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Full Length Research Paper

Effects of benzylaminopurine and naphthalene acetic acid on proliferation and shoot growth of pineapple (*Ananas comosus* L. Merr) *in vitro*

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This study was conducted to evaluate the pineapple regeneration and shoot growth as affected by 6-benzylaminopurine (BAP) at 2.0 mg/l and naphthalene acetic acid (NAA) at 0.2 mg/l *in vitro*. BAP and NAA at the concentration of 2.0 and 0.2 mg/l were used in this study. BAP at 2.0 mg/l significantly affected the production of shoots per explant, shoot length and weight. Total shoot length was higher in BAP (2 mg/l) than in control (MS medium without hormone) and NAA (0.2mg/l) after 10, 20, 30, 40, 50 and 60 days incubation period. Total shoot length was highest in BAP in all incubation periods. Total shoot weight was higher in BAP (2 mg/l) and lower in NAA (0.2 mg/l) as compared to MS medium without hormone. The results showed that BAP at the concentration of 2 mg/l was effective for pineapple shoot growth and development.

Key words: Pineapple regeneration, shoot growth, 6-benzylaminopurine, naphthalene acetic acid.

INTRODUCTION

Micropropagation or tissue culture of shoot tips or crown has been successfully carried out in pineapple (Hammad and Taha, 2008a). As a result, millions of pineapple propa-gules can be produced by tissue culture of the crown or shoot tips per year. They reported the rate of multiplication and the total number of plantlet produced using plant growth regulators. A total number of plantlets production ranging from 40 (Dewald et al., 1988), 280 (Devi et al., 1997), 5000 (Zepeda and Sagawa, 1981), 40000 (Liu et al., 1989) to 100000 (Sripaoraya et al., 2003) from single explant per year has been reported. Most researchers used a combination of 6-benzylaminopurine (BAP) and other plant growth regulators like naphthalene acetic acid (NAA) or indole acetic acid (IAA) or indole butyric acid (IBA). Propagation of pineapple can be obtained *in vitro* with BAP alone (Be and Debergh, 2006), mixture of hor-mones like BAP and NAA (Firoozabady and Gutterson, 2003), IBA (Boxus et al., 1991), IAA (Hamad and Taha, 2008a), 2,4-dichlorophenoxy acetic acid (2,4-D) (Liu et al., 1989), combination of BAP and two auxins such as NAA and IAA (Mathews and Rangan, 1979), IAA and IBA (Teixeira et al., 2006) and NAA and IBA (Soneji et al., 2002). Application of BAP alone can be cost effective and can be more useful than a combination of two or three hormones. Currently, from the literature, BAP at a con-centration of 1.0 (Be and Debergh, 2006), 1.5 (Almeida et al., 2002), 2.0 (Bhatia and Ashwath, 2002), 2.5 (Smith et al., 2002), 3.0 (Firoozabady and Gutterson, 2003) and 4.0 mgL^{-1} (Omokoio et al., 2001) has been recommended for multiplication of pineapple.

From previous studies, the optimum concentration of BAP reported ranged between 1.0 and 4.0 mgL^{-1}. But most of the literature showed that shoot formation occur-red from the 1st generation of the crown of pineapple. Sujatha and Reddy (1998) reported that the use of a wider concentration range in caster bean increased castor proliferation rate five times. However, a wider concentration range and mixture of hormones are not

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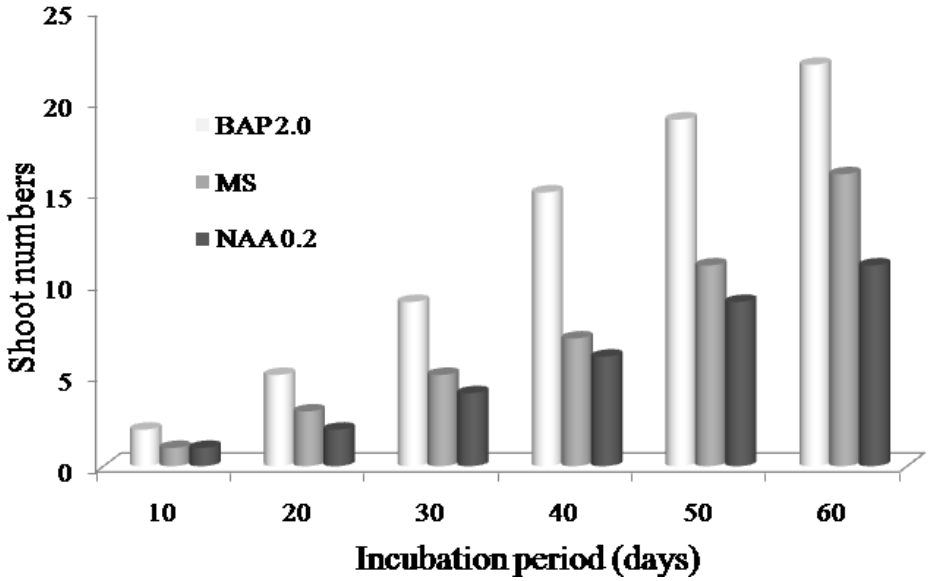


Figure 1. Number of shoots produced by using different growth regulators. Duncan multiple range test was used for analysis at 0.05 level of significant.

recommended nowadays by environmentalists even though it shows better shoot formation. This is due to pollution and to the fact that it is not cost effective, and has been altogether avoided.

At present, shoot formation at different BAP and NAA concentrations in all the previous studies in pineapple have not been conclusive. Therefore, the objectives of this study were to investigate the effect of BAP and NAA, singly used, at the concentration of range of 2.0 and 0.20 mg/l on the number shoot formation, weight and length of pineapple after 3rd generation (2nd subculture) *in vitro*.

MATERIALS AND METHODS

Medium preparation

MS (Murashige and Skoog, 1962) medium was prepared (1 L) from stock solutions and supplemented with sucrose at 30 g/L. The medium was adjusted to pH 5.7 before adding agar at 7.0 gL^{-1}. The beaker containing the medium was placed over a magnetic stirrer hot plate and heated to boiling to dissolve the agar and then dispensed equally (20 ml jar^{-1}) into 24 glass jars (5 x 15 cm) with screw rim and plastic lid which were autoclaveable. The medium was then autoclaved at 121°C and 1.5 kg cm^{-2} for 25 min. After that the autoclave was stopped and allowed to cool down. The medium was then divided into 30 beakers (25 ml each). Hormone was not added (control) to the first 10 beakers and BAP at 2.0 mg/L was added to the beakers no. 11 to 20 and NAA 0.2 mg/l was added to the beakers no. 21 to 30.

The terminal growth point, about 1.5 cm in size, of 20 smooth cayenne pineapples (*Ananas comosus* L. Merr.) fruits were removed from the fruit crown and placed in a beaker. It was washed thoroughly with water and sterilized with clorox (20%) for 25 min. The explants were then rinsed twice in distilled water for 5 min, trimmed to 5 mm^3 and cultured in cylindrical glass jar (Hamad and Taha, 2008b) with a rimmed neck and plastic cover containing 25 ml of hormone free MS medium (10 jars), medium with BAP at 2.0 mg/l (10 jars) and NAA 0.2 mg/l (10 jars), respectively. The cultures were transferred to incubation room and kept under constant temperature of 25°C and photoperiod of 16 h of light was provided by fluorescence lamps. The experiments was designed as complete randomize block design (CRBD) and the means significance was tested at p = 0.05 by Duncan`s multiple range test. All the experiments were conducted in Tissue Culture Laboratory, Biotechnology Division, the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia. After one month, contaminant-free cultures were sub-cultured firstly and after two month, the contaminant-free subcultures (secondly) were sub-cultured on solidified MS (hormone free), MS enriched with 2.0 mg/l BAP and MS enriched with 0.2 mg/l NAA. The multiple callus formation started after 10 days.

Data collection and analysis

Data was collected after 10, 20, 30, 40, 50, 60 days of incubation. The process was repeated 6 times for each of the incubation period. The average number and length of shoots per explant and the total number of shoots produced were calculated and used for evaluation of the different treatments. The shoots were removed from the cultures, weighed, separated into individual shoot so as to count the number and measure the length and weight of shoots.

RESULTS AND DISCUSSION

Figure 1 shows that the shoot number increased with increase in the incubation days in all treatments. The shoot number was higher in BAP at 2.0 mg/l than in control and NAA 0.2 mg/l for all the incubation days. The total shoot number was 23 per explant in BAP at 2.0 mg/l followed by 17 and 12 per explant in control (hormone free MS medium) and NAA at 0.2 mg/l. There was a significant difference between MS medium and MS medium treated with BAP and NAA.

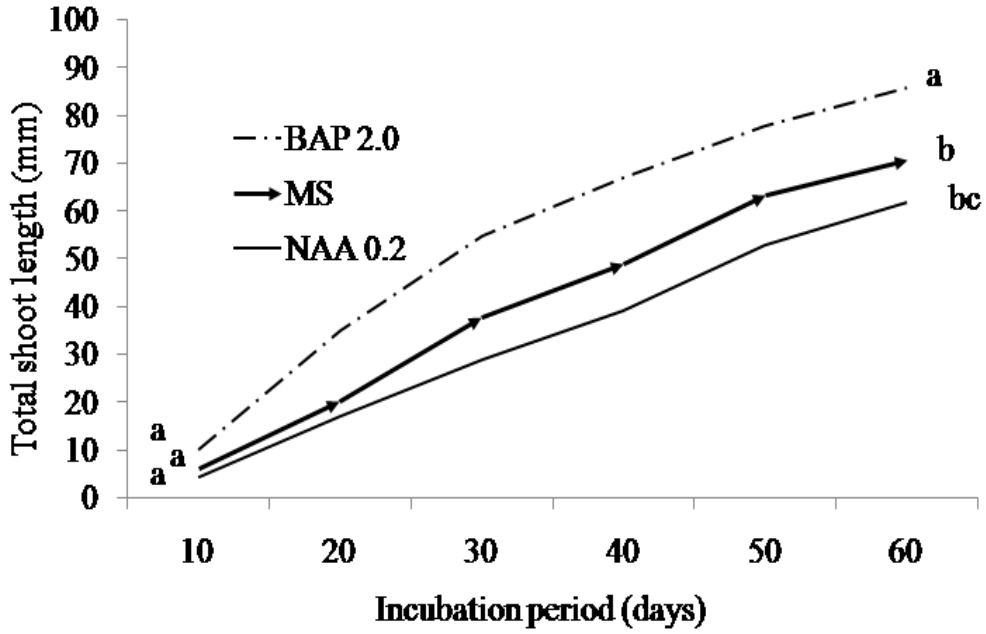


Figure 2. Total shoot length by using growth regulators. Duncan multiple range test was used for analysis at 0.05 level of significance.

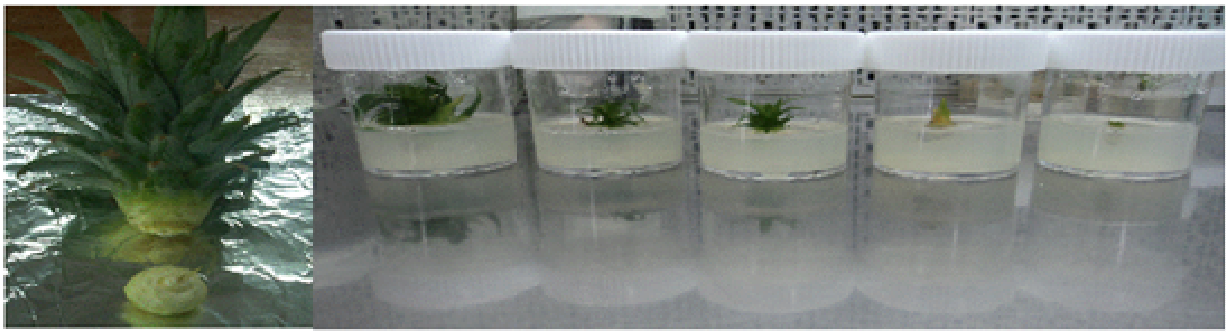
Table 1. Total shoot weight of different incubation days in different media. Duncan multiple range test was used for analysis at 0.05 level of significant.

Medium	Incubation period (day)					
	10	20	30	40	50	60
MS + BAP 2.0 mg/L	0.2^{a}	0.6^{a}	1.11^{a}	1.32^{a}	1.56^{a}	1.66^{a}
MS	0.1^{a}	0.3^{b}	0.98^{a}	1.2^{a}	1.3^{b}	1.41^{b}
MS + NAA 0.2 mg/L	0.1^{a}	0.2^{b}	0.89^{a}	1^{a}	1.12^{b}	1.2^{b}

Figure 2 shows that the shoot length was higher in BAP at 2 mg/l than in MS medium and NAA at 0.2mg/l.Shoot length increased with an increase in the incubation days for all the treatments. However, it was highest in BAP at 2 mg/l. There was a significant difference of shoot length between the explants in MS medium and the hormone treated explants. A significant difference was observed after DMRT analysis at 0.05 levels of signifi-cance among all the replicates. The results showed that BAP treatment had a better effect on the explants as compared to the other treatments. However, there was no significant difference between the MS medium (control) and NAA treated explants. Table 1 shows that the total shoot weight was maximum in the BAP treated explants at 10, 20, 30, 40, 50 and 60 days of incubation. There was no significant difference between the BAP treated explant and MS medium as well as NAA treated explants at 10, 20, 30 and 40 days of incubation. The difference shown by DMRT statistical analysis was at a 0.05 level of significance. Figure 3 shows the culture of the crown of pineapple and the subculture from explants at different growth hormone concentration (BAP at 2 mg/l, MS medium and NAA at 0.2mg/L).

Hammad and Taha (2008a and b) reported that in 4 cycles of culture, shoot number was highest in BAP at 1.5 mg/L after 4 subcultures. This work exhibited better results after a 2nd subculture. It might be due to the shoot or explant which reflected a better proliferation potentiality or BAP activation at the 2nd cycle stage than after four cycles. A different total shoot per explant number, but similar average shoot per explant number were also observed in Paeony (Harris and Mantell, 1991). It seems that the total number of shoot production, shoot length and weight, rather than the shoot number, shoot length and shoot weight/explant, should be emphasized as a means for evaluating the factors involved in prolixferation.

The shoot length and weight were 95, 70, 60 mm and 1.66, 1.41 and 1.2 g in BAP, MS medium amd NAA treated hormone, respectively. This result showed a better performance than previously reported data by Hamad and Taha (2009). According to their report, the tallest (25



Crown Cultures from crown (1st culture) in the plastic jar

Cultures from crown outside plastic jar (1st culture)

1st subculture from explants (2nd culture)

2nd subculture from explants (3rd culture) after 30 days

Figure 3. Photo showing the culture of crown of pineapple and subculture of explants in different growth hormones

mm) and heaviest (0.67 g) shoots were obtained in hormone free and at 0.25 mg/L and the shortest (7 mm) and lightest (0.13 g) shoots were obtained at 1.25 mg/L. The shoot length decreased as the BAP concentration increased up to 0.5 mg/L, while shoot weight decreased as concentration increased up to 1.25 mg/L in the 4th cycle of culture. Our results showed better response and it might be due to the better growth effect in the 2nd cycle.

The optimal concentration of BAP for the multiplication of pineapple (*A. comosus* L. Merr.) cv. smooth cayenne

in solid MS appeared to be 1.75, 2.0, 2.25 and 3.5 mgL^{-1}. Each resulted in the highest number of shoot formed (12 shoots/explant), equal shoot length of 8 mm and about 0.18 g/explant (average weight/explant) and over 1.5 g per culture (total weight/explant) per 60 days of incubation.

The main goal of tissue culture is to optimize the multiplication of plantlets *in vitro*. The 12 shoots per explant of pineapple obtained in agar solidified MS enriched with BAP at 1.75, 2.25 and 3.5 mgL^{-1} in this study was higher than the previously reported number of 10 (Sripaoraya et al., 2003) and 7 shoots per explant (Bhatia and Ashwath, 2002) obtained in response to BAP at 2.0 mgL^{-1}. It was also higher than the 9 (Be and Debergh, 2006), 7 (Aydieh et al., 2000) and 3 shoots per explant (Zepeda and Sagawa, 1981) obtained in response to BAP at 1.0 mgL^{-1} and the 10 shoots (Firoozabady and Gutterson, 2003) in response to 3.0 mgL^{-1}. The result was not in agreement with the use of BAP at 1.5 mgL^{-1} as suggested by Almeida et al. (2002) and 2.5 mgL^{-1} as suggested by Smith et al. (2002). The pineapple tissue culture is usually done for propagation purposes and the best treatment is judged by the rate of shoot formation. Other parameters such as total or average fresh weight are rarely reported. As the goal was propagule production, neglecting of weight is understandable. However, for the use of biomass for animal feeding, reporting weight would be more important than any other parameters. There was a significant difference in the total weight per explant (weight/culture). Pereze et al. (2003) obtained higher fresh weight, protein content and protease activity on response to BAP at 0.5 mg/L. Be and Debergh (2006) reported that although, BAP at 1.0 mgL^{-1} and a combination of BAP at 1.0 plus IBA at 0.5 mgL^{-1} induced equal number of shoots, the latter treatment doubled the total fresh weight/culture. Firoozabady and Gutterson (2003) counted the number of shoots in specific unit of weight and used the ratio for estimation of shoot number from the total weight obtained using a bioreactor system. Salehi and Khosh-Khui (1997) suggested a model by which the shoot length after one week in culture could be used for the estimation of the number of shoots that miniature rose would produce after four weeks of incubation.

Conclusion

It can be concluded that BAP at 2.0mg/L is the best concentration to effectively get higher shoot number of pineapple from crown explants, in the 2nd subculture, at the vegetative stage of two months period.

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