In Vitro Regeneration From Protocorms In *Dendrobium nobile* Lindl.

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Abstract

An experiment was conducted on *in vitro* regeneration of *Dendrobium nobile* Lindl. The protocorm like bodies (PLBs) of *Dendrobium nobile* Lindl. were obtained from Than Lwin Orchid Garden, Bahan Township, Yangon Region. This experiment was carried out in the tissue culture laboratory, Htone Bo Agricultural Research Farm Taunggyi, Department of Agricultural Research during January to April 2023. In this paper, the plantlets were initiated from Protocorm likes bodies *Dendrobium nobile* Lindl. The uniformly developed protocorms (*in vitro* origin) having shoot initials were cultured on Murashige and Skoog (MS) medium, supplemented with various combinations of coconut water, BAP and NAA. Six treatments such as T_1 (MS only), T_2 (MS + Coconut water 200ml/1liters), T_3 (MS + 1mg.L^{-1} B AP), T_4 (MS + 3mg.L^{-1}BAP), T_5 (MS + 1mg.L^{-1}BAP + 0.1mg.L^{-1}NAA) and T_6 (MS + 3mg.L^{-1}BAP + 0.1mg.L^{-1}NAA) were undertaken. The research was laid out in a Completely Randomized Design (CRD), 6 treatments of medium type for shoot induction and 5 replication, each was represented by one bottle with one explants. Length of plantlets (cm), number of shoot emergence and number of leaves per explants were measured in after culture. The highest Length of plantlets, number of shoots and leaves were found when 3mg.L^{-1} BAP (T_4) was supplemented into MS medium. From the present result, it may be recommended that MS medium supplemented with 3mg.L^{-1} BAP (T_4) may be used for rapid shoot induction and regeneration of *Dendrobium nobile* Lindl.

Keywords: Regeneration, *Dendrobium nobile*, protocorm-like body

Introduction

The genus *Dendrobium* is known for its commercial valueas cut flowers in most countries and as traditional medicine of China and India. Several ethno medicinal properties (viz. anti-diabetic,anti-pyretic, immune regulatory, anti-cancer, cure to skindisease, anxiety, panic and stomach ache) and important phytochemicals compound (viz. benzyl derivatives, phenanthrenederivatives, alkaloids, flavonoids, pigments, sesquiterpenoids, anti-tumour, anti-mutagenic and anti-pyretic) have beenrecorded and reviewed in many *Dendrobium* species (Teixeira, 2013)

Protocorm-Like Bodies (PLBs) are also widely used in the orchid industry as it can be multiplied indefinitely and also grows into a complete plantlet. PLB is defined as a spherical tissue mass that resemble the early stage of orchid embryo development which is induced from initial explants. A large number of PLB can be obtained within a short period of time when appropriate medium was used. Each formed PLBs can be further cut into pieces and subcultured to give rise to several protocorm for further multiplication. They can proliferate and regenerate rapidly then grow into complete plantlets when cultured on appropriate medium and placed at proper cultural conditions(Arditti, 2008).

The aims and objectives are to investigatethe plantlet production from protocorm-like bodies (PLBs) potentially *in vitro*, to study regeneration of the *Dendrobium cariiniferum* in *in vitro* propagation, to attempt transplanting of cultured plantlets from glass vials to the pot and to grow the micropropagated plants in the natural environment.

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Materials and methods

Source of Plant material

The protocorm like bodies (PLBs) of *Dendrobium nobile* Lindl. (Daung-myi-thitkwa) were obtained from Than Lwin Orchid Garden, Bahan Township, Yangon Region.

Figure1. Protocorm like bodies of *Dendobiumnobile*

Collection and Identification of *Dendrobium nobile* Lindl.

An epiphytic orchid *Dendrobium nobile* Lindl. was collected from Taunggyi area.

Subculture

The PLBs of *Dendrobium nobile* Lindl. were sub-cultured until the plantlets.As the protocorm explants were obtainedthrough *in vitro* cultures, no sterilization was required.

Protocorm like bodies (PLBs) were cultured on MS (Murashige and Skoog, 1962) medium for two month and then the germinated plantlets of *Dendrobium nobile* Lindl. until the length of plantlets size approximately 0.2 cm. The plantlets of *Dendrobium nobile* Lindl. were excised from in a culture room. The plantlets were cultured into prepared culture bottles.

Culture Bottles

All culture bottles were washed with soap detergent, then rinsed throughly with hot distilled water. Then they are dried in oven at 110°C for 30 minutes. Culture bottles with the capacity of 50 ml each containing 20 ml of nutrient medium were used.

Culture condition

All culture were aseptically maintained at 25 ± 2°C under continuous illumination of about 120 foot candles from 4- feet white fluorescent tube and 25%-35% relative humidity. There experiment were carried out at the tissue culture laboratory, Htone Bo Agricultural Research Farm Taunggyi, Department of Agricultural Research during January to April 2023.

Preparation of culture medium

The composition of culture medium were listed in table 1.

Table 1. Composition of MS (Murashige and Skoog, 1962) Medium

	Components		Amount per liter (mg/l)
Macronutrients	1.	NH_4NO_3	1650
	2.	KNO_3	1900
	3.	$CaCl_2\ 2H_2O$	440
	4.	$MgSO_4\ 7H_2O$	370
	5.	$K_2H_2PO_4$	170
	6.	$FeSO_4 7H_2O$	27.8
		Na_2EDTA	37.3
Micronutrients	7.	$MnSO_4\ 4H_2O$	22.3
		$ZnSO_4\ 7H_2O$	8.6
		H_3BO_3	6.2
		KI	0.83
		$CuSO_4\ 5H_2O$	0.025
		$Na_2MoO_4 2H_2O$	0.25
		$CoCl_2 2H_2O$	0.025
	8.	Nicotinic acid	0.1
		Pyridoxin HCl	0.1
		Thiamine HCl	0.02
		Glycine	0.4
	Sucrose		30g
	Agar power		6g
	pH		5.7

Method of Media Preparation

According to the Murashige and Skoog (1962), method of 1000ml medium preparation applied in the present study is as follows;

1. 300ml distilled water was poured into 3000ml beaker.
2. Proper amount of stock solution were added.
3. Sugar 30g was added and stirred.
4. Agar powder 6 g was slowly added and stirred.
5. Before the addition of agar, the pH value was adjusted to be 5.7 with 0.1N NaOH and 0.1N HCl
6. The volume of solution was made up to 1000ml and the level was marked.
7. The solution was gently heated until it starts to boil.
8. Then medium was heated and stirred until agar was completely dissolved and becomes amber-colored.
9. The medium was dispensed into culture bottles.
10. The culture bottles were covered and autoclaved.
11. The media were sterilized in an autoclave at 120-121°C and 1.2 kg/cm^2 for 30 minutes.
12. Then the bottles were cooled and used.

Experimental Design

The experiment was laid out using Completely Randomized Design (CRD) with five replications. Total of six treatments were included in the study. The treatments were T_1 (MS only), T_2 (MS + Coconut water 200ml/1liters), T_3 (MS + 1mg.L^{-1} B AP), T_4 (MS + 3mg.L^{-1}BAP), T_5 (MS + 1mg.L^{-1}BAP + 0.1mg.L^{-1}NAA) and T_6 (MS + 3mg.L^{-1}BAP + 0.1mg.L^{-1}NAA), respectively.

Data Collection and Statistical Analysis

Data were collected at weekly intervals. The length of plantlets (cm), number of shoot emergence and number of leaves per explants were measured in the study. The collected data were statistically analyzed using IRRISTAT (Version 403) developed by International Rice Research Institute (IRRI), the Philippines. All treatments means were compared using least significant difference (LSD) at 5 % level of significance (Gomez and Gomez, 1984).

Results

Scientific Name : *Dendrobium nobile* Lindl.
Myanmar name : Daung-myi-thitkwa
Family : Orchidaceae
Distribution : Kachin State, Kayin state, Mon State, Shan State
Flowering period : May – June

Figure 2. Habit of *Dendrobium nobile* Lindl.

Length of plantlets (cm)

The length of plantlets (cm)had significantly differences in *Dendrobium nobile* Lindl. plantlets among all treatments. Sixty days after culture, T_4 (MS+3mg.L^{-1}BAP) treated *Dendrobium nobile* Lindl. plantlets were the highest in plant height (1.35 cm) (Table 2 and Figure 3).

Table 2. Effect of different concentrations of various hormones onthe length of plantlets of *Dendrobium nobile* Lindl.

Treatments	Length of plantlets (cm)						
	14DAC	21DAC	28DAC	35 DAC	42DAC	49DAC	60DAC
T_1	0.46	0.36	0.63	0.33	0.33	0.33	0.43
T_2	0.53	0.53	0.53	0.57	0.57	0.57	0.70
T_3	0.93	1.0	1.03	1.03	1.03	1.03	1.17
T_4	0.77	1.10	1.10	1.10	1.10	1.10	1.35
T_5	0.77	0.80	0.77	0.80	0.87	0.87	0.93
T_6	0.67	0.97	1.00	1.03	1.03	1.03	1.13
F-test	ns	ns	ns	*	*	*	*
5% LSD	0.43	0.53	0.51	0.53	0.53	0.54	0.60
CV %	52.5	56.2	50.7	55.4	55.4	55.8	53.8

Each value represented the mean from 5 replications. Mean differences in each column was determined by LSD at 5% level of significant. ns = non significant, * = significant.

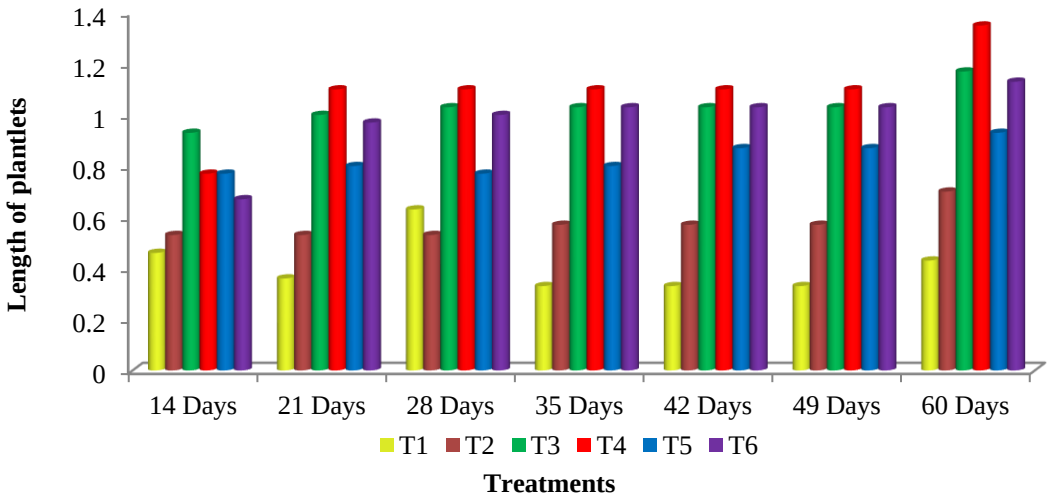


Figure 3. Effect of different concentrations of various hormones onthe length of plantlets of *Dendrobium nobile* Lindl.

Number of leave

The number of leaves increased with days after inoculation. Maximum number of leaves was obtained at 60 DAC from these treatments compared to control.

The highest number of leaves per explants (4.83) was noticed at 3mg.L^{-1}BAP, whereas the lowest one was (1.20) in control (Table 3 and Figure 4).

Table 3. Effect of different concentrations of various hormones onthe number of leave of *Dendrobium nobile* Lindl.

Treatments	Number of leave						
	14DAC	21DAC	28DAC	35 DAC	42DAC	49DAC	60DAC
T_1	1.10	1.27	1.90	1.17	1.17	1.17	1.20
T_2	1.53	1.57	1.63	1.63	1.63	1.63	1.77
T_3	2.60	3.17	3.50	3.70	3.70	3.70	3.73
T_4	2.33	3.03	3.10	3.23	3.23	4.13	4.83
T_5	2.30	2.63	2.87	3.53	3.53	4.43	4.45
T_6	1.53	2.47	2.73	2.87	2.87	3.47	3.58
F-test	ns	ns	ns	ns	ns	*	ns
5% LSD	1.59	1.82	1.82	2.21	2.21	2.46	2.37
CV %	70.4	65.1	58.5	69.3	69.3	67.0	60.5

Each value represented the mean from 5 replications. Mean differences in each column was determined by LSD at 5% level of significant. ns = non significant, * = significant.

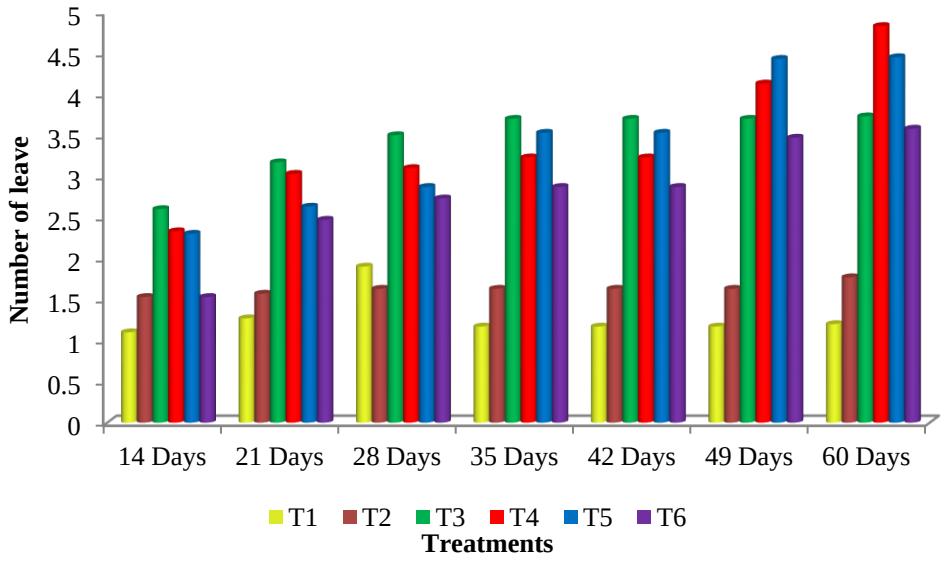


Figure 4. Effect of different concentrations of various hormones onthe number of leave of *Dendrobium nobile* Lindl.

Number of shoot

There was a significant influence of different concentrations of BAP found in the number of shoots per explants initiated in this experiment.

It was observed that MS medium supplemented with 3mg.L^{-1}BAP showed highest number (2.10) of shoot induction at 56 days after inoculation, whereas the lowest number of shoots (0.38) at 60 days was found with hormone free medium in control (Table 4 and Figure 5).

Table 4. Effect of different concentrations of various hormones onthe number of shoot of *Dendrobium nobile* Lindl.

Treatments	Number of shoot						
	14DAC	21DAC	28DAC	35 DAC	42DAC	49DAC	56DAC
T_1	0.00	0.10	0.10	0.10	0.10	0.10	0.38
T_2	0.00	0.33	0.10	0.16	0.20	0.30	0.66
T_3	0.00	0.23	0.33	0.40	0.40	0.40	0.90
T_4	0.00	0.30	0.43	0.56	0.66	1.17	2.10
T_5	0.00	0.13	0.20	0.23	0.23	0.50	0.63
T_6	0.00	0.13	0.27	0.26	0.26	0.43	0.53
F-test	ns	ns	ns	ns	ns	ns	*
5% LSD	0.00	0.27	0.40	0.49	0.56	0.93	0.82
CV %	0.0	148.0	143.4	143.3	152.9	163.0	79.5

Each value represented the mean from 5 replications. Mean differences in each column was determined by LSD at 5% level of significant. ns = non significant, * = significant.

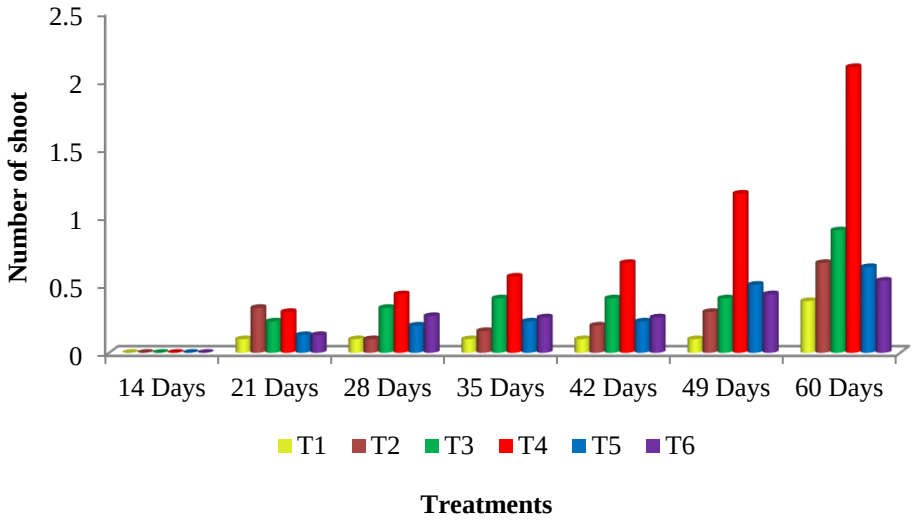


Figure 5. Effect of different concentrations of various hormones onthe number of shoot of *Dendrobium nobile* Lindl.

Discussion and Conclusion

The plantlets of *Dendrobium nobile* Lindl. were cultured in MS medium supplemented with different combinations and concentrations of growth regulators coconut water 200ml/1liters, 1mg.L^{-1} BAP, 3mg.L^{-1} BAP, 1mg.L^{-1}BAP + 0.1mg.L^{-1}NAA and 3mg.L^{-1}BAP + 0.1mg.L^{-1}NAA.

These studies indicated that medium supplemented with appropriate concentration of BAP has increased the rate of shoot proliferation and plantlet formation. The plantlets were cultured on the basal media of MS (Murashige and Skoog, 1962) concentration of BAP (1mg.L^{-1} and 3mg.L^{-1}). The highest growth of plantlets and number of shoot was obtained 3mg.L^{-1} BAP in MS (Murashige and Skoog, 1962) medium.

Arditti (2008) stated that the success of early attempts to culture cells and tissue explants could be depend on the addition of plant growth regulators into the culture media. The potential effects of BAP on *Dendrobium* have been studied on shoot regeneration (Talukder *et al.,* 2003), flower induction (Nambiar *et al.,* 2012), protocorm like bodies (PLBs) regeneration and plantlet formation (Khatun *et al.,* 2010). These studies indicated that medium supplemented with appropriate concentration of BAP has increased the rate of shoot proliferation and plantlet formation.

In conclusion the studied orchids are most beautiful and having immense commercial value. *In vitro* protocorm like bodies (PLBs) derived propagation technique is a simple approach for multiplication of orchids. It could be effectively applied for large scale propagation intended for future conservation, reintroduction in their natural ecological niche and mercantile aspects.

The present investigation was carried out for the conservation of rare and endangered orchid species by mass propagation.

Hence, this in vitro seed germination technique by using hormonal supplement might be beneficial for the ex-situ conservation of this orchid species and fulfilling its commercial demand.

T_1 T_2 T_3

T_4 T_5 T_6

Figure 6. Plantlet regeneration and multiplication of *Dendrobium nobile* at 21 days after culture

T_1 T_2 T_3

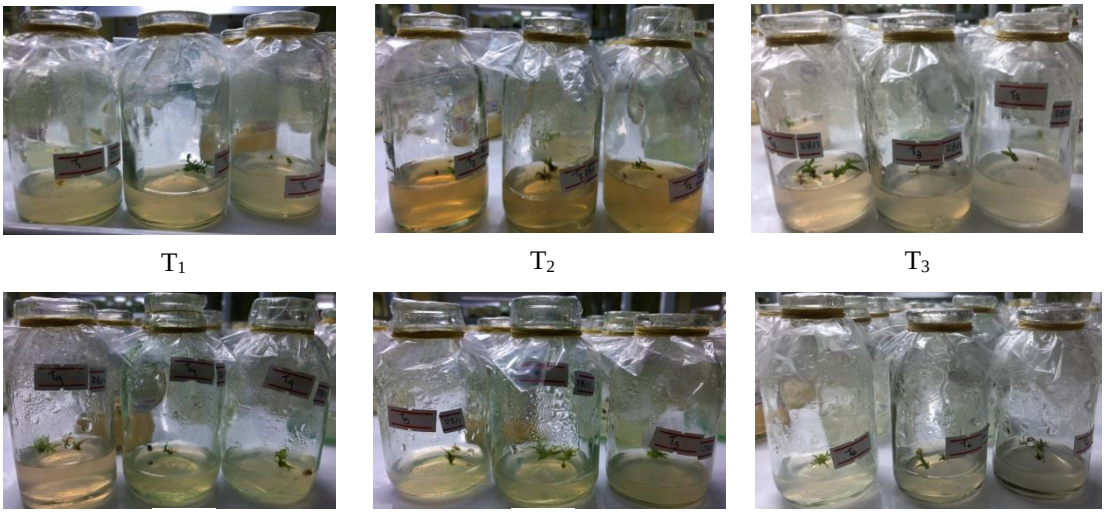
T_4 T_5 T_6

Figure 7. Plantlet regeneration and multiplication of *Dendrobium nobile* at 28 days after culture

T_1 T_2 T_3

T_4 T_5 T_6

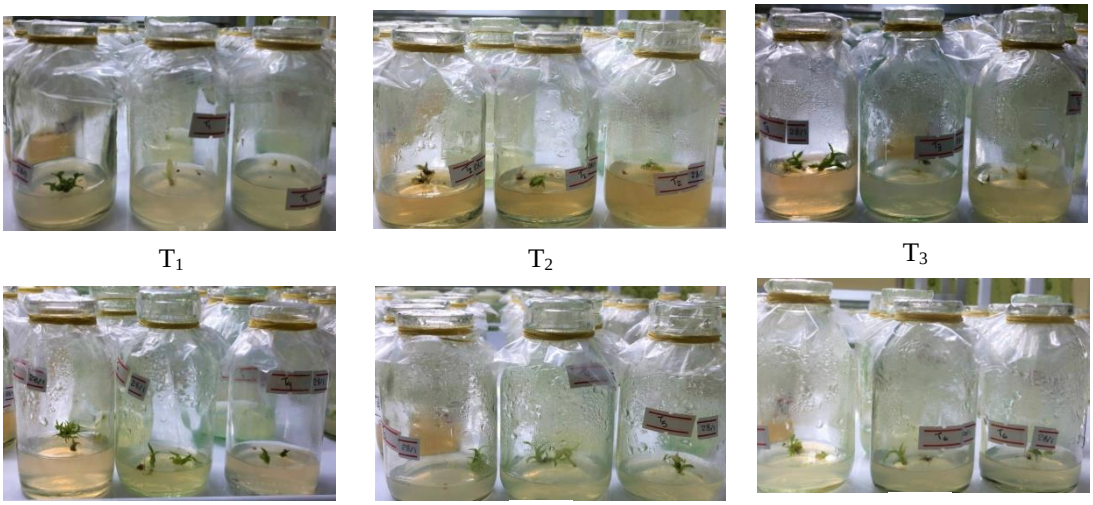
Figure 8. Plantlet regeneration and multiplication of *Dendrobium nobile* at 35 days after culture



T_1 T_2 T_3

T_4 T_5 T_6

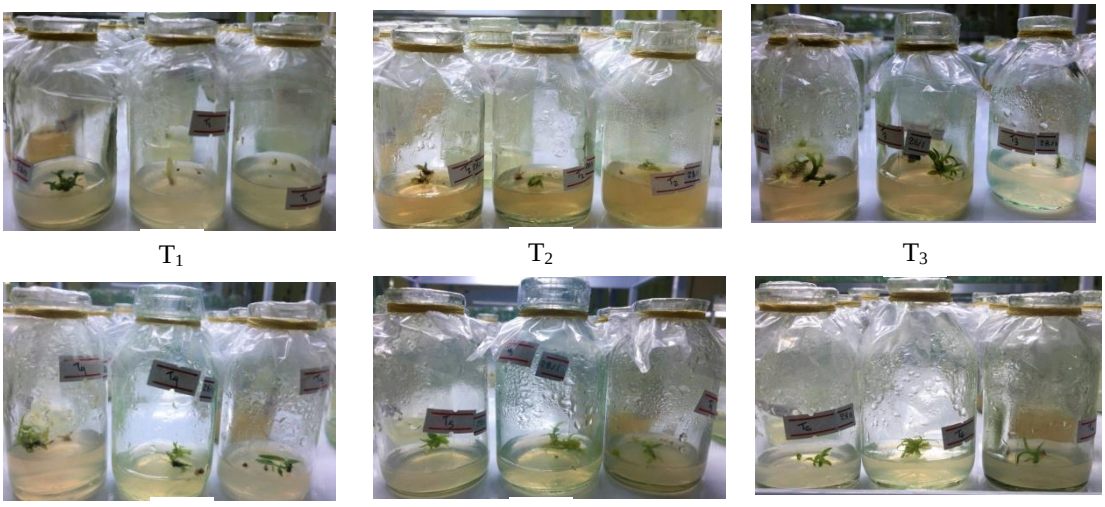
Figure 9. Plantlet regeneration and multiplication of *Dendrobium nobile* at 42 days after culture



T_1 T_2 T_3

T_4 T_5 T_6

Figure 10. Plantlet regeneration and multiplication of *Dendrobium nobile* at 49 days after culture



T_1 T_2 T_3

T_4 T_5 T_6

Figure 11. Plantlet regeneration and multiplication of *Dendrobium nobile* at 60 days after culture

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