ENHANCING SHOOT GROWTH IN BANANA TISSUE CULTURE: THE EFFECT OF BENZYLAMINO PURINE AND PHOTOPERIODS

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Abstract. Banana has a high economic value and is in great demand by consumers, but efforts to increase banana production require large quantities of healthy and uniform seeds. Generative propagation of bananas is not possible because they do not produce seeds, while vegetative propagation using sucker is hampered by the limited availability of plant materials. Therefore, we studied an alternative technology for propagating banana seeds by tissue culture technique. This study aimed to obtain an effective concentration of growth regulator (Benzylamino Purine) and photoperiod to encourage *in vitro* shoot multiplication in 'Barangan' banana. Plant materials used were *in vitro* shoots of 'Barangan' banana cultured on MS basal medium. The concentration of Benzylamino Purine tested were 0 (control); 2.5 mg·L^{-1}, 5.0 mg·L^{-1}, 7.5 mg·L^{-1}, and 10.0 mg·L^{-1}; and photoperiod of 16 hours per day, 24 hours per day and total darkness. The trial used a factorial completely randomized design with 4 replications, and each experimental unit consisted of 4 cultures. The results showed that both Benzylamino Purine concentrations and photoperiods had a significant effect on the time of shoot initiation, number of shoots, number of leaves, number of roots and the longest root length. Benzylamino Purine concentration of 10.0 mg·L^{-1} and a photoperiod of 16 hours per day was the best combination to support shoot multiplication in 'Barangan' banana explants in an *in vitro* system. To obtain more optimal results and determining the efficiency of the multiplication rate, this study needs to be continued by testing BAP concentration beyond 10.0 mg·L^{-1} and 16-hours photoperiod.

Key words: BAP; Benzyladenine; *in vitro* culture; *Musa acuminata*; plant propagation.

INTRODUCTION

Banana (*Musa acuminata*) is one of the horticultural crops with good economic prospects in Indonesia. Bananas are in great demand by domestic and foreign consumers, either as table fruit or in the form of processed products. Apart from its sweet and refreshing taste, bananas also contain high and complete nutrition, such as sugar, protein, fat, fiber, starch, tannic acid, vitamin A, vitamin B, vitamin C, vitamin D, calcium, phosphorus, iron, sodium, potassium, magnesium, and zinc [24].

One type of banana that has high market demand and has the opportunity to be developed is the 'Barangan' banana. 'Barangan' bananas have a distinctive, sweet taste and fragrant aroma, and have many benefits, especially for providing balanced nutrition. In 100 g of 'Barangan' banana there are 110 calories, 25.8 g of carbohydrates, 1.2 g of protein, 3 g of vitamin C and potassium [45].

The high market demand for 'Barangan' bananas either in Jambi Province and or in Indonesia has resulted in the need to expand the planting area. This effort requires the availability of seeds of uniform size, healthy and in large quantities. These things are the main limiting factors in the intensification and extensification of banana cultivation [42].

Conventional vegetative propagation of bananas takes time and the number of seeds produced is limited. In addition, the use of vegetative propagules allows the spread of pathogens from one generation to the next. Besides, the non-uniform age of the offspring causes an increase in production costs. Therefore, technological breakthrough is needed to provide quality and healthy banana seeds in large quantities and in a short time. The appropriate technology to overcome this obstacle is vegetative propagation through tissue culture techniques [14, 21, 44, 45].

One of the determining factors for the success of plant propagation using tissue culture technique is the use of plant growth regulator (PGR) [27, 28, 47]. The type and concentration of PGR depends on the purpose and culture stage. Apart from that, the conditions of the culture environment, especially lighting, also influence the development of cultured explants [12, 18, 32, 33].

Cytokinin is a growth regulator that is needed in tissue culture media and is applied in concentrations adjusted to the desired growth [3]. Cytokinin plays a role in cell division, callus proliferation, shoot formation, morphogenesis, organogenesis, and embryogenesis. One of the cytokinins, namely Benzylamino Purine (BAP), is a type of cytokinin that is commonly used in tissue culture [46]. It is a first generation cytokinin influencing plant growth and development. Study on the use of BAP in banana tissue culture has been widely carried out [20, 22, 23, 29]. However, to our knowledge reports on the use of BAP in 'Barangan' banana tissue culture are still limited in number.

Apart from growth regulators, light especially photoperiod, is also very important for plant growth and morphogenesis during *in vitro* culture. The importance of light in tissue culture lies in its effect on photomorphogenesis, not photosynthesis [16]. However, the mechanism by which light affects culture growth is not yet fully understood [36]. Allegedly, the light received by phytochrome pigments is translated into hormone metabolism. Galston [15] claimed that the presence of blue light receptor known as riboflavin in culture medium might inhibit tissue growth in the

presence of light but not in the absence of light. This inhibition, however, could be reversed by the addition of auxin. This has been proven in root formation in micro-cuttings of *Eucalyptus ficifolia* [17] and *Malus pumila* [40].

Present investigation was carried out with the objectives: 1) to determine the effect of BAP on the growth and development of 'Barangan' banana shoot explants in an *in vitro* system under different photoperiod conditions, and 2) to obtain BAP concentrations and photoperiod duration that can stimulate shoot proliferation and root formation on 'Barangan' banana shoots in an *in vitro* system.

MATERIALS AND METHODS

Plant material used in this study was *in vitro* shoots of 'Barangan' banana (*Musa acuminata*) resulting from previous *in vitro* culture. The media used was the composition of Murashige and Skoog (MS) [35]. The variables that will be tested was BAP at various concentrations and different photoperiod regimes.

The equipment used in this trial were culture flasks, Erlenmeyer glasses, Beaker glasses, measuring flasks, pipettes, analytical balance, pH meter, autoclave, magnifying glass, oven, laminar air flow cabinets, hot plates with magnetic stirrer, spirit lamp, and dissecting kits (tweezers, scalpel handle and scalpel knife). Apart from that, in culture room equipment were also needed such as a room thermometer and hygrometer as well as a photoperiod control timer. Light source came from a white fluorescent light of Tube Luminescent (TL) lamp with an intensity of approximately 50 $\mu mol \cdot m^{-2} \cdot s^{-1}$ which was installed on the culture racks.

The culture media and equipment were sterilized by autoclaving for approximately 20 minutes at 121 ^{o}C and 1 atmosphere [5, 13]. Meanwhile, the explants were surface sterilized following laboratory protocol [4] prior to inoculation.

The experiment used a Completely Randomized Design with BAP and photoperiod as tested variables. The BAP concentrations tested were 0.0 $mg \cdot L^{-1}$, 2.5 $mg \cdot L^{-1}$, 5.0 $mg \cdot L^{-1}$, 7.5 $mg \cdot L^{-1}$, and 10.0 $mg \cdot L^{-1}$. The photoperiods under which explants grown were 16-hours per day, 24-hours per day and continuous dark. Thus, there are 15 treatment combinations. Each treatment was repeated 3 times, so that there were 45 experimental units. Each experimental unit consisted of 4 culture flasks, so in total there were 180 cultures.

The variables observed are: 1) shoot formation and development which includes the time the shoots emergence, the number of shoots, and the number of leaves, 2) root formation and development which includes the number of roots and the length of the longest root.

Data collected during the trial were analyzed statistically using Analysis of Variance by the aid of Microsoft Excel Spreadsheet [31], and continued with the Least Significant Difference test to see differences among treatment means [9].

RESULTS

Analysis of variance showed that there was a significant effect of BAP and photoperiod on the time of shoot emergence (*P-value* = 0.00). The differences in the effect of various BAP concentrations and different photoperiod duration on the time of shoot emergence are presented in Tables 1, 2 and 3.

Table 1. The effect of various BAP concentrations on the time of shoot emergence in 'Barangan' banana explants cultured *in vitro* under different photoperiod conditions (days after culture).

BAP Concentrations	Photoperiods		
	16-hours per day	24-hours per day	Continuous dark
0.0 $mg \cdot L^{-1}$	32.11 ± 2.12 b A	61.67 ± 0.96 a A	32.11 ± 2.12 b BC
2.5 $mg \cdot L^{-1}$	26.67 ± 0.51 c B	63.11 ± 1.37 a A	38.33 ± 0.96 b A
5.0 $mg \cdot L^{-1}$	26.44 ± 0.11 c B	61.44 ± 0.68 a A	39.00 ± 2.52 b A
7.5 $mg \cdot L^{-1}$	21.67 ± 1.73 c C	48.67 ± 1.53 a B	35.11 ± 1.13 b AB
10.0 $mg \cdot L^{-1}$	16.00 ± 0.69 b D	36.56 ± 0.29 a B	38.00 ± 2.46 a A

Notes: values followed by the same lowercases in the same rows and the same uppercases in the same columns are not significantly different ($LSD_{0.05}$ = 4.28).

Table 2. The effect of various BAP concentrations on the time of shoot emergence in 'Barangan' banana explants (days after culture).

BAP Concentrations	Time to shoot emergence
10.0 $mg \cdot L^{-1}$	30.19 ± 3.63 a
7.5 $mg \cdot L^{-1}$	35.15 ± 3.97 b
0.0 $mg \cdot L^{-1}$	41.96 ± 5.01 c
5.0 $mg \cdot L^{-1}$	42.30 ± 5.17 c
2.5 $mg \cdot L^{-1}$	42.70 ± 5.40 c

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 1.92).

Table 3. The effect of different photoperiods on the time of shoot emergence in 'Barangan' banana explants (days after culture).

Photoperiods	Time to shoot emergence
16-hours per day	24.58 ± 1.53 a
Continuously dark	36.51 ± 1.01 b
24-hours per day	54.29 ± 2.78 c

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 2.47).

On the number of shoots parameter, analysis of variance showed that there was a highly significant effect of BAP (*P-value* = 0.00). However, neither photoperiod nor its interaction with BAP showed a significant effect (*P-values* were 0.48 and 0.10 respectively). The differences in the effects of BAP concentrations and length of photoperiods on the number of shoot formation are presented in Tables 4, 5 and 6.

BAP concentrations and photoperiod had significant effect on leaf number, both singly (*P-value* = 0.01) and in their interaction (*P-value* = 0.01). The differences in the influence of BAP concentrations and photoperiod duration on leaf number are presented in Tables 7, 8 and 9 respectively.

BAP and photoperiod showed significant effect on the number of roots, both singly (*P-value* = 0.01) and their interaction (*P-value* = 0.01). The differences in the effect of BAP concentrations and length of photoperiods on the number of roots are presented in Tables 10, 11 and 12.

The longest root length was significantly influenced by BAP (*P-value* = 0.00), photoperiod (*P-value* = 0.01) and the interaction of the two (*P-value* = 0.00). The differences in the influence of BAP concentrations and length of photoperiod on the longest root length based on the LSD test are presented in Tables 13, 14 and 15.

Table 4. The effect of various BAP concentrations on the number of shoot formation in 'Barangan' banana explants cultured *in vitro* under different photoperiod conditions.

BAP Concentrations	Photoperiods		
	16-hours per day	24-hours per day	Continuous dark
0.0 mg·L^{-1}	1.78 ± 0.40	1.33 ± 0.00	1.33 ± 0.00
2.5 mg·L^{-1}	1.67 ± 0.19	1.00 ± 0.00	1.33 ± 0.19
5.0 mg·L^{-1}	1.00 ± 0.00	1.11 ± 0.11	1.67 ± 0.19
7.5 mg·L^{-1}	2.11 ± 0.40	2.22 ± 0.11	1.78 ± 0.22
10.0 mg·L^{-1}	1.66 ± 0.22	2.33 ± 0.19	2.00 ± 0.33

Table 5. The effect of various BAP concentrations on the number of shoot formation in 'Barangan' banana explants.

BAP Concentrations	Number of shoot formation
10.0 mg·L^{-1}	2.19 ± 0.14 a
7.5 mg·L^{-1}	2.04 ± 0.15 ab
2.5 mg·L^{-1}	1.48 ± 0.12 c
5.0 mg·L^{-1}	1.33 ± 0.12 c
0.0 mg·L^{-1}	1.26 ± 0.14 c

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.28).

Table 6. The effect of different photoperiods on the number of shoot formation in 'Barangan' banana explants.

Photoperiods	Number of shoot formation
24-hours per day	2.22 ± 0.16
Continuously dark	2.33 ± 0.11
16-hours per day	2.00 ± 0.11

Table 7. The effect of various BAP concentrations on the number of leaf formation in 'Barangan' banana explants cultured *in vitro* under different photoperiod conditions.

BAP Concentrations	Photoperiods		
	16-hours per day	24-hours per day	Continuous dark
0.0 mg·L^{-1}	2.00 ± 0.33 a C	2.11 ± 0.29 a B	1.44 ± 0.11 b A
2.5 mg·L^{-1}	2.67 ± 0.33 a C	1.89 ± 0.29 b B	1.56 ± 0.11 b A
5.0 mg·L^{-1}	1.89 ± 0.48 a C	2.00 ± 0.00 a B	1.67 ± 0.19 a A
7.5 mg·L^{-1}	4.44 ± 0.97 a B	3.11 ± 0.48 b B	2.00 ± 0.19 b A
10.0 mg·L^{-1}	6.33 ± 1.26 a A	5.78 ± 0.68 b A	1.78 ± 0.48 b A

Notes: values followed by the same lowercases in the same rows and the same uppercases in the same columns are not significantly different ($LSD_{0.05}$ = 1.53).

Table 8. The effect of various BAP concentrations on the number of leaf formation in 'Barangan' banana explants.

BAP Concentrations	Number of leaf formation
10.0 mg·L^{-1}	4.63 ± 0.84 a
7.5 mg·L^{-1}	3.19 ± 0.48 b
2.5 mg·L^{-1}	2.04 ± 0.21 c
0.0 mg·L^{-1}	1.85 ± 0.17 c
5.0 mg·L^{-1}	1.85 ± 0.16 c

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.68).

Table 9. The effect of different photoperiods on the number of leaf formation in 'Barangan' banana explants.

Photoperiods	Number of leaf formation
16-hours per day	3.47 ± 0.54 a
24-hours per day	2.98 ± 0.42 a
Continuously dark	1.69 ± 0.11 b

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.88).

Table 10. The effect of various BAP concentrations on the number of root formation in 'Barangan' banana explants cultured *in vitro* under different photoperiod conditions.

BAP Concentrations	Photoperiods		
	16-hours per day	24-hours per day	Continuous dark
0.0 mg·L^{-1}	1.33 ± 0.19 a DE	1.44 ± 0.11 a CD	1.33 ± 0.00 a A
2.5 mg·L^{-1}	2.00 ± 0.67 a CD	1.56 ± 0.56 a BC	1.56 ± 0.11 a A
5.0 mg·L^{-1}	2.11 ± 0.29 a BC	1.11 ± 0.11 a DE	1.44 ± 0.11 a A
7.5 mg·L^{-1}	2.89 ± 1.06 a B	2.67 ± 0.51 a B	1.67 ± 0.00 a A
10.0 mg·L^{-1}	6.11 ± 1.13 a A	6.11 ± 1.25 a A	1.67 ± 0.33 b A

Notes: values followed by the same lowercases in the same rows and the same uppercases in the same columns are not significantly different ($LSD_{0.05}$ = 1.71).

Table 11. The effect of various BAP concentrations on the number of root formation in 'Barangan' banana explants.

BAP Concentrations	Number of root formation
10.0 mg·L^{-1}	4.63 ± 0.89 a
7.5 mg·L^{-1}	2.41 ± 0.39 b
2.5 mg·L^{-1}	1.70 ± 0.26 bc
5.0 mg·L^{-1}	1.56 ± 0.18 c
0.0 mg·L^{-1}	1.37 ± 0.07 c

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.76).

Table 12. The effect of different photoperiods on the number of root formation in 'Barangan' banana explants.

Photoperiods	Number of root formation
16-hours per day	2.89 ± 0.54 a
24-hours per day	2.58 ± 0.55 a
Continuous dark	1.53 ± 0.07 b

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.99).

Table 13. The effect of various BAP concentrations on the longest root length in 'Barangan' banana explants cultured *in vitro* under different photoperiod conditions (cm).

BAP Concentrations	Photoperiods		
	16-hours per day	24-hours per day	Continuous dark
0.0 mg·L^{-1}	1.67 ± 0.33 a C	1.78 ± 0.29 a C	2.11 ± 0.11 a A
2.5 mg·L^{-1}	2.56 ± 0.59 a C	1.67 ± 0.00 a C	2.11 ± 0.29 a A
5.0 mg·L^{-1}	2.56 ± 0.73 a C	1.89 ± 0.29 a C	2.00 ± 0.51 a A
7.5 mg·L^{-1}	2.78 ± 0.40 ab BC	3.44 ± 0.78 a B	1.89 ± 0.29 b A
10.0 mg·L^{-1}	4.44 ± 0.40 b A	7.78 ± 1.06 a A	3.00 ± 0.51 b A

Notes: values followed by the same lowercases in the same rows and the same uppercases in the same columns are not significantly different ($LSD_{0.05}$ = 1.48).

Table 14. The effect of various BAP concentrations on the longest root length in 'Barangan' banana explants.

BAP Concentrations	Longest root length (cm)
10.0 mg·L^{-1}	5.07 ± 0.79 a
7.5 mg·L^{-1}	2.70 ± 0.35 bc
5.0 mg·L^{-1}	2.15 ± 0.29 cd
2.5 mg·L^{-1}	2.11 ± 0.23 cd
0.0 mg·L^{-1}	1.85 ± 0.15 d

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.66).

Table 15. The effect of different photoperiods on the longest root length in 'Barangan' banana explants.

Photoperiods	Longest root length (cm)
24-hours per day	3.31 ± 0.66 a
16-hours per day	2.80 ± 0.31 a
Continuous dark	2.22 ± 0.18 b

Notes: values followed by the same letters are not significantly different ($LSD_{0.05}$ = 0.85).

DISCUSSION

The response showed by explants cultured under *in vitro* system is not always the same one among another, and much depend upon the type of explants used, environmental conditions, medium composition, and the presence of plant growth regulators in culture medium. The combination of two or more of such factors often becoming critical factor and very crucial in inducing and promoting growth response from cultured explants. Laslo and Vicaș [26] suggested that the direction of explant growth and development was very much influenced by the ratio of endogenous against exogenous growth regulators. In addition, Zhao *et al.* [43] claimed that the incorporation of exogenous growth regulators into culture medium might affect the activity of those hormones already presence within cultured tissues. Therefore, it is important to pay serious attention to the types and concentrations of exogenous plant growth regulators applied to culture medium for inducing growth and development of explant to the desired direction.

Environmental conditions such as photoperiod also plays important role in directing explant growth and development. The photoperiod desired by *in vitro* culture is a manifestation of *in vivo* plant growth and

development. Plants that normally respond to a certain photoperiod during *in vivo* growth will also respond to the same photoperiod during *in vitro* culture [34]. By considering the complete photomorphogenic response properties of plants, it can be predicted that short-day species such as *Chrysanthemum* will be more readily cultured in the vegetative phase under long photoperiods [25].

In general, the photoperiod required for *in vitro* culture ranges from 14-hours to 16-hours per day. However, certain plants are responsive to a shorter photoperiod of 8-hours per day, for example *Alocasia amazonica* [19]. Meanwhile, other plants require continuous periods of light or dark to produce a certain response. For example, 5.0 µM BAP under dark condition was essential for organogenesis in *Phaseolus angilaris* [33]. In addition, dark preconditioning of shoot explants of *Habenaria radiata* before culture increased survival rates of explants and bud formation [32]. Darkness significantly speed up the conversion of somatic embryos into plantlets in *Phoenix dactylifera*, reduced the duration from 24 to 12 weeks [2]. On the other hand, plant regeneration from nodal segment of *Nicotiana tabacum* was much better in light rather than dark condition [38].

Our results revealed that, in general the application of BAP at various concentrations on shoot explants cultured under different photoperiods resulted in significant effect on time to shoot emergence, number of shoots, number of leaves, number of roots and length of longest root. However, number of proliferated shoots did not significantly affected by photoperiod (*P-value* = 0.48) nor its combination with BAP (*P-value* = 0.10). Moreover, among BAP concentrations tested, the application of 10.0 $mg{\cdot}L^{-1}$ resulted in the shortest time to shoot emergence, largest number of shoots, leaves and roots, and the longest roots. This BAP 10.0 $mg{\cdot}L^{-1}$ is significantly different to other levels. Meanwhile, among photoperiods tested, the application of 16-hours photoperiod produced the shortest time to shoot emergence, and the largest number of leaves and roots. However, there is no different effect of obtained on the number of shoots. In addition, photoperiod of 24-hours per day produced the longest root length. Visually, the effect of various BAP concentrations and different photoperiod in tissue culture of banana 'Barangan' is presented in Figure 1.

Figure 1 shows that shoot proliferation much depended upon the concentration of BAP in culture medium and photoperiod condition. The application of BAP up to 10.0 $mg{\cdot}L^{-1}$ produced more shoot growth under photoperiod of 16-hours per day. Furthermore, under photoperiod of 24-hours per day, the use of BAP of 7.5 $mg{\cdot}L^{-1}$ produced more shoot proliferation. However, when BAP concentration was raised to 10.0 $mg{\cdot}L^{-1}$ the shoots grew abnormally, indicated by leaves that have long, and separated leaf sheath and they did not form pseudostem. This results coincided with report of Abbas *et al.* [1] on tissue culture of ginger (*Zingiber offinale*), where the application of 9 $mg.L^{-1}$ BAP was the best conditions for microrhizomes induction under 16-hours photoperiod. A photoperiod of 16-hours light was used effectively in tissue culture of *Viola wittrockiana*, *Sequoia sempervirens, Stevia rebaudiana*, and *Nymphaea lotus thermalis* [6-8].

George and Sherrington [16] and Zulkarnain *et al.* [48] stated that successful shoot formation requires media and growth regulators in the form of cytokinins with or without auxin. In connection with this, Kalimuthu *et al.* [20] suggested that the best shoot proliferation on 'Cavendish' banana was found in the treatment of BAP 3.0 $mg{\cdot}L^{-1}$ + NAA 0.2 $mg{\cdot}L^{-1}$, followed by the combination of BAP 4.0 $mg{\cdot}L^{-1}$ + NAA 0.2 $mg{\cdot}L^{-1}$. Meanwhile, Utami *et al.* [39] claimed that shoot growth was fastest after culture initiation and the highest average number of leaves was obtained when BAP 2.5 mg L^{-1} and NAA 0.5 mg L^{-1} were used in tissue culture of 'Ambon Hijau' banana. Further, Saputri *et al.* [37] reported that BAP concentration of 6.0 $mg{\cdot}L^{-1}$ was able to induce the fastest shoot growth with the highest number of shoots in 'Barangan' banana tissue culture. Budi [10] also reported the formation of highest shoot number in banana explants by the application of 1.5 $mg{\cdot}L^{-1}$ BAP.

Concerning artificial light, Cavallaro *et al.* [11] reported that in general plants require photoperiod of 16-hours per day such as *Nicotiana tabacum, Solanum lycopersicum, Cymbidium finlaysonianum, Euphorbia milii,* and *Gerbera jamesonii.* Other species grew best under 12-hours photoperiod such as *Lilium oriental, Brassica napus* and *Oryza sativa,* while some varieties of *Vitis vinifera* and *Solanum tuberosum* grew best under 10-hours photoperiod. In addition to this, the 16-hours photoperiod was also proven to be the best for micropropagation of *Cunninghamia lanceolata* [41] It was reported that plantlets subjected to photoperiod 16-hours had longer root, higher height, rooting rate, root number, and the higher levels of chlorophyll content. However, in *Vaccinium floribundum* the best growth of seedlings was found under 24-hours of photoperiod as reported by Meneses *et al.* [30]. Our study revealed that 16-hours or 24-hours per day photoperiods were appropriate for proliferation and growth of 'Barangan' banana plantlets under *in vitro* condition, but 16-hours photoperiod is preferable. Growth inhibition in cultured tissues could be related to the activity of phytochrome pigment. *In vitro* investigation by Galston [15] confirmed that application of small amount of riboflavin, a blue light receptor pigment, resulted in marked growth inhibition if peas tissue were exposed to light. In contrast, there was no such inhibition occurred in the absence of light. This inhibition could be partially reversed by the addition of relatively large quantities of IAA. Thus, it appeared possible that riboflavin in some way caused the photo-inactivation of auxins.

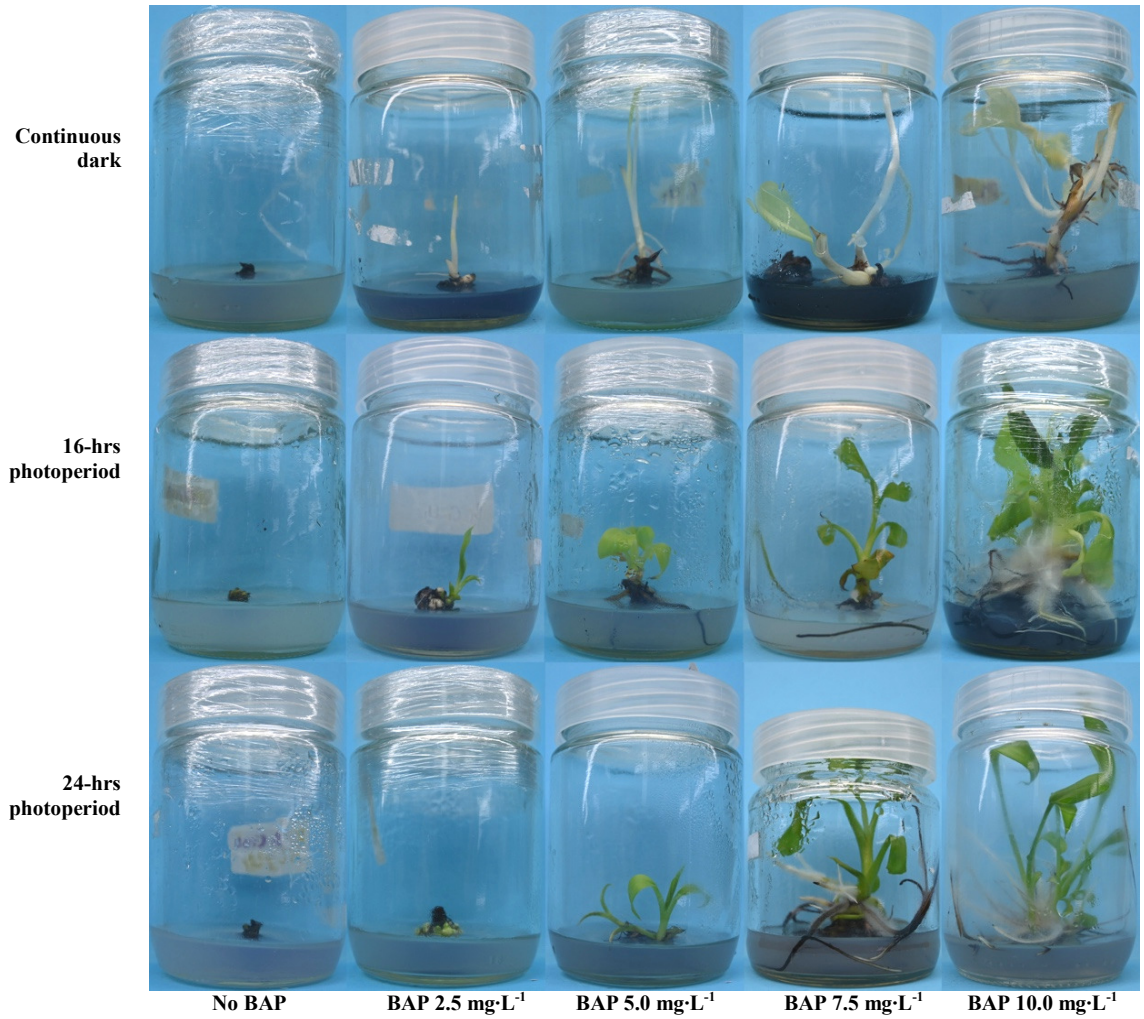

Figure 1. The effect of various BAP concentrations and different photoperiods on the *in vitro* culture of banana 'Barangan' shoot explants (90 days after culture).

It can be concluded that the acquisition of shoot multiplication via tissue culture of *Musa acuminata* 'Barangan' was related to the raise of BAP concentration in culture medium and length photoperiod under which explants were maintained. Although shoot number was not photoperiod dependent, the morphogenesis was undoubtedly much depended upon the presence of BAP in culture medium along with maintaining the cultures under 16-hours photoperiod. The best performance shoot multiplication was acquired when explants were cultured on medium supplemented with BAP 10.0 $mg \cdot L^{-1}$ with light condition of 16-hours per day photoperiod. In addition, BAP 10.0 $mg \cdot L^{-1}$ and 16-hours photoperiod also support rooting but not root length.

To obtain more optimal results and determining the efficiency of the multiplication rate, this study needs to be continued by testing BAP concentration beyond 10.0 $mg \cdot L^{-1}$ and 16-hours photoperiod.

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