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Forest Department

Study on Nodal Cutting Method of Medicinal Orchid: *Dendrobium puchellum* Roxb. ex Lindl, *Dendrobium aphyllum* Roxb.f, *Dendrobium crystallinum* Rchb.f, *Dendrobium moschatum* Buch.Ham.Sw and *Dendrobium primulinum* Lindl.



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ဆေးဖက်ဝင်သစ်ခွမျိုးများဖြစ်သည့် ဆင်မမျက်ကွင်း ၊ ခမောက်ကြိုး ၊ စက္ကူပန်း၊ ဝါဆိုပန်း နှင့် သင်းကြူကြူ တို့အား အဆစ်ဖြတ်ပိုင်းမျိုးပွားနည်းစနစ်ဖြင့် စမ်းသပ်လေ့လာခြင်း

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သစ်တောမျိုးရိုးဗီဇ နှင့် ဇီဝနည်းပညာဌာနစု၊ သစ်တောဖွံ့ဖြိုးရေးဌာနခွဲ၊
သစ်တောသုတေသနဌာန၊ ရေဆင်း

စာတမ်းအကျဉ်း

သစ်ခွပင်၏ အဆစ်ဖြတ်ပိုင်းများမှ အညွှန့် နှင့် အမြစ် ထွက်ရှိ၍ အပင်သစ်တစ်ပင်အဖြစ် ရှင်သန်ကြီးထွားရန်အတွက် လိုအပ်သည့်အခြေခံအချက်အလက်များကို လေ့လာဖော်ထုတ်သိရှိနိုင်ရန်အတွက် ဤသုတေသနလုပ်ငန်းကို အကောင်အထည်ဖော် ဆောင်ရွက်ခဲ့ခြင်းဖြစ်ပါသည်။ ဤသုတေသနတွင် လေ့လာသောသစ်ခွမျိုးများသည် ဆေးဘက်ဝင်သစ်ခွမျိုးများဖြစ်ပြီး၊ မျိုးပွားရာတွင် ရှင်သန်နိုင်မှုတို့ကိုသိရှိစေရန်အတွက် ဟိုမုန်းကင်း၊ ဗစ်တာမင်နှင့် ဖာတီစတက် တို့နှင့် အတူ အခြေခံအစာလွှာနှစ်မျိုးဖြစ်သော (၁)အုန်းဆံခွံ နှင့် (၂) မီးသွေးခဲ၊ အုတ်နီခဲ၊ အုန်းဆံခွံ တို့ကိုအသုံးပြု၍ စမ်းသပ်မှု(၃)မျိုးဖြင့် စမ်းသပ်ဆောင်ရွက်ခဲ့ပါသည်။ သစ်ခွမျိုးစိပ်တို့၏ လုပ်ဆောင်နိုင်စွမ်းကို သိရှိနိုင်စေရန် သီတင်းပတ်တိုင်း စာရင်းအချက်အလက် ကောက်ယူ လေ့လာခဲ့ပါသည်။ စတင်မွေးမြူခြင်းအဆင့်တွင် ဟိုမုန်းကင်းနှင့်အတူ အခြေခံအစာလွှာနှစ်မျိုး တို့ဖြင့်စမ်းသပ်ခဲ့ရာ ခမောက်ကြိုး နှင့် သင်းကြူကြူသစ်ခွမျိုးတို့မှာ အခြေခံအစာလွှာ(၁)တွင် ပိုမိုဖြစ်ထွန်း၍ ဆင်မမျက်ကွင်း၊ ဝါဆိုပန်း နှင့် စက္ကူပန်း သစ်ခွမျိုးတို့မှာ အခြေခံ အစာလွှာ (၂) တွင်ရှင်သန်မှုနှုန်းအကောင်းဆုံးဖြစ်ကြောင်းစမ်းသပ်တွေ့ရှိရပါသည်။ ပွားများမွေးမြူခြင်းအဆင့် နှင့် ဒဏ်ခံနိုင်ရည်အဆင့်တို့တွင် အခြေခံအစာလွှာနှစ်မျိုးနှင့်အတူ ဟော်မုန်းကင်း နှင့် ဟော်မုန်း အားတိုးဆေးတို့ကိုအသုံးပြုရာတွင် စာရင်းအင်းနည်းလမ်းအရ အညွှန့်ထွက်ရှိမှု နှင့် အပင် အရှည်မှာ များစွာကွာခြားမှုမရှိကြောင်း စီစစ်လေ့လာသိရှိရပါသည်။ ရာသီအလိုက်ရှင်သန်မှုနှုန်းမှာ ၇၉ မှ ၉၅ ရာခိုင်နှုန်း အထိရှိ၍ နွေရာသီတွင် မိုးရာသီ နှင့် ဆောင်းရာသီထက် ပိုမိုဖြစ်ထွန်းကြောင်း စမ်းသပ်တွေ့ရှိရပါသည်။

Study on Nodal Cutting Method of Medicinal Orchid: *Dendrobium puchellum* Roxb. ex Lindl, *Dendrobium aphyllum* Roxb.f, *Dendrobium crystallinum* Rchb.f, *Dendrobium moschatum* Buch.Ham.Sw and *Dendrobium primulinum* Lindl.

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Abstract

This research paper is carried out for exploring favorable conditions for rooting juvenile shoots of *Dendrobium* species. It is a very potential orchid species for producing medicinal purposes. In this research, Vitamin (B1) and FERTI-START with the two media, coconut fiber (M1) and charcoal, small brickbat and coconut fiber (M2) were used for shoot and root growth performance. Three experiments were conducted for investigating the effectiveness of without hormone with two media, without hormone and hormonal treatments between two media and seasonal variation on growth response of nodal cuttings. These activities are done every week to determine using treatments and media for overall performance of five species. The survival percentage was influenced by without hormone and two different media. Based on the results of each orchid, *Dendrobium aphyllum* and *Dendrobium primulinum* were found the best performance on M1 than M2 among all species within three seasons in induction stage. The effects of shooting and rooting hormone with different durations influenced on each medicinal orchid species for three seasons. According to the (ANOVA), there were not statistically significant difference in shoot formation and length (inches) among all measurements in multiplication and hardening stage. After the process of hardening, the newly transplanted plantlets survived up to 79-95% exhibiting normal growth. As observed, these plantlets established well and grew favorably during hot season.

Key Words: Medicinal species, nodal cutting, hormone, seasonal variation, vegetative propagation

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1. Introduction

Dendrobium species is a genus of mostly epiphytic and lithophytic orchids in the family Orchidaceae. It is very large genus, containing more than 1,800 species that are found in diverse habitats throughout much of south, east and South East Asia, including China, Japan, India, the Philippines, Indonesia, Australia, New Guinea, Vietnam and many of the islands of the Pacific. Orchids in this genus have roots that creep over the surface of trees or rocks, rarely having their roots in soil. Up of six leaves develop in a tuft at the tip of a shoot and from one to a large number of flowers are arranged along an unbranched flowering stem. Several attempts have been made to separate Dendrobium into smaller genera, but most have not been accepted by the World Checklist of Selected Plant Families. The threat to Dendrobium species is illegal collecting and damage arising from bark stripping to host trees. Repeated burning, clearing and draining have resulted in habitat alteration and degradation. This species is including in CITES Appendix I by IUCN Red List. (J.A. Roberts & et.al.2019)

Dendrobium segments to form protocom-like bodies (PLBs) are further developed into plantlets that can be grown into mature plants in farmlands or greenhouses. Recent statistics indicate that there are more than 130 companies now producing Dendrobium herbs for commercial purposes in china. *Dendrobium* plants are rich in polysaccharides, and the amounts present in mature plants are controlled by many genetic and environmental factors, in particular species differences (Zhao etal., 2011; Xu etal., 2008), growth duration, and harvest time (Jin,2012; Li and Zhu, 2010), blossoming (Zhu et al., 2010), plant intensities (Zheng et al., 2012), growing temperature, and humidity (Jiang et al., 2007; Zhu et al., 2010).

Plant of the Dendrobium genus, one of the largest in the Orchidaceae, manifest a diversity of medicinal effects encompassing antiangiogenic, immunomodulating, antidiabetic, cataractogenesis-inhibiting, neuroprotective, hepatoprotective, anti-inflammatory, antiplatelet aggregation, antifungal, antibacterial, antiherpetic,, antimalarial, aquaporin-5 stimulating, and hemagglutininating activities and also exert beneficial action on colonic health and alleviate

symptoms of hyperthyroidism. The active principles include awide range of proteinaceous and non-proteinaceous molecules.

However, successful propagation of difficult-to-root species can be achieved if the type of cutting (hardwood, softwood, or root), date of collection (seasonal growth development), stock plant or cutting manipulation (pruning, wounding, etc.), rooting treatment (auxin type and concentration, rooting media), and greenhouse parameters (mist-bed system, supplemental lighting, temperature, etc.) are carefully considered. Vegetative propagation methods are also very useful tools in propagating forest tree, especially for cloning of desired quality trees. Vegetative propagation methods (including rooted cuttings) will be required in order to produce genetically Dendrobium species.

The Chinese continues to use orchids for medicinal purposes until today, most commonly in the form of medicinal tea. Dried dendrobium is believed to possess medicinal properties that can help treat cancer, strengthen the immune system, and improve eyesight. Orchids, like any pot plant, absorb carbon dioxide from their surrounding environment and release oxygen back into the air. As well as their uniform natural beauty. Orchids have also been claimed to offer health benefits. Some cultures even believe that orchid flowers as aheler of fever, diabetes, kidney and lugs. The use of a health plant (vigorous and disease-free) as the propagation stock is a good start of vegetative propagation. The fundamental consideration in growing any orchids is to know their natural habitats, including the elevation, seasonal changes in temperature, rainfall amounts, and light intensity.

In addition, it is important to know whether they grow in soils, on trees, or on rocks. A better understanding of the local climates would provide an important appreciation of different and critical demanding cultural requirements of various species. The natural habitats of different orchids can differ widely, and different orchids have a temperature range in which they can grow. Below or above the temperature range, at the extreme temperatures, the plants will soon become weak. Orchids can be generally grouped into three different temperature categories, i.e., cool-, intermediate-, or warm-growing, depending on their temperature requirements.

Orchids have been used in many parts of the world in traditional healing system as well as in the treatment of a number of diseases since the ancient time. Though Orchidaceae is regarded as a largest family of plant kingdom, few studies have been done regarding their medicinal properties. Linking of the indigenous knowledge of medicinal orchids to modern research activities provides a new reliable approach, for the discovery of novel drugs much more effectively than with random collection. Many of these orchids face the extreme danger of

extinction due to over- exploitation and habitat loss. Vegetative propagation could be one of the most suitable alternative tools to minimize the pressure on natural population of medicinal orchids and their sustainable utilization.

2. Objectives

The objectives of this research are as follows;

(1) To determine effect of plant growth regulator in five medicinal orchids species by nodal cutting method

(2) To investigate the seasonal variation influencing of the development of shoots and roots from collective cutting.

3. Literature Review

3.1 Distribution and habitat

Dendrobium orchids are widely distributed worldwide, from tropical Asian countries, such as India, Sri Lanka, the Phillippines, Australia, the Pacific Islands, and Papua New Guinea, where the biggest diversity was noted. Dendrobium orchids hail from awide variety of native settings, including tropical cloud forests where they grow a epiphytes on tree limbs high in the forest canopy. Others thrive in environs with strongly alternating wet-dry seasons. In their native habitats, many dendrobium orchids grow attached to deciduous trees. Orchids in the genus Dendrobium have adapted to awide variety of habitats, from the high altitudes in the Himalayan mountain to lowland tropical forests and even to the dry climate of the Australian desert. Orchids are nature's most extravagant group of flowering plants distributed throughout the world from tropics to high alpine. They exhibit incredible range of diversity in size, shape and color of their flowers. Though orchids are grown primarily as ornamentals, many are used as herbal medicines, food, and other have cultural value by different cultures and tribes in different parts of the world.

3.2 Description

Dendrobium orchids are primarily epiphytic, growing in nature on the branches and trunks of trees. They are sympodial, producing new stems (pseudobulbs) at the base of the previous year's stems. They have awide distribution and great variation in growth characteristics and floral structure. Terrestrial monocotyledonous plants that usually have showy 3-petaled flowers with the middle petal enlarged into a lip and differing from the others in shape and color. Holding in flower foods before packing and shipping is not necessary as a good hydration or bleach solution can be equally effective, flowers harvested in the winter last longer than those harvested in late summer. Flowes are 1-2 inches across, with 3 colored sepals, 2 petals and a 3rd

petal forming a lip, a spur extends from the back of the flower. Stems are 12-24 inches long bearing up to 15 flowers. Plant is an epiphytic perennial, in nature it grows on tree branches. Plant is classed as a monocotyledon, leaves mostly parallel veined.

3.3 Preparation of the Media

No propagation method is going to work if the right media for growth is not used. In propagation, the air content of shooting media should be between 20 and 45 volume percent to promote soot formation and growth. The volume percent in media should not drop below 15 volumes percent (Gislerod, 1983). This ensures adequate oxygen availability for the developing soot systems. Increases in air within the media increases the oxygen diffusion rate (ODR). This increase is what will aid the root systems in acquiring the optimum amount of oxygen needed. The contents of media can vary for different regions and different species. Two Type of media used in that experiment was sterilized M1 and M2, which were the most favorable media for shooting of orchid cuttings.

3.4 Affecting factors on nodal cutting method

The success of nodal cutting method is influenced by many factors. These factors include temperature, light, nutrient and plant growth regulator containing in basal media, and part of plant. The common definitions of these groups are the following: warm growers need approximately 20–30 °C during the day and 20–25 °C at night; intermediate growers need approximately 15–20 °C during the day and 10–15 °C at night; and cool growers need daytime temperatures approximately 10–15 °C days and 5–10 °C at night. Most orchids would grow very well in the range between 60 % and 80% humidity with good air circulation. Some species native to cloudy forests prefer a little higher humidity around 90%.

4. Materials and Methods

There were three experiments conducted in this study:

Experiment I: Study on the effectiveness of without hormone and different media on growth response of cuttings of five medicinal orchid species at three seasons in induction stage.

Experiment II: Study on effectiveness of without hormone and hormonal treatments with different duration and media on growth response of cuttings in multiplication stage

Experiment III: Study on Effect of rooting hormone and different duration and media on the species of nodal cutting in hardening stage.

4.1 Study area

Nodal segments of one year old of medicinal orchid of Dendrobium: Sinma myatqueen (*Dendrobium puchellum*), Kamaukkyue (*Dendrobium aphyllum*), Satku pan (*Dendrobium crystallinum*), Waso pan (*Dendrobium moschatum)* and ThinkyuKyu (*Dendrobium primunilum*) were used as experimental materials. There were collected from Southern Shan State: Pinloung, Kalaw, Ywa NganTownship. This study area lies between approximately 19˚ 56' N and 95˚ 56' E at an elevation of about 600 m abs. The experiments were carried out in Mist Chamber of Yezin, Forest Research Institute.

4.2 Preparation of cutting materials

The cuttings were collected from juvenile stem of one-year-old seedlings raised in the nursery. For each experimental unit or plot, 20 cuttings were tested. The cuttings selected were normally 15-25 cm long and 0.3-2.0 cm in diameter. There was a requirement for critical leaf area which ensures the optimal balance between the positive effects of photosynthesis and the negative effects of transpiration. Usually one or two leaves near the tip and sometimes together with active bud were left and the base of the cutting is treated with suitable auxins before being put into sterile medium under a mist propagator. Cuttings were taken from selected seedlings in the evening to protect burning from sunlight during daytime.

4.3 Preparation of planting media.

Orchids were planted in two media: coconut fiber (M1) and charcoal, small brickbat and coconut fiber (M2) and raised in plastic mist chamber. All the explants were maintained at 25-32º C (Tem:) and 60-80 % (RH) in it.

4.4 Preparation of the growth hormone

In that experiment, shooting and rooting hormone for different duration were used to determine the most suitable one for shooting and rooting of stem cuttings of orchid species. Vitamin (B1) and Ferti-Start Hormone Solution are containing Thiamine hydrochloride and chelated iron, it may promote cell division in the growing stage of plant. Hence, it is mainly applied to facilitate mature, step-up output, break quiescence, and encourage germination and cultivator. The stock solution of Vitamin (B1) and Ferti-start were prepared the amount of 10 % of this solution was mixed with distilled water to get 1000 ml concentration. Dipping methods were used in these experiments.

4.5 Study on Effect of Seasonal Variation

In the seasonal variation experiment, the seasons were divided into three periods. The experiments were conducted in three different seasons. For hot season, the experiment was conducted during April, 2020. For rainy season, the experiment was conducted during August, 2020 and for cold season, during November, 2020.

4.6 Data Collection and Analysis

The experiment was conducted using Completely Randomized Block Design (CRBD) with 4 replications and without hormone and 4 treatments. The observations were recorded regularly for the survival percentage, shoot and length formation of each treatment. Vitamin (B1) and FERTI-START with the two media, coconut fiber (M1) and charcoal, small brickbat and coconut fiber (M2) were used for shoot and length performance. These activities are done every week to determine using treatments and media for overall performance of five species. There were planted 4 explants with each without hormone and hormonal treatments for one replication. For each species, 20 explants were used with one medium for one replication. Therefore, the total number of sampled explants was 800. Data collection has been processed and accumulated by using Microsoft Office Excel 2003. Collected data has been statistically analyzed by using statistical software. Analysis of Variance (ANOVA) was done by using statistix version 8.0. Mean values were separated by using Least Significant Differences (LSD) test as 0.05 level.

Experimental Design

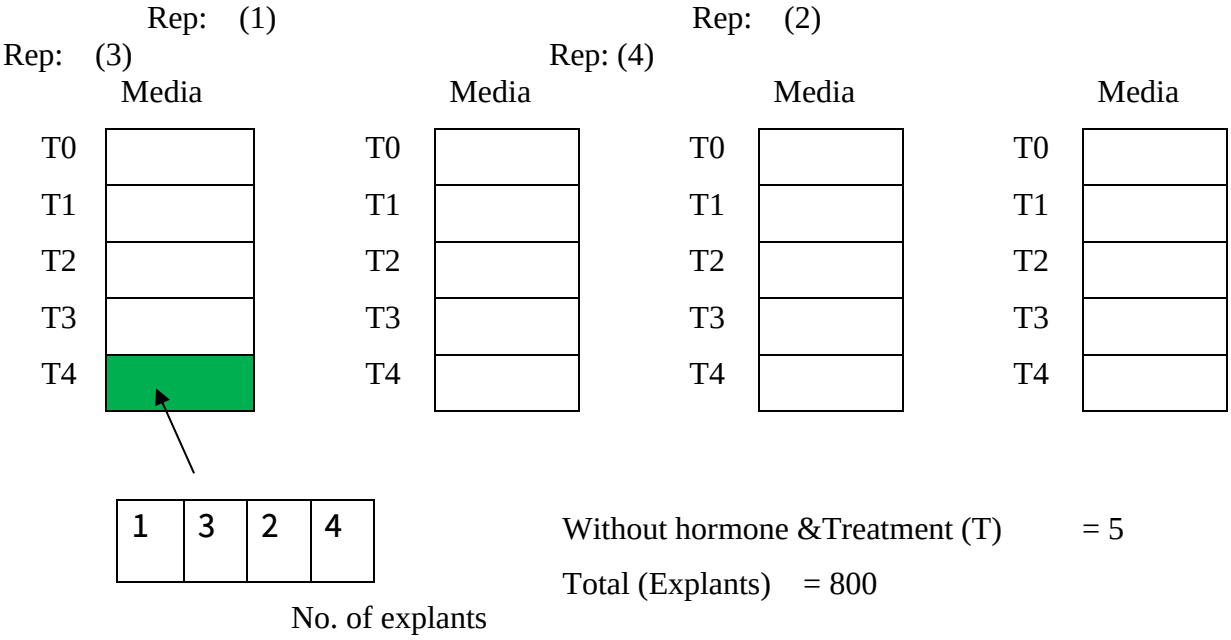


Table (1) Preparation of the two Media (I and II) with without hormone for experiment (I)

No.	Basal Media	Component	Weight(pound)
1	M1	coconut fiber	30
2	M2	charcoal, small brickbat and	30

	coconut fiber

Table (2) Without hormone and hormonal treatment (B1) with different duration in two Media (I and II) for experiment (II)

No. of Treatment	B1 (ml /l)	Duration (min:)
T_0 (without hormone)	-	-
T_1	10	5
T_2	10	10
T_3	10	15
T_4	10	20

Table (3) Without hormone and hormonal treatment (Ferti-start) with different duration in two Media (I and II) for experiment (III).

No. of Treatment	Ferti-start (ml /l)	Duration (min:)
T_0 (without hormone)	-	-
T_1	10	5
T_2	10	10
T_3	10	15
T_4	10	20

5. Results and Discussions

5.1. Experiment I: Study on the effectiveness of different media for nodal cutting method of five medicinal orchid species in initial stage

Without hormone and two different media were used in initial stage. The effects of explants position were described on survival percentage of one year old of nodal segments with terminal positions of each orchid species. The survival percentage was influenced by without hormone and two different media. The result of maximum survival percentage was found 100% and minimum rate was 55% for species 1. Maximum rate of 95% and minimum rate of 60% were obtained for species 2. And for specie 3, maximum rate was 90% but 20% minimum rate. For species 4, maximum rate was showed by 100% and as minimum 75 %.Maximum percentage was 95 and minimum was 40% for species 5. Based on the results of each orchid, Species 2 and

5 were found the best performance on medium 1 than medium 2 among all species within three seasons (Table 4). Highest survival rate was obtained during summer season followed by winter season and then rainy season in this experiment.

This result is in agreement with K. Rajneesh, *et. al*., 2009 reported that nodal cutting took from one year old branches for nodal testing. P. Sharma, 2011 reported that 2-3 nodal segments of one year old were survived than two year old nodal cutting. In contrast to the finding of Dr. A, Sarita (2012) who reported that the highest survival percent was influenced by different levels of the hormone concentrations and duration in initial stage.

Table (4) Survival percentage of five medicinal orchid species at two different media in initial stage

No.	Species	Media	Summer (SV %)	Rainy (SV %)	Winter (SV %)
1	*D. puchellum*	M1	55	70	70
		M2	100	80	85
2	*D. aphyllum*	M1	95	95	90
		M2	75	60	85
3	*D. crystallinum*	M1	20	40	75
		M2	75	90	80
4	*D. moschatum*	M1	75	90	75
		M2	100	95	85
5	*D. primulinum*	M1	95	70	90
		M2	70	40	55

5.2. Experiment II: Study on effectiveness of Without Hormone and Hormonal Treatments on growth response of cuttings in multiplication stage

Based on the results of each species, the effects of hormonal variation were revealed on survival percentage (SV%). Based on the results of each treatment, T1 was found as the best among others in summer and T_0, T1, T2 and T3 was observed the best performance but T4 was the lowest in rainy season, T2 and T3 were found at M_2 medium in winter. For species 2, T_0, T1, T2 and T3 were conducted as good performance in hot season and also T1 and T2 the good

performance in rainy season.T0, T1, T3 were used as the best but T2 was found very lower at M_1 medium in winter.

For species 3, T4 was found as the best among others in summer and T2 and T3 were observed the best performance in rainy season and T2 and T4 were found as the best at M_2 medium in winter. For species 4, T_0 and T1 produced good results but T2 and T3 showed bad performance in summer season. In rainy season, T_0, T1, T3 and T4 were the best but T2 was the lowest performance. T1, T2 and T3 were showed good performance but T4 was the bad at M2 medium in winter. For species 5, T0, T1 and T3 were conducted good performance but T2 and T4 was the bed in hot season. T_0 was the highest but T3 was the lowest performance in rainy season. T2 was the best but T0 and T1 was the bad performance at the M1 medium in winter. (Figure 1)

The clumps of shoots were excide from nodal segments after 4 weeks of growth on initiation medium and supplemented with without hormone and different duration and concentrations of hormone. According to the (ANOVA), there was no statistically significant difference in the different levels of treatments. Mean value of number of shoot ranged from 2.92 to 3.84 (Table 5)

This result is similar that in survival percentage, Strosse, et al. (2008) who reported that there was decreased the growth when increasing plant growth regulator concentration. Sarita Arya & et .al, 2008, reported that regenerated shoots were grown in medium supplemented with ferti-stat for further multiplication. Mohamed et al. (2007) stated that plant growth regulator supplements into the nutrient medium are one of the most influential factors affecting efficiency of vegetative propagation. Moreover, Kishor and Devir (2009) reported that exogenous application of different cytokinins, has become obligatory for induction of multiple shoot in many plants.

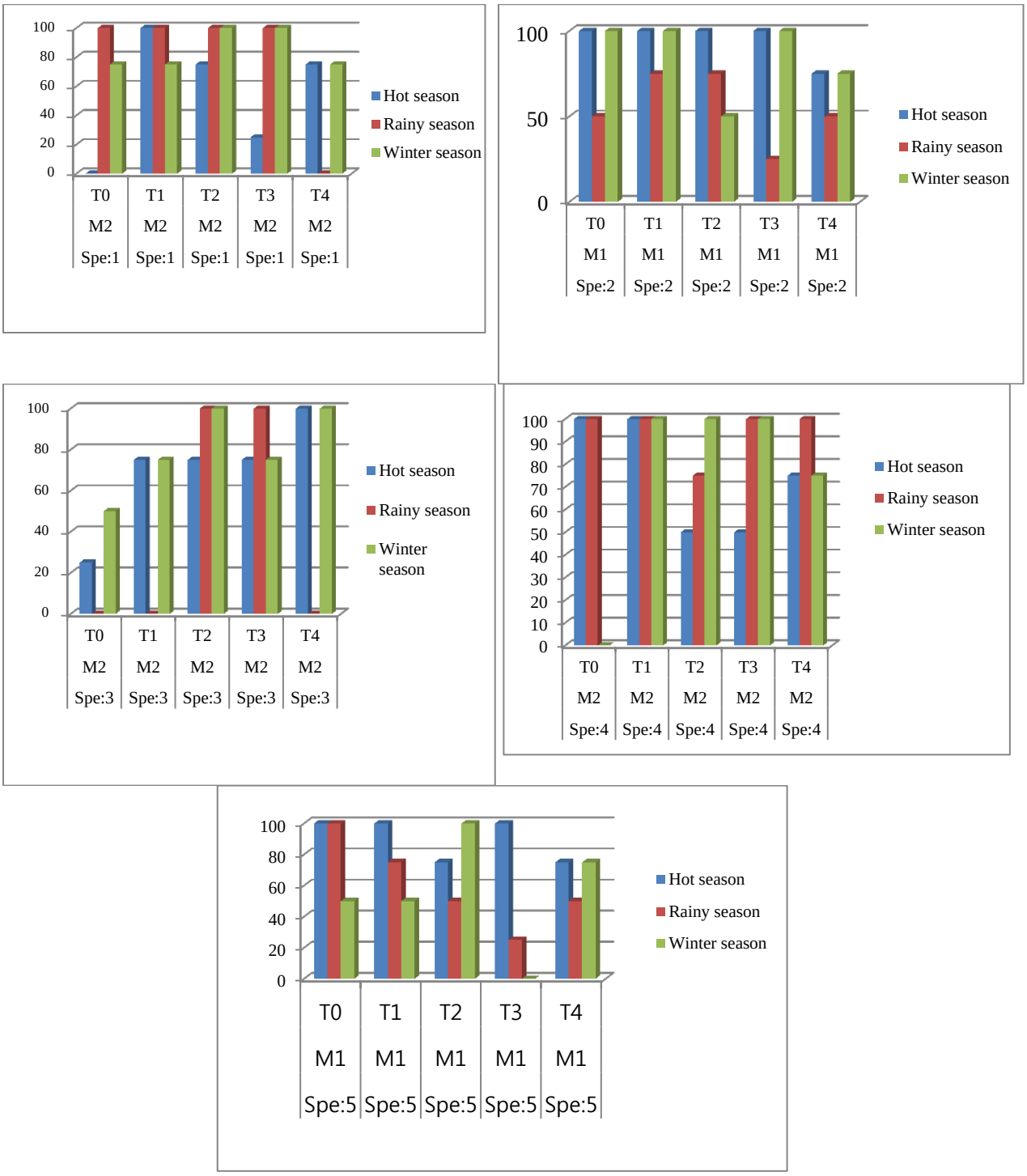


Figure (1) The effect of without hormone and hormonal treatment and two different media on five medicinal orchid species of survival percentage for three seasons in multiplication stage

Table (5) Effect of without hormone and hormonal treatment on shoot formation of medicinal orchid species in three seasons

Species	Summer	Rainy	Winter
		(Treatment)	
D. puchellum	3.8000A	3.0000A	3.2000 A
D. aphyllum	4.0000A	3.2000A	3.0000 A
D. crystallinum	3.8000A	2.8000 A	3.8000 A
D. moschatum	4.0000A	3.2000 A	3.4000 A
D. primulinum	3.6000A	2.4000 A	3.8000 A
Grand Mean	3.84	2.92	3.44
CV%	9.74	72.49	20.14

ns = not significant

The clumps of shoots were excide from nodal segments after 4 weeks of growth on initiation medium and supplemented with free hormone and different duration and concentrations of hormone. According to the (ANOVA), there was no statistically significant difference in the different levels of treatments. Mean value of number of shoot ranged from 2.92 to 3.84. (Table 5)

This result is similar that in survival percentage, Strosse, et al. (2008) who reported that there was decreased the growth when increasing plant growth regulator concentration. Sarita Arya & et .al, 2008, reported that regenerated shoots were grown in medium supplemented with vitamin for further multiplication. Mohamed et al. (2007) stated that plant growth regulator supplements into the nutrient medium are one of the most influential factors affecting efficiency of vegetative propagation. Moreover, Kishor and Devir (2009) reported that exogenous application of different cytokinins, has become obligatory for induction of multiple shoot in many plants.

5.3 Experiment III: Study on Effect of rooting hormone and different media on the species of nodal cutting in hardening stage

The effects of hormonal variation were revealed on survival percentage (SV %) in hardening stage. Based on the results of each treatment, for species 1, all treatments were showed good performance at M_2 medium in summer. For species 2, T0, T1 and T2 were found the best performance at M1 medium in three seasons. For species 3, T1, T2, T3 and T4 were found in summer and T2 and T3 were observed in rainy season but T_0 was found as the best at M_2 medium in winter. For species 4, T3 produced good results in all seasons. For species 5, T0 and T3 were conducted good performance at M1 medium in all seasons (Figure 2)

In this experiment, there were two factors of treatment; hormonal treatment as main plot factor and season as subplot factor. According to ANOVA table, there was not significant at 5 % level (Appendix II).The effects of hormone and durations influenced on each medicinal orchid species for three seasons. Among different seasons when the experiment was conducted, highest survival rate was found during hot season (95%) followed by cold season (85%) and then rainy season (79%).

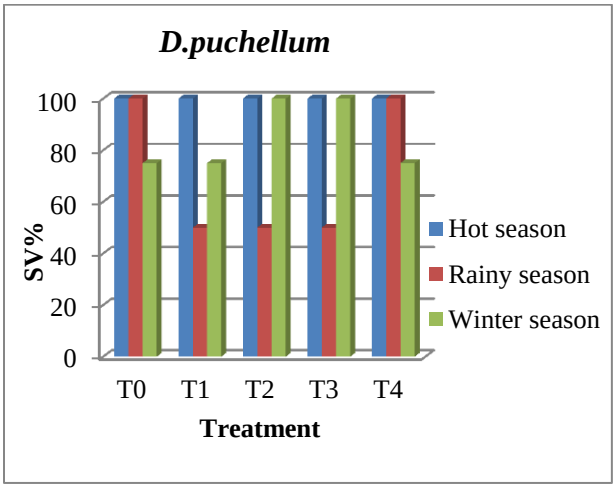
There were rapid growth of the cuttings by dry season resulting in almost all the cuttings rooting by fourth week of planting. Whereas in the rainy season, there was gradual rooting of cuttings up to the eight week. ANOVA revealed that there were no statistically significant differences in high formation among all measurements. But among different seasons when the experiment was conducted, mean range was found during rainy season (30.56) inches followed by winter (29.32) inches and then hot season (21.84) inches as shown in table 6. Regarding to shoot development of experimental units, there was found that active pairs of shoot developed after about one week. But root development was found only after about 45 days for most cuttings and after 60 days for some. Non-survived cuttings were wiled and died within one week. Fangus formation was found in some cuttings and in this experiment, it was not recognized as surviving. Only cuttings of which their shoots and roots developed well were counted for data collection. After following the process of hardening and acclimatization, the newly transplanted plantlets survived up to 79-95% exhibiting normal growth. As observed, these plantlets established well and grew favorably during hot season.

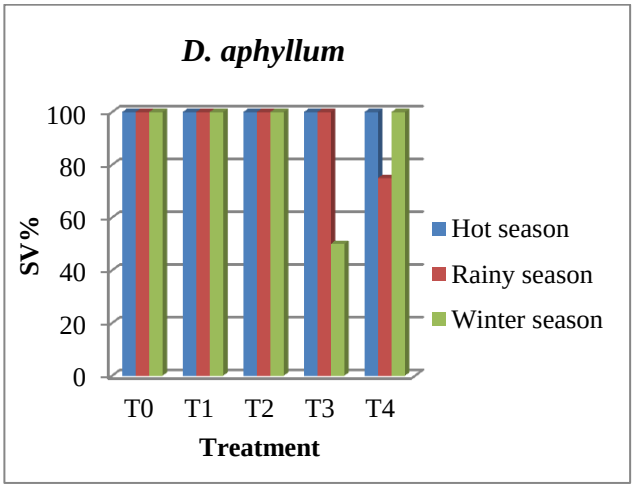
This observation was similar with that of Dick and Magingo (2012) reported that rooting ability of cuttings varied among node positions. In contract to the finding of Hentig and Gruber's reported that adventitious root formation was influenced by NAA dosage for nodal cuttings. Zhang, *et.al.,* (2010) stated that IBA powder may be more effective in plantlet rooting of *Dendrobium* species.The shoots were individually cut about 1.5 inches above the base and discarded while the 'propagules' (shoot cut) were separated again and placed in medium containing IBA at powder for induction of roots.

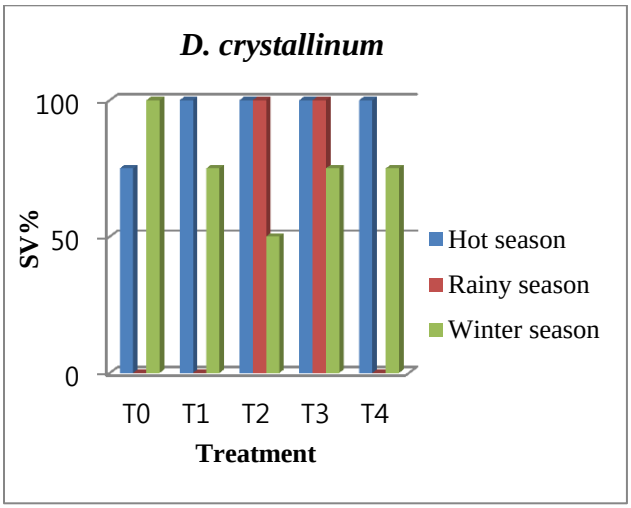
Table (6) Effect of without hormone and hormonal treatment on length (inches) of medicinal orchid species in three seasons

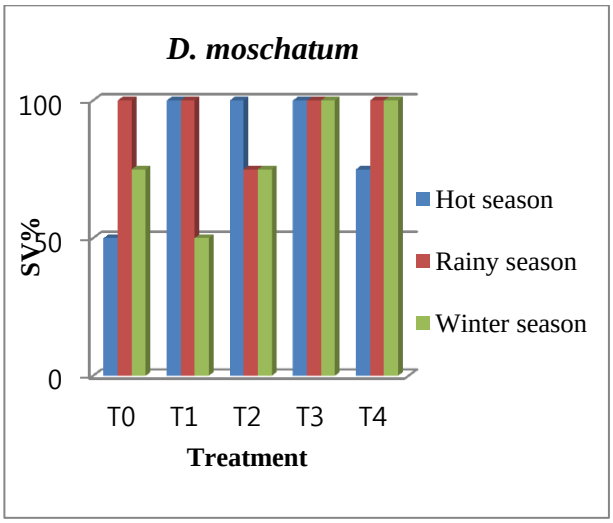
Species	Summer	Rainy	Winter
		Treatment	
D. puchellum	23.000 A	23.800 A	26.200 A
D. aphyllum	30.200 A	38.000 A	25.000 A
D. crystallinum	22.000 A	36.800 A	27.600 A
D. moschatum	17.200 A	28.200 A	30.800 A
D. primulinum	16.800 A	26.000 A	37.000 A
Grand Mean	21.84	30.56	29.32
CV%	78.62	95.55	86.10

ns = not significant









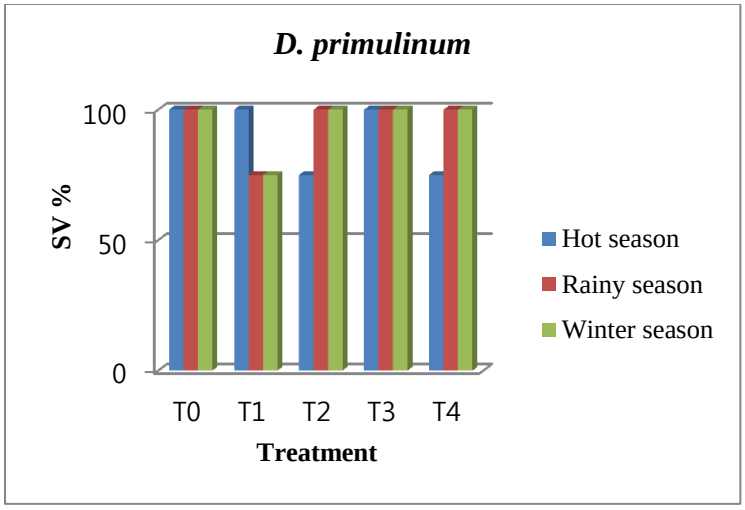


Figure (2) The effect of without hormone and hormonal treatment and two different media on five medicinal orchid species of survival percentage for three seasons in hardening stage

6. Conclusions and Recommendations

6.1 Conclusions

1. The result of experiments indicated that medicinal orchid could be raised through nodal segments.
2. Survival percentage of medicinal orchid species was affected by without hormone and under media (M1 and M2), species 2 and 5 were found the best performance on medium 1 than medium 2 among all species within three seasons in initial stage.
3. *Dendrobium* species can be regenerated via single-node cuttings, either without hormone treatment. Or with even better results using low concentrations of Vitamin (B1) and Ferti-Start for growth formation.
4. Among different seasons when the experiment was conducted, highest survival rate was found during hot season (95%) followed by cold season (85%) and then rainy season (79%) in hardening stage.

6.2 Recommendations

1. Further study should be tested again in different concentration level of hormone treatment to indentify the detail informations.
2. According to the results of these experiments, it was found that shoot and height growth formation were not statistically significant difference in the different levels of treatments under favourable environmental conditions. Consequently, further experiments should be conducted under different environmental conditions for producing the maximum percentage.
3. Other test such as cutting position and different age of cutting and test for condition between cuttings, such as different hormone and media should be conducted.

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collection of mother plant

Shooting and Rooting Hormone

Preparation of basal media

Multiplication stage (6 month)

Hardening stage (4 month)

Hardening stage (8 month)

Plate1. Procedure established from collection of mother plant to hardening stage of medicinal orchids

Appendix I

Experiment (II)

Table (7) ANOVA for shoot formation of five orchid species in two media at summer season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	0.56000	0.14000	1.00	0.4307
Error	20	2.80000	0.14000		
Total	24	3.36000			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 3.8400 CV 9.74

Table (8) ANOVA for shoot formation of five orchid species in two media at rainy season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	2.2400	0.56000	0.13	0.9717
Error	20	89.6000	4.48000		
Total	24	91.8400			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 2.9200 CV 72.49

Table (9) ANOVA for shoot formation of five orchid species in two media at winter season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	2.5600	0.64000	1.33	0.2921
Error	20	9.6000	4.48000		
Total	24	12.1600			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 3.4400 CV 20.14

Appendix (II)

Experiment (III)

Table (10) ANOVA for length (inches) of five orchid species in two media at summer season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	590.96	147.740	0.50	0.7353
Error	20	5896.40	294.820		
Total	24	6487.36			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 21.840 CV 78.62

Table (11) ANOVA for length (inches) of five orchid species in two media at rainy season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	831.8	207.940	0.24	0.9100
Error	20	17054.4	852.720		
Total	24	17886.2			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 30.560 CV 95.55

Table (12) ANOVA for length (inches) of five orchid species in two media at winter season

Source	*DF*	*SS*	*MS*	*F*	*P*
V001	4	462.6	115.660	0.18	0.9453
Error	20	12744.8	637.240		
Total	24	13207.4			

***DF* Degree of Freedom, *SS* Sum of Squares, *MS* Mean Sum of Squares, *F* F test, *P* no significant level: p > .05000**

Grand Mean 29.320 CV 86.10