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Effects of Explants Position and Plant Growth Regulators on *In Vitro* Regeneration of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

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ဝါးဘိုးမျက်ဆံကျယ်ဝါးမျိုး၏အဆစ်အပိုင်းများနှင့်ဟိုမုန်းတို့၏အကျိုးသက်ရောက်မှုအပေါ် တစ်သျှူးမွေးမြူခြင်းနည်းဖြင့်စမ်းသပ်လေ့လာခြင်းသုတေသန

ခင်ပပရွှေ၊ သုတေသနလက်ထောက် -၂

စာတမ်းအကျဉ်းချုပ်

ဤသုတေသနလုပ်ငန်းသည် ဝါးဘိုးမျက်ဆံကျယ် ဝါးမျိုး၏ တစ်နှစ်သား နှင့် နှစ်နှစ် သား အရွယ်အဖူးပါ အဆစ်အပိုင်းများကိုသုံး၍ တစ်သျှူးမွေးမြူခြင်းဖြင့် စမ်းသပ် ထားပါသည်။ စမ်းသပ်မှုအဆင့်တိုင်းကို ကျပန်းရွေးချယ်ခြင်းနည်းကိုသုံး၍ အခြေခံဆေး ဖျော်စပ်မှု အနေဖြင့် Murashige and Skoog (MS) 1962 နည်းကိုအသုံးပြုထားပါသည်။

စမ်းသပ်ချက်(၁)အနေဖြင့် စတင်မွေးမြူခြင်းစနစ်ကို လုပ်ဆောင်ပါသည်။ ဤအဆင့် တွင် တစ်နှစ်သားနှင့် နှစ်နှစ်သားအရွယ် ဝါးဘိုးမျက်ဆံကျယ်ဝါးမျိုး၏ အဖူးပါ အဆစ် အပိုင်း သုံးပိုင်း(ထိပ်၊အလယ်၊အခြေ)တို့အား ၃စင်တီမီတာအရွယ်ဖြတ်၍ ၀.၁% နှင့် ၀.၂% မာကျူရစ် ကလိုရိုဒ်ဖျော်ရည်ထဲတွင် ၅မိနစ် နှင့် ၁၀မိနစ်စီစိမ်၍ငင်း၊ ၁% ဆိုဒီယမ် ဟိုက်ပိုကလိုရိုဒ် ဖျော်ရည်ထဲတွင် (၁၀၊ ၁၅၊ ၂၀) မိနစ်စီစိမ်၍ငင်း ပိုးသတ်ကင်းစင်မှု ပြုလုပ်ပြီး ဟိုမုန်း ကင်း သော အာဟာရပြင်ပေါ်တွင် စမ်းသပ်မွေးမြူမှု ပြုလုပ်ပါသည်။ ဤစမ်းသပ်ချက်တွင် ၀.၂% မာကျူရစ် ကလိုရိုဒ်ဖျော်ရည်ထဲတွင် ၁၀မိနစ်စိမ်၍ ပိုးသတ် ကင်းစင်မှု ပြုလုပ်ထားသော တစ်နှစ်သားအရွယ် အလယ်အဆစ်အပိုင်းသည် (၈၀%) ရှင်သန်မှုရှိ၍ ထိပ်နှင့် အခြေအဆစ် အပိုင်းများထက် ရှင်သန်မှုကောင်းမွန်ကြောင်း တွေ့ရှိရသည်။

စမ်းသပ်ချက် (၂) တွင် တစ်နှစ်သားအရွယ် အလယ်အဆစ်ပိုင်းများကိုသုံး၍ ၃% နှင့် ၂% သကြားပမာဏရှိ ဟိုမုန်းအသုံးပြုထားသော အာဟာရပြင်ပေါ်တွင် စမ်းသပ်မှု (၇) မျိုး (T1 - T7) ဖြင့်ပွားများမွေးမြူမှုကို လုပ်ဆောင်ပါသည်။ ယင်းတို့အနက် T1 လုပ်ဆောင် ချက်သည် ရှင်သန်မှု နှင့် အညွှန့်ထွက်ရှိမှုအား ပိုမိုကောင်းမွန်သည့်အပြင် T4 လုပ်ဆောင်ချက် သည် အညွှန့်အချင်းထွက်ရှိမှု အပေါ်တွင် အကျိုးသက်ရောက်မှု ကောင်းမွန် ကြောင်း တွေ့ရှိရ သည်။

စမ်းသပ်ချက် (၃) အနေဖြင့် ဓါတ်ခွဲခန်းအတွင်း အမြစ်မွေးမြူခြင်းအဆင့်ကို လုပ်ဆောင် ပါသည်။ ဤစမ်းသပ်ချက်တွင် အခြေခံအာဟာရပြင်နှင့်အတူ (၈၀၊ ၁၀၀၊ ၁၂၀) မိုင်ခရိုမိုလ်ရှိ အင်ဒိုလီဗျူတရစ်အက်ဆစ် အမြစ်ထွက်ဟိုမုန်းကို အသုံးပြုပါသည်။ (၁၀၀) မိုင်ခရိုမိုလ်ရှိ အင်ဒိုလီဗျူတရစ်အက်ဆစ် အမြစ်ထွက်ဟိုမုန်းဖြင့် စမ်းသပ်ချက်တွင် (၆၀) ရာခိုင်နှုန်းကျော် အမြစ်ထွက်ရှိကြောင်း တွေ့ရှိရသည်။

Effects of Explants Position and Plant Growth Regulators on *In Vitro* Regeneration of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

By

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Abstract

An efficient *in vitro* regeneration protocol of a multipurpose bamboo species *Dendrocalamus hamiltonii* Nees et Arn. Ex Monro has been demonstrated using single node cuttings taken from the lateral branches of one year and two year old culms. Different micro propagation stages were involved in this study. For each stage, randomized complete block design (RCBD) was employed and Murashige and Skoog (MS) 1962 used as basal medium.

In experiment (1), three portions (distal, middle and base) of nodal segments of one year and two year old were cultured on a basal medium of Murashige and Skoog (MS) medium without any plant growth substance. Nodal segments (about 3.0 cm in length) were surface sterilized for 0.1% and 0.2% $HgCl_2$ for 5 and 10 minutes and 1.0 % NaOCl for 10, 15, 20 minutes to optimize the sterilent concentration and duration of surface sterilization. Treatment with 0.2% $HgCl_2$ for 10 minutes duration provided maximum level of survival percent (80 %) for middle position of one year old.

In experiment (2), the effects of plant growth regulators and 3% and 2% sucrose concentration on *in vitro* shoots multiplication were investigated. The treatments were seven replications and PGRs levels such as from T1 to T7. T1 showed better in growth performances at survival percentage and shoot formation percentage.T4 was found the best followed by T1 generally performed better than others at shoot diameter.

In experiment (3), the *in vitro* for rooting was investigated. The treatments were three PGRs levels (80, 100, 120) μM of IBA on Murashige and Skoog (MS) medium. Rooting efficiency was also markedly enhanced (> 60%) when the propagules, following shoot cut, were placed on to MS medium supplemented with 100 μM IBA for 2 weeks and then transferred to IBA free medium.

Key words: *Dendrocalamus hamiltonii* Micropropagation, PGRs

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Effects of Explants Position and Plant Growth Regulators on *In Vitro* Regeneration of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

1. Introduction

In Myanmar, bamboo is widely used from ancient time to present. Bamboos are quite common for scaffolding and house building, weaving materials. In rural areas, the bamboo has been using for house building without other building materials including timber. In addition, rural people have being highly depended on bamboo and bamboo products for their subsistence and incomes.102 species of bamboos are recorded in Myanmar. Almost all recorded species are locally used for construction and items of daily utilities but the young shoots of only 13 species are edible (PD 146/02 Rev- 1(1).

Dendrocalamus hamiltonii is one of edible species. Its young shoots are eaten both fresh and boiled shoot and used to make pickle bamboo shoot. The wide market for both fresh and pickle bamboo shoot of this species has been already in Myanmar. These pickle bamboo shoots are commercially produced and widely marketable in Myanmar. The pickle shoot of *D. hamiltonii* is more delicious and tender than others. In addition, the adult culms of *D. hamiltonii* are strong and commonly used for construction of house and bridges, basket making, fence, water vessel and fuel in rural areas. Thus *D. hamiltonii* is a multipurpose bamboo species.

Since supply of natural bamboo resources is not enough for increasing consumption, it is necessary to cultivate for sustainable utilization. However, seed supply is major limiting factor in bamboo cultivation because bamboos seldom flower and even when they do flower, most of seed are not fully developed or have weak viability. Therefore bamboos can be reproduced by vegetative means (Zhang et al., 2010). Tissue culture is a modern technique of vegetative propagation methods. In this experiment micro propagated shoots were initiated from nodal part of lateral branch from one year old culms of *D. hamiltonii* for *in vitro* regeneration.

2. Objectives

1. To determine effect of explants position on *in vitro* regeneration of *D. hamiltonii* Nees et Arn. Ex Munro
2. To determine effect of plant growth regulators on *in vitro* regeneration of *D. hamiltonii* Nees et Arn. Ex Munro
3. To produce planting materials of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro through *in vitro* culture

3. Literature Review

3.1 Origin and distribution of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

Dendrocalamus hamiltonii Nees et Arn. Ex Munro is commonest Himalayan *Dendrocalamus* species and apparently found along the entire Himalayan range. It prefers hill terrains and is mostly found in gullies in subtropical forest types, especially along the outer ranges of hills (Stapleton, 1994). It naturally distributes in tropical and subtropical climatic zone viz. India, Bhutan, Nepal, Myanmar and Thailand (Salam and Pongen 2008). In Myanmar, it naturally grows in Kachin State, Sagaing Region, Magwe Region and Mandalay Region. (Nyan Tun, 2005).

3.2 Identification features of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

This species is evergreen or deciduous large bamboo. It forms densely clumped with tall and erect stem curved downwards at the top. Its culms or stems are large. Each culm are12 - 20 m or up to 25 m tall and 10 - 18.5 cm diameter. Culms are usually naked below, much branched above. Culm surface is dull green and densely covered with whitish brown to light brown furry wax at first, remaining dull. Internodes are 30 - 50 cm long and wall is 1.25 cm thick. Nodes are marked with root scares.

3.3 Flowering of *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro

Bamboos flower once in their lifetime and the flowering is gregarious. Bamboos in a particular locality flower simultaneously. But the flowering period of a particular bamboo forest is unpredictable. It couldn't know when the plants will flower. It may take 10, 20 to 60 years for maturity and then flower. Bamboos die after flowering and production of huge quantities of seeds (Bareja, 2010). Like other bamboos, *Dendrocalamus hamiltonii* Nees et Arn. Ex Munro usually flowers sporadically every year, sometimes gregariously and the flowering cycle is reported to be 30 - 40 years (Salam & Pongen, 2008).

3.4 Conventional bamboo propagation

Bamboo can be propagated through sexual or asexual methods. Sexual method is propagation by using of seed, as in annual crops like rice, corn and beans. However, this method is unreliable and rarely known because seeds of bamboo are not available. Besides, it may take a

century or even more for certain species to produce seeds, and the exact period for seed production is impossible to predict (Bareja, 2010).

3.5 Background history of bamboo *in vitro* propagation

In bamboo propagation, conventional methods of vegetative propagation cannot meet to the present scale of demand for propagules and the alternative is the use of tissue culture techniques (Nadgir *et al.,* 1984 and Ramanayake, 2006). This is a recently developed technique where parts of the plants consisting of either seeds, inflorescences, stem - node sections, meristem domes, or leaves from underground shoots are used to initiate the cultures (Prutpongse & Gavinlertvatana, 1992 and Bareja, 2010).

4. Materials and Methods

1. The experiments were carried out at the Plant Tissue Culture Laboratory, Department of Horticulture and Agricultural Biotechnology, Yezin Agricultural University from May 2013 to July 2014.
2. Nodal segments of one year and two years old culms of wabo-myet-san-gye (*Dendrocalamus hamiltonii*) were used as experimental materials. They were collected from the clumps cultivating in Moe-Swe Research Station, Pyinmana.
3. Three positions (distal, middle and basal) of nodal segments (about 3.0 cm in length) were cut and surface sterilized.
4. In experiment (1), the experiment was used hormone free medium with seven treatments.

Number of Treatment	$HgCl_2$ (%)	NaOCl (%)	Duration (min)
1	0.1		5
2	0.1		10
3	0.2		5
4	0.2		10

5	1.0	10
6	1.0	15
7	1.0	20

5. In experiment (2), the effects of plant growth regulators with 3% and 2% sucrose concentrations on *in vitro* shoots multiplication were investigated. The treatments were seven replications and PGRs levels such as from T1 to T7.

Basal Medium	**Treatment**	**BAP (µM)**	**NAA (µM)**
	T1	0	0
	T2	5	1
	T3	7	1
MS	T4	9	1
	T5	5	2
	T6	7	2
	T7	9	2

6. In experiment (3), the treatments were three PGRs levels (80, 100, 120) µM of IBA on ½ Murashige and Skoog (MS) medium.

Basal Medium	Treatment	IBA (µM)
	T1	80
½ MS	T2	100
	T3	120

7. A Randomized Complete block Design (RCB) was laid out at every stage of culture. Data collected has been processed and accumulated by using Microsoft Office Excel 2003. Collected data has been statistically analyzed by using statistical software: Analysis of Variance (ANOVA) was done by using Statistix version 8.0. Mean values were separated by using Least Significant Differences (LSD) test as 0.05 level.

5. Results and Discussions

5.1 Experiment 1: Effects of various concentrations of Mercuric Chloride ($HgCl_2$) and Sodium Hypochlorite (NaOCl) on different positions at different durations on initial culture of *D. hamiltonii*

5.1.1 Survival percentage

The effects of Mercuric Chloride ($HgCl_2$) and Sodium Hypochlorite (NaOCl) concentrations and durations of surface sterilization were described on survival percentage of one year and two year of nodal segments with three positions of Wa-bo-myet-san-gye (Figure 5.1.a). Survival percentage was influenced by different levels of the $HgCl_2$ and NaOCl concentrations and durations. Based on the results of each position, the middle position of one year old stem was found the best but the poorest at the basal position of two year old stem. The effect of middle position of the node on one year old stem of explants was cultured as second time explants.

In the second experiment, results of survival revealed that 0.1% ($HgCl_2$) for 5 minutes, 10 minutes and 0.2% ($HgCl_2$) for 5 minutes were dead except 0.2 % ($HgCl_2$) for 10 minutes. The best survival percentage was found the 0.2 % ($HgCl_2$) for 10 minutes duration but the poorest in 1.0 % NaOCl for 15 minutes (Figure 5.2.a). With regard to the performance of the increase in concentration of $HgCl_2$ and treated time, middle position of the nodal segment of one year old was found as the best among others. The middle position with one year old was used for shoot multiplication.

5.1.2 Shoot formation percentage

The effects of $HgCl_2$ and NaOCl concentrations and durations of surface sterilization were described on shoot formation percentage of one year and two year of nodal segments of Wa-bo-myet-san-gye (Figure 5.1.b). The best shoot formation percentage was showed the middle position of one year old stem and the poorest at the base position of two year old stem.

The results revealed that the best shoot formation percentage was observed in 0.2 % ($HgCl_2$) for 10 minutes duration and the poorest shoot formation percentage was showed in 1.0 % NaOCl for 15 minutes duration (Figure 5.2.b).

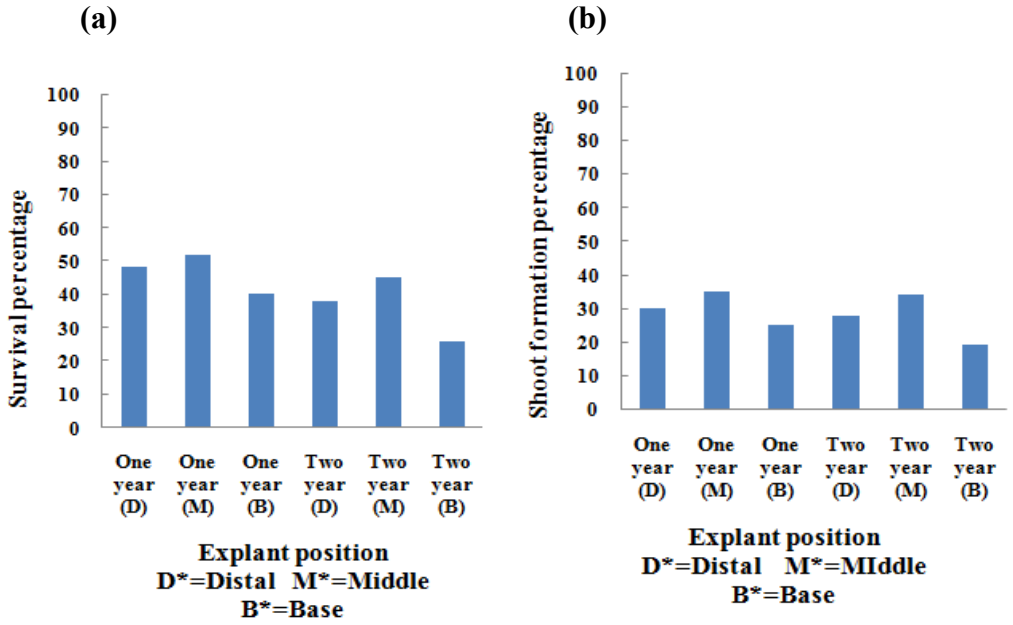


Figure 5.1. Effects of one year and two year of bamboo stem and position of the node on the stem on survival percentage and shoot formation percentage of explants cultured *in vitro*

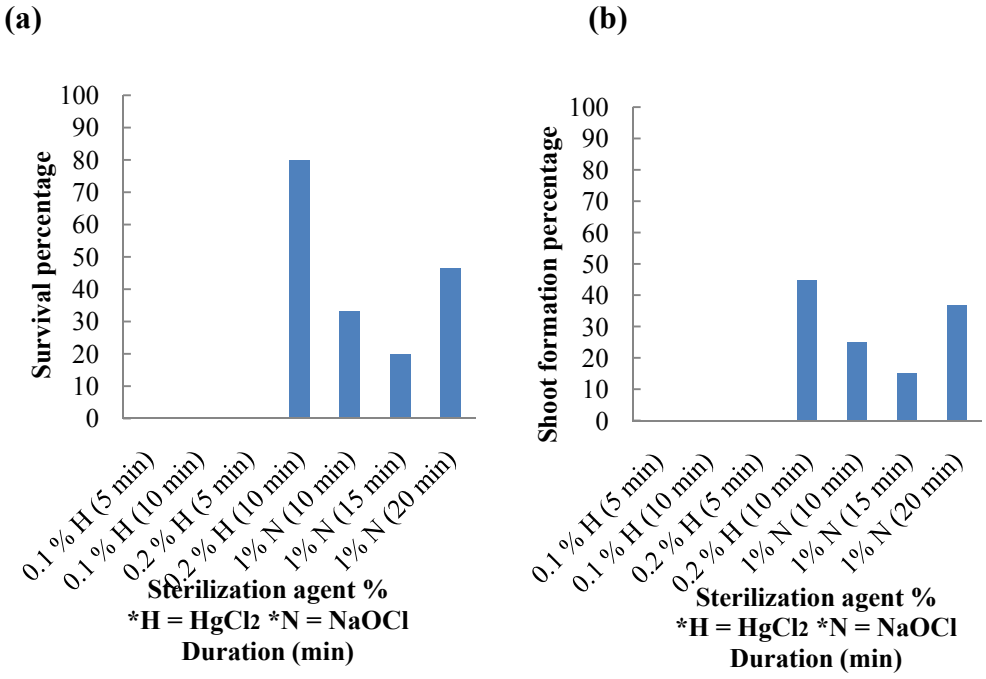


Figure 5.2. Effects of different sterilization methods on surface sterilization of the explants of middle position of one year old stem on survival percentage and shoot formation percentage at 2 weeks in *in vitro*

5.2 Experiment 2: Effects of different concentrations and combination of 6 - Benzylaminopurine (BAP) and Napthaleneacetic acid (NAA) and 3 % and 2 % sucrose (w/v) concentration on shoot multiplication of *D. hamiltonii*

5.2.1 Effects of Plant Growth Regulators (BAP, NAA) and 3 % and 2 % (w/v) sucrose concentration on survival percentage

The effects of different concentration of BAP and NAA and 3 % and 2 % sucrose (w/v) concentration were revealed on survival percentage of *D. hamiltonii*. Based on the results of each treatment, the T1, T2 and T5 were found the best followed by T7, T3, T6 and T4 in 3% sucrose (w/v) concentration (Figure 5.3.a). Mean value of survival percentage ranged from 5.51 (55.1 percent) to 6.53 (65.3 percent).

Based on the results of each treatment, the T1 was also the best followed by T7, T2, T3, T5, T6 and T4 in 2% sucrose (w/v) concentration (Figure 5.3.b). The mean value of survival percentage ranged from 7.76 (78 percent) to 9.39 (94 percent).

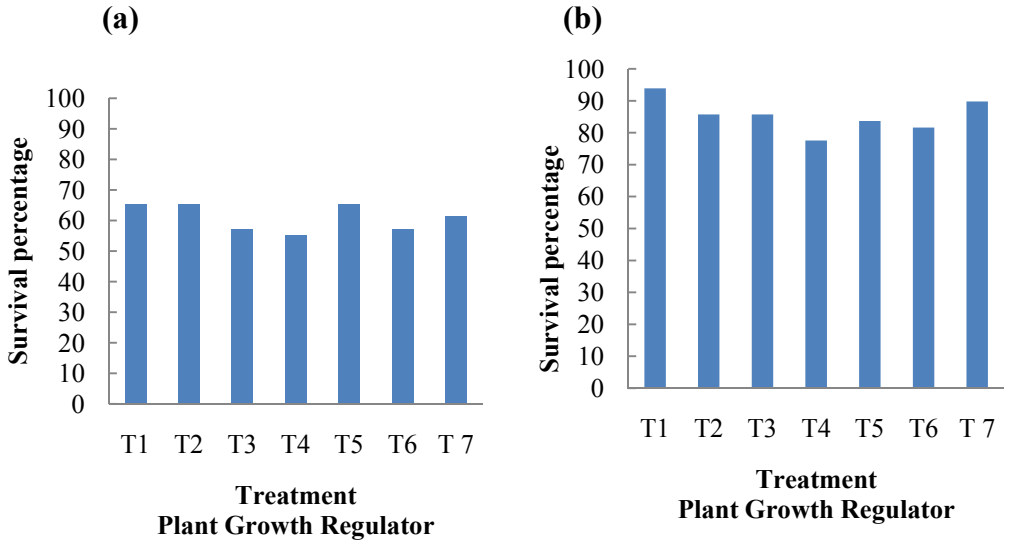


Figure 5.3 Effects of Plant Growth Regulators (PGRs) combinations and (a) 3% and (b) 2% sucrose (w/v) concentration on survival percentage of middle position of one year old stem at 2 weeks in *in vitro*

5.2.2 Effect of Plant Growth Regulators (BAP, NAA) and 3% and 2 % sucrose (w/v) concentration on shoot formation percentage

The effects of different concentration of BAP and NAA and 3% and 2% sucrose (w/v) concentration were also affected on shoot formation percentage of *D. hamiltonii*. Based on the results of each treatment, T1 and T3 were found to have more shoots formation followed by T7. However, T2 and T4 were the poorest followed by T5 and T6 in Figure 5.4 (a). The mean value of shoot formation ranged from 1.22 (12.2 percent) to 2.86 (29 percent) for over all treatments.

The effects of different concentrations of BAP and NAA and sucrose 2 % on shoot formation percentage were also described (Figure 5.4 b). Results indicated that T1 and T7 were the best while the T3, T2 and T4 were the poorest followed by T5 and T6. The mean value of shoot formation ranged from 5.1 (51 percent) to 8.57 (86 percent) for over all treatments.

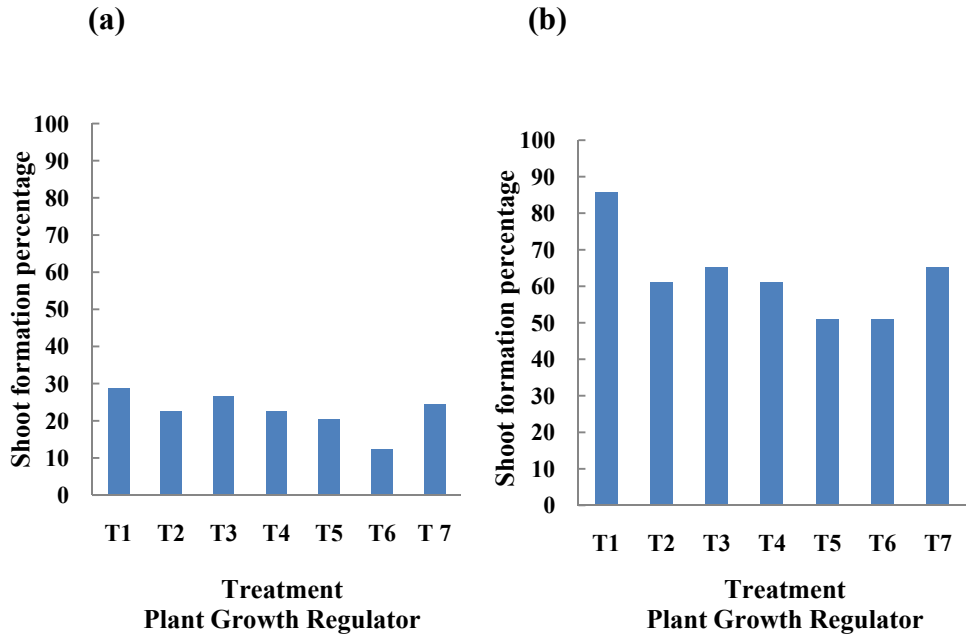


Figure 5.4 Effects of Plant Growth Regulators (PGRs) combinations and (a) 3% and (b) 2% sucrose (w/v) concentration on shoot formation percentage of middle position of one year old stem at 2 weeks in *in vitro*

5.2.3 Effect of Plant Growth Regulators (BAP, NAA) and 3 % and 2 % sucrose (w/v) concentration on number of shoots per explant

During culture initiation of proliferation medium, a few explants started multiple shoot formation while most of the explants simply grew in size. The effects of different concentrations of BAP and NAA and 3 **%** and 2 % sucrose (w/v) concentration were influenced on number of shoots per explant of *D. hamiltonii*. Based on the results of each treatment, there was no significant effect of Plant Growth Regulator in variation of treatments (Table 5.1) and (Table 5.2).

5.2.4 Effect of Plant Growth Regulators (BAP, NAA) and 3% and 2 % sucrose (w/v) concentration on shoot length (cm).

The effects of different concentrations of BAP and NAA and 3% and 2 % sucrose (w/v) concentration were influenced on shoot length (cm) of *D. hamiltonii*. The results showed that significant effect of different concentrations of BAP and NAA and 3% sucrose (w/v) concentration on shoot length. T7 was the best followed by T4 while the T3 the poorest followed by T1 (Table 5.1). The mean value of shoot length ranged from 0.7 to 1.8 in this study.

The results indicated that T6 the best followed by T5. The T4 the poorest followed by T2 and T3 (Table 5.2). Analysis of Variance on treatment measured showed significantly. The mean value of shoot length ranged from 1.69 to 2.55 in this study.

5.2.5 Effect of Plant Growth Regulators (BAP, NAA) and 3% and 2 % sucrose (w/v) concentration on shoot diameter (cm).

The effects of different concentrations of BAP and NAA and 3% and 2 % sucrose (w/v) concentration were described on shoot diameter (cm) of *D. hamiltonii.* Based on the results of each treatment, T4 was the best followed by T1. However, the T6 was the poorest followed by T3. Analysis of variance on treatment measured showed significantly ($P \geq 0.1$) differences among PGRs in each treatment shown in Table (5.1). The mean value of shoot diameter ranged from 0.08 to 0.43 in this study.

Results indicated that T1 was the best followed by T5 and T6. However, T4 was the poorest followed by T7 (Table 5.2). There was highly significant effect of PGR in variation of treatments in this study. The mean value of shoot diameter ranged from 0.15 to 0.22.

Table 5.1. Number of shoots per explant, Shoot length (cm) and shoot diameter (cm) as affected by different levels of BAP and NAA and 3% sucrose (w/v) at 6 weeks in *in vitro*

Plant Growth Regulator Treatment	Number of shoot per explant	Shoot length (cm)	Shoot diameter (cm)
T1	2.20 a	0.76 b	0.35 ab
T2	2.32 a	0.92 ab	0.20 bc
T3	2.25 a	0.70 b	0.12 c
T4	2.36 a	1.79 a	0.43 a
T5	2.30 a	1.39 ab	0.20 bc
T6	2.29 a	0.94 ab	0.08 c
T7	2.19 a	1.81 a	0.18 bc
Pr > F	0.66 ^{ns}	0.03*	0.01*
CV (%)	73.71	68.80	82.69

ns = not significant, * = significant

Table 5.2. Number of shoots per explant, Shoot length (cm) and Shoot diameter (cm) as affected by different levels of BAP and NAA and 2% sucrose (w/v) at 6 weeks in *in vitro*

Plant Growth Regulator Treatment	Number of shoot per explant	Shoot length (cm)	Shoot diameter (cm)
T1	2.23 ab	2.21 ab	0.22 a
T2	2.35 a	1.70 b	0.18 bc
T3	2.34 a	1.89 b	0.18 bc
T4	2.39 a	1.69 b	0.15 c
T5	2.33 ab	2.52 a	0.21 ab
T6	2.32 ab	2.55 a	0.20 ab
T7	2.24 ab	1.98 ab	0.16 c
Pr > F	0.05 ^{ns}	0.02*	0.002**
CV (%)	27.74	26.14	19.64

^{ns} = not significant, * = significant, ** = highly significant

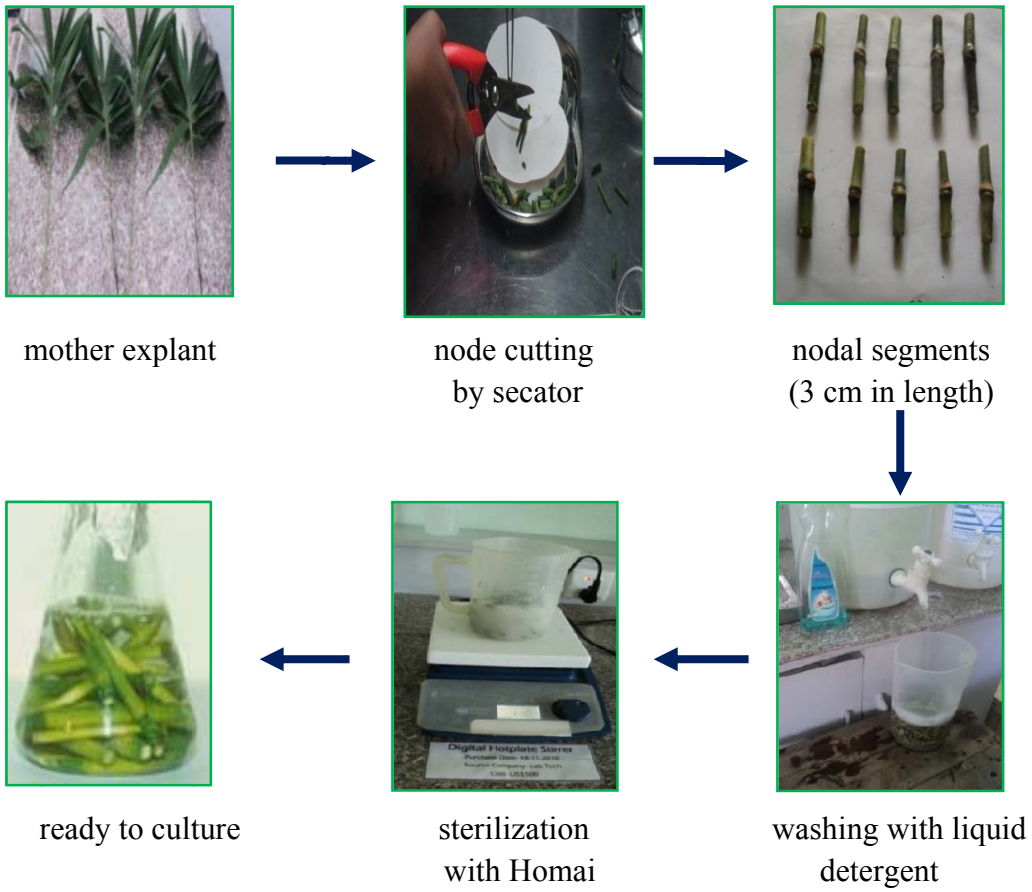
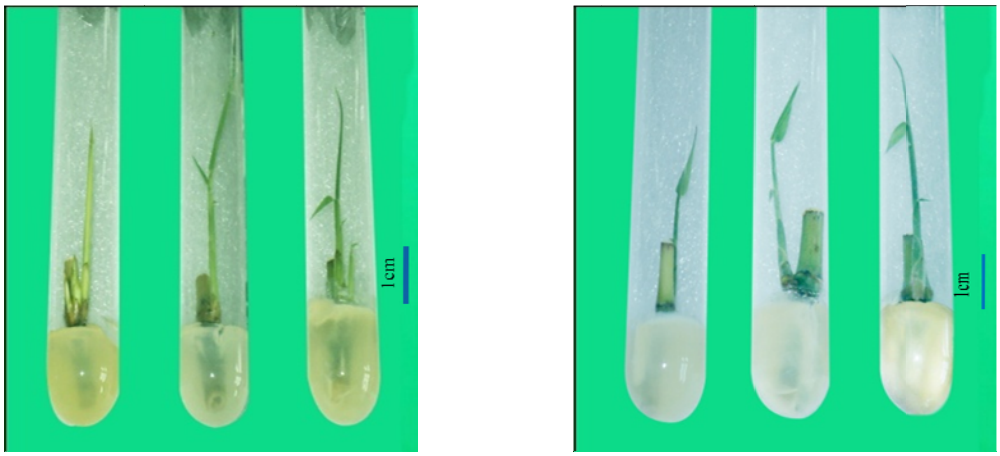
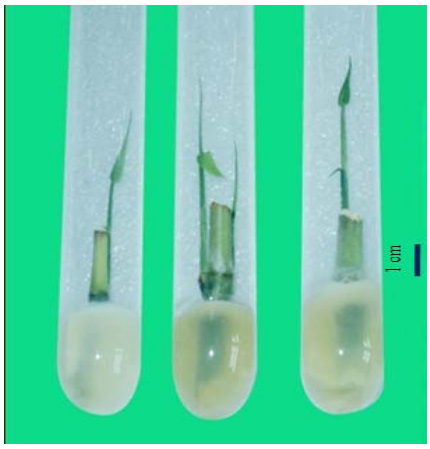


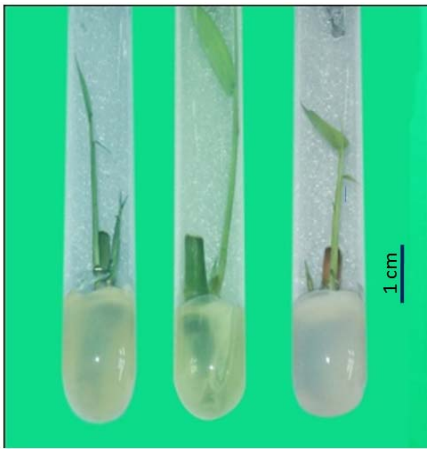
Plate 5.1 Explants preparation and inoculation of *D. hamiltonii*



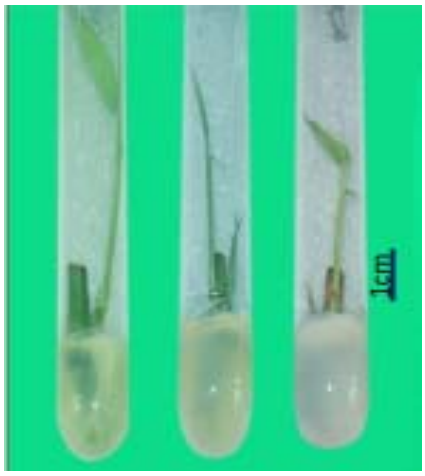
(A) BAP 5 µM NAA 1 µM (B) BAP 7 µM NAA 1 µM



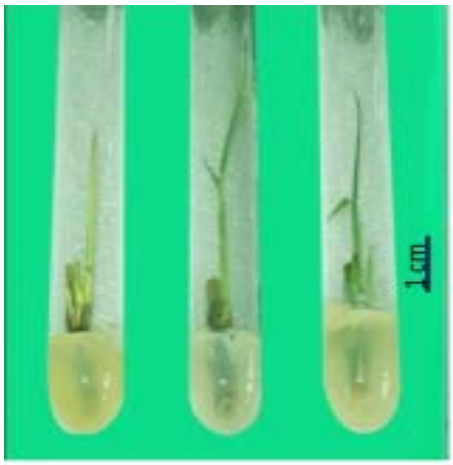
(C) BAP 9 µM NAA 1 µM



(D) BAP 5 µM NAA 2 µM

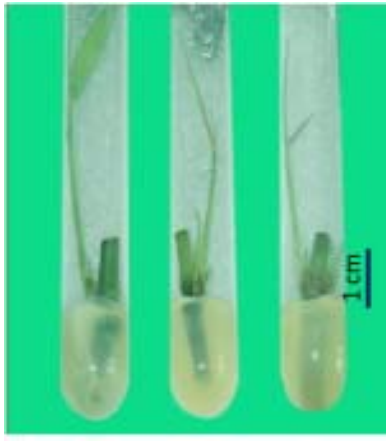


(E) BAP 7 µM NAA 2 µM



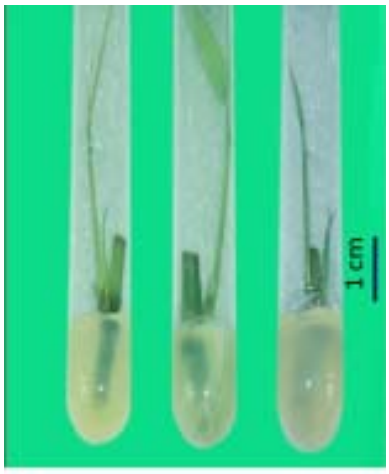
(F) BAP 9 µM NAA 2 µM

Plate 5.2 Shoot multiplication on MS media supplemented with 3 % sucrose and Plant Growth Regulators

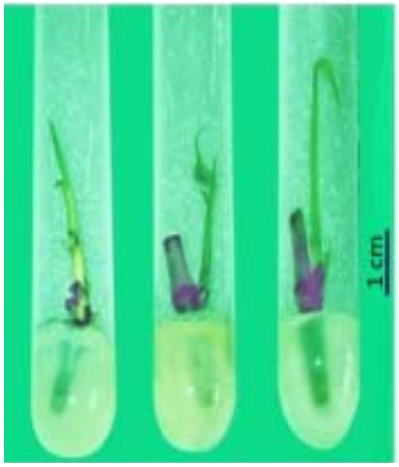


(A) BAP 5 µM NAA 1µM

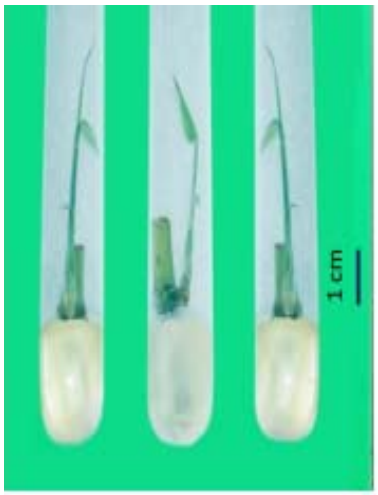
(B) BAP 7 µM NAA 1 µM



(C) BAP 9 µM NAA 1µM



(D) BAP 5 µM NAA 2 µM



(E) BAP 7 µM NAA 2µM

(F) BAP 9 µM NAA 2 µM

Plate 5.3 Shoot multiplication on MS media supplemented with 2 % sucrose (w/v) and Plant Growth Regulators

5.3. Experiment 3: Effects of different concentrations of 3 - Indolebutyric acetic (IBA) and 2% sucrose (w/v) concentration on *in vitro* rooting of *D. hamiltonii*

5.3.1 Effects of different concentrations of 3 - Indolebutyric acid (IBA) and 2% sucrose (w/v) concentration on survival percentage

The effects of different concentrations of 3- Indolebutyric acetic (IBA) and 2% sucrose concentration were influenced on *in vitro* rooting of survival percentage of *D. hamiltonii*. Based on the results of each treatment, T2 was the best but T3 was the poorest performance (Figure

5.5. a). There was highly significant effect of PGRs in variation of treatments in this study. Mean values of survival percentage ranged from 6.71 (67 percent) to 9.57 (96 percent) (Table 5.3).

5.3.2 Effects of different concentrations of 3 - Indolebutyric acid (IBA) and 2% sucrose (w/v) concentration on root formation percentage

The effects of different concentrations of IBA were also affected on root formation percentage. With regard to the performance of PGRs, T2 was found as the best among others and T3 was observed as the poorest for root formation performance (Figure 5.5. b). There was highly significant effect of PGRs in variation of treatments in this study. Mean values of root formation percentage ranged from 3.43 (34.3 percent) to 6.14 (61.4 percent) (Table 5.3).

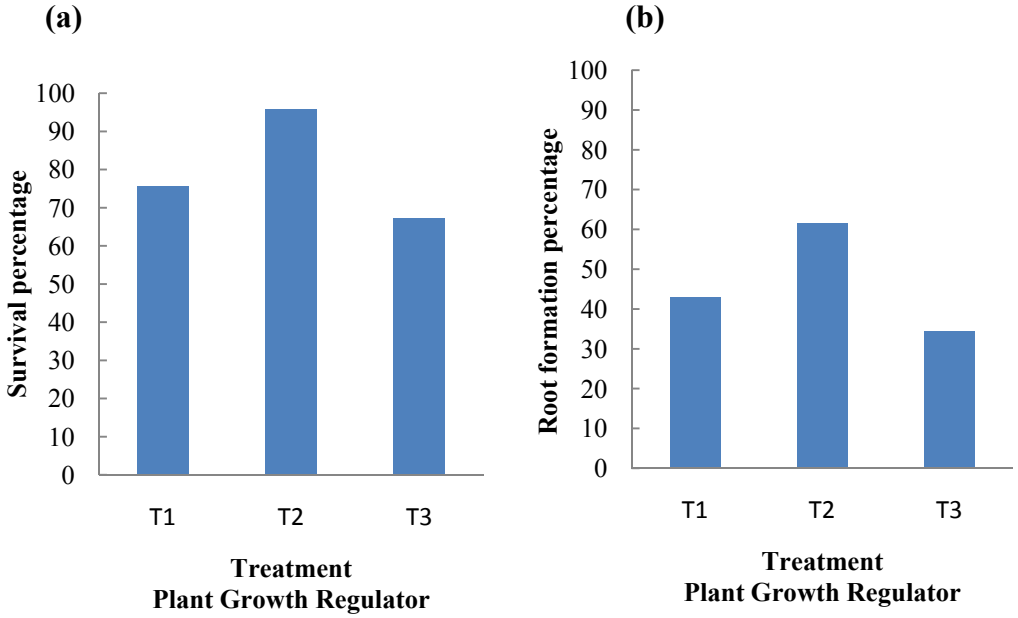


Figure 5.5 Effect of Plant Growth Regulators (PGRs) combination and 2% sucrose (w/v) concentration on (a) survival percentage and (b) root formation percentage of middle position of one year old stem at 2 weeks in *in vitro*

Table 5.3 Survival percentage and root formation percentage as affected by different levels of IBA and 2% sucrose (w/v) at 2 weeks in *in vitro*

Plant Growth Regulator Treatment	Survival percentage	Root formation percentage
T1	7.57 b	4.29 b
T2	9.57 a	6.14 a
T3	6.71 c	3.43 c
LSD (0.05)	0.25	0.34
Pr > F	0.001**	0.001**
CV %	2.74	6.40

** = highly significant

Table 5.4 Number of roots per explant, root length (cm) and root diameter (cm) as affected by different levels of IBA and 2% sucrose (w/v) at 6 weeks in *in vitro*

Plant Growth Regulator Treatment	**Number of roots per explant**	**Root length (cm)**	**Root diameter (cm)**
T1	1.39 a	2.94 a	0.15 a
T2	1.6 ab	3.18 a	0.11 b
T3	1.74 a	3.15 a	0.14 a

Pr > F	0.08 ^{ns}	0.21^{ns}	0.02*****
CV %	16.83	8.31	15.64

^{ns} = not significant, * = significant

5.3.3 Effects of different concentrations of 3 - Indolebutyric acid (IBA) and 2% sucrose (w/v) concentration on number of roots per explants

The effects of different concentrations of IBA were influenced on number of roots per explant of *D. hamiltonii*. According to the ANOVA, the result showed that no significant effect of different concentrations of IBA and 2 % sucrose (w/v) concentration on number of roots per explant (Table 5.4).

5.3.4 Effects of different concentrations of 3 - Indolebutyric acetic (IBA) and 2 % sucrose (w/v) concentration on root length (cm)

The effects of different concentrations of IBA were also described on root length (cm) of *D. hamiltonii*. According to the ANOVA, the results obtained that no significant difference at ($P > 0.05$) was observed in root length as affected by different plant growth regulators (Table 5.4).

5.3.5 Effects of different concentrations of 3 - Indolebutyric acetic (IBA) and 2% sucrose (w/v) concentration on root diameter (cm)

The effects of different concentrations of IBA were described on root diameter (cm) of *D. hamiltonii*. According to the ANOVA, the results indicated that significant ($p < 0.05$) differences among treatments were also found for root diameter. Based on the results of each treatment, T1 was found the best performance followed by T3. T2 was the poorest performance among the treatments. Mean values of roots diameter ranged from 0.11 to 0.15 (cm). (Table 5.4)

(A) IBA 80 µM

(B) IBA 100 µM

(C) IBA 120 µM

Plate 5.4 Roots formation on MS media supplemented with various concentration of IBA and 2% sucrose (w/v) after two month

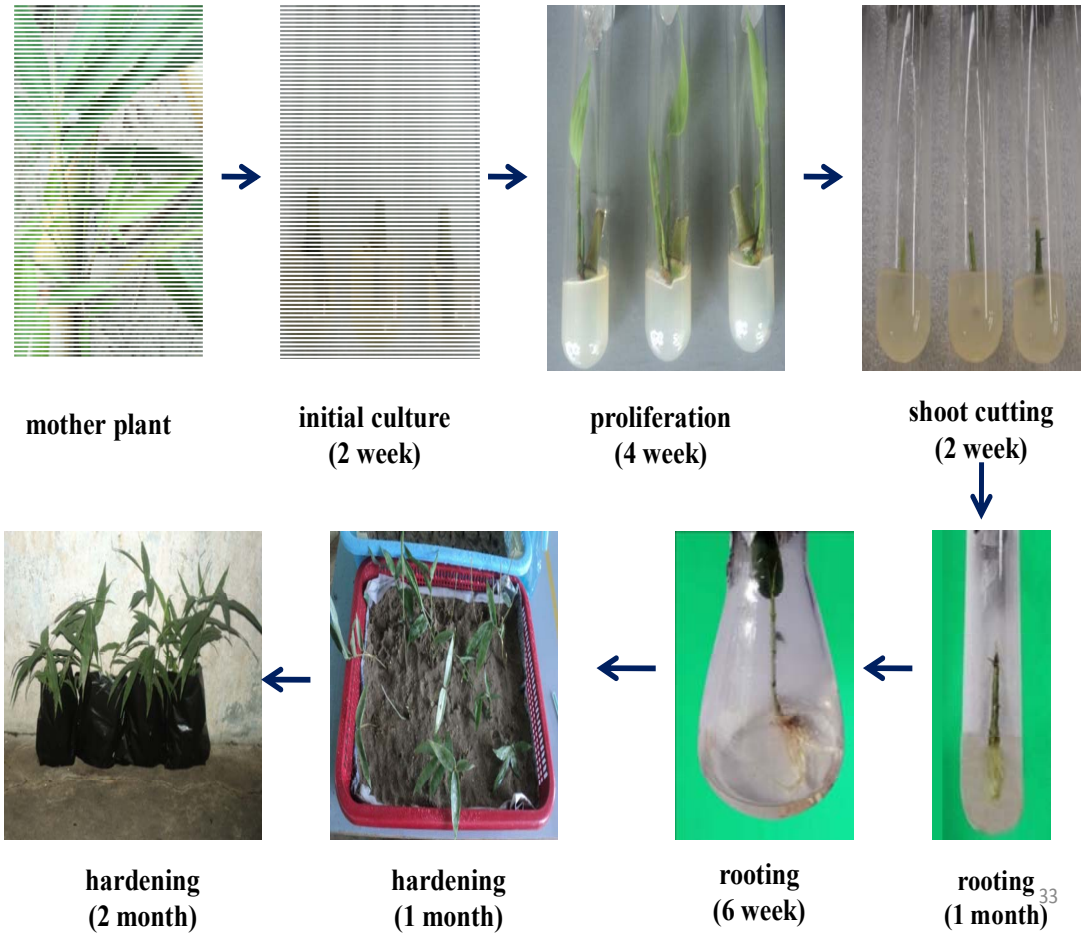


Plate 5.5 Procedure established from initial stage to acclimatization stage of *D. hamiltonii*

6. Conclusions and Recommendations

6.1 Conclusions

According to the results in this study showed one year old of middle position of nodal segments with axillary bud as effective means to propagate. Plant Growth Regulators (PGRs) were found to significantly the efficiency of *in vitro* regeneration depending upon the type of this species studied. Among the specific conclusions drawn from this study include:

1: Survival percentage of *D. hamiltonii* micropropagation was affected by $HgCl_2$ concentration and duration in initial culture stage, $HgCl_2$ 0.2 % for 10 minutes was increased in survival percentage. Shoot formation percentage was also dependent on $HgCl_2$ concentration and duration.

2: The results of the comparative assessment revealed that hormone free medium showed better in growth performance at survival percentage and shoot formation percentage. It would serve as general guideline reduces the production cost.

3: Based on the results of this experiment, combination of BAP 9 μM and NAA 1 μM was found the best followed by without hormone medium generally performed better than others at shoot diameter.

4: Optimal root formation percentage and survival percentage were revealed in IBA 100 μM medium in two weeks and continuous applied hormone free medium.

5: There were significant between the different level of IBA concentration in 80 and 100 μM media. When treating with 80 μM IBA medium was observed that the best and 100 μM IBA medium was showed that the poorest performance in root diameter.

6.2 Recommendations

Based on results obtained in the present study, the following recommendations are made and should be considered for future research work.

1: Other tests such as various segments from less than one year old should be tested.

2: Effect of Mercuric Chloride ($HgCl_2$) for some duration on sterilization performance of other bamboo species of nodal segments should be tested in more detail.

3: Control treatment for shoot multiplication and sand media for rooting should be carried out.

4: Effect of sucrose content on shoot multiplication and rooting performance should be further investigated.

5: Further experimentation for sterilization of *in vitro* culture with full facilities is needed.

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APPENDIX

Appendix 1. Composition of full and half strength of Murashige and Skoog's - 1962 (MS) medium.

Components	Concentration in medium ($gm.L^{-1}$)	
	FMS	**HMS**
NH_4NO_3	1.65	0.825
KNO_3	1.90	0.95
$CaC1_2.2H_2O$	0.44	0.22
$MgSO_4.7H_2O$	0.37	0.185
KH_2PO_4	0.17	0.085
$FeSO_4.7H_2O$	27.8	13.9
NA_2 EDTA	37.3	18.65
H_3BO_3	6.2	6.2
$MnSO_4.4H_2O$	22.3	22.3
$ZnSO_4.4H_2O$	8.6	8.6
KI	0.83	0.83
$Na_2MoO_4.2H_2O$	0.25	0.25
$CuSO_4.5H_2O$	0.025	0.025
$CoCl_2.6H_2O$	0.025	0.025
Nicotinic acid	0.5	0.5
Pyridoxin. HCl	0.5	0.5
Thiamine. HCl	0.1	0.1
Glycine	2	2
Myoinositol	0.1	0.1