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Full Length Research Paper

Effect of benzylaminopurine (BAP) pulsing on *in vitro* shoot multiplication of *Musa acuminata* (banana) cv. Berangan

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MS (Murashige and Skoog) media supplemented with benzylaminopurine (BAP) showed that the number of bud formation in shoot cultures of *Musa acuminata* cv.Berangan during the initiation stage increased proportionately with the concentrations used (11, 22 and 33 µM). However, the highest concentration of BAP (33 µM) simultaneously increased the formation of abnormal shoots. After the first apical bud appeared, explants were transferred to MS medium with lower concentrations of BAP either with or without indole acetic acid (IAA). Proliferation media supplemented with IAA showed enhanced shoot multiplication and elongation but did not help to reduce the abnormality index that occurred.

Key words: micropropagation, adventitious buds, banana, Musa acuminata

INTRODUCTION

Banana originated from the South East Asian region, where the greatest diversity of edible bananas are found (Stover and Simmonds, 1987). Bananas account for approximately 22% of the fresh fruit production and are ranked as the second most important fruit crop (Pua, 2007). The different types of banana have not been fully exploited since Cavendish types mainly dominate the market place globally.

For commercialization, it is important that consistent supplies of good quality bananas are produced. This could be achieved through clonal planting materials obtained through tissue culture propagation technique. This technique provides high rates of multiplying genetically uniform, pest and disease-free planting materials. Propagation of banana through *in vitro* techniques has been reported by several workers using different explants sources and methods (Jalil et al., 2003; Madhulatha et al., 2004; Strosse et al., 2006; Wong et al., 2006; Venkatachalam et al., 2006, 2007; Resmi and Nair, 2007; Shirani et al., 2009).

In tissue culture, plant growth regulators (PGR) are critical media components in determining the developmental pathway of the plant cells. Cytokinins such as benzylaminopurine (BAP) and kinetin are generally known to reduce the apical meristem dominance and induce both axillary and adventitious shoots formation from meristematic explants in banana (Madhulatha et al., 2004). The most established banana shoot-tip culture system was achieved by using BAP as a supplement to Murashige and Skoog (MS) basal media (Murashige and Skoog, 1969). The effectiveness of BAP over other cytokinins in inducing multiplication of shoot tip cultures has been reported in different cultivars of bananas (Rahman et al., 2006; Resmi and Nair, 2007; Farahani et al., 2008; Buah et al., 2010). BAP has a marked effect in stimulating the growth of axillary and adventitious buds and foliar development of shoot tip cultures (Abeyarante and Lathiff, 2002; Buah et al., 2010). Meanwhile, combinations of

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Abbreviations: PGR, Plant growth regulators; **BAP,** benzylaminopurine; **IAA,** indole acetic acid; **MS,** Murashige and Skoog; **IBA,** indole-3-butyric acid.

Table 1. Effect of different concentration of BAP on proliferation of adventitious buds.

Concentration of BAP (µM)	Fresh weight (g)	Bud formation (%)
11	0.88± 0.25 a	45
22	2.54± 0.36 b	75
33	4.04± 0.45 c	90

Values are the mean ± SD. Values with different letters are significantly different at the 0.05 level.

BAP with auxins such as indole acetic acid (IAA) or indole-3-butyric acid (IBA) were also used for *in vitro* multiplication of bananas (Dhed'a et al., 1991; Resmi and Nair, 2007). Although BAP stimulates shoot proliferation in bananas, it is also known to have mutagenic effects at high concentration producing off type plantlets (Bairu et al., 2008). Appearance of the off-type plantlets from *in vitro* multiplication process is considered a great disadvantage. The purpose of this study is to develop a regime for the use of BAP that is widely used to increase the multiplication rate for plantlets and concurrently control its mutagenic effect so as to decrease the percentage of morphologically abnormal plantlets formation.

MATERIALS AND METHODS

Plant materials

Young suckers of *Musa* var. 'Berangan' were collected from a healthy true-to-type mother plants. After removing the leaves and the roots, the suckers were thoroughly washed with tap water to remove adhering soil. The samples were trimmed to a size of 8 to 10 cm in length and 5 to 6 cm in diameter by removing the layers of the developing leaves. Then, the suckers were placed under running tap water for 30 min before the shoot apices were transferred to a laminar flow. The shoot apices explants were sequentially treated with 50% (v/v) Chlorox (the active ingredient is 5.25% sodium hypochlorite) mixed with few drops of Tween 20 for 30 min in order to sterilize the surface. Then they were dipped into 95% (v/v) ethanol for 2 min followed by a mixture of 100% (v/v) Chlorox, Tween 20 and 0.1% (w/v) $HgCl_2$ solution for 5 min. After these treatments, explants were rinsed with sterilized distilled water and left to dry for 15 min. Subsequently, the explants were trimmed to a size of 3 to 4 cm in length and 2 to 3 cm in diameter.

Initiation media

The culture initiation media was prepared by adding 10 mg/l ascorbic acid (as an antioxidant), 87 µM sucrose and 2 g /l gelrite as solidifying agent to MS basal medium (Murashige and Skoog 1962) and supplemented with different concentrations of BAP (11, 22 and 33 µM). Cultures were maintained on each BAP concentration until the first individual bud appeared. Subsequently, each explant was cut into four parts and kept in the culture initiation media for another 30 days. During the initial phase, cultures were subcultured three times to reduce browning due to the presence of phenolic compounds. The fresh weight and percentage of buds obtained from each treatment were recorded.

Proliferation media

The explants were subsequently transferred to MS medium with either the same or lower concentrations of BAP than the initiation media and supplemented either with or without IAA (Table 2). Transfers to fresh medium and subculturing were carried out every 30 days up to 3 cycles. In the propagation stage, the shoots per explant were counted and the average shoot length from randomly selected sample was determined.

Abnormality index was calculated by taking the ratio of abnormal to normal shoots. Plantlets were categorized as normal or abnormal based on morphological appearance such as hyperhydricity and presence of undifferentiated tissue. Data were analyzed using SPSS 14.0 version and the mean difference were contrasted using Duncan's multiple range tests at the 0.05 level of significance.

Culture conditions

The pH of all culture media was adjusted to 5.8 prior to addition of gelrite at 2.5 g/l and autoclaving. All culture media were autoclaved at 121°C for 20 min. Cultures were maintained at 28°C under 16-h photoperiod with light intensity of 31.4 µmol $m^{-2} s^{-1}$.

RESULTS AND DISCUSSION

The effects of different concentration regimes of BAP on bud initiation and shoot multiplication either with or without IAA were investigated. After the initial bud was subcultured, multiple adventitious buds were produced from the base of the explants after 30 days. Table 1 shows the fresh weight and percentage of buds obtained on media tested. The frequency of bud formation doubled and the fresh weight increased about four times higher in media with BAP at 33 µM when compared to media supplemented with 11 µM BAP.

Explants with newly formed buds were transferred to media with either similar or lower concentrations of BAP at the proliferation stage. Results indicated that BAP at 22 µM induced the highest number of normal and elongated shoots, although more shoots proliferated on initiation medium supplemented with BAP at 33 µM (Table 2). At 11 and 6.5 µM BAP, the lowest number of elongated shoots was obtained. With the addition of IAA to BAP supplemented media, shoot proliferation and elongation were generally enhanced with an optimal concentration of 22 µM BAP and 2 µM IAA where the number of shoots was 20.25 ± 2.5 and shoot length was 10.60 ± 1 cm.

The abnormality index of proliferated shoots varied with different concentrations of BAP either with or without IAA (Figure 1). The results indicated that with decreasing BAP concentration in the proliferation stage, abnormality index significantly decreased. There was no significant difference in abnormality index between BAP and combination of BAP with IAA. The highest abnormality index was recorded in media with 33 µM BAP. The cultures appeared as a clump of corms and produced small stunted shoot clusters (Figure 2). The results indicated that decreasing BAP to 22 µM significantly caused lower abnormality

Table 2. Shoot proliferation and elongation response to different combination of BAP with (+) and without (0) IAA in *Musa accuminata* cv. Berangan.

Initiation media	Proliferation media		Number of shoot	Mean shoot length	Multiplication rate
BAP (µM)	BAP (µM)	IAA (2 µM)			
33	33	+	14 ±1.4^{d}	7.73±0.6 ^{f}	3.5±0.3^{d}
		0	12.75±3.7^{d}	6.43±0.9^{de}	3.18±0.9^{d}
33	22	+	20.25±2.5 ^{f}	10.60±1 ^{h}	5.06±0.6^{f}
		0	17.25±2.2^{e}	9.33 ±0.6^{g}	4.31 ±0.5^{e}
33	11	+	7.75±1.2 ^{c}	6.52±0.7^{e}	1.93±0.3^{c}
		0	7.25±2.6 ^{c}	5.8±0.7^{cde}	1.81±0.6^{c}
22	22	+	12.25±1.7^{d}	9.99±0.9^{gh}	3.06±0.4^{d}
		0	11±1.6 ^{d}	8.9±1^{g}	2.72±0.4^{d}
22	11	+	7.5±2.5 ^{c}	5.26±1^{bcd}	1.87±0.6^{c}
		0	6.75±1.5^{bc}	5.7 ±0.5^{bcd}	1.68±0.3^{bc}
11	11	+	5 ±0.81^{abc}	4.7±0.6^{abc}	1.25±0.2^{abc}
		0	5.25±0.95^{abc}	4.32±0.8^{ab}	1.31±0.2^{abc}
11	6.5	+	4±0.81 ^{ab}	3.93±1.8^{a}	1±0.2^{ab}
		0	3.5±1.2 ^{a}	3.98±0.2^{a}	0.87±0.3^{a}

Values are the mean ± SD. Values with different letters are significantly different at the 0.05 level.

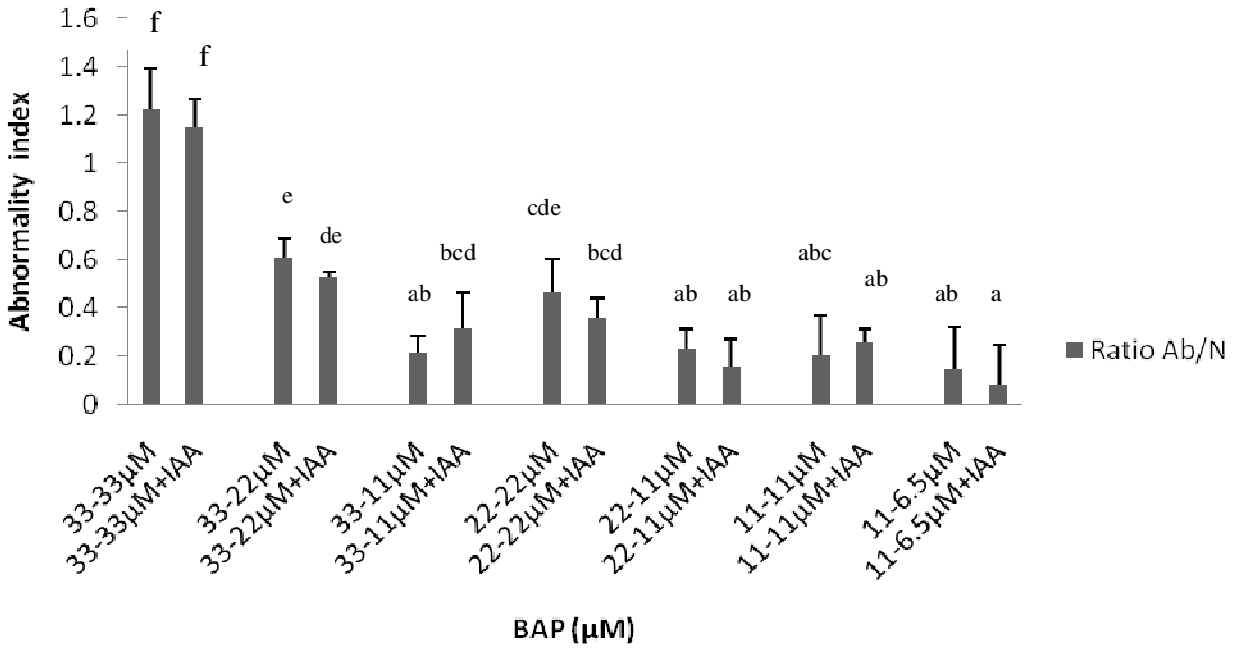

Figure 1. Effect of BAP pulsing with and without IAA on abnormality. Vertical bars indicate standard deviation (n = 4). Values with different letters are significantly different at the 0.05 level.

index than 33 µM where normal shoot induction was the highest.

This study had shown that increasing the concentration of BAP during the initiation stage enhanced the fresh weight and percentage of buds formation. The importance of the application of high BAP concentration to initiate bud formation from explants were reported by Zaffari et al. (2000) and Subramaniam et al. (2008) in Cavendish banana cultivar Brasilian (AAA). Previous researchers (Vuylsteke and De Langhe, 1985; Venkatachalam et al., 2007; Bairu et al., 2008) indicated that 5 mg/l (22.2 µM) BAP was the optimum concentration for most banana cultivars. However, Arinaitwe et al. (2000) reported that *in vitro* bud initiation from banana was cultivar dependent.

Upon subculture for shoot proliferation, the highest number of normal and elongated shoots was derived

Figure 2. A, B: Abnormality index caused by BAP. C: normal and elongated plantlet.

from media supplemented with 2 µM IAA and 22 µM BAP. Generally, the results have shown that shoot multiplication and elongation was significantly better in the presence of IAA than BAP alone. Dhed'a et al. (1991) reported that combinations of BAP with IAA or IBA were effective for *in vitro* multiplication of bananas and plantains. Resmi and Nair (2007) reported high shoot multiplication but a reduction in the length of shoots in media with a combination of BAP and IAA in triploid cultivar by using inflorescence explants. Venkatachalam et al. (2007) reported a reduction in the number as well as length of shoot that occurred with exposure to high levels of BAP alone (44.44 µM) in banana cv. Nanjanagudu Rasabale (AAB). Synergistic effects of plant growth regulators have influenced the cultural response in banana shoot proliferation and elongation.

In this study, the abnormality index increased with increasing concentrations of BAP with the highest value at 33 µM BAP (Figure 1). Vidya and Nair (2002) reported that occurrence of somaclonal variants in red banana (AAA) is due to the presence of high concentration of BAP in the culture medium. This study suggested that high concentration of BAP after bud initiation was not essential for shoot propagation due to the reduction in the number of shoots and high incidences of abnormality. High concentrations of BAP did not allow recovery of the explants in tissue cultures in becoming complete normal plants due to the habituation effect of BAP. In addition, similar response was observed in terms of abnormality index even with the incorporation of IAA to the medium (Figure 1). D'Amato (1978) reported that application of high concentrations of growth hormones for clonal propagation is often disadvantageous since they may cause various chromosomal abnormalities resulting in the production of non true-to-type plants. In this regard, there are numerous reports which show the shortcomings of using high level of cytokinin that produces abnormality and affects its genetic variability (Martin et al., 2006; Shirani et al., 2009).

It can be concluded that the application of high concentration of BAP increased bud formation during the initiation stage. However, it might be deleterious to the cultures due to high abnormality index. Incorporation of IAA to the media enhanced shoot proliferation and elongation but

could not reverse the abnormality index that has occurred. The application of BAP in the media needs to be carefully monitored in establishing optimized culture systems for *in vitro* propagation to obtain normal plantlets.

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