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Study of commercially viable *Vanda coerulera* , *and Dendrobium chrysotoxum*, *D.devonianum* on multiplication stage from protocorms to plantlets by in vitro seed culture methods

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List of Abbreviation

1. CW= coconut water
2. AC= activated charcoal
3. BH= Banana Homogenenate
4. NAA= naphthaleneacetic acid
5. BAP= 6-benzylaminopurine
6. DAC= days after culture
7. KC= (Knudson, 1946) Basal medium of Knudson C
8. PDA= pollination days after
9. MS= Murashige and Skoog (1962)
10. DAP= days after pollination
11. WAPD= weeks after plantlets development

Study of commercially viable *D.chrysotoxum* Lindl, *D.devonianum* Rch.b.f, and *vanda coerulera* Griff ex Lindl on multiplication stage from protocorm to plantlet by *in vitro* culture methods

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ABSTRACT

Three kinds of orchid species; *Dendrobium chrysotoxum*, *D. devoniaum*, and *Vanda coerulera* were carried out multiplication stage of tissue culture process in the biotechnology laboratory of Forest Research Institute (FRI) , Forest Department. They produced a large number of protocorms (the portions that do not sprout leaves, shoots, and roots), and only a small number of plantlets. All three species were treated with basic hybridization stock solution (MS) and Hyponex. The parameters; weight, plantlet numbers, length, root sizes (maximum and minimum) were tested with six nutritional treatments. In this paper, it was found that T1 is the best treatment for fresh weight, number of plantlets, plantlet length, maximum and minimum (root size) of *Vanda coerulera* and *D. chrysotoxum*. In *D. devoniaum*, T3 is the best treatment for the parameters of weight and plantlet number, and T1 is the best media for the parameters of root sizes (maximum and minimum) and length of plantlets. This study supports to explore the nutritional proportions of the best media for each species in order to fulfill the aim of sustainable mass production for the targeted orchid species by *in vitro* seed culture method.

Key word: Media, Multiplication, Orchid, Tissue Culture.

စီးပွားရေးအရအလားအလာရှိသော မိုးလုံးမှိုင်း၊ ရွှေတူမောက်ခမ်းဝါနှင့် ကြောင်ခေါင်းပန်းများကို ပွားများမွေးမြူခြင်းအဆင့်မှ အပင်ငယ်အဆင့်သို့ ကူးပြောင်းခြင်းအား တစ်သျှူးနည်းဖြင့်လေ့လာခြင်း

နွယ်နွယ်ဝင်း၊ သုတေသနလက်ထောက်-၂
အိအိဇင်၊ သုတေသနလက်ထောက်-၂
ခင်ပပရွှေ၊ လက်ထောက်သုတေသနအရာရှိ

စာတမ်းအကျဉ်း

သစ်တောသုတေသနဌာန၊ ဇီဝနည်းပညာဓာတ်ခွဲခန်းအတွင်း သစ်ခွမျိုးစိတ်များကို တစ်သျှူးနည်းဖြင့် ပွားများမွေးမြူထားပါသည်။ ထိုပွားများမွေးမြူထားသော သစ်ခွမျိုးစိတ်များအနက် *Vanda corulera* တို့၏ Protocorm၊ *Dendrobium chrysotoxum* နှင့် *D.devoniaum* တို့၏ Protocorm (အရွက်၊ အညွှန့်နှင့် အမြစ် မထွက်သည့်အပိုင်း) အမြောက် အများ ဖြစ်ပေါ်နေပြီး Plantlets (အပင်ငယ်) ထွက်ရှိမှုမှာ အနည်းငယ်သာ ရှိနေပါသည်။ ၎င်းမျိုးစိတ် (၃)မျိုးအား အခြေခံမျိုးပွားစာ MS (Murashige and Skoog) နှင့် Hyponex ကို အသုံးပြုခဲ့ပါသည်။ အကောင်းဆုံး အခံဖျော်ရည် အမျိုးအစားနှင့် အကောင်းဆုံး မျိုးပွားစာ အာဟာရအချိုး အစားအား လေ့လာသိရှိစေရန် အပင်ငယ်များ၏အလေးချိန်၊ အပင်၏ အရေ အတွက်၊ အပင်၏အမြင့်၊ အပင်၏အကြီးဆုံးအမြစ် နှင့် အပင်၏အငယ်ဆုံး အမြစ် (အရွယ်) တို့ကို အာဟာရမျိုးပွားစာ (Treatment) (၆)မျိုးဖြင့် စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။ စမ်းသပ် မှုများအရ *Vanda coerulera* တွင် T1 သည်အပင်ငယ်၏အလေးချိန်၊ အပင်အရေအတွက်၊ အရှည်၊ အကြီးဆုံးနှင့် အငယ်ဆုံးအမြစ်(အရွယ်) တို့အတွက် အကောင်းဆုံးဖြစ်ကြောင်းနှင့် *D. chrysotoxum* တွင် T1 သည် အပင်ငယ်၏အလေးချိန်၊ အပင်အရေအတွက် ၊အပင်၏ အရှည်၊ အကြီးဆုံးနှင့် အငယ်ဆုံးအမြစ် (အရွယ်) တို့အတွက် အကောင်းဆုံး ဖြစ်ကြောင်း တွေ့ရှိခဲ့ရ ပါသည်။ ထို့အပြင် *D. devoniaum* သစ်ခွမျိုးစိတ်အတွက် T3 သည် အပင်ငယ်၏ အလေးချိန်၊ အပင်ငယ်၏ အရေအတွက်တို့တွင် အကောင်းဆုံးဖြစ်ကြောင်းနှင့် *D.devoniaum* သစ်ခွမျိုးစိတ်အတွက်T1သည်အပင်၏ အကြီးဆုံးအမြစ်၊ အပင်၏အငယ်ဆုံးအမြစ် (အရွယ်) နှင့် အပင်ငယ် ၏ အရှည်တို့တွင် အကောင်းဆုံးဖြစ်ကြောင်း တွေ့ရှိခဲ့ရပါသည်။ လေ့လာတွေ့ရှိချက်များအရ သစ်ခွမျိုးစိတ်များ အား ထာဝစဉ်တည်တံ့စေရန်နှင့် မျိုးရင်း မတူသော သစ်ခွမျိုးစိတ်များကို တစ်သျှူးနည်းဖြင့် မျိုးစိတ်တစ်ခု

ချင်းစီ၏ အပင်ငယ်အမြောက်အများ ထုတ်လုပ်နိုင်ရန်အတွက် အကောင်ဆုံးမျိုးပွား စာအာဟာရ အချိုးအစားကို သိရှိခဲ့ပါသည်။

Study of commercially viable *Vanda coerulera, and Dendrobium chrysotoxum*, *D.devonianum* on multiplication stage from protocorms to plantlets by in vitro seed culture methods

1. Introduction

There are many genera and species in the family Orchidaceae with 600 - 800 genera and 25,000 – 35,000 species (De, L.C., & Medhi, R.P, 2015). According to the Myanmar Forest Department, (841) native orchids have been officially recorded (Tun, N. 2014). Although a lot of orchid species have already grown in the various environmental conditions, orchids are very sensitive to habitat degradation. Certain species of orchids are now severely depleted due to widespread deforestation through excessive logging, forest fire, over-use of natural resources, over-collection, the loss of suitable habitats and the growing size of human populations altering landscapes in order to use the land for buildings. Therefore, the number of orchids has been decreasing. Moreover, some orchid species are rare and endangered.

Plants belonging to Orchidaceae are listed in International Union of Conservation of Nature (IUCN) Red List of Threatened Species. In fact, *Vanda coerulera*, *Dendrobium chrysotoxum* and *D.devoninum* have been recently included in Appendix II of nearly threatened species of plants and animals (CITES, 2019).

Vanda coerulea Griff ex Lindl is popularly known as blue Vanda due to its blue color flower, a unique characteristic, is an epiphytic orchid with monopodial growth. The flowers are large in size and have beautiful color and the blue color being rare in the orchid word, is famous worldwide in hybridization (Roy et al., 2011). *Vanda coerulea* Griff ex Lindl can be found as the full bloom plant throughout the entire season.

Dendrobium is the second largest genus of Orchidaceae Family (Publishers, Gayathri publishers, & M. Maridass, 2010). It is the member of Simpodiod group. *D.* species can be grown almost throughout Myanmar. Although *Dendrobium* species are epiphyte, some can be grown on lime stones and rocks. Among them, *D. devoianum* of 1/5 inches in diameter and 2 feet in length stem is flexible and brown (Tun, N. 2014). Although the primary stem stands erect, it hangs downwards as the stem is small and long. The stem has nodes with 4 inches and long leaves that resemble grasses (Tun, N. 2014). A foliage leaf sprouts out per node. After the leaves fall, a single flower blooms per node. Sepals and petals are white with mauve -coloured rim (Tun, N. 2014). The lip is large with 2 yellow marks on white-color and the edge is covered with mauve-coloured soft hairs. The 10-15 flowers per plant blooms by hanging down (Tun, N. 2014).

D. chrysotoxum has stocky pseudobulb that like a greenish-yellow mace which is 6 inches to 1-foot-high inflorescence emerges among the leaves at tip and produces 15-20 flower (Tun, N. 2014). The stem with strips at the upper and the lower parts is yellow. At the top of the stem, there are 4-6 leaves which are 6-8 inches long and 1-inch-wide (Tun, N. 2014). The inflorescence having the length of 6 inches to 1 foot emerges among the leaves at the tip and produces 15-20 flowers. Sepals and petals are golden yellow and the lip tip is dark orange-yellow.

Effective conservation strategies should be devised to prevent further loss of the already depleted orchid population. The *in vitro* approach through the application of plant

tissue culture technology provides an excellent opportunity for effective conservation by mass-propagating orchids within a certain amount of time. Therefore, *Vanda coerulea, Dendrobium devonianum* and *D. chrysotoxum* were tested the multiplication process in the laboratory. These are the reasons why this research was conducted in order to evaluate the best possible way for mass production.

1.1 Literature Review

Distribution and identification features of Orchidacea *Vanda coerulera* Griff ex Lindl, *Dendrobium chrysotoxum* and *D. devoniaum*

Among monopodial orchid, *Vanda coerulea* Griff ex Lindl is popularly known as Blue Vanda due to its blue color flower, a unique characteristic, is an epiphytic orchid with monopodial growth habit and is commonly occurred at elevations of 1000-15000m (Roy, et al. 2011). The flowering time of the orchid is in July and August (Wu, K., et.al, 2014). It is well-grown in Taunggyi, Kalaw, Loilem, Pindaya, YwaNgan, Hopone, Aung Ban, Yat Sauk of Shan region, and around Pyin Oo Lwin Naung Cho and Mogok of Mandalay Division (Tun, N. 2014).

Dendrobium is the second largest genus of Orchidaceae, which is composed of approximately 1500 species scattered in the world, and there are the most popular and high valued orchids in the market (Chen & Ji, 1998) & (Yu et al., 2015). In Myanmar, there are over 100 species of *Dendrobium* (Tun, N. 2014). *D. devoianum* can be seen naturally growing in Kachin state, Shan state, and blooms in August and September (Tun, N. 2014). It can be found out throughout Bago Division, Mandalay Division, Kachin state, Chin state, and flowers blossom from March to May (Tun, N. 2014). *D. chrysotoxum* is mostly found at an elevation of 700 to 1200 m in deciduous forests of Northeast India, Bhutan, China, Thailand, and Laos (Kumar, S., et al., 2014). D.chrysotoxum is commonly known as "Golden orchid", and extremely popular in local floriculture market due to its arching inflorescence besotted with 15–20 highly attractive honey fragrant yellowish bright cultured flowers (Kumar, S., et al., 2014).

Effect of activated charcoal, tryptone, peptone, coconut water, and banana on the growth of plantlet

The intent was to determine the morphogenetic effect of activated charcoal (AC) on protocorm and subsequent plantlet growth (Roy et al., 2011). Organic additives such as tryptone, peptone, coconut water (CW), Banana Homogenenate (BH), and others are commonly added to orchid media to promote seed germination, seedling growth and rooting which generally consist of low molecular weight proteins, amino acids, vitamins and plant growth substances, which are able to enhance plant growth by providing plant cells with a readily available source of nitrogen (Wu et al., 2014). CW, when combined with peptone, was optimum for seed germination. 1/4 MS containing 20% CW and 1.0 gl^{-1} peptone was most suitable for germination than other concentrations of CW in combination (Wu et al, 2014)

Four different media were used as treatment and these were:

M1	=	Hyponex medium (3 g L^{-1}Hyponex +8 g L^{-1} peptone + 2 g L^{-1} charcoal + 8 g L^{-1} Agar + 30 g L^{-1} Sucrose)
M_2	=	MS medium, Murashige and Skoog (1962)
M_3	=	OKF medium (3 g L^{-1} OKF +8 g L^{-1} Peptone + 2 g L^{-1} Charcoal + 8 g L^{-1} Agar + 30 g L^{-1} Sucrose)
M_4	=	Knudson C medium (Knudson, 1964), used as check (Alam, Rashid, Hossain, Salam, & Rouf, 2002).

These four different media were used from the article entitled " *In vitro* Seed Propagation of Dendrobium (*Dendrobium transparens*)." (Alam, et al, 2002).

Combination effect of NAA, and BAP for *Vanda* species.

The protocorms from *in vitro* germination cultured on KC basal media with addition of NAA and BAP combinations. The combinations of these hormones invariably induced multiple new protocorms formation to a variable extent. After 45 days of culture, protocorms started to initiate first shoot. Proliferation mostly occurred after protocorms develop its first shoot (Decruse et al., 2003). Treatment with 2.0 mg l-1 BAP + 0.5 mg l-1 NAA gave an earliest response to proliferate at 60 DAC with proliferation percentage of 65 ± 20.88%, and average production of 3 ± 0.77 new protocorms per responsive protocorms. First shoot of the new protocorms was observed at 90 DAC with 0.5 -1.0 mm length (Decruse et al., 2003). The new protocorms was then producing it second leaf after 120 days of culture. After 180 days of culture, all treatments showed 100% proliferation but differ in production of new protocorms. Treatment with 2.0 mg l-1 BAP + 0.5 mg l-1 NAA also produced an average of 9 ± 2.2 new protocorms per explant(Decruse et al., 2003). By now the new protocorms produce of two leaves with 2 -3mm length (Decruse et al., 2003). The combinations of NAA and BAP are also well-known to induce enhanced bud proliferation in shoot tips of Cattleya (Melissa et al., 1994), shoot tips and flower stalk buds of *Phalaenopsis* and *Doritaenopsis* (Tokuhara and Mii, 1993), foliar meristem of *Renanthera imshootiana* and *V.coerulea* (Seeni and Latha, 1992, 2000) and axillary buds from stem node of *V.spathulata* (Decruse et al., 2003).

Culture multiplication for *D.chrysotoxum* (Lindl.)

Protocorm (2 mm long) sourced from 26 weeks old cultures, maintained on basal 1/2MS medium, were used as explants Saraanjeet & K,et, al 2011).. The complex growth supplement such as banana Homogenenate (BH-25gl-1, 50gl-1, and 75gl-1w/v, were used individually in the medium. Banana Homogenenate was obtained from ripened fruits which purchased from market Saraanjeet & K,et, al 2011). Required quantities of pulp was weighted, homogenised in a blender and added to the medium Saraanjeet & K,et, al 2011).. All the media, except undefined medium i.e. PDA, was fortified with sucrose (Daurala sugar Works, India) as source of nutrition and gelled with 8% (MS and 1/2MS) and 0.9% (KC and M) agar

powder (Hi Media). The PH of the media was adjusted to 5.7 after adding the growth regular and organic growth supplement and dispensed in the test tube of size (25mm x 150mm) Saraanjeet & K,et, al 2011). The media was autoclaved at 120°C for 20 min at 1.06 kg/cm2 at pressure of 1.06kg/2cm for 15 min. Autoclaved medium was kept at 37 C to check any further contamination. The above media components were used for the multiplication of *D.chrysotoxum* (Lindl.) (Saraanjeet & K,et, al 2011).

2. Objection

➢ To know the best proportion of stock solution type and the effect of hormone during the multiplication stage from protocorms to plantlets by *in vitro* seed technique.

3. Materials and Methods

In this experiment, protocorms of three native orchid species; (1) *Vanda coerulea* Griff ex Lindl, (2) *Dendrobium chrysotoxum*, (3) *Dendrobium devoninum* which derived from *in vitro* seed culture were used with a mean fresh weight of 0.31±0.02g. These protocorms were weighted using the two-digit electronic balance in a contaminant free laminar flow. Additionally, six different media were used as treatments and denoted as T1, T2, T3, T4, T5, and T6 respectively. Four media out of six were used MS medium (Murashige and Skoog), MS-based media with different proportions of chemical components in each.

Six different media were used as treatment and these were:

T1 = 1/4MS+0.001 mg l^{-1} α-naphthalen-eacetic acid (NAA)+ 10% coconut water (CW)+ 100 g l^{-1} ripe banana homogenate (BH)+ 1.0 gl^{-1} peptone+10 gl^{-1} sucrose+1.0 gl^{-1} activated charcoal (AC)+ 8 g l^{-1} agar (Wu et al., 2014).

T2 = 3 gl^{-1} Hyponex N06.5,6,19+2.0 gl^{-1} activated charcoal (AC)+ 8.0 gl^{-1} peptone +30 g l^{-1} sucrose+ 8 g l^{-1} agar (Alam,et al, 2002).

T3 = 1/2MS+30 gl^{-1} sucrose+20% coconut water (CW) + 6 g l^{-1} agar the regular media for multiplication stage in FRI lab.

T4 = 1/2MS+30 gl^{-1} sucrose+0.01 mgl^{-1} α-naphthalen-eacetic acid (NAA) + 15% coconut water (CW) + 8 g l^{-1} agar the regular media for multiplication stage in FRI lab.

T5 = 3 gl^{-1} Hyponex N06.5,6,19 + 20 gl^{-1} sucrose+1.0 gl^{-1}

peptone + 0.5 mg l^{-1} α-naphthalen-eacetic acid NAA+ 10% coconut water (CW)+ 150 g l^{-1} banana homogenate (BH)+ 8 g l^{-1} agar (Wu et al., 2014).

T6 = MS (basal media) + 30 gl^{-1} sucrose+8 g l^{-1} agar (Without hormone).

Moreover, the pH of the medium was adjusted to 5.8 before gelling with agar which was dissolved by boiling the medium. The molten medium was dispensed about 40ml into a sterile culture bottle (85 × 65 mm) and covered with aluminum foil. These culture bottles were autoclaved at 120°C for 20 min at 1.05 kg/cm^2 pressure. The culture vessels were incubated at 22 ± 2°C under an 8-h photoperiod of 336.1 Lux (fluorescent tubes-TL-D36W Philips, Snow White, Extreme Cool Daylight, made in POLAN). The study was carried out in a completely randomized design with three replications. Plantlet growth of *Dendrobium*

chrysotoxum, *D.devoninum* was assessed after four month period. Furthermore, plantlet growth of *Vanda coerulea* was assessed after six month period. After that period, the parameters of fresh weight, number of plantlets, plantlet height, maximum and minimum (root size) were assessed accordingly. All experiments consist of three independent replicates with 10 culture bottles per replicate, with 0.31±0.02g protocorm in each bottle. There replications were applied for each treatment.

Figure 1: Weight measurement of protocorms with electric 2-balance digital equipment

Figure 2: Light measuring instrument

3.1. Data analysis

Data was analyzed by using statistical product and service solutions (SPSS) version 20. All the variables for every treatment were compared by using Kruskal-Walls one way ANOVA (Non-Parametric Method). Pairwise comparison was applied to compare among treatments. The types of treatments are six for one species. Total numbers are (180) treatments for one species, and there are three replications for each treatment.

Figure 3: length and weight measurement for *Vanda ceorulera*

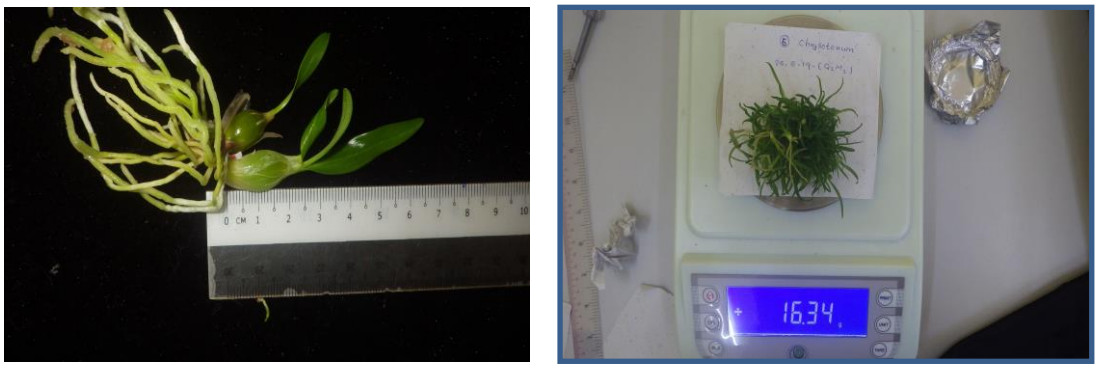

Figure 4: Measurement of length, root size and weight for *Dendrobium chrysotoxum*

Figure5: Length and weight measurement for *D.devoniaum*

4. Results and Discussion

4.1. Effect of different media on *Dendrobium chrysotoxum in vitro* seed culture

Table 1: Comparison on survival rate and capacity among treatments for three orchid species

Treat-ments	Moderate (%) of *V. coerulera*	Rooting (%) of *V. coerulera*	Moderate (%) of *D. hrysotoxum*	Rooting (%) of *D. chrysotoxum*	Moderate (%) of *D. devoniaum*	Rooting (%) of *D. devoniaum*
T1	93	73	67	73	70	70
T2	97	63	43	13	50	47
T3	93	50	57	40	70	70
T4	93	57	83	83	67	70
T5	97	87	83	83	60	60
T6	93	65	83	10	67	26

4.1.1. Fresh weight of plantlet for *Dendrobium chrysotoxum*

According to A Kruskal-Wallis test, there were significant differences in all treatments (Figure 6 & Table 2). There is a strong significant differences between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 6 & Table 2).The mean fresh weight of *Dendrobium chrysotoxum* is 7.57±4.25 in treatment T1 (Table 2). The effect of T1 and T5

is higher than that of other treatments (Figure 6, Table 2 & Table 3). By Dunn's pairwise tests, T1 is significantly higher than T2, T3, and T6 at the adjusted significant level ($p<0.005$) (Table 3). There was no evidence of a difference among the other pairs (Table 3).

D.chrysotoxum was tested with six treatments for the fresh weight as shown in the (Figure 6 & Table 2). Among them, T1 is the best treatment among the treatments in all tests (see Figure 7). However, T2 was the worst among the treatments in the fresh weight of *D.chrysotoxum* while (Alam,et al, 2002) found out that T2 is the second best media for the multiplication of *Dendrobium transparens* plantlets from the seed culture. This is because of the difference in species in both tests. T3 and T4 are the regular media for multiplication stage in FRI lab. T5 is better than T2, T3, T4, and T6 in fresh weight. The result is the same with the finding of (Wu et al., 2014) in which T5 was found to be most suitable for plantlet growth of *Renantherra*.

Table 2: Effect of six treatments for *D. chrysotoxum* according to respective parameters after the four-month period

Treatment	Fresh Weight (g)	Number of plantlet (plant)	Maximum Root Size (cm)	Minimum Root size (cm)	Height of plantlet (cm)
T1	7.43±268	101.90±33.99	4.00±1.03	1.85±1.145	69.23±25.79
T2	2.97±1.96	50.46±31.28	.562±1.51	.30±.97	27.94±18.57
T3	4.55±5.70	48.41±46.22	3.41±1.00	.94±.39	45.27±44.82
T4	4.30±1.89	40.88±21.69	1.90±.79	.62±.21	44.82±13.01
T5	7.86±5.98	64.28±41.85	3.68±1.87	1.05±.65	54.44±43.33
T6	3.57±2.51	15.27±21.51	.07±.14	.00±.01	3.67±5.24

Table 3: Effective of treatments on growth for the fresh weight of *Dendrobium chrysotoxum* after the four-month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T2-T6	-7.533	12.634	-.596	.551	1.000
T2-T3	-4.735	12.284	-.385	.700	1.000
T2-T1	41.600	11.878	3.502	.000	.007
T3-T6	-2.798	11.811	-.237	.813	1.000
T2-T5	-30.520	11.401	-2.677	.007	.111
T2-T4	-15.060	11.401	-1.321	.164	1.000
T6-T1	34.067	11.388	2.991	.003	.042
T3-T1	36.865	10.999	3.352	.001	.012
T6-T5	22.987	10.889	2.111	.035	.522
T6-T4	8.327	10.889	.765	.444	1.000
T3-T5	-25.785	10.481	-2.460	.014	.208
T3-T4	-11.125	10.481	-1.061	.289	1.000
T4-T1	25.740	10.002	2.573	.010	.151
T5-T1	11.080	10.002	1.108	.268	1.000
T6-T5	-14.660	9.430	-1.555	.120	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

(g)

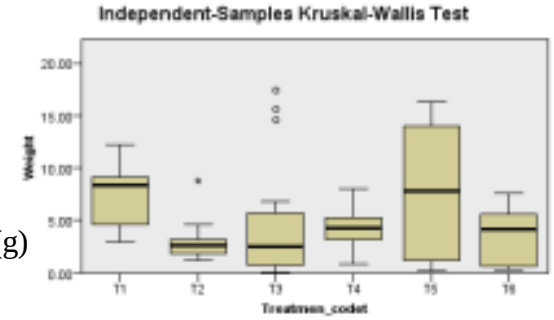

Figure 6: Effect of six treatments for the fresh weight of *Dendrobium chrysotoxum* after four-month period

4.1.2. The number of plantlets for *Dendrobium chrysotoxum*

According to A Kruskal-Wallis test, there were significant differences among treatment (Figure 7 & Table 2). There is a strong significant differences between the mean ranks of at least one pair of groups ($p < 0.001$) Table (Figure 7 & Table 2). The mean plantlets number of *Dendrobium chrysotoxum* is 101.90±33.99 in treatment T1 T (Table 2).

By Dunn's pairwise tests, T1 is significantly higher than that of T6, T4, T3 and T2 at the adjusted significant level ($p<0.005$) Table (4). T5 is significantly higher than T6 at the adjusted significant level ($p<0.005$) Table (4). There was no evidence of a difference between the other pairs Table (4).

In *D. chrysotoxum*, comparing the number of plantlets among all the treatments, T1 is the most suitable treatment for the number of plantlets among all the other treatments. The result shows the same outcome with the finding of (Wu et al., 2014) in which T1 was significantly higher than all other treatments or the highest percentage of the number shoot for 75 days culture of *Renantherra imschootiana* Rolfe growth. Moreover, T5 is more proper than T2, T3, T4, and T6 (basal media) as shown in (Figure 7 & table 4). The result is the same with the finding of (Wu et al., 2014) in which T5 was found as the most suitable for plantlet growth *in vitro* since it resulted in the number of shoots for 150 day culture of *Renantherra imschootiana* Rolfe growth in vitro was significantly affected by NAA and BH concentrations (Wu et al., 2014).

The latter treatment (T2) is the least nutrient giving medium for the plantlets number in *D. chryosotoxnum* while (Alam, et al, 2002) found out that T2 is the second best media for the multiplication of *Dendrobium transparens* plantlets from the seed culture. T3 and T4 are the regular media for the multiplication stage in FRI lab. The result of T2 is the same with the finding of (Alam M., et al., 2002) in which the maximum survival plant (76%) for *Dendrobium transparens* was observed in MS medium that was statistically similar to that of Hyponexmedium or T2 (71%) (Alam M., et al., 2002).

Table 4: Effectiveness of treatments on growth for the number of plantlets of *Dendrobinum chrysotoxum* after the four-month period

Each node shows the sample average rank of Treatmen_codet.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T4	27.660	10.887	2.531	.011	.170
T6-T3	29.847	11.809	2.527	.011	.172
T6-T2	34.777	12.632	2.753	.006	.089
T6-T5	43.780	10.887	4.021	.000	.001
T6-T1	71.000	11.386	6.236	.000	.000
T4-T3	2.287	10.479	.218	.827	1.000
T4-T2	7.217	11.399	.633	.527	1.000
T4-T5	-16.220	9.429	-1.720	.085	1.000
T4-T1	43.440	10.001	4.344	.000	.000
T3-T2	4.930	12.282	.401	.688	1.000
T3-T5	-13.933	10.479	-1.330	.184	1.000
T3-T1	41.153	10.997	3.742	.000	.003
T2-T5	-9.003	11.399	-.790	.430	1.000
T2-T1	36.223	11.876	3.050	.002	.034
T5-T1	27.220	10.001	2.722	.006	.097

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

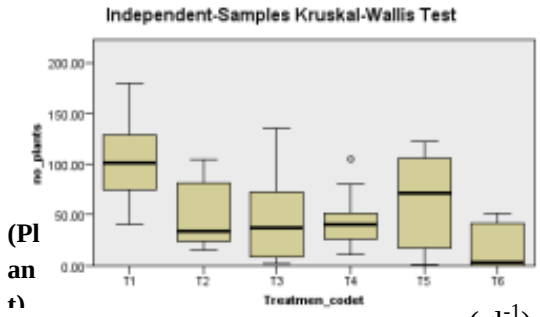

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Figure 7: Effect of six treatments for the number of plantlets of *Dendrobinum chrysotoxum* after four-month period

4.1.3. The maximum root size of plantlets for *Dendrobium chrysotoxum*

According to A Kruskal-Wallis test, there were significant differences among treatments (Figure 8 & Table 2). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 8 & Table 2). The mean maxim root size of *Dendrobium chrysotoxum* is 4.00±1.03 in treatment T1 Table (2). By Dunn's pairwise tests, T1 is significantly higher than T6, T2, and T4 at the adjusted significant level ($p<0.005$) (Table 5). T5 is significantly higher than T2, T4, T3, and T6 at the adjusted significant level ($p<0.005$) Table (5). T3 is significantly higher than T2 and T6 at the adjusted significant level ($p<0.005$) Table (5). There was no evidence of a difference between the other pairs Table (5).

In *D. chrysotoxum*, comparing the maximum root-size among six different treatments, T1 was found as most suitable treatment for maximum root size among all the treatments (see Figure (8).The result shows the same outcome with the finding of (Wu et al., 2014) in which T1was found significantly higher than 10-test media at 150 days after culture for length of the longest root of *Renantherra imschootiana* Rolfe (Wu et al., 2014). T5 is more suitable than T2, T3, T4, and T6 in all tested treatments for maximum root size of *D. chrysotoxnum* Figure (8), Table (7). The result shows the same outcome with the finding of (Wu et al., 2014) in which T5 was found as the most suitable length of the longest root for 12 weeks after plantlets development for Plantlet of *Renantherra imschootiana* Rolfe growth. Whereas (Alam. M., et al., 2002) found that T2 is the second suitable media for the root size of *Dendrobium transparens* plantlets from seed culture. T6 which contains MS basal media was not found as effective in all treatments for the maximum root size of *D. chrysotoxum* (see Figure 8).

Table 5: Effect of six treatments for maximum root of size of *D. chrysotoxum* for the four-month period

Each node shows the sample average rank of Treatmen_codet.

Sample1 Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	8.397	12.547	.669	.503	1.000
T6-T4	36.547	10.814	3.287	.001	.015
T6-T3	62.814	11.730	5.355	.000	.000
T6-T5	63.687	10.814	5.889	.000	.000
T6-T1	71.067	11.310	6.284	.000	.000
T2-T4	-27.149	11.322	-2.398	.016	.247
T2-T3	-54.416	12.199	-4.461	.000	.000
T2-T5	-55.289	11.322	-4.883	.000	.000
T2-T1	62.669	11.796	5.313	.000	.000
T4-T3	27.267	10.409	2.620	.009	.132
T4-T5	-28.140	9.365	-3.005	.003	.040
T4-T1	36.520	9.933	3.676	.000	.005
T3-T5	-.873	10.409	-.084	.933	1.000
T3-T1	8.253	10.923	.756	.450	1.000
T5-T1	7.380	9.933	.743	.458	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

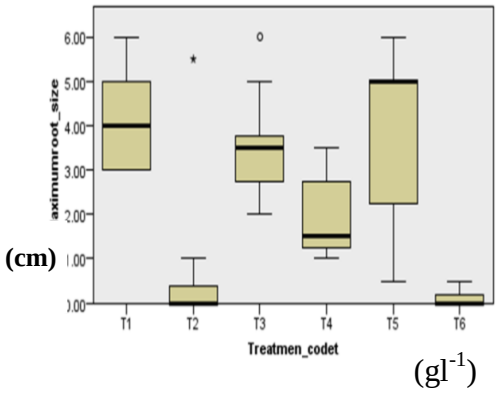


Figure 8: Effect of six treatments for maximum root size of *D. chrysotoxum* after four-month period

4.1.4. The minimum root size of plantlets for *Dendrobium chrysotoxum*

According to A Kruskal-Wallis test, there were significant differences among treatments (Figure 9 & Table 2). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 9 & Table 2). By Dunn's pairwise tests, the mean minimum root size of *Dendrobium chrysotoxum* is 1.85±1.15 in the T1

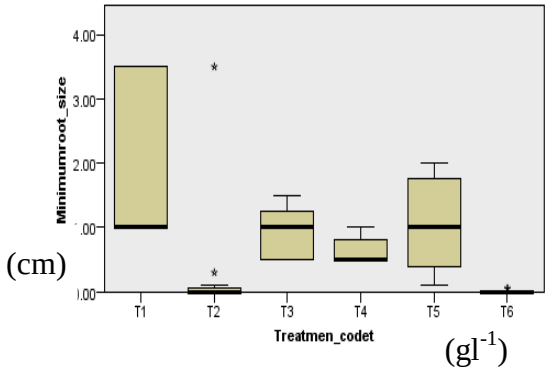
treatment Table (2). T1 and T5 are higher than other treatments (Table 2). T1 is significantly higher than T4, T2 and T6 at the adjusted significant level (P<0.005) Table (6). Moreover, T3 is significantly higher than T2, and T6 at the adjusted significant level (p<0.005) Table (6). And T5 is significantly higher than T6 and T2 (p<0.005). T2 is significantly higher than T6 (p<0.005) Table (6). There was no evidence of a difference between the other pairs Table (6).

For minimum root-size of *D. chrysotoxum*, it was tested with six treatments *in vitro* culture from protocorm to plantlets (Table 6&Figuer9). T1 was found as the most suitable among all the treatments for the minimum root size of *D. chrysotoxum* (Table 6&Figuer9). T1was found significantly higher than 10-test medias at 150 day after culture for length of the shortest root of *Renantherra imschootiana* Rolfe (Wu et al., 2014).Treatment T5 was the second appropriate among all the treatments for the minimum root-size of *D.chrysotoxum* (see Figure 9 and Table 6).The result shows the same outcome with the finding of (Wu et al., 2014) in which Treatment T5 was found to be most suitable for plantlet of *Renantherra imschootiana* Rolfe growth *in vitro* since it resulted in length of the shortest roots for plantlet growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). T6 which contains MS basal media was not found as effective in all treatments for the minimum root-size of *D. chrysotoxum* (see Figure 9 and Table 6).

Table 6: Effectiveness of treatments on growth for minimum root of size of *D. chrysotoxum* for the four-month period

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	8.641	12.348	.700	.484	1.000
T6-T4	39.953	10.643	3.754	.000	.003
T6-T3	57.951	11.544	5.020	.000	.000
T6-T5	58.093	10.643	5.458	.000	.000
T6-T1	77.483	11.131	6.961	.000	.000
T2-T4	-31.312	11.143	-2.810	.005	.074
T2-T3	-49.310	12.006	-4.107	.000	.001
T2-T5	-49.452	11.143	-4.438	.000	.000
T2-T1	68.842	11.609	5.930	.000	.000
T4-T3	17.998	10.244	1.757	.079	1.000
T4-T5	-18.140	9.217	-1.968	.049	.736
T4-T1	37.530	9.776	3.839	.000	.002
T3-T5	-.142	10.244	-.014	.989	1.000
T3-T1	19.532	10.750	1.817	.069	1.000
T5-T1	19.390	9.776	1.983	.047	.710

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(cm) (gl^{-1})

Figure 9: Effect of six treatments for minimum root size of *D. chrysotoxum* after four-month period

4.1.5. The length of plantlets for *D. chrysotoxum*

According to A Kruskal-Wallis test, there were significant differences among the treatments (Table 2 & Figure 10). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Table 2 & Figure 10). The mean height of *Dendrobium chrysotoxum* is 69.23±25.79 in treatment T1 Table (2) &Table (6). By Dunn's pairwise tests, T1 and T5 are higher than other treatments (Figure 10 & Table 7). T1 is significantly higher than T3, T5, T4, and T6 at the adjusted significant level ($p<0.005$) Table (7). T2 is significantly higher than T4, and T6 at the adjusted significant level ($p<0.005$) Table (7). Moreover, T3 is higher than T4 at the adjusted significant level ($p<0.005$) Table (7). There was no evidence of a difference between the other pairs Table (7).

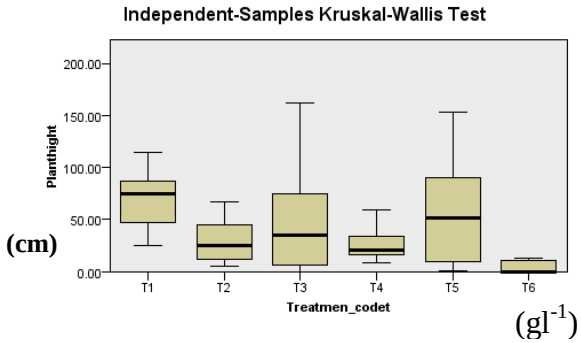
In *D.chryosotoxum,* the length of plantlet was tested with six treatments as shown in (Table 2 & Figure 10). Amongst, Treatment T1 was found as the most suitable combination among all the treatments for the length of plantlet (see Figure 10). Similarly, T1 was the most suitable among the treatments for the plantlet height. T6 which constitutes MS basal media was the least effective medium for the plantlet height (see Figure 10). T5 treatment is more suitable than T2, T3, T4, and T6 treatment (basal media) (Figure 10& table 7) while treatment T2 is the second suitable of *Dendrobium transparens* plantlets from seed culture (Alam,M.,et al., 2002). T1 was significantly higher than all other treatments the highest shoot formation at 75 day after culture in stage 5 of plantlets of *Renanthera imschootiana* Rolfe (Wu et al., 2014). T3 and T4 are the regular media for multiplication stage in FRI lab. The tallest plantlets (2.22, 2.92,3.40, and 4.06cm at 3,6,9, and 12 weeks after plantlets development respectively) were observed in MS medium , followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68,2.06 and 2.52cm at 3,6,9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam,M.,et al., 2002).

Table 7: Effect of six treatments for length of plantlets of *D.chrysotoxum* after the four months period

Each node shows the sample average rank of Treatmen_codet.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T4	36.340	10.887	3.338	.001	.013
T6-T2	38.308	12.631	3.033	.002	.036
T6-T3	46.088	11.808	3.903	.000	.001
T6-T5	55.320	10.887	5.081	.000	.000
T6-T1	74.350	11.386	6.530	.000	.000
T4-T2	1.968	11.398	.173	.863	1.000
T4-T3	9.748	10.479	.930	.352	1.000
T4-T5	-18.980	9.428	-2.013	.044	.662
T4-T1	38.010	10.000	3.801	.000	.002
T2-T3	-7.781	12.281	-.634	.526	1.000
T2-T5	-17.012	11.398	-1.493	.136	1.000
T2-T1	36.042	11.875	3.035	.002	.036
T3-T5	-9.232	10.479	-.881	.378	1.000
T3-T1	28.262	10.996	2.570	.010	.152
T5-T1	19.030	10.000	1.903	.057	.856

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(cm)

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Figure 10: Effect of six treatments for length of plantlets of *D. chrysotoxum* after the four-month period

4.2. Effect of Different media on *Dendrubium devoniaum in vitro* seed culture

4.2.1. The fresh weight of *Dendrubium devoniaum*

According to Kruskal-Wallis test, there is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 11 & Table 8). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 11 & Table 8). The mean fresh weight of *Dendrobium devoniaum* is 11.98±6.35 in T3 treatment (table 8). T3 and T5 are higher than other treatments (Figure 11& Table 9). By Dunn's pairwise tests, T3 is significantly higher than T2 and T6 at the adjusted significant level ($p<0.005$) Table (9). T5 is significantly higher T6 at the adjusted significant level ($p<0.005$) Table (9). Moreover, T4 is higher than T6 at the adjusted significant level ($p<0.005$). Additionally, T1 is higher than T6 at the adjusted significant level ($p<0.005$) Table (9). There was no evidence of a difference between the other pairs Table (9).

In *D. devoninum*, the fresh weight was tested with six different treatments *as shown* in the (Figure 11 &table 9). Amongst, Treatment T3 was found as the most suitable treatment among the treatments for the fresh weight of *D. devoninum* (see Figure 11 and Table 9). T3 is regular media in FRI lab form multiplication to plantlets. Furthermore, Treatment T1 found as more suitable than Treatment T2, T4, T5 and T6; MS basal media, which was found as the least effective medium for the fresh weight of *D. devoninum* (see figure 11, and Table 9) Figure (6). Treatment T5 is third suitable in all treatments for the fresh weight of *D.devoninum (Table 9, and 8 & figure 11).* Treatment T2 is the second suitable for seed germination and plantlets of *Dendrobium transparens* percent (Alam, M., et al., 2002). Treatment T5 was found to be most suitable for plantlet growth *in vitro* since it resulted in the highest fresh weight for Plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). The tallest plantlets (2.22, 2.92,3.40, and 4.06cm at 3,6,9, and 12 weeks after plantlets development respectively) were observed in MS medium, followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68,2.06 and 2.52cm at 3,6,9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam,M.,et al., 2002).

Table 8: Effect of six treatments for *D. devoniaum* according to respective parameters after the four-month period

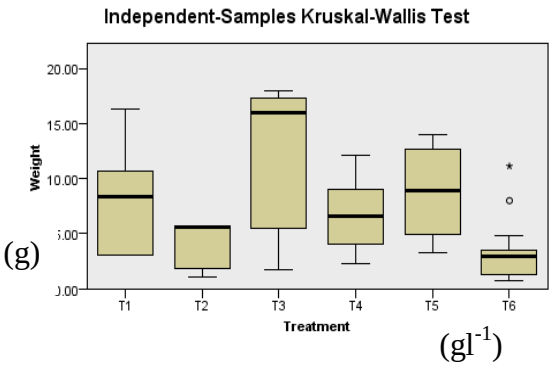
Treatment	FreshWeight (g)	Number of plantlet(plant)	Maxinimum-Root of Size (cm)	Minimum-Root of size (cm)	length of plantlet (cm)
T1	7.57±4.25	141.43±49.43	4.80±.71	2.72±.60	109.00±31.61
T2	3.87±2.40	63.00±7.44	4.47±2.01	.23±.18	14±7.25
T3	11.98±6.35	135.91±35.64	0.±2.01	22.57±38.08	75.67±45.96
T4	6.52±2.85	77.15±34.10	1.52±1.38	.63±.48	49.40±25.25
T5	8.64±3.97	91.94±22.95	4.22±.39	1.91±.54	76.11±19.00
T6	3.094±2.48	55.62±19.37	.22±.07	.10±.07	22.24±8.89

Table 9: Effectiveness of treatments on growth for the fresh weight of ***Dendrobium devoninum*** after the four-month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	12.310	11.496	1.071	.284	1.000
T6-T4	35.102	10.410	3.372	.001	.011
T6-T1	37.238	10.282	3.622	.000	.004
T6-T5	46.508	10.702	4.346	.000	.000
T6-T3	56.238	10.282	5.470	.000	.000
T2-T4	-22.793	11.610	-1.963	.050	.744
T2-T1	24.929	11.496	2.169	.030	.452
T2-T5	-34.198	11.873	-2.880	.004	.060
T2-T3	-43.929	11.496	-3.821	.000	.002
T4-T1	2.136	10.410	.205	.837	1.000
T4-T5	-11.406	10.825	-1.054	.292	1.000
T4-T3	21.136	10.410	2.030	.042	.635
T1-T5	-9.270	10.702	-.866	.386	1.000
T1-T3	-19.000	10.282	-1.848	.065	.969
T5-T3	9.730	10.702	.909	.363	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(g) (gl^{-1})

Figure 11: Effect of six treatments for the fresh weight of *Dendrobium devoninum* after the four-month period

4.2.2. The number of plantlets for *Dendrubium devoninum*

According to A Kruskal-Wallis test, there were significant differences among treatments (Figure 12 & table 10). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 12 & table 10). The mean plantlet number of *D.devoninum* is 141.43±49.43 in treatment 1 (Table 8).T1 and T3 are higher than other treatments (Table 8, and 10& Figure 12). By Dunn's pairwise tests, T1 is significantly higher than T2, T4, and T6 at the adjusted significant level ($p<0.005$) Table (18). Moreover, T3 is significantly higher than T2, T4, and T6 at the adjusted significant level ($p<0.005$). T5 is significantly higher than T6 at the adjusted significant level ($p<0.005$) (Table 10). There was no evidence of a difference between the other pairs (Table 10).

In *D. devoniaum*, comparing the number of plantlets among all the treatments, T3 is the most suitable treatment among all the other treatments for the number of plantlets of *D.devoniaum*(Table 8, and 10& Figure 12). Moreover, Treatment T3 is regular media for multiplication stage in FRI lab for *Dendrobium spp*. T1 is more proper than T2, T5, T4, and T6 as shown in Figure (7) and Table (12). But, T1 was significantly higher than all other treatments or the highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (Wu et al., 2014). Futhermore, T5 treatment is more suitable than T2, T4, and T6 treatment (basal media) and the latter treatment (T6) is the least nutrient giving medium for the plantlets number in *D. chryosotoxnum* (Table 8, and 10& Figure 12).

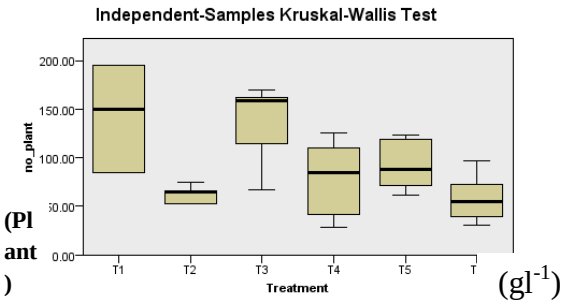
Therefore, T3 treatment is the first suitable, T1 is the second suitable and T5 is the third suitable in all treatments for the number of plantlets of *D. devoninum* (Table 8, and 10& Figure 12)*)*. Treatment T2 is the second suitable for *Dendrobium transparens* percent (Alam,M.,et al., 2002).Treatment T5 was found to be most suitable for plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* since it resulted in the tallest shoots for Plantlet growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). The tallest plantlets (2.22, 2.92,3.40, and 4.06cm at 3,6,9, and 12 weeks after plantlets development respectively) were observed in MS medium, followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68,2.06 and 2.52cm at 3,6,9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam,M.,et al., 2002).

Table 10: Effectiveness of treatments for on growth number of plantlet of ***Dendrobium devoninum*** after four- month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	4.429	11.495	.385	.700	1.000
T6-T4	20.143	10.409	1.935	.053	.795
T6-T5	35.754	10.701	3.341	.001	.013
T6-T1	59.786	10.281	5.815	.000	.000
T6-T3	60.714	10.281	5.905	.000	.000
T2-T4	-15.714	11.609	-1.354	.176	1.000
T2-T5	-31.325	11.872	-2.639	.008	.125
T2-T1	55.357	11.495	4.816	.000	.000
T2-T3	-56.286	11.495	-4.897	.000	.000
T4-T5	-15.611	10.824	-1.442	.149	1.000
T4-T1	39.643	10.409	3.808	.000	.002
T4-T3	40.571	10.409	3.898	.000	.001
T5-T1	24.032	10.701	2.246	.025	.371
T5-T3	24.960	10.701	2.332	.020	.295
T1-T3	-.929	10.281	-.090	.928	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(Plant) (gl^{-1})

Figure 12: Effect of six treatments for plantlet number of *D. devoninum* after the four-month period.

4.2.3. The maximum root size of plantlets for *Dendrobium devoninum*

According to A Kruskal-Wallis test, there were significant differences among treatments (Figure 13& Table 11). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 13& Table 11). The mean root size (maximum) of *D. devonimum* is 4.80±0.71 in the treatment T1 (Table 8). By Dunn's pairwise tests, T1 and T3 are higher than of other treatments (Figure 13& Table 11,and 8). T1 is significantly higher than T4, T2, and T6 at the adjusted significant level ($p<0.005$) (Table 11). T3 is significantly T2, T4, and T6 at the adjusted significant level ($p<0.005$) (Table 11).

Moreover, T5 is significantly higher than T2, T4, and T6 at the adjusted significant level ($p<0.005$). There was no evidence of a difference between the other pairs (Table 11).

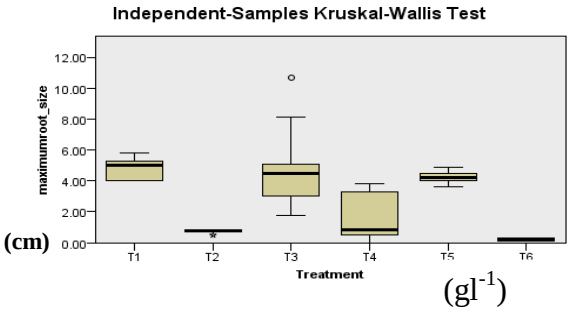
D. devoninum had been tested with six treatments for the maximum root- size in vitro form protocorm to plantlet (see Figure 13 & Table 11). No other treatment in all treatments as suitable as Treatment T1 for the maximum root-size of *D. devoninum.* Treatment T3 was more suitable than T2, T4, T5, and T6 MS media (basal media) and the latter treatment (T6) is the least nutrient giving medium for the maximum number in vitro form protocorms to plantlets of *D.devoninum* (see Figure 13 & Table 11). Treatment T2 is the second suitable for seed germination and plantlet of percent (Alam, M., et al., 2002). T1 was significantly higher than all other treatments or The highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (Wu et al., 2014).Treatment T5 was found to be most suitable for plantlet growth *in vitro* since it resulted in the longest roots and highest fresh weight for Plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). T3 and T4 are regular media were use in FRI lab in multiplication stage from protocoms to plantlets.

Table 11: Effectivess of treatments on growth for maximum root size of ***D.devoninum*** *in vitro* culture after four-month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	23.786	11.468	2.074	.038	.571
T6-T4	29.650	10.385	2.855	.004	.065
T6-T5	66.278	10.676	6.208	.000	.000
T6-T3	71.048	10.257	6.926	.000	.000
T6-T1	79.952	10.257	7.795	.000	.000
T2-T4	-5.864	11.582	-.506	.613	1.000
T2-T5	-42.492	11.844	-3.588	.000	.005
T2-T3	-47.262	11.468	-4.121	.000	.001
T2-T1	56.167	11.468	4.898	.000	.000
T4-T5	-36.628	10.799	-3.392	.001	.010
T4-T3	41.398	10.385	3.986	.000	.001
T4-T1	50.302	10.385	4.844	.000	.000
T5-T3	4.770	10.676	.447	.655	1.000
T5-T1	13.675	10.676	1.281	.200	1.000
T3-T1	8.905	10.257	.868	.385	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(cm) (gl^{-1})

Figure 13: Effect of six treatments for the maximum root size of ***D.devoninum*** in vitro culture after the four- month period

4.2.4. The minimum root size of plantlets for *Dendrobium devoninum*

A Kruskal-Wallis test provided very strong evidence of a difference ($p < 0.001$) between the mean ranks of at least one pair of groups (Table 12& Figure 14). Dunn's

pairwise tests were carried out for the fifteen pairs of groups table (12).The mean root size (minimum) of *D.devoninum* is 22.57±38.08 in the treatment (Table 8). T1 and T3 are higher than of other treatment (Table 12& Figure 14). T1 is significantly higher thanT4, T2, and, T6 at the adjusted significant level ($p<0.005$ table (12).T1 is significantly higher than T2, and T6 at the adjusted significant level ($p<0.005$) (Table 12). T5 is significant higher than T2, and T6 at the adjusted significant level ($p<0.005$) (Table 12). There was no evidence of a difference between the other pairs (Table 12).

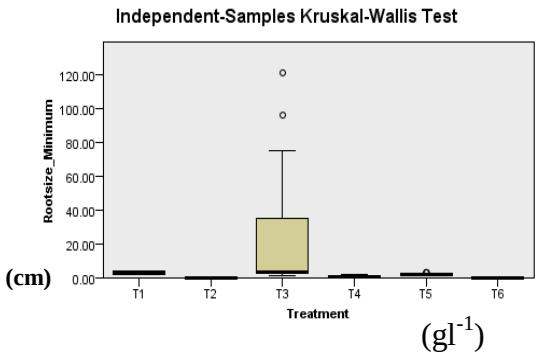
For minimum root-number of *D.devoninum, it was tested with six treatments in vitro* culture as shown in (Table 12& Figure14). Amongst, no other treatment was as suitable as treatment T1 for minimum root size of *D.devoninum* (Figure 14& Table (12). Accordingly, treatmentT5 is found the second suitable in all medium for minimum root- size of *D.devoninum in vitro* culture from protocorm to plantlets (Figure 14& Table 12). *Moreover,* T3 is found the third suitable in six medium for the minimum root-size of *D.devoninum*(Table 12& Figure14). Moreover, T6 is the least nutrient giving medium *for the root-size of the minimum root-size in vitro form protocorm to plantlet* (Table 12& Figure14). Treatment T2 is the second suitable for seed germination and plantlet of *Dendrobium transparens* percent (Aam, M., et al., 2002).T1 was significantly higher than all other treatments or the highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (5). Treatment T5 was found to be most suitable for plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* since it resulted in the longest roots and for Plantlet growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). T3 and T4 are regular media in my lab form multiplication or protocoms technique to plantlets.

Table 12: Effectiveness of treatments on growth for minimum root-size of ***D.devoninum*** *in vitro* culture after four-month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T2	10.000	11.433	.875	.382	1.000
T6-T4	27.500	10.353	2.656	.008	.119
T6-T5	54.278	10.644	5.099	.000	.000
T6-T3	74.810	10.226	7.315	.000	.000
T6-T1	75.810	10.226	7.413	.000	.000
T2-T4	-17.500	11.547	-1.516	.130	1.000
T2-T5	-44.278	11.808	-3.750	.000	.003
T2-T3	-64.810	11.433	-5.668	.000	.000
T2-T1	65.810	11.433	5.756	.000	.000
T4-T5	-26.778	10.766	-2.487	.013	.193
T4-T3	47.310	10.353	4.569	.000	.000
T4-T1	48.310	10.353	4.666	.000	.000
T5-T3	20.532	10.644	1.929	.054	.806
T5-T1	21.532	10.644	2.023	.043	.646
T3-T1	1.000	10.226	.098	.922	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(cm) (gl^{-1})

Figure14: Effect of six treatments for of the minimum root size of ***D.devoninum*** in vitro culture after the four- month period

4.2.5. The length of plantlet for *D.devoninum*

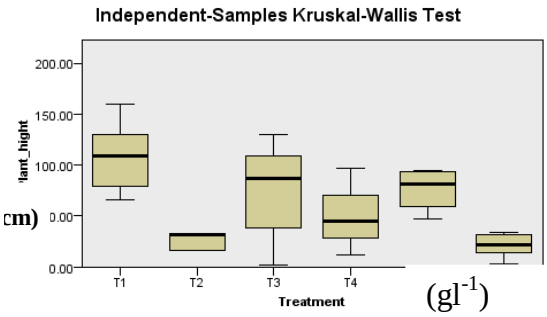
According to A Kruskal-Wallis test, there were significantly differences among treatments (Figure 15 &Table13). There is a strong significance between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 15 &Table13). The mean high of plantlet of *D.devoninum* is (109.00±31.61) in T3 treatment (Table8). T1 and T3 are the higher than other treatment (Figure 15 &Table13). T1 is significantly higher than T4, T2, and T6 at the adjusted significant level ($p<0.005$) (Table 13), Furthermore, Treatment T3 is significantly higher than T2, and T6 at the adjusted significant level ($p<0.005$) (Table 13). Treatment T5 is significantly higher than T2, and T6, at the adjusted significant level ($p<0.005$) (Table 13). There was no evidence of a difference between the other pairs (Table 13).

For plant height of *D.devoninum, it was tested six treatments in vitro* culture (see Figure 15 &Table13)Amongst, Treatment T1 was the most suitable among all the treatments for plantlet height of *D. devoninum*(Figure 15 &Table13).*Treatment T5 is the second suitable* among all the treatments for plantlet height of *D. devoninum* (Figure 15 & Table13).*Treatment T3 is more suitable than T4, T2, and T6* MS (basal media), which was the least effective medium among the treatments for the plant height of *D.devoninum* (Figure 15 &Table13). Treatment T2 is the second suitable for seed germination and plantlet Of *D.transparens* percent (Alam, M., et al., 2002).T1 was significantly higher than all other treatments or the highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (Wu et al., 2014). Treatment T5 was found to be most suitable for plantlet of growth *in vitro* since it resulted in the tallest shoots, for plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA, and BH concentrations (Wu et al., 2014).T3 and T4 are regular media in my lab form multiplication or protocoms technique to plantlets. The tallest plantlets (2.22, 2.92,3.40, and 4.06 cm at 3, 6, 9, and 12 weeks after plantlets development respectively) were observed in MS medium , followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68,2.06 and 2.52cm at 3,6,9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam, M., et al., 2002)

Table 13: Effectiveness of treatments on growth for plantlet-length of *D.devoninum* after the four-month period

Sample1-Sample2	Statistic	Error	Statistic	Sig.	Adj.Sig.
T6-T2	8.643	11.497	.752	.452	1.000
T6-T4	27.421	10.411	2.634	.008	.127
T6-T3	44.333	10.283	4.311	.000	.000
T6-T5	50.905	10.703	4.756	.000	.000
T6-T1	69.476	10.283	6.756	.000	.000
T2-T4	-18.779	11.611	-1.617	.106	1.000
T2-T3	-35.690	11.497	-3.104	.002	.029
T2-T5	-42.262	11.874	-3.559	.000	.006
T2-T1	60.833	11.497	5.291	.000	.000
T4-T3	16.912	10.411	1.624	.104	1.000
T4-T5	-23.483	10.826	-2.169	.030	.451
T4-T1	42.055	10.411	4.040	.000	.001
T3-T5	-6.571	10.703	-.614	.539	1.000
T3-T1	25.143	10.283	2.445	.014	.217
T5-T1	18.571	10.703	1.735	.083	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(gl^{-1})

Figure15: Effect of six treatments for plantlet-length of *D.devoninum* after the four-month period

4.3. Comparison of differences between *Dendrobium chrysotoxum* and *D.devoninum* according to respective parameters after the four-month period

There were a significant difference between fresh weight, number of plantlet, maximum root size, minimum root size, and height of plantlet of *Dendrobium chrysotoxu* (m:5.41, sd:4.33), (m:55.43, sd:42.25), (M:2.75, sd:2.18), (m:0.86, sd:0.87), and (m:39.67, sd:36.78), fresh weight, number of plantlet, maximum root size of plantlets , minimum root size plantlets , and length of plantlet of *D.devoninum* (M:7.11, sd:4.95), (F(1,228):7.538,p<.05), *M:96.28,sd:46.57), (F(*1,228): 48.527,p<.05) , (M:5.07, sd:18.02), (F(1,288): P>.05), and (M:61.55, sd:40.86), (F(1,288) :P>.05 (Table 14).

Table 14: Comparison of different between *Dendrobium chrysotoxum* and ***D.devoninum*** according to respective parameters after the four-month period

Species name	Fresh Weight (g)	Number plantlet (plant)	Maximimum-Root Size (cm)	Minimum Root size (cm)	Length of plantlet (cm)
D. chrysotoxum	5.41±4.33	55.43±42.25	2.49±1.88	.86±.87	39.66±36.08
D.devoninum	7.10±4.96	96.28±6.57	2.75±2.18	18.01±2.97	50.68±40.00

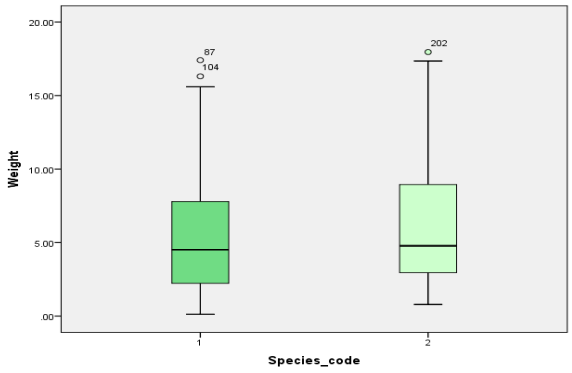


Figure 16 Comparisons of different between *Dendrobium chrysotoxum* and *D.devoninum for fresh weight after* four-month period.

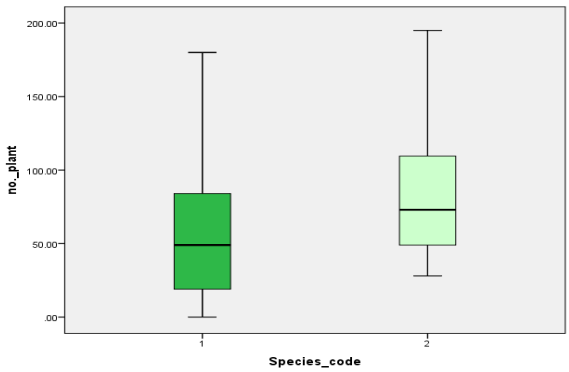


Figure 17: Comparisons of different between *Dendrobium chrysotoxum* and *D.devoninum for* number of plantlet *after* four-month period.

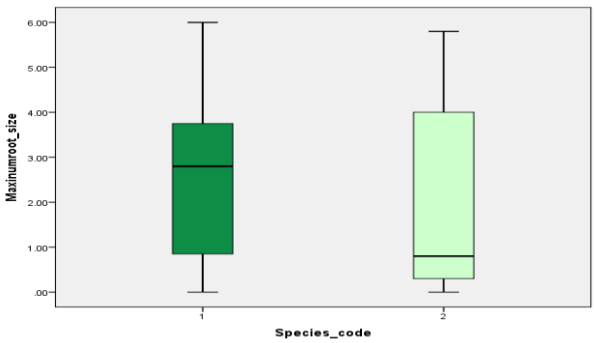


Figure 18: Comparisons of different between *Dendrobium chrysotoxum* and *D.devoninum for* Maximum root size *after* four-month period.

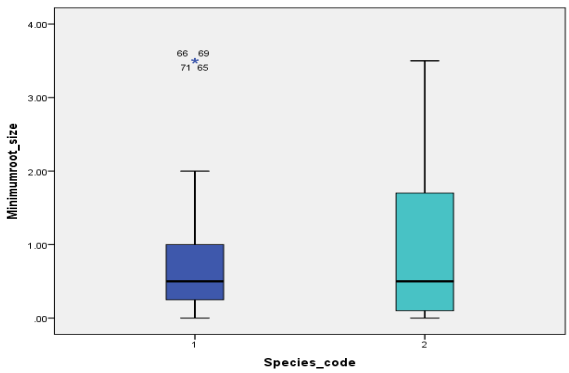


Figure 19: Comparisons of different between *Dendrobium chrysotoxum* and *D.devoninum for* Minimum root **size** *after* four-month period.

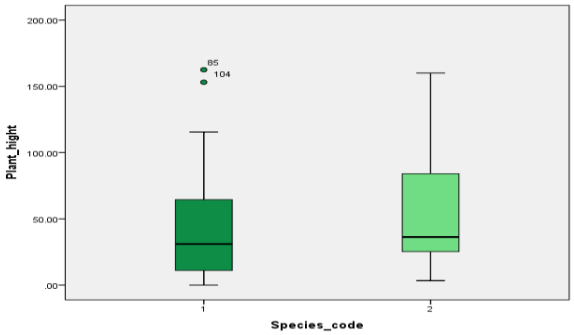


Figure 20: Comparisons of different between *Dendrobium chrysotoxum* and *D.devoninum for* plantlets length *after* four- month period.

In this paper, it was found that the fresh weight of *D.devoninum* is a slightly better than the fresh weight of the *D. chrysotoxum table (15),* Figure (16). Moreover, comparing the difference between *D.chrysotoxum* and *D.devoniaum,* T1 was the most suitable treatment for the fresh weight of *D.chrysotoxum* among all the treatments. Treatment T3 was the best for the fresh weight of *D.devoniaum* among the treatments table (15). In this study, it was found that the number of plantlets of *D.devoninum* was higher than the number of plantlets of D.chrysotoxum table (16) & figure (17). Furthermore, in contrast, T1 treatment was the best among all experimental treatments to obtain the maximum number of plants in Dendrobium chrysotoxum, and T3 treatment was the best among all experimental treatments to get the highest number of plants in *D.devoniaum.* Thus, differences in the ratio of the most suitable hormone (Hormone) and stock media to the maximum number of plantlets, even though they are the same genus, they differ in species. In this paper, For *D.chrysotoxum,* and *D.devoniaum,* the best treatment for the maximum root size of plantlets, the minimum root size, and the height of plantlets is T1 Figure (17, 18, and 19) Table (15).

In this study, a comparative study of *D.chrysotoxum* and *D.devoninum* found that there was little difference between the fresh weight, maximum root-size of plantlet, and minimum root-size of plantlet in all parameters table (15) & Figure (16,18 and 20). Also, there was a significant discrepancy between the number of seedlings and the height of seedlings. In addition, a comparative study of *D.chrysotoxum* and *D. devoninum* found that in all variables, the number of young plants differed significantly due to differences in the ratio of stock solution. In *D.chrysotoxum* and *D.devoninum,* the most appropriate treatment for the height of plantlets was found as T1. In addition, *D.chrysotoxum* and *D.devoninum* are characterized by differences in stem length. The stem of the *D.devoninum* is slender and elongated, and the stem of the *D.chrysotoxum* orchid is in a dilapidated state. Therefore, the height of *D.devoninum* seedlings was significantly higher than that of *D.chrysotoxum* seedlings.

4.4. Effect of Different media on *Vanda coerulera in vitro* seed culture

Table 15: Effect of six treatments on *vanda coerulera* according to respective parameters after the six-month period

Treatment	Fresh Weight(g)	Number of plantlet(plant)	Maximum-Root of Size (cm)	Minimum-Root of size (cm)	Height of plantlet (cm)
T1	5.169±2.84	58.57±38.05	2.40±1.13	0.50±0.18	101.71±78.65
T2	2.51±1.35	44.59±35.15	1.34±.87	0.38±.0.22	67.77±56.57
T3	3.19.±3.34	36.79±30.44	1.11±.87	0.28±0.29	55.16±.67.30
T4	.79±.87	12.50±13.42	.72±.90	.18±.21	10.83±13.56
T5	1.97±2.26	27.97±34.35	1.50±1.11	.39±.27	37.98±57.09

4.4.1. The fresh weight of plantlets for *Vanda coerulera*

A Kruskal-Wallis test provided very strong evidence of a difference ($p < 0.001$) between the mean ranks of at least one pair of groups (Table 16 & Figure (21). Dunn's pairwise tests were carried out for the fifteen pairs of groups (Table 16). The mean fresh weight of *Vanda coerulera* is (5.167±2.84) in T1 treatment (Table 15). T1 and T3 are higher than other treatment (Table 15, 16 & Figure 21). T1 is significantly higher than T4, T5, and T6 at the adjusted significant level ($p<0.005$) (Table 16). Furthermore, treatment T3 is significantly higher than T4, at the adjusted significant level ($p<0.005$) (Table 16). Treatment T2 is significantly higher than T4 at the adjusted significant level ($p<0.005$) (Table 16). There was no evidence of a difference between the other pairs (Table 16).

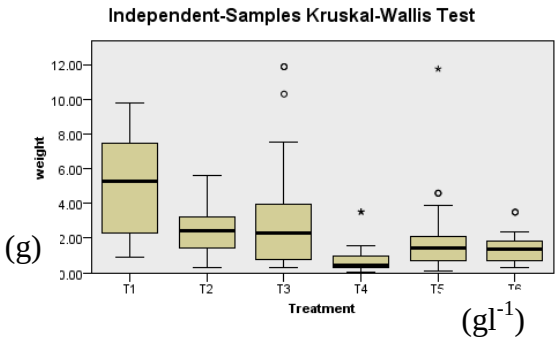
Vanda coerulera had been tested with six treatments for the fresh weight in vitro from protocorms to plantlets (Table 15, 16 & Figure (21). No other treatment in all treatments as suitable as Treatment T1 for the fresh weight of *Vanda coerulera* (Table 15, 16 & Figure 21). The result shows the same outcome with the finding of (Wu et al., 2014) in which T1 was significantly higher than all other treatments or the highest percentage of plantlet formation (95.67%) for 75 days culture for Plantlet of *Renantherra imschootiana* Rolfe growth. Treatment T2 was more suitable than T4, T5, T3, and T6 (16) (Table 15, 16 & Figure 21). Treatment T5 was found to be the second suitable for plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* since it resulted in highest fresh weight for Plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014). Moreover, the latter treatment (T4) is the least nutrient giving medium for the fresh weight in vitro from protocorms to plantlets of Vanda coerulera (see Figure & Table). Treatment T4 is less suitable than Treatment T6 (basal medium) which *giving medium* for the fresh weight in vitro from protocorm to plantlet of *Vanda coerulera* (see Figure12 & Table 18). Treatment T2 is the second suitable for seed germination and plantlent of *D.transparens* percent (Alam,M.,et al., 2002). Treatment T2 is the second suitable for seed germination and plantlent of *D.transparens* percent (Alam,M.,et al., 2002).

Table 16: Effectiveness of treatments on growth for fresh weight *coerulera* after six months period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T4-T6	-29.625	13.153	-2.252	.024	.365
T4-T5	-36.731	13.039	-2.817	.005	.073
T4-T3	56.786	13.153	4.317	.000	.000
T4-T2	63.145	13.039	4.843	.000	.000
T4-T1	93.661	13.153	7.121	.000	.000
T6-T5	7.106	13.039	.545	.586	1.000
T6-T3	27.161	13.153	2.065	.039	.584
T6-T2	33.520	13.039	2.571	.010	.152
T6-T1	64.036	13.153	4.868	.000	.000
T5-T3	20.055	13.039	1.538	.124	1.000
T5-T2	26.414	12.925	2.044	.041	.615
T5-T1	56.930	13.039	4.366	.000	.000
T3-T2	6.359	13.039	.488	.626	1.000
T3-T1	36.875	13.153	2.803	.005	.076
T2-T1	30.516	13.039	2.340	.019	.289

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(g) (gl^{-1})

Figure 21: Effect of six treatments for Fresh weight of *Vanda corulera* after the six-month period

4.4.2. The number of plantlets for *Van*
4.4.3. *da coerulera*

A Kruskal-Wallis test provided very strong evidence of a difference ($p<0.001$) between the mean ranks of at least one pair of groups (Table 17, and Figure 22). Dunn's pairwise tests were carried out for the fifteen pairs of groups (Table 17). The mean number of plantlets of *Vanda coerulera* is 58.57±38.05 in the T1 treatments (Table 15). T1 and T2 are higher than the other treatments (Table 15, 17, and Figure 22). T1 is significantly higher than T5, T4, and T6 at the adjusted significant level ($p<0.005$) (Table 17). Furthermore, treatment T2 is significantly higher than T4, and T6 at the adjusted significant level ($p<0.005$) (Table 17). Treatment T5 is significantly higher than T6 at the adjusted significant level ($p<0.005$) (Table 17). And TreatmentT3 is significantly higher than T6 at the adjusted significant level ($p<0.005$) (Table 17).

For number of plantlets of *Vanda coerulera, it was tested with six treatments in vitro* culture from *protocorms to plantlets of Vanda coerulera (see Figure22 & Table 15, 17)***.** Amongst, no other treatment was as suitable as treatment (T1) for number of plantlets of *Vanda coerulera* **(**Figure 22& Table 15). The result shows the same outcome with the finding of (Wu et al., 2014) in which T1 was significantly higher than all other treatments or the highest percentage of the number shoot for 75 days culture of *Renantherra imschootiana* Rolfe growth. T1 was significantly higher than all other treatments or the highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (Wu et

al., 2014).T2 was found the second suitable in six treatments for number of plantlets in vitro form protocorm to plantlet of *Vanda coerulera* Figure (17) and Table (14). The tallest plantlets (2.22, 2.92,3.40, and 4.06 cm at 3, 6, 9 and 12 weeks after plantlets development respectively) were observed in MS medium , followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68, 2.06 and 2.52cm at 3, 6, 9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam, M., et al., 2002). T3 is found the third suitable suitable in six treatments for number of *plantlets in vitro* form protocorm to plantlet *of Vanda coerulera* Figure (17) and Table (18).*T3* treatment media usually use for *Dendrobium spp*. on multiplication stage in biotechnology laboratory of Forest Research Institute (FRI). Moreover, T6 is the least nutrient giving medium *for* number of plantlets in vitro form protocorms to plantlets of *Vanda coerulera* Figure (17) Table (18). Treatment T5 was found to be most suitable for plantlet growth *in vitro* since it resulted in the number shoots, for Plantlet of *Renanthera imschootiana* Rolfe growth in vitro was significantly affected by NAA and BH concentrations (Wu et al., 2014). T3 and T4 are regular media in my lab multiplication stage from protocoms to plantlets.

4.4.4. maximum root of size of plantlets for*Vanda coerulera*

According to A Kruskal-Wallis test, there were significantly differences among treatments (Figure 23 &table 18). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 23 &table 18). Dunn's pairwise tests were carried out for the fifteen pairs of groups (Table 18) The mean size (maximum root) of plantlets of *Vanda coerulera in* is 2.40±1.13 in the T1treatments (Table 15).T1and T5 are higher than other treatments (Table 15,18, and Figure 23). Treatment T1 is significantly higher than T5, T2, T3, T4, and T6 at the adjusted significant level ($p<0.005$) (Table 18). Likewise, Treatment T5 is higher than T4 and Treatment T2 is higher than T5 at the adjusted significant level ($p<0.005$) (table 18). There was no evidence of a difference between the other pairs (Table 18).

Vanda coerulera had been tested with six treatments for the maximum root size in vitro form protocorms to plantlets (see Figure23 & Table 18, 15). No other treatment in all treatments as suitable as Treatment T1 for the maximum root size of *Vanda coerulera* in vitro culture after *six-month period* (see Figure 23 & Table 15, and 18). The result shows the same outcome with the finding of (Wu et al., 2014) in which T1was found significantly higher than 10-test media at 150 days after culture for length of the longest root of *Renantherra imschootiana* Rolfe (Wu et al., 2014).

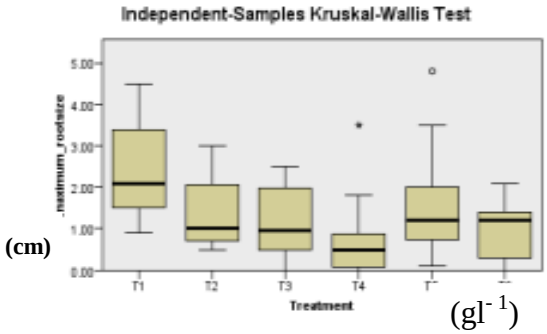
Accordingly, Treatment T5 was more suitable than T4, T2, T3, and T6 MS media *(*basal media*) (see Figure23 & Table 15, 18).* Moreover, Treatment T4 is found the latter nutrient giving medium for *the maximum-root of size Vanda ceorulera* in vitro form protocorm to plantlet after six-month period (see Figure 23& Table15) Treatment T5 was found to be most suitable for plantlet of *Renanthera imschootiana* Rolfe growth in vitro since it resulted in the tallest shoots, for Plantletof *Renanthera imschootiana* Rolfe growth in vitro was significantly affected by NAA and BH concentrations (Wu et al., 2014). As a result, t*reatment T6 (*basal media) is more suitable than Treatment T4 MS media. Basal media giving medium for the maximum root- size in vitro form protocorm to plantlet of Vanda

coerulera (see Figure18 & Table19). Treatment T2 is the second suitable for seed germination and plantlet of *D.transparens* percent (Alam, M., et al., 2002). T3 and T4 are usually media in my FRI form multiplication or protocoms to plantlets.

Table 18: Effectiveness of treatments on growth for maximum-root of size plantlet of *Vanda coerulera* after six months period

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T4-T6	-23.982	13.134	-1.826	.068	1.000
T4-T3	29.018	13.134	2.209	.027	.407
T4-T2	39.103	13.021	3.003	.003	.040
T4-T5	-44.828	13.021	-3.443	.001	.009
T4-T1	84.714	13.134	6.450	.000	.000
T6-T3	5.036	13.134	.383	.701	1.000
T6-T2	15.121	13.021	1.161	.246	1.000
T6-T5	20.845	13.021	1.601	.109	1.000
T6-T1	60.732	13.134	4.624	.000	.000
T3-T2	10.086	13.021	.775	.439	1.000
T3-T5	-15.810	13.021	-1.214	.225	1.000
T3-T1	55.696	13.134	4.241	.000	.000
T2-T5	-5.724	12.906	-.444	.657	1.000
T2-T1	45.611	13.021	3.503	.000	.007
T5-T1	39.887	13.021	3.063	.002	.033

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.



(cm) (gl^{-1})

Figure 23: Effect of six treatments for maximum root -size of plantlet of *Vanda corulera* after six months period

4.4.5. The minimum root size of plantlets for *Vanda coerulera*

Aaccording to A Kruskal-Wallis test, there were significantly differences among treatments Table (19, 15& Figure 24). There is a strong significant difference between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure24). The mean root size (minimum) of plantlets of *Vanda coerulera* is 0.502.40±0.18 in the T1 treatments (Table 15). Dunn's pairwise tests were carried out for the fifteen pairs of groups (Table19). T1 and T2 are higher than other treatments (Table 19, 15, and Figure 24). By Dunn's pairwise tests, Treatment T1 is higher than T3, T4, and T6 at the adjusted significant level ($p<0.005$) (Table 19). Moreover, treatment T2 is higher than T4, and treatment T5 is higher than T4 at the adjusted significant level ($p<0.005$) (Table 19).There was no evidence of a difference between the other pairs (Table 19).

For the minimum root size of *Vanda coerulera, it was tested with six treatments in vitro* culture from protocorms to plantlets as shown in *(see Figure24 & Table 15, 19).* Amongst**,** no other treatment was as suitable as treatment T1 for the minimum root size of *Vanda corulera* **(**Figure 24&Table 15, 19). T1was found significantly higher than 10-test media at 150 day after culture for length of the shortest root of *Renantherra imschootiana*

Rolfe (Wu et al., 2014). T2 is found the second suitable in six treatments for minimum root size of *Vanda corulera* in vitro culture from protocorms to plantlets Figure (19) and Table (18). Traetment T2 is the second suitable for seed germination and plantlets of *D.transparents* percent (Alam, M., et al., 2002). *As result of, T3 is found the third suitable* in six treatments for the minimum root size of *Vanda corulera* in vitro culture from protocorms to plantlets (Figure 24 and Table 15, 19). T3 and T4 are regular media in the biotechnology laboratory of Forest Research Institute (FRI) form multiplication or protocoms to plantlets Moreover, T4 is the least nutrient giving medium *for the minimum root size in vitro form protocorms to plantlets* (Figure 24& Table 15.Treatment T5 was found to be most suitable for plantlet growth *in vitro* since it resulted in the longest roots and for plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014).

Table 19: Effectiveness of treatments on growth for minimum root of size plantlet of *Vanda ceorulera* after six months period

Each node shows the sample average rank of treatment_code.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T4-T6	-14.964	13.019	-1.149	.250	1.000
T4-T3	22.607	13.019	1.736	.082	1.000
T4-T5	-44.848	12.906	-3.475	.001	.008
T4-T2	48.555	12.906	3.762	.000	.003
T4-T1	75.696	13.019	5.814	.000	.000
T6-T3	7.643	13.019	.587	.557	1.000
T6-T5	29.884	12.906	2.315	.021	.309
T6-T2	33.591	12.906	2.603	.009	.139
T6-T1	60.732	13.019	4.665	.000	.000
T3-T5	-22.241	12.906	-1.723	.085	1.000
T3-T2	25.948	12.906	2.010	.044	.666
T3-T1	53.089	13.019	4.078	.000	.001
T5-T2	3.707	12.793	.290	.772	1.000
T5-T1	30.849	12.906	2.390	.017	.253
T2-T1	27.142	12.906	2.103	.035	.532

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

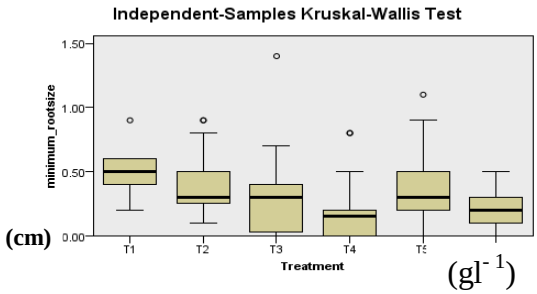


Figure 24: Effect of six treatments for minimum root-size of plantlet of *Vanda corulera* after six months period

4.4.6. The length of plantlets for *Vanda coerulera*

According to A Kruskal-Wallis test, there were significantly differentance among treatments (Table 20& Figure25). There is a strong significance between the mean ranks of at least one pair of groups ($p < 0.001$) (Figure 25). The mean the high of plantlets of *Vanda coerulera* is 101.71±78.65 which is higher than other treatments (Table 15). Treatments T1, and T2 are Higher than other Treatment Table (15, 20 &Figure 25). Dunn's pairwise tests were carried out for the fifteen pairs of groups (Table 20) Treatment T1 is higher than T3, T5,

T6, and T4 at the adjusted significant level ($p<0.005$) (Table 20). Moreover, Treatment T2 is higher than T4, and T6 at the adjusted significant level ($p<0.005$). And, Treatment T3 is higher than T4 at the adjusted significant level ($p<0.005$) (Table 20). There was no evidence of a difference between the other pairs (Table 20).

Vanda corulera had been tested with six treatments for the height of plantlets in vitro form protocorm to plantlet (see Figure 25 & Table15, 20). No other treatment in all treatments as suitable as Treatment T1 for the high of plantlets of *Vanda coerulera* in vitro culture from protocorms to plantlets at *a period of six months* (see Figure25 & Table15). Treatment T2 was more suitable than T4, T5, T3, and T6 MS media (basal media) and the latter treatment (T4) (see Figure25 & Table 15). Which is the least nutrient giving medium for the fresh weight in vitro form protocorms to plantlets of Vanda coerulera (see Figure25 & Table15). T1 was significantly higher than all other treatments the highest shoot formation at 75 days after culture in stage 5 of plantlets of *Renanthera imschootiana* Rolfe (Wu et al., 2014). T1 was significantly higher than all other treatments or the highest percentage of plantlet of *Renanthera imschootiana* Rolfe formation (95.67%) for 75 days culture (Wu et al., 2014). Treatment T6 *MS media* (basal media is more suitable than TreatmentT4 *giving medium* for the fresh weight in vitro form protocorm to plantlet of *Vanda coerulera* (see Figure 16 & Table17, 18).As result of, T2 is the second suitable in six treatments media for the height of plantlet of *Vanda corulera* in vitro culture from protocorm to plantlets Figure (20) and Table (17, 18). Traetment T2 is the second suitable for seed germination and plantlent of *D.transparens* percent (Alam, M., et al., 2002). Treatment T5 was found to be most suitable for plantlet of *Renanthera imschootiana* Rolfegrowth *in vitro* since it resulted in the tallest shoots, for Plantlet of *Renanthera imschootiana* Rolfe growth *in vitro* was significantly affected by NAA and BH concentrations (Wu et al., 2014).T3 and T4 are regular media in FRI lab form multiplication stage from protocoms to plantlets. The tallest plantlets (2.22, 2.92,3.40, and 4.06cm at 3,6,9, and 12 weeks after plantlets development respectively) were observed in MS medium , followed by Hyponex medium or T2 while OKF_1 medium produced the dwarf plantlets (1.26,1.68,2.06 and 2.52cm at 3,6,9, and 12 WAPD respectively) for *Dendrobium transparens* (Alam, M., et al., 2002).

Table20: Effectiveness of treatments ongrowth for length of plantlet of *Vanda coerulera* after six-month period

Each node shows the sample average rank of Treatment.

Sample1-Sample2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj.Sig.
T6-T4	11.946	13.146	.909	.363	1.000
T6-T5	40.842	13.032	3.134	.002	.026
T6-T3	49.786	13.146	3.787	.000	.002
T6-T2	63.411	13.032	4.866	.000	.000
T6-T1	81.821	13.146	6.224	.000	.000
T4-T5	-28.896	13.032	-2.217	.027	.399
T4-T3	37.839	13.146	2.878	.004	.060
T4-T2	51.465	13.032	3.949	.000	.001
T4-T1	69.875	13.146	5.315	.000	.000
T5-T3	8.943	13.032	.686	.493	1.000
T5-T2	22.569	12.917	1.747	.081	1.000
T5-T1	40.979	13.032	3.144	.002	.025
T3-T2	13.626	13.032	1.046	.296	1.000
T3-T1	32.036	13.146	2.437	.015	.222
T2-T1	18.410	13.032	1.413	.158	1.000

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same.
Asymptotic significances (2-sided tests) are displayed. The significance level is .05.

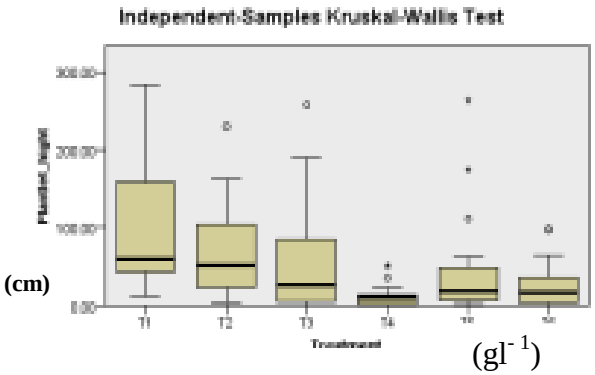


Figure 25: Effect of six treatments for height of plantlet of *Vanda coerulera* after six-month period

Figure 26: Data collected on orchid Tissue culture,*Vanda corulera* seedling or plantlets grown outside

Figure 27: After collection of data, the seedling or plantlets of *D.chrysotoxum* grown outside

5. Conclusion

Although the orchid species are similar, the protocorm is a small plant, and it is more profitable to use different proportions of nutrient content to produce large numbers of orchid tissue**.** In this study, it was found that orchids of different species, even though they are the members of the same genus, it can only be successful if we use different amounts of stock solution and hormones in the propagation process for different species. If the orchid species were not of the same genus, the basal media used to determine the presence or absence of suitable orchid species, and the time and date of the protocom to become a plantlet varied.
In this experiment, the treatments T1 the first, T5 the second are the best suitable for all parameters of *D.chrysotoxum* orchid species. The treatments of T3 is the best suitable treatment for the fresh weight, and number of plantlets of *D.devoniaum* , and the treatments T1 is the best suitable treatments for the length of plantlets, maximum root of size, and minimum root size of plantlets of *D.devoniaum.* Moreover, T1 is the best suitable for all parameters of *Vanda coerulera.* Theatment T2 is the second best suitable for Fresh weight, number of plantlets, minimum root size and the length of plantlets of *Vanda coerulera,* and T5 is second best suitable for the maximum root size of plantlets of *Vanda coerulera* orchids. In addition, it was found out that they are the best stock combination for the mass production of targeted orchid plantlets. Furthermore, one important finding is that it can be cost-effective in the mass production of targeted orchid species in Myanmar if we use supplemental natural components such as coconut water and banana with major chemical hormones rather than solely depend on the vast amount of chemical hormones. By doing this, this study will highlight the importance of reintroduction programs in their native lands for the restoration of targeted orchid species in their respective ecosystems. *D.devonianum, Vanda coerulea, & D. chrysotoxum* should be conserved with *In-situ* and *Ex-situ* method for sustainable development.

6. Recommendation

Organic fertilizers such as peptone, CW, BH, PH, AC together with essential chemicals other should be used to promote the growth and rooting of plantlet. Therefore, instead of using other expensive chemical hormones, the organic fertilizers should be used in the final stages of protocorms to plantlets for the purpose of commercialization and mass production.

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9. References

Alam, M.K, et al., Rashid, M., Hossain, M.,Salam, M., & Rouf, M. (2002*). In vitro* Seed Propagation of Dendrobium (Dendrobium transparens) Orchid as Influenced by Different Media. *Biotechnology (Faisalabad)*, *1*(2), 111-115. doi:10.3923/biotech.2002.111.115

Chen,Q. X., & Ji, Z. H. (1998). Encyclopedia of China orchid. Beijing, China. *China ForestryPublishing House*.

CITES.n (2019, November 26). *Checklist of CITES species*. https://checklist.cites.org/.

De, L. C., & Medhi, R. P. (2015). (PDF) ORCHID-A DIVERSIFIED COMPONENT OF FARMING SYSTEMS ... Retrieved December 10, 2020, from https:// www. researchgate. net/ publication/335443632_ORCHID-A_DIVERSIFIED_ COMPONENT_OF_FARMING_SYSTEMS_FOR_PROFITABILITY_AND_LIVELI HOOD_SECURITY_OF_SMALL_AND_MARGINAL_FARMERS

Decruse, D., et al. (2008). Effect of NAA and BAP on protocorm proliferation of Borneo ... Retrieved November 18, 2020, from https://www.researchgate.net/publication/259920952 _Effect_of_NAA_and_BAP_on_protocorm_proliferation_of_Borneo_Scented_Orchid

HAW, S., Sinicae, F., Changlin, T., Hua-shing, K., Tsin, W., KUANG Ko-zen & LI Pei-chun (eds), Qiyun, Z. (1999, January 01). Flora Reipublicae Popularis Sinicae -Tomus 57 (1). Retrieved November 06, 2020, from https://www.abebooks.com/book-search/title/flora-reipublicae-popularis-sinicae/used/

Maridass, M., et al. (2010, August 15). In Vitro Propagation of Dendrobium nanum through rhizome ... Retrieved November 6, 2020, from https://www. researchgate. net/ publication/ 273845533_In_Vitro_Propagation_of_ Dendrobium_ nanum_ through_ rhizome_bud_culture

Publishers, G., Gayathripublishers, & M. Maridass, M., Et al. (2010, August 15). Vol no.12 pp50 54. Retrieved November 06, 2020, from https://issuu.com/gayathripublishers/docs/vol._no._1_2_pp50-54

Roy, A., Patel, R., Patel, V., Sajeev, S., & Deka, B.C. 2011). Asymbiotic seed germination, mass propagation and seedling development of Vanda coerulea Griff ex.Lindl. (Blue Vanda): An in vitro protocol for an endangered orchid. *Scientia Horticulturae*, *128*(3), 325–331. https://doi.org/10.1016/j.scienta.2011.01.023

Kumar, S., et al. (2014, May 3). Ethno-medicinal Uses of some Orchids of Nagaland, North-east India. Retrieved November 25, 2020, from https:// scialert. net/ fulltext/? doi=rjmp. 2014.126.139

Saraanjeet, K., et al., & K, K. (2011, May 6). *In Vitro* Propagation of Dendrobium chrysotoxum (Lindl.). Retrieved November 18, 2020, from http://www.globalsciencebooks.info/Online/GSBOnline/images/2011/FOB_5(1)/FOB_ 5(1)50-56o.pdf

Tun, N. 2014. Wild Orchid of Myanmar.Vol.2, PP.412

Wu, K., et.al. (2014*). In Vitro* Propagation and Reintroduction of the Endangered Renanthera imschootiana Rolfe. *PLoS ONE,9*(10). doi:10.1371/journal.pone.0110033

Yu, Z., et al., Gong, C., Lu, B., Yang, L., Sheng, Y., Ji, L., & Wang, Z. (2015, January 01). *Dendrobium chrysotoxum* Lindl. Alleviates Diabetic Retinopathy by Preventing Retinal Inflammation and Tight Junction Protein Decrease. Retrieved November 25, 2020, from https://www.hindawi.com/journals/jdr/2015/518317