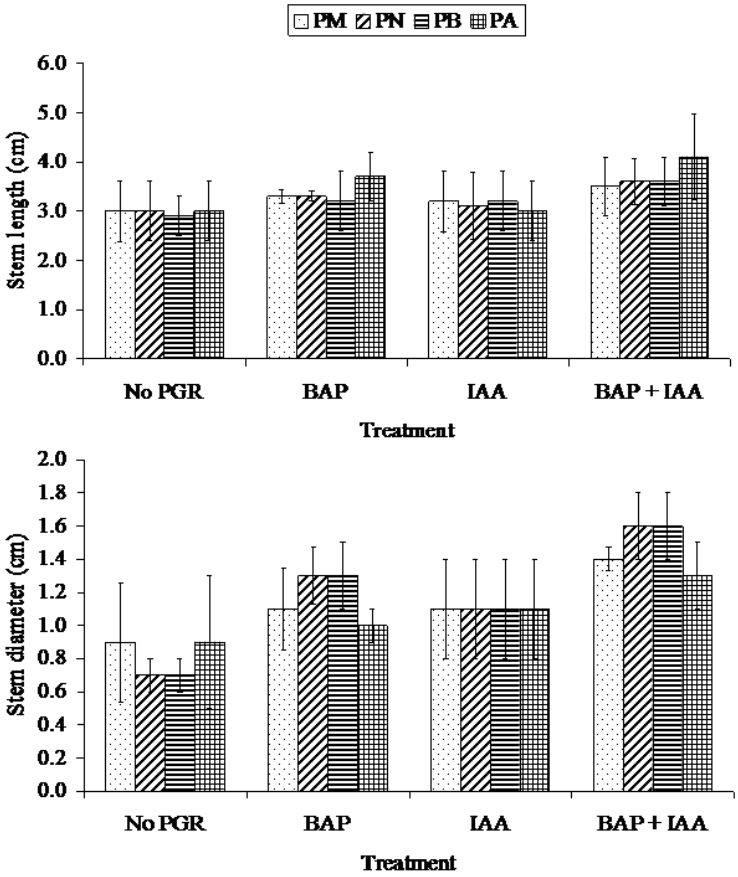

Figure 4: Mean stem length and stem diameter of plants regenerated from scalps of banana cultivars Pisang Mas (PM), Pisang Nangka (PN), Pisang Berangan (PB) and Pisang Awak (PA) after 30 days of culture on medium lacking plant growth regulators (no PGR), or supplemented with 1 mg L^{-1} BAP or 0.2 mg L^{-1} IAA alone, or in combination. Bars represent SEM, n = 90 scalps.

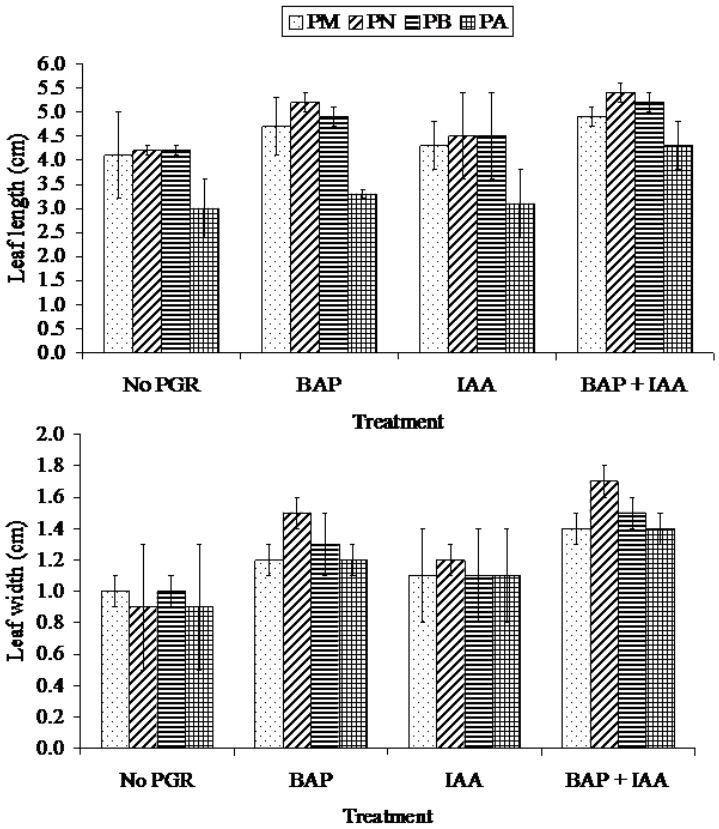

Figure 5: Mean leaf length and width of plants regenerated from scalps of banana cultivars Pisang Mas (PM), Pisang Nangka (PN), Pisang Berangan (PB) and Pisang Awak (PA) after 30 days of culture on medium lacking plant growth regulators (no PGR), or supplemented with 1 mg L^{-1} BAP or 0.2 mg L^{-1} IAA alone, or in combination. Bars represent SEM, n = 90 scalps.

Highly proliferating nodule-like meristems were induced from scalps of all the Malaysian banana cultivars investigated. The induction rates of such nodule-like meristems varied amongst cultivars, with induction rates increasing with increasing BAP concentrations. Highly proliferating nodule-like meristems have been induced from shoot tip-derived scalps of other banana cultivars and genotypes (Panis & Thinh 2001; Rahman *et al.* 2004; Strosse *et al.* 2006; Sadik *et al.* 2007). Concentrations of BAP which maintained nodule-like meristems were genotype-dependent, in agreement with previous reports. Thus, BAP at 2.2 or 22 mg L^{-1}, with 0.2 mg L^{-1} IAA, induced nodule-like meristematic structures in several banana cultivars and genotypes (Panis & Thinh 2001; Strosse *et al.* 2006). Strosse *et al.* (2006) reported that 2.2 mg L^{-1} BAP with 0.2 mg L^{-1} IAA inhibited the outgrowth of shoots, but maintained highly proliferating nodule-like meristems in 18 banana cultivars with AA, AAA, AAA-h, AAB and ABB genomes. Highly proliferating nodule-like meristems could be

maintained in bananas with ABB, AAB and AAA genomes if the BAP concentration was increased to 22 mg L^{-1}, in combination with 0.2 mg L^{-1} IAA. Priyono (2001) induced 70% of banana cultures to form nodule-like meristems when exposed to 10–15 mg L^{-1} BAP. However, Rahman *et al.* (2004) reported that only 29% of shoot tips of the banana cultivar BARI-1 developed highly proliferating nodule-like meristems on a culture medium with 5 mg L^{-1} BAP. Scalps could be used to provide a continuous supply of highly proliferating nodule-like meristems by reducing the growth of shoots from the scalps.

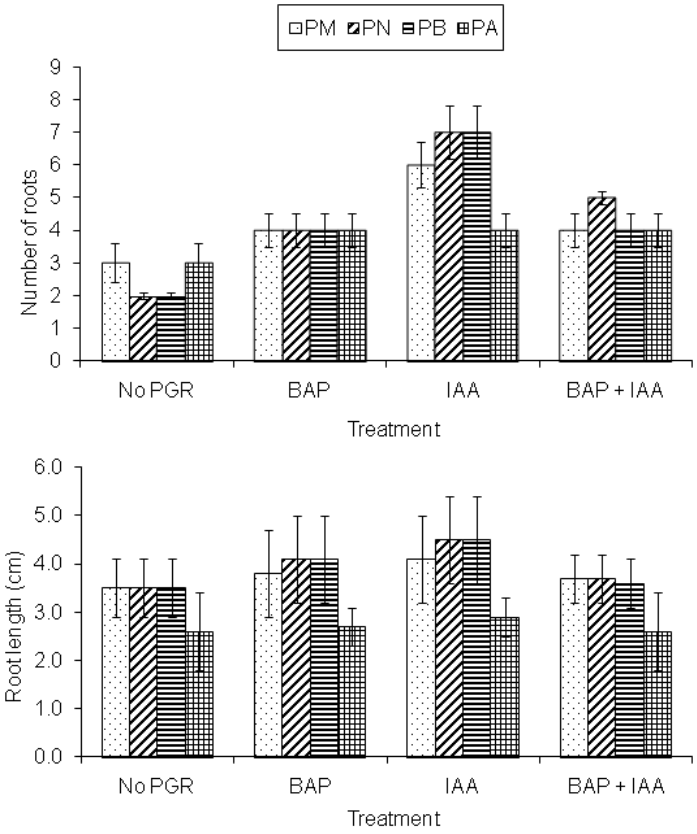

Figure 6: Mean number and length of roots of plants regenerated from scalps of banana cultivars Pisang Mas (PM), Pisang Nangka (PN), Pisang Berangan (PB) and Pisang Awak (PA) after 30 days of culture on medium lacking plant growth regulators (no PGR), or supplemented with 1 mg L^{-1} BAP or 0.2 mg L^{-1} IAA alone, or in combination. Bars represent SEM, n = 90 scalps.

The current study also reports plant regeneration from scalps of the Malaysian banana cultivars investigated. Rapid regeneration and improved growth of plants were obtained on a culture medium containing 1 mg L^{-1} BAP and 0.2 mg L^{-1} IAA. The plants developed roots on the same medium, although optimal rooting was obtained following transfer of regenerated plants to a culture

medium containing 0.2 mg L^{-1} IAA. Previous researchers reported that plant regeneration and rooting from banana scalps can be achieved on MS medium lacking plant growth regulators, or containing 0.2 mg L^{-1} IAA, indole-3-butyric acid (IBA) and α-naphthalene acetic acid (NAA), or with 0.1%–0.25% (w/v) activated charcoal (Gubbuk & Pekmezci 2004; Smith *et al.* 2005). Gubbuk *et al.* (2005) showed that rooting of regenerated banana shoots was optimal in the presence of 0.1%–0.25% (w/v) activated charcoal and 0.2 mg L^{-1} IBA, the rooting response was cultivar-dependent. Other researchers recommended 0.2–0.4 mg L^{-1} thidiazuron combined with 0.2 mg L^{-1} IAA for both plant regeneration and rooting in some bananas (Gubbuk & Pekmezci 2004; Gubbuk *et al.* 2005).

CONCLUSION

This investigation reports shoot multiplication, induction of highly proliferating nodule-like meristems and plant regeneration of the Malaysian banana cultivars Pisang Mas, Pisang Nangka, Pisang Berangan and Pisang Awak. It is the first report on induction of highly proliferating nodule-like meristems and plant regeneration from scalps of Malaysian banana cultivars. The culture protocols developed in this study provide a baseline for future work on micropropagation, genetic improvement and conservation of the four Malaysian bananas. Furthermore, this information will support indirectly banana research and sustainable banana cultivation and productivity, which will benefit all banana stakeholders such as producers, traders and consumers, particularly those in Malaysia, but more generally on an international basis.

ACKNOWLEDGEMENTS

The authors thank the Sarawak Foundation, Malaysia for financial support to Philip Sipen, and Ms. Ines van den Houwe of the *Musa* Germplasm Transit Centre, Bioversity International, Katholieke Universitiet Leuven, Belgium for supplying shoots of Malaysian bananas.

REFERENCES

Abeyaratne W M and Lathiff M A. (2002). *In vitro* propagation of Rathambala (*Musa* AAA) and the occurrence of phenotypic variations in the pseudostem. *Annals of Sri Lanka Department of Agriculture* 4: 191–197.

Acereto-Escoffie P O M, Chi-Manzanero B H, Echeverria-Echeverria S, Grijalva R, Kay A J, Gonzalez-Estrada T, Castano E and Rodriguez-Zapata L C. (2005). *Agrobacterium*-mediated transformation of *Musa acuminata* cv. “Grand Nain” scalps by vacuum infiltration. *Scientia Horticulturae* 105(3): 359–371.

Agrawal A, Swennen R and Panis B. (2004). A comparison of four methods for cryopreservation of meristems in banana (*Musa* spp.). *Cryo-Letters* 25(2): 101–110.

Arvanitoyannis I S, Mavromatis A G, Grammatikaki-Avgeli G and Sakellariou M. (2008). Banana: Cultivars, biotechnological approaches and genetic transformation. *International Journal of Food Science and Technology* 43(10): 1871–1879.

Assani A, Bakry F, Kerbellec F, Haicour R, Wenzel G and Foroughi-Wehr B. (2003). Production of haploids from anther culture of banana [*Musa balbisiana* (BB)]. *Plant Cell Reports* 21(6): 511–516.

Aurore G, Parfait B and Fahrasmane L. (2009). Bananas, raw materials for making processed food products. *Trends in Food Science and Technology* 20(2): 78–91.

Chai M, Ho Y W, Liew K W and Asif J M. (2004). Biotechnology and *in vitro* mutagenesis for banana improvement. In J S Mohan and R Swennen (eds.). *Banana improvement - cellular, molecular biology and induced mutation*. Enfield, NH, USA: FAO/IAEA/INIBAP, Science Publishers Inc., 59–77.

FAOSTAT (2009). *The Food and Agriculture Organization Corporate Statistical Database.* http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567# ancor. (accessed 8 September 2009).

Ghosh A, Ganapathi T R, Nath P and Bapat V A. (2009). Establishment of embryogenic cell suspension cultures and *Agrobacterium*-mediated transformation in an important Cavendish banana cv. Robusta (AAA). *Plant Cell, Tissue and Organ Culture* 97(2): 131–139.

Gubbuk H and Pekmezci M. (2004). *In vitro* propagation of some new banana types (*Musa* spp.). *Turkey Journal of Agriculture and Forestry* 28(5): 355–361.

Gubbuk H, Onus A C and Pekmezci M. (2005). Improvement on *in vitro* propagation of dwarf cavendish banana (*Musa* spp.) types using thidiazuron and active charcoal. *Tropical Agriculture* 82(3–4): 219–224.

Heslop-Harrison J S and Schwarzacher T. (2007). Domestication, genomics and the future for banana - review. *Annals of Botany* 100(5): 1073–1084.

Jalil M, Khalid N and Othman R Y. (2003). Plant regeneration from embryogenic suspension cultures of *Musa acuminata* cv. Pisang Mas. *Plant Cell, Tissue and Organ Culture* 75(3): 209–214.

Kulkarni V M, Ganapathi T R, Suprasanna P and Bapat V A. (2007). *In vitro* mutagenesis in banana (*Musa* spp.) using gamma irradiation. In J S Mohan and H Haggman (eds.). *Protocols for micropropagation of woody trees and fruits*. The Netherlands: Springer, 543–559.

Murashige T and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum* 15(3): 473–497.

Nahamya P. (2000). *Development of embryogenic cell suspensions for East African Highland Bananas*. Msc. diss., Makerere University Kampala.

Panis B, Withers L A and De Langhe E. (1990). Cryopreservation of *Musa* suspension cultures and subsequent regeneration of plants. *Cryo-Letters* 11(3): 337–350.

Panis B and Thinh N T. (2001). *The International Network for the Improvement of Banana and Plantain* (*INIBAP) Technical Guideline 5 - Cryopreservation of* Musa *Germplasm*. Montpellier, France: IPGRI.

Priyono. (2001). Micropropagation of banana (*Musa paradisiaca*) through cormlet initiation by *in vitro* culture of apical meristem slices. *Jurnal Ilmu Dasar* 2: 41–48.

Rahman M Z, Nasiruddin K M, Amin M A and Islam M N. (2004). *In vitro* response and shoot multiplication of banana with BAP and NAA. *American Journal of Plant Science* 3(4): 406–409.

Sadik K, Rubaihayo P R, Magambo M J S and Pillay M. (2007). Generation of cell suspensions of East African highland bananas through scalps. *African Journal of Biotechnology* 6(11): 1352–1357.

Shapira O, Khadka S, Israeli Y, Shani U and Schwartz A. (2009). Functional anatomy controls ion distribution in banana leaves: Significance of Na seclusion at the leaf margins. *Plant, Cell Environment* 32(5): 476–485.

Sipen P, Anthony P and Davey M R. (2011a). Cryopreservation of scalps of Malaysian bananas using a pregrowth method. *Cryo-Letters* 32(6): 197–205.

Sipen P, Chubo J K, King P J H, Huat O K and Davey M R. (2011b). Genetic improvement of banana using conventional and *in vitro* technologies. *Journal of Crop Improvement* 25: 697–727.

Smith M K, Hamill S D, Becker D K and Dale J L. (2005). *Musa* spp. – banana and plantain. In R E Litz (ed.). *Biotechnology in agriculture series no. 29: Biotechnology of fruit and nut crops*. Wallingford, UK: CABI, 365–391.

Snedecor G W and Cochran W G. (1989). *Statistical methods*, 8th ed. Ames, Iowa, USA: Iowa State College Press.

Strosse H, Schoofs H, Panis B, Andre E, Reyners K and Swennen R. (2006). Development of embryogenic cell suspensions from shoot meristematic tissue in bananas and plantains (*Musa* spp.). *Plant Science* 170(1): 104–112.

Strosse H, Andre E, Sagi L, Swennen R and Panis B. (2008). Adventitious shoot formation is not inherent to micropropagation of banana as it is in maize. *Plant Cell, Tissue and Organ Culture* 95(3): 321–332.

Xiao W, Huang X, Gong Q, Dai X M, Zhao J T, Wei Y R and Huang X L. (2009). Somatic hybrids obtained by asymmetric protoplast fusion between *Musa* Silk cv. Guoshanxiang (AAB) and *Musa acuminata* cv. Mas (AA). *Plant Cell, Tissue and Organ Culture* 97(3): 313–321.