Micro-tuber Induction of Elephant Yam (*Amorphophallus oncophyllus*)*

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Abstract

Micro-tuber induction of elephant yam (*Amorphophallus oncophyllus* Prain ex Hook.f.) was carried out for rapid propagation. In vitro shoots with two-to-three leaves were individually cultured for 8 weeks on modified Murashige and Skoog (MS) medium solidified with 5.5 g/l agar and supplemented with 2.69 or 5.37 µM 1-naphthalene acetic acid (NAA) alone or in combination with 2.22 or 4.44 µM N6-benzyladenine (BA). High concentration of NAA at 10.74, 16.11, 21.48, and 26.85 µM in combination with 8.88 µM BA were also tested for micro-tuber induction. It was found that at the low concentrations of NAA and BA, 2.69 µM NAA in combination with 4.44 µM BA provided the highest shoot fresh weight (0.32 g), micro-tuber fresh weight (0.28 g) and micro-tuber diameter (0.85 cm). For high concentrations of NAA and BA, 10.74, 16.11 or 21.48 µM NAA in combination of 8.88 µM BA showed no significant differences in shoot fresh weight and micro-tuber fresh weight but 10.74 µM NAA with 8.88 µM BA gave the highest micro-tuber diameter (0.65 cm). Then, MS medium supplemented with NAA (2.69, 5.37, 10.74, and 21.48 µM) in combination with BA (4.44, 2.22, 8.88, and 8.88 µM), respectively, were chosen to produce micro-tubers under light and dark conditions. Micro-tubers induced under dark condition from many combinations of plant growth regulators obtained more fresh weight and bigger size than those induced under light condition. However, 2.69 µM NAA and 4.44 µM BA under dark condition gave the highest micro-tuber fresh weight (0.45 g) and diameter (0.99 cm). All micro-tubers at the diameter of 0.5 cm or more were grown successfully with 100% survival rates.

Keywords: elephant yam, tuber induction, *Amorphophallus* sp., micropropagation

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* This research aimed to produce micro-tubers from shoots of *A. oncophyllus* in order to use as propagating materials.

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Introduction

Elephant yam or elephant foot yam (*Amorphophallus* sp.), a member of Araceae family, is a perennial herbaceous tuber crop distributed in tropical and subtropical regions mainly in the South East Asia (Angayarkanni et al., 2007). Elephant yam was found up to 68 species in Thailand including *A. oncophyllus*. *A. oncophyllus* is a species that mainly containing glucomannan, neutral polysaccharide (Sugiyama et al., 1972; Sakai, 1979; Harmayani et al., 2014) with promising biodegradable and biocompatibility activities and has been reported to be highly potential for use in food and pharmaceutical industries (Gateprasert, 2004; Zhang et al., 2005).

There were many reported on micropropagation and *in vitro* plant regeneration from corm segments (Irawati and Nyman, 1986), leaf (Kohlenbach and Becht, 1998), corm-derived callus (Liu et al., 2001; Yan et al., 2005), petiole-derived callus (Hu et al., 2008) of many species of elephant yam (*Amorphophallus* spp.). Induction of many *in vitro* storage organs (tuber, corm and bulb) were established on many plants such as taro (Hussain and Tyagi, 2006), caladium (Sugaram et al., 2007; Thepsithar et al., 2009), cyclamen (Yamaner and Erdag, 2008), gladiolus (Saha et al., 2013), saffron (Cavusoglu et al., 2013), glory lily (Kumar et al., 2015), and lachenalia (Bach et al., 2015). For *Amorphophallus* spp., corm or tuber was induced from petiole-derived callus (Hu et al., 2006), and plantlets (Wang et al., 2006). Moreover, genetic stability from *in vitro* regenerated plants was investigated from *A. albus* (Hu et al., 2008). Factors affected tuber or corm induction were growth regulators, sucrose, medium conditions and culture conditions (Hu et al., 2006; Hosseini et al., 2013; Hussain and Tyagi, 2006; Thepsithar et al., 2009; Saha et al., 2013).

This research reported effects of plant growth regulators (NAA and BA) and light conditions on micro-tuber induction from shoots of *A. oncophyllus* in order to use as propagating materials.

Materials and Methods

Plant Material

Single shoots with two-to-three leaves, about 1.0 cm height, (Figure 1a), from multiple shoot induction medium, Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) supplemented with 8.88 µM BA were used as explants for all experiments without subculturing onto plant growth regulator-free MS medium.

Media and Culture Conditions

A medium used for micro-tuber induction was MS medium containing 30 g/l sucrose and 5.5 g/l agar (Hardy Diagnostics Criterion^{TM}) supplemented with various concentrations of 1-Naphthalene acetic acid (NAA) and N6-Benzyl adenine (BA). The pH of all media was adjusted to 5.7 prior to adding agar and autoclaving. Explants were cultured in 240-ml glass jars containing 50 ml of culture medium for 8 weeks. Cultures were incubated at 24±1°C under a 16-h photoperiod at 35 – 40 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool-white fluorescent lamps and/or under dark conditions.

Statistical Analysis

Shoot fresh weight, micro-tuber fresh weight and number diameter of micro-tuber were recorded after 8 weeks of culturing. Data were analyzed by Duncan's New Multiple Range Test at p = 0.05 (Duncan, 1955).

Results and Discussion

In vitro propagation of elephant foot yam (*Amorphophallus campanulatus* Blume) from corm explants was established using MS medium supplemented with 5.37 μM NAA and 8.88 μM BA for callus induction, and plant regeneration from callus was induced by MS medium supplemented with 10.74 μM NAA and 4.44 μM BA (Akhond and Ali, 1998). From preliminary test of multiple shoot induction medium of *A. oncophyllus*, the best medium was MS medium supplemented with 8.88 μM BA (data not shown). Single shoots from this medium were used as initial explants for micro-tuber induction. For *Dioscorea* spp. (Kenyan yam), MS medium supplemented with 2.22 μM BAP and 0.12 μM IAA was the suitable for plantlet regeneration from shoots (Mwirigi et al., 2010).

Effects of NAA and BA at low concentration on micro-tuber induction

Shoot explants were cultured on MS medium supplemented with 2.69 or 5.37 μM NAA alone or in combination with low concentrations of BA at 2.22 or 4.44 μM. No micro-tuber was found from MS medium without plant growth regulator (Table 1; Figure 1b) or MS medium supplemented with NAA alone but callus was formed at the basal end of shoots (Table 1, Figure 1c). MS medium containing NAA in combinations with BA provided micro-tubers. However, 2.69 μM NAA in combination with 4.44 μM BA gave the highest shoot and micro-tuber fresh weight (0.32 and 0.28 g, respectively) as well as micro-tuber diameter (0.85 cm) (Table 1; Figure 1d).

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Table 1. Effects of NAA and BA at low concentrations on micro-tuber induction of *Amorphophallus oncophyllus* cultured on MS medium for 8 weeks

PGRs (µM)		Growth of shoots as explants [1/]		
NAA	BA	Shoot FWs (g)	Micro-tuber FWs (g)	Micro-tuber diameter (cm)
0.00	0.00	0.07±0.01 [b]	0.00±0.00 [b]	0.00±0.00 [c]
2.69	0.00	0.18±0.03 [ab]	0.00±0.00 [b]	0.00±0.00 [c]
2.69	2.22	0.21±0.05 [ab]	0.16±0.04 [ab]	0.65±0.06 [b]
2.69	4.44	0.32±0.09 [a]	0.28±0.25 [a]	0.85±0.10 [a]
5.37	0.00	0.13±0.02 [b]	0.00±0.00 [b]	0.00±0.00 [c]
5.37	2.22	0.16±0.03 [ab]	0.12±0.03 [ab]	0.58±0.09 [b]
5.37	4.44	0.21±0.10 [ab]	0.16±0.09 [ab]	0.48±0.05 [b]

[1/] n = 16, Values (mean ± S.E.) in the same column are not significantly different by Duncan's new multiple range test at p = 0.05

Effects of NAA and BA at high concentration on micro-tuber induction

Shoot explants were cultured on MS medium supplemented with 10.74, 16.11, 21.48, 26.85 µM NAA in combination with high concentration of BA at 8.88 µM (the best concentration of plant growth regulator for multiple shoot induction). There were no significant differences in shoot fresh weight obtained from NAA in combination of BA (0.22 – 0.31 g). MS medium containing 10.74 or 16.11 µM in combination with 8.88 µM BA gave the highest micro-tuber fresh weight (0.22 or 0.20 g, respectively) and micro-tuber diameter (0.65 or 0.56 cm, respectively). The high concentration of NAA at 21.48 and 26.85 µM reduced tuber fresh weight and tuber size (Table 2).

Table 2. Effects of NAA and BA at high concentrations on micro-tuber induction of *Amorphophallus oncophyllus* cultured on MS medium for 8 weeks

PGRs (µM)		Growth of shoots as explants [1/]		
NAA	BA	Shoot FWs (g)	Micro-tuber FWs (g)	Micro-tuber diameter (cm.)
0.00	0.00	0.05±0.01 [b]	0.00±0.00 [c]	0.00±0.00 [c]
10.74	8.88	0.29±0.04 [a]	0.22±0.07 [a]	0.65±0.08 [a]
16.11	8.88	0.25±0.01 [a]	0.20±0.05 [a]	0.56±0.04 [ab]
21.48	8.88	0.31±0.07 [a]	0.17±0.07 [ab]	0.53±0.05 [b]
26.85	8.88	0.22±0.04 [a]	0.13±0.04 [b]	0.25±0.02 [c]

[1/] n = 16, Values (mean ± S.E.) in the same column are not significantly different by Duncan's new multiple range test at p = 0.05

Effects of light condition in combination with NAA and BA on micro-tuber induction

MS medium containing 2.69, 5.37, 10.74 and 21.48 µM NAA in combination with 4.44, 2.22, 8.88 and 8.88 µM BA, respectively, which provided the good results (Table 1 and 2) were chosen for studying effects of light conditions (light and dark) on micro-tuber induction. Generally, micro-tubers induced under dark condition obtained more fresh weight (0.24 – 0.45 g) and the bigger size (0.63 – 0.99 cm) than those (0.17 – 0.35 g and 0.55 – 0.87 cm) induced under light condition, but not significantly different in the same concentrations of NAA and BA. However, 21.48 µM NAA in combination with 8.88 µM BA promoted significantly more micro-tuber fresh weight under dark condition. There was no statistical difference in micro-tuber fresh weight (0.22 and 0.24 g) and micro-tuber diameter (0.68 and 0.69 cm) obtained from 10.74 NAA with 8.88 BA cultured in light or dark condition, respectively (Table 3; Figure 1e). However, 2.69 µM NAA in combination with 4.44 µM BA under dark condition provided the highest micro-tuber fresh weight (0.45 g) and micro-tuber diameter (0.99 cm) (Table 3; Figure 1f). For fresh weight of shoots, dark condition gave yellow leaves and more shoot fresh weight on the same combination of NAA and BA, but not significantly different. However, under dark condition the combination of 5.37 µM NAA with 2.22 µM BA provided significant more shoot fresh weight (Table 3).

Table 3. Effects of light conditions in combinations with NAA and BA on micro-tuber induction of *Amorphophallus oncophyllus* cultured on MS medium for 8 weeks

PGRs (µM)		conditions	Growth of shoots as explants [1/]		
NAA	BA		Shoot FWs (g)	Micro-tuber FWs (g)	Micro-tuber Diameter (cm)
2.69	4.44	light	0.39±0.04 ab	0.35±0.15 ab	0.87±0.10 ab
		dark	0.44±0.08 a	0.45±0.10 a	0.99±0.13 a
5.37	2.22	light	0.23±0.03 c	0.18±0.07 bc	0.63±0.08 bc
		dark	0.43±0.08 ab	0.36±0.09 ab	0.71±0.09 b
10.74	8.88	light	0.28±0.05 bc	0.22±0.04 b	0.68±0.08 b
		dark	0.40±0.07 ab	0.24±0.06 b	0.69±0.07 b
21.48	8.88	light	0.31±0.04 b	0.17±0.02 c	0.55±0.05 c
		dark	0.38±0.09 ab	0.35±0.08 ab	0.63±0.06 bc

[1/] n = 16, Values (mean ± S.E.) in the same column are not significantly different by Duncan's new multiple range test at p = 0.05

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Micro-tubers, more than 0.5 cm in diameter, from 8-week cultures, were grown in plastic pots containing sterile soil and covered with plastic bags to obtain moist chamber for 2 weeks and placed in the greenhouse. Then the plastic bags were taken off and the plants were watered once a day. Survival rate of micro-tuber regenerated shoots was 100% after 4 weeks.

Figure 1. Micro-tuber induction of *Amorphophallus oncophyllus* cultured on MS medium supplemented with plant growth regulators for 8 weeks (bar = 0.5 cm)

a) A single shoot with 2-3 leaves as an initial explant

b) MS without plant growth regulator (no micro-tuber)

c) MS + 5.37 μM NAA under light condition (callus with no micro-tuber)

d) MS + 2.69 μM NAA + 4.44 μM BA under light condition (micro-tuber)

e) MS + 10.74 μM NAA + 8.88 μM BA under light condition (micro-tuber)

f) MS + 2.69 μM NAA + 4.44 μM BA under dark condition (micro-tuber)

Generally, BA and sucrose were the important factors for storage organ induction *in vitro*. Liquid MS medium containing 10 μM BA with 60 g/L sucrose was suitable for caladium (Thepsithar et al., 2009; Sugaram et al., 2007). The similar results were obtained from bulblet production of *Narcissus papyraceus* using 60 – 90 g/l sucrose in culture medium (Hosseini et al., 2013).

For tuber induction of *Amorphophallus* spp., it was found that MS medium supplemented with 4.44 μM BA in combination with 2.69 μM NAA were the appropriate medium for tuber induction from shoots of *A. oncophyllus*. Wang et al. (2006) reported that MS medium supplemented with 5.37 μM NAA and 1.70 μM PP_{333} (paclobutrazol) at 25 – 30°C under 1,500 – 2,000 lux light was the best conditions for tuber induction from 3 – 4 cm long plantlets of *A. rivieri*. However, tubers were induced from petiole-derived callus (Hu et al., 2006) in *A. albus*. Compact nodular calli were induced from MS medium supplemented with 5.37 μM NAA and 4.44 μM BA, then, tubers were induced on MS medium supplemented with 2.69 μM NAA and 8.88 μM BA with 6% (w/v) sucrose (Hu et al., 2006). Similarly, Hu and Li (2008) established callus from petiole segments on MS medium supplemented with 5.37 μM NAA and 8.88 μM BA and transferred onto MS medium supplemented with 2.69 μM NAA and 2.22 μM BA. The calli were induced into adventitious bud differentiation and corm-like structure on MS medium supplemented with 2.69 μM NAA and 8.88 μM BA.

For lighting and photoperiod, darkness was the suitable environment for bulb formation of *Brunsvigia undulate* (Rice et al., 2011). Hu et al. (2006) presented that short day (8 h) or long-day (16 h) photoperiods did not affect corm formation and growth significantly for *A. albus*, but dark condition provided slightly better tuber fresh weight and tuber size than light condition did for *A. oncophyllus*. In contrast, the greatest numbers of bulblet and size of bulblet were obtained from the medium supplemented with 60 g/l sucrose under 54 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$ and 90 g/l sucrose under 108 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$, respectively (Hosseini et al., 2013).

Conclusions

The study demonstrated that MS medium supplemented with the combination of 2.69 μM NAA and 4.44 μM BA which cultured under the dark condition was the best effective condition for micro-tuber induction of elephant yam (*Amorphophallus oncophyllus*). The development of appropriate technique for *in vitro* micro-tuber induction of *A. oncophyllus* is necessary for mass propagation, breeding program and germplasm collections.

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