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Study on Multiplication of Four Orchid Species by Stem Cutting Method




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Abstract

This research was carried out in order to find out the basic information necessary for the growth of new plants from orchid stem cuttings to the stage of seed bearing. Four kinds of orchids, namely, *Vanda coerulea* Griff. ex Lindl., *Dendrobium moschatum* (Banks) SW., *Dendrobium chrysotoxum* Lindl and *Paphiopedilum charlesworthii* (Rolfe) Pfitz experimented and researched by vegetative reproduction and seed cultivation. The *Vanda coerulea* Griff ex. Lindl orchids are economically important. The *Dendrobium moschatum* (Buch-Ham) SW and *Dendrobium chrysotoxum* Lindl are used medicinally. The *Paphiopedilum charlesworthii* (Rolfe) Pfitz orchids are rare orchids. In this experiment, research was conducted on three types of basic nutrients and hormone free and hormone use methods and on the production of new shoots, the height of the new plants produced, roots formation, flowering and fruit set by self-pollination. There are two types of pollination, namely self-pollination and cross-pollination. In this study of orchids species, self-pollination was used by own hand. According to the statistical calculations, the economically important *V. coerulea* orchid had higher flowering and fruiting rates than other orchids. From the results of this research and experiment, it will be possible to find out a way to save time and money, and on the one hand, it will be possible to protect the orchid species from extinction.

Key Words: hormone, media, seasonal variation, vegetative propagation, pollination

သစ်ခွမျိုးစိတ်လေးမျိုးတို့အား ပင်ပိုင်းမျိုးပွားခြင်းနည်းဖြင့် ပွားများမွေးမြူလေ့လာခြင်း

ခင်ပပရွှေ၊ လက်ထောက်သုတေသနအရာရှိ
သစ်တောသုတေသနဌာန

စာတမ်းအကျဉ်း

သစ်ခွပင်၏ပင်စည်ဖြတ်ပိုင်းများမှ အပင်သစ်များထွက်ရှိလာ၍ မျိုးစေ့တင်ရှိမှု အဆင့်တိုင်အောင် ရှင်သန်ကြီးထွားရန်အတွက် လိုအပ်သည့်အခြေခံအချက်အလက်များကို လေ့လာဖော်ထုတ် သိရှိနိုင်ရန်အတွက် ဤသုတေသနလုပ်ငန်းကို အကောင်အထည်ဖော် ဆောင်ရွက်ခဲ့ခြင်းဖြစ်ပါသည်။ ဤသုတေသနတွင် မိုးလုံးမှိုင်း၊ ဝါဆိုပန်း၊ ရွှေတူမောက် ခမ်းဝါနှင့် မြွေမင်းသား သစ်ခွမျိုးတို့ကို ပင်ပိုင်းမျိုးပွားခြင်းနည်းဖြင့် စမ်းသပ်လေ့လာ၍ သုတေသန ပြုဆောင်ရွက်ခဲ့ပါသည်။ မိုးလုံးမှိုင်း သစ်ခွမျိုးသည် စီးပွားရေးအရအရေးပါ၍၊ ဝါဆိုပန်းနှင့် ရွှေတူမောက်ခမ်းဝါ သစ်ခွမျိုးတို့သည် ဆေးဖက်ဝင် သစ်ခွမျိုးများဖြစ်ကြပြီး မြွေမင်းသား သစ်ခွမျိုးသည် ရှားပါး သစ်ခွမျိုးဖြစ်ပါသည်။ ထိုသို့စမ်းသပ်လေ့လာရာတွင် အခြေခံမျိုးပွားစာ (၃)မျိုးနှင့်အတူ ဟော်မုန်းမပါနှင့် ဟော်မုန်း အသုံး ပြုမှုနည်း (၂)မျိုးတို့ဖြင့် အညွှန့်သစ်ထွက်ရှိမှု၊ ထွက်ရှိခဲ့သော အပင်သစ်တို့၏ အမြင့် တိုင်းတာမှု၊ အမြစ် ဖွဲ့စည်းမှု၊ ပန်းပွင့် ပွင့်မှုနှင့် မျိုးစေ့တင်ရှိမှုတို့ကို စမ်းသပ်သုတေသန ပြုလုပ်ခဲ့ပါသည်။ မျိုးစေ့တင်ရှိရန်အတွက် ပန်းပွင့်များကို ဝတ်မှုန်ကူးပေးသောနည်းဖြင့် စမ်းသပ်ရာတွင် ပင်တည်းဝတ်မှုန်ကူးပေးခြင်းနှင့် ပင်ခြားဝတ်မှုန်ကူးပေးခြင်းဟူ၍ နည်း(၂)မျိုးရှိပါသည်။ ယခု မျိုးစေ့တင်ရှိမှုကို လေ့လာရာတွင် ကိုယ်တိုင်လက်ကိုအသုံးပြု၍ ပင်တည်း ဝတ်မှုန် ကူးခြင်းနည်းကို အသုံးပြုခဲ့ပါ သည်။ ဤသုတေသနစမ်းသပ်ချက်မှ စီးပွားရေးအရ အရေးပါသော မိုးလုံးမှိုင်းသစ်ခွမျိုးသည် အခြားသစ်ခွမျိုးများထက် ပန်းပွင့် ပွင့်နှုန်းများသကဲ့သို့ မျိုးစေ့တင်ရှိမှု နှုန်းသည်လည်း များသည်ကို တွေ့ရှိခဲ့ရပါသည်။ ဤစမ်းသပ်ချက်ရလဒ်မှ အချိန်နှင့် ငွေကုန် သက်သာမည့်နည်းလမ်းကို သိရှိနိုင် သည့်အပြင် သစ်ခွမျိုးစိတ်များ မျိုးသုဉ်းပျောက်ကွယ်မှုကို တစ်ဖက်တစ်လမ်းမှ ကာကွယ်ပေးနိုင် မည်ဖြစ်ပါသည်။

Study on Multiplication of Four Orchid Species by Stem Cutting Method

1. Introduction

Orchids are one of the most diverse and widely cultivated plant families, with over 25,000 species worldwide. (Chin, S.W and Chen, F.C, 2021) The introduction of multiplication techniques for orchid species is pivotal in the field of horticulture and conservation. Among the various methods employed, vegetative propagation and self-pollination stand out as effective strategies for enhancing the growth and sustainability of these exquisite plants. Vegetative propagation involves the asexual reproduction of orchids, allowing for the production of genetically identical offspring from a parent plant. This method not only ensures consistency in desirable traits such as flower color and size but also enables rapid multiplication, which is essential for commercial cultivation and conservation efforts. Techniques such as division and keiki (offset) production are commonly utilized to achieve successful vegetative propagation. (H, agsater & et.al, 2009)

On the other hand, self-pollination is a natural reproductive strategy that allows orchids to produce seeds without the need for cross-pollination. This method can be particularly advantageous in isolated environments where pollinators may be scarce. By facilitating self-fertilization, orchids can maintain their populations and adapt to changing ecological conditions. Both vegetative propagation and self-pollination play significant roles in the multiplication of orchid species. Understanding and utilizing these methods effectively contribute to the preservation of biodiversity and meet the growing demands of orchid enthusiasts and commercial growers alike. The propagation of orchid species is a crucial aspect of horticulture, enabling the cultivation and preservation of these exquisite plants. Two primary methods of orchid propagation are stem cuttings and seed propagation, each with its unique advantages and challenges.

Vanda coerulea Griff. ex Lindl. orchid is an economically important species. It is commonly known as the Blue Vanda, a species of orchid native to Southeast Asia, particularly found in regions such as Myanmar, Thailand, and Laos. This epiphytic orchid is renowned for its striking blue to violet flowers, which can reach up to 10 cm in diameter and typically bloom during the warmer months. The plant features long, narrow leaves that can grow up to 60 cm in length and thrives in warm, humid environments with bright indirect light. *V. coerulea* Griff ex. Lindl is often cultivated for ornamental purposes due to its stunning floral display and is popular among orchid enthusiasts. (Sutapa Dutta & et. al, 2011)

Dendrobium moschatum (Banks) SW. is medicinal orchid species. It is commonly referred to as the fragrant *Dendrobium*, a species of orchid belonging to the family Orchidaceae. It is native to various regions of Southeast Asia, including India, Nepal, Bhutan, and parts of Myanmar and Thailand. This species is characterized by its slender stems and vibrant flowers. The plant typically thrives in warm and humid environments and prefers well-draining substrates, often found growing on trees or rocks in its natural habitat. It is particularly valued for its fragrant blooms, which can emit a sweet scent, making it a popular choice among orchid enthusiasts and collectors. (K, Hubert, 2014)

Dendrobium chrysotoxum Lindl is also medicinal orchid species. It is commonly known as the Golden Yellow *Dendrobium*, a species of orchid native to Southeast Asia, particularly found in regions such as Thailand, Myanmar, and Malaysia. This epiphytic orchid is characterized by its striking golden-yellow flowers that typically bloom in clusters, making it a popular choice among orchid enthusiasts and collectors. The plant generally features slender, cane-like stems and can grow up to 1 meter in height. It thrives in warm, humid environments

and prefers well-drained potting media. Cultivating *D.chrysotoxum* Lindl requires attention to light conditions; it favors bright but indirect sunlight. Regular watering during the growing season, along with a balanced fertilizer regimen, supports healthy growth and flowering.

Paphiopedilum charlesworthii (Rolfe) Pfitz orchid species is a rare species. It is commonly known as Charlesworth's Paphiopedilum, is a species of orchid belonging to the family Orchidaceae. This particular orchid is native to the tropical regions of Southeast Asia, specifically found in areas such as Myanmar and Thailand. The plant is characterized by its unique slipper-shaped flowers. The blooms are not only visually striking but also have a delightful fragrance, making them a popular choice among orchid enthusiasts. (De, L.C, 2014)

In this research paper, we will explore vegetative propagation and self-pollination used in the cultivation of (commercial, medicinal and rare) orchids.

Vegetative propagation is a method used to produce new plants using plant segments. This method allows for the rapid propagation of desirable traits and characteristics, making it possible to produce plants with consistent quality and appearance. In the case of (commercial, medicinal and rare) orchids, vegetative propagation can be achieved through techniques such as stem cuttings, leaf cuttings, or division.

Self-pollination is a method used to transfer pollen from the male reproductive organ (stamen) to the female reproductive organ (pistil) within the same flower. This method can occur naturally through the movement of pollen grains within the flower's reproductive structure. Self- pollination can be advantageous in certain situations, such as when cross-pollination is not possible or when the desired genetic traits are already present within the plant.

Both of these methods can be used in the cultivation of orchids to produce new plants (Keiki) with desirable traits and characteristics. By understanding and utilizing these methods, researchers and orchid enthusiasts can continue to explore and develop new varieties of orchids, furthering our knowledge of these fascinating plants.

Problem statement

The problem statement for the multiplication of orchid species through stem cutting method can be articulated as follows:

Orchids are a diverse family of flowering plants, known for their complex reproductive structures and unique growth requirements. The successful propagation of orchid species is critical for conservation efforts, horticultural practices, and commercial cultivation. However, traditional methods of propagation, such as division or tissue culture, can be labor-intensive and costly.

Stem cutting method involves taking sections of the stem from a parent plant to produce new individuals. The primary challenge with stem cuttings in orchids is that many species do not readily root from cuttings due to their specialized root systems and growth habits. Factors such as the timing of cutting, environmental conditions (humidity, temperature), and appropriate rooting hormones must be optimized to enhance success rates.

2. Objectives

The objectives of this research are as follows;

(1) To determine effect of plant growth regulator in four orchids species by vegetative propagation and self-pollination.

(2) To investigate the seasonal variation influencing of the development of shoots and roots from collective stem cutting by three basal media.

3. Literature Review

Orchids are among the most diverse and widely distributed flowering plants, and their propagation is crucial for both conservation and commercial purposes. This literature review focuses on methods of orchid propagation: stem cuttings and seed propagation, examining their effectiveness, advantages, and challenges.

Stem cutting is a vegetative propagation technique that involves taking a section of the stem from a parent plant to grow new individuals. Research has shown that this method can be effective for certain orchid species, particularly those belonging to the genera such as Dendrobium_and Cattleya. According to Smith et al. (2020), successful rooting from stem cuttings can be influenced by several factors including cutting length, hormonal treatments (such as auxins), and environmental conditions like humidity and temperature.

Advantages:

- Rapid multiplication of desirable traits from parent plants.

- Reduced time to flowering compared to seed propagation.

Challenges:

- Limited applicability; not all orchid species respond well to stem cuttings.

- Risk of disease transmission if proper sterilization techniques are not employed.

Self-pollination is a method of reproduction in plants that involves the transfer of pollen from one part of a flower to another. It can occur within the same flower (autogamy) or between different flowers on the same plant (geitonogamy).

Seed propagation is the traditional method for orchid cultivation, relying on the germination of seeds under controlled conditions. Orchid seeds are known for their small size and lack of nutrient reserves; thus, they require specific symbiotic relationships with mycorrhizal fungi for successful germination. Jones (2019) highlighted that while seed propagation allows for genetic diversity, it often requires a longer time frame before seedlings mature into flowering plants.

Advantages:

- High potential for genetic variation, which can lead to new hybrids.

- Greater adaptability in various environmental conditions due to diverse genetic backgrounds.

Challenges:

- Longer time required for germination and growth compared to vegetative methods.

- Complexity in managing fungal symbionts necessary for seed germination.

While stem cuttings may offer quicker results with specific genera, seed propagation remains essential for maintaining genetic diversity within orchid populations. Future research should focus on optimizing conditions for both methods to enhance efficiency and success rates in orchid cultivation.

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 four orchid species at three seasons.

Experiment II: Study on effectiveness of without hormone and hormonal treatments with different duration and media on growth response of stem cuttings.

Experiment III: Study on Effect of rooting hormone and different duration and media on fruit set by manual self-pollination.

4.1 Experimental area

Stem cutting of two-year-old of orchid species: *Vanda coerulea* Griff. ex Lindl**.** (Moe Lone Hmaine), *Dendrobium moschatum* (Banks) SW. (Waso Pan), *Dendrobium chrysotoxum Lindl*. (Shwe Tu Mouk Khan War), *Paphiopedilum charlesworthii* (Rolfe) Pfitz (Mway Min Thar), were used as experimental materials. There were collected from Southern shan state: Pinloung, Kalaw and Taunggyi Township. Southern shan state: temperatures and humidity can range from 19 ◌°C to 26˚ C and 70-80 % (https//www; timeanddate com>weather). This 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 materials and planting media

The cuttings were collected from juvenile stem of two-year-old seedlings raised in the nursery. For each experimental unit or plot, 21 cuttings were tested. 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.

Orchids were planted in three media: (1) coconut fiber and moss (M1), coconut fiber and charcoal (M2), small brickbat, coconut fiber and moss (M3) and were raised in mist chamber. All the explants were maintained at 20-25º C (Tem:) and 70-80 % (RH) in it.

4.3 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.

4.4 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 summer season, the experiment was conducted during April, 2023. For rainy season, the experiment was conducted during August, 2023 and for winter season, during November, 2023.

4.5 Data Collection and Analysis

The experiment was conducted using Factorial design with 4 replications and without hormone and 2 treatments. The observations were recorded regularly for the survival percentage, shoot and length formation of each treatment. Vitamin (B1) and Ferti-start with the three media were used for shoot and length performance. These activities are done every week to determine using treatments and media for overall performance of four species. There were planted 7 explants with each without hormone and hormonal treatments for one replication. For each species, 21 explants were used with one medium for one replication. Therefore, the total number of sampled explants was 252. 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 (One species)

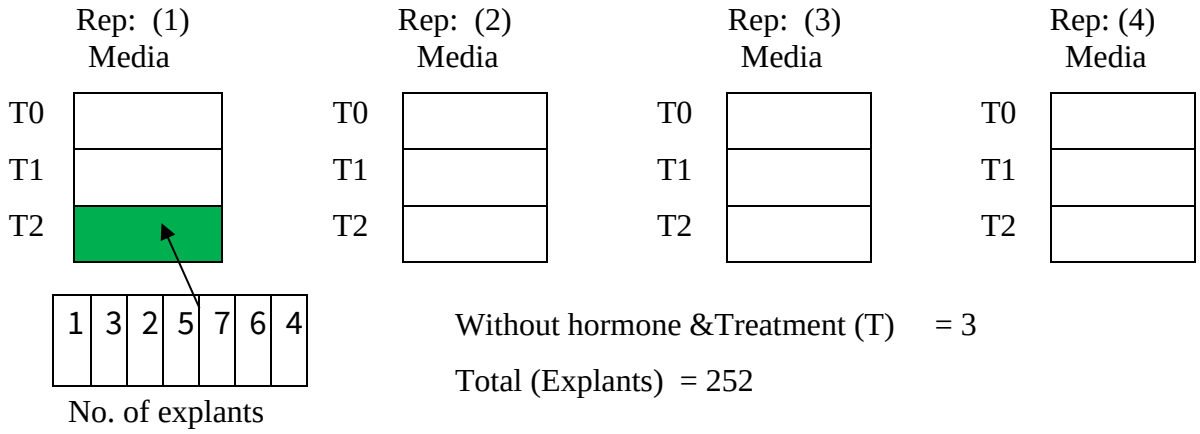

Table 1. Preparation of the three Media (I, II and II) with without hormone for experiment (I)

No.	Basal Media	Component	Weight(pound)
1	M1	coconut fiber and moss	30
2	M2	coconut fiber and charcoal	30
3	M3	small brickbat, coconut fiber and moss	30

Table 2. Without hormone and hormonal treatment (B1) with different duration in three Media (I, II and III) for experiment (II)

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

Table 3. Without hormone and hormonal treatment (Ferti-start) with different duration in three Media (I, II 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

5. Results and Discussions

Experiment I: Study on the effectiveness of without hormone and different media on growth response of cuttings of four orchid species at three seasons.

New shoots were counted in three media types (M1, M2, M3) of growth and development in summer, rainy and winter weather. As *V. coerulea*, in M1 madia, 28 % in summer and winter and 86 % in rainy season. 56 % in summer and 86% in raining season and 28% in winter season in M2 media. In M3 media, 42% in summer and winter season and 86 % in rainy season. Therefore, it was found that the survival percentage was the same in all three types of media in the raining season. It was found that *V. coerulea* orchid species survived more in the rainy season than in the dry season in all three types of media.

As for *D. moschatum*, in M1 media, it was found that 86% survival in rainy season, 70% in summer and 42 % in winter. In M2 media, the survival rate in the rainy season is the best, but the survival rate in winter and summer season was the same. In M2 media, survival rate was 70 % in rainy season and 42 % in the summer and winter season. In M3 media, 70 % survived in the rainy season, but 42 % survived in the summer and 14 % in the winter season, which is a low survival rate. Therefore, it was found that M1 media had the best survival rate in rainy season and M3 media had the lowest survival rate was winter.

As *D. chrysotoxum* orchid, in M1 media, it was found that 100% survival in the rainy season, 70 % survival in summer and 42 % survival in winter. M2 media found a good survival rate in all three seasons with 100 % survival in each. In M3 media, it was found that the survival rate was the same in rainy season and 70 % in winter, and 42 % survival in summer. Therefore, M2 media had the best survival in all three seasons and should be used for this orchid species.

As a rare species of orchid, *P. charlesworthii* orchid, M1 media showed good survival rate of 100 % in summer and rainy season, and 42 % survival rate in winter. M2 media found a good survival rate of 100 % in all three climates. As for M3 media, it was found that 86 % survived in summer and rainy season, and 42 % survived in winter. Therefor, M2 media was a better survival rate than other media in all three types of weather and should be used. (Table 4)

This result is in agreement with K. Rajneesh, *et. al*., 2009 reported that stem cutting took from two year old branches for vegetative propagation testing. P. Sharma, 2011 reported that stem of two year old were survived than one year old stem 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 keiki of orchid species at three different media in three seasons

No.	Species	Media	Summer (SV %)	Rainy (SV %)	Winter (SV %)
1.	*V. coerulea* Griff ex. Lindl**.** (Moe Lone Hmaine)	M1	28	86	28
		M2	56	86	28
		M3	42	86	42
2.	*D. moschatum* (Buch - Ham) SW. (Waso Pan)	M1	70	86	42
		M2	42	70	42
		M3	42	70	14
3.	*D. chrysotoxum Lindl*. (Shwe Tu Mouk Khan War)	M1	70	100	42
		M2	100	100	100
		M3	42	70	70
4.	*P. charlesworthii* (Rolfe) Pfitz (Mway Min Thar),	M1	100	100	42
		M2	100	100	100
		M3	86	86	42

Experiment II: Study on effectiveness of without hormone and hormonal treatments with different duration and media on growth response of stem cuttings.

Orchids species were used in hormone-free and hormone- treated ways to study new plant production. According to the statistical calculation, there was no significant difference between the three seasons for *V. coerulea* orchid species. (Table 5) In the same way, there were no significant differences between the *D. moschatum, D. chrysotoxum* and *P. charlesworthii* orchids using hormone-free and hormone-based methods. (Table 6,7) According to the P value, *P. charlesworthii* was highly significant in winter (Table 8).

Table 5. ANOVA for effect of without hormone and hormonal treatment on new shoot of *V. coerulea* orchid species in three seasons

Treatment	Summer	Rainy	Winter
T0	0.2857 a	0.8571 a	0.5714 a
T1	0.5714 a	0.8571 a	0.4286 a
T2	0.4286 a	0.8571 a	0.7143 a
Grand Mean	0.4286	0.8571	0.5714
CV%	121.21	44.10	158.99
Pr > F	0.5978 ^{ns}	1.0000 ^{ns}	0.8425 ^{ns}

ns = not significant

Table 6. ANOVA for effect of without hormone and hormonal treatment and three media on new shoot of *D. moschatum* orchid species in three seasons

Treatment	Summer	Rainy	Winter
T0	0.7143 a	3.4286 a	0.4286 a
T1	1.0000 a	2.4286 ab	0.4286 a
T2	1.0000 a	1.0000 b	0.1429 a
Grand Mean	0.9048	2.2857	0.3333
CV%	150.62	94.19	146.39
Pr > F	0.9030 ^{ns}	0.1342 ^{ns}	0.4647 ^{ns}

ns = not significant

Table 7. ANOVA for effect of without hormone and hormonal treatment and three media on new shoot of *D. chrysotoxum* orchid species in three seasons

Treatment	Summer	Rainy	Winter
T0	1.7143 a	3.2857 a	0.4286 b
T1	1.4286 a	2.8571 a	2.0000 a
T2	0.4286 a	1.4286 a	1.5714 ab
Grand Mean	1.1905	2.5238	1.3333
CV%	98.71	69.89	85.57
Pr > F	0.1279 ns	0.1481 ns	0.0502 ns

ns = not significant

Table 8. ANOVA for effect of without hormone and hormonal treatment and three media on new shoot of *P. charlesworthii* orchid species in three seasons

Treatment	Summer	Rainy	Winter
T0	1.8571 a	2.7143 a	0.4286 b
T1	2.0000 a	2.8571 a	1.2857 a
T2	1.5714 a	1.7143 a	0.4286 b
Grand Mean	1.8095	2.4286	0.7143
CV%	56.13	43.09	72.72
Pr > F	0.7280 ns	0.1120 ns	0.0082 **

ns = not significant ** = highly significant

As a measurement of the height of the new plants, measurements were carried out in all three types of media in all three seasons. In this measurement, *V. coerulea* orchid species had the highest growth in M2 media in the rainy season and slow growth in M3 media in winter. As for *D. moschatum*, it was found that M2 media had the highest growth rate in the rainy season, but M3 media had a lower growth rate in the winter season.

In the *D. chrysotoxum* orchid, M2 media had the highest growth in winter, but M3 media showed lowest growth in summer. And then, M1 media showed fast growth in rainy season, but M3 media showed slow growth in winter. As *P. charlesworthii* orchid, M1 media showed fast growth in rainy season, but M3 media showed slow growth in winter. Therefore, *V. coerulea* and *D. moschatum* orchids should be planted in M2 media during the rainy season, *D. chrysotoxum* orchids should be planted in M2 media in winter, and *P. charlesworthii* orchids should be planted in M1 media in rainy season for height growth performance. (Figure 1)

This result is similar that in survival percentage, Strosse, et al. (2008) who reported that there was decreased the height growth when increasing plant growth regulator concentration. Sarita Arya & et al, 2008, reported that regenerated heights 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.

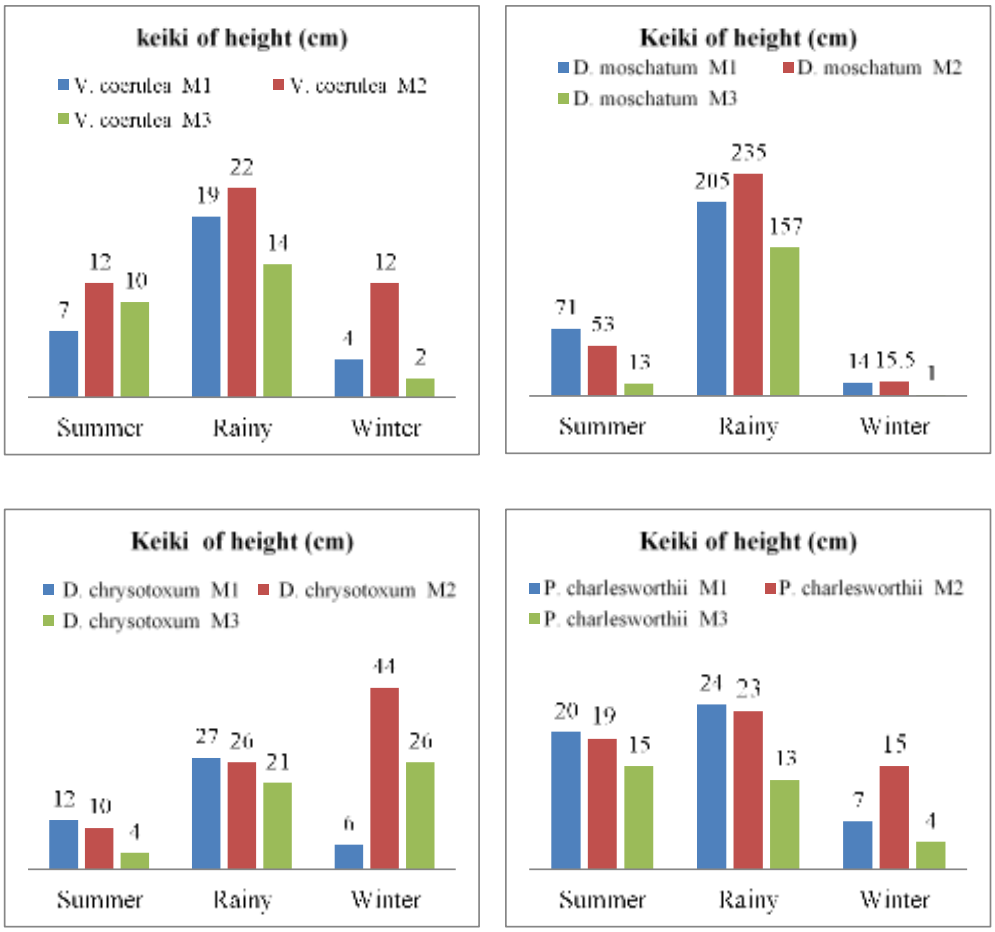

Figure 1. New plant (keiki) of orchid species of height at three different media in three seasons

As for the formation of new roots, it was studied in three types of media. According to the experimental results, *V. coerulea* was found that the rate of new root production in M2 media was strong in rainy season, and the rate of new root production was low in M3 media in winter. As *D. moschatum* orchid variety, it was found that M2 media had very good new root production in rainy season, but no production rate in all three media types in winter. As for *D. chrysotoxum* orchid, many new roots were produced in the rainy season in M1 media, but no new roots were produced in the M2 media. As *P. charlesworthii* orchid, M2 media had a good rate of new root production in the rainy season, but it was found that M3 media had no new root production in winter. Therefor, it was found that *V. coerulea, D. moschatum, P. charlesworthii* orchid species had strong new root production in the rainy season in M2 media, but no new root production in winter in M3 nedia. As *D. chrysotoxum,* , M1 media had a good new root production rate in the rainy season, but it was found that M2 media did not produce any new roots even in that season.(Figure 2)

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 ferti-start dosage for nodal cuttings. Zhang, *et.al.,* (2010) stated that vitamin B1may be more effective in plantlet rooting of *Dendrobium* species.The shoots were individually cut about 1.5 feet above the base and discarded while the 'propagules' (stem cut) were separated again and placed in medium containing IBA at powder for induction of roots.

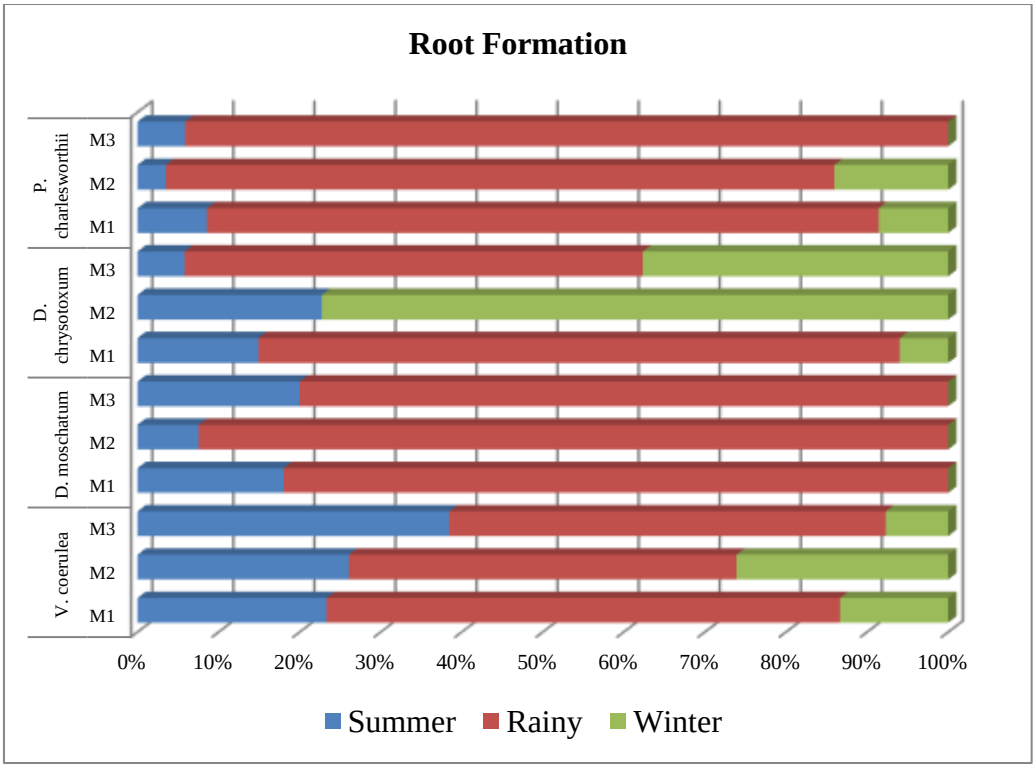

Figure 2. New plant (keiki) of orchid species of root formation at three different media in three seasons

At the hardening stage, it was found that when compared between treatments, *V. coerulea* and *P. charlesworthii* were significantly higher at T0 and it was found that *D. moschatum* and *D. chrysotoxum* orchids had better survival in T0 and T1.According to the P value, *D. moschatum* was significant in rainy season. (Table 9)

The results of the study was consistent with Dick and Magingo (2021) they found that the highest shoot length was observed in BAP 5-9 µM and NAA 2 µM. In hardening stage, Anand, A., & et al., 2000 observed that media supplemented with 10 ml/l Ferti-start concentrations were induced to suppress shoot and root elongation in rainy season.

Table 9. ANOVA for effect of without hormone and hormonal treatment and three media on hardening of orchids species in rainy season

Treatment	***V. coerulea***	***D. moschatum***	***D. chrysotoxum***	***P. charlesworthii***
T0	1.0000 a	1.0000 a	1.0000 a	1.0000 a
T1	0.7143 a	1.0000 a	1.0000 a	0.8571 a
T2	0.7143 a	0.5714 b	0.8571 a	0.8571 a
Grand Mean	0.8095	0.8571	0.9524	0.9048
CV%	49.22	CV 36.00	22.91	34.11
Pr > F	0.3242 ^{ns}	0.0260*	0.3874 ^{ns}	0.6147 ^{ns}

ns = not significant * = significant

In the analysis of flower blooming, we conducted an experiment in three types of basic breeding materials using hormones and hormone stimulation. After the hardening stage, the flowers often bloom in summer, especially in May, and data were collected during that season.

When compared between treatments, in *V. coerulea* T2 had better result than the other treatments, and the flowering rate was better. *D. moschatum* orchids were found to have a

higher rate of flowering than the other treatments when the hormone was applied. It was found that T1 had a strong flowering rate for this orchid, but T2 had a significantly lower flowering rate. It was found that the *P. charlesworthii* had a high rate of flower production at T1 and a week flower production in the absence of hormone. Therefore, in order to improve the flowering rate of *V. coerulea* species, it was recommended to try to plant the orchids in T2, *D. moschatum* orchids without hormones, and *D. chrysotoxum* and *P. charlesworthii* in T1. According to the P value, *D. chrysotoxum* was heighly significant (Table 10)

Stem cuttings offer a relatively straightforward and efficient method for propagating certain orchid species, particularly those that are easier to root. This method allows for the rapid production of clones that retain the desirable traits of the parent plant, ensuring consistency in flower quality and growth characteristics. (Dick & Magingo, 2012)

Table 10. ANOVA for effect of without hormone and hormonal treatment and three media on flower of orchids species in summer season

Treatment	***V. coerulea***	***D. moschatum***	***D. chrysotoxum***	***P. charlesworthii***
T0	1.8571 a	0.7143 a	0.7143 b	0.2857 b
T1	1.7143 a	0.2857 a	2.7143 a	0.8571 a
T2	3.5714 a	0.4286 a	0.2857 c	0.4286 ab
Grand Mean	2.3810	0.4762	1.2381	0.5238
CV%	168.58	105.83	85.74	90.00
Pr > F	0.6361^{ns}	0.2937 ^{ns}	0.0010**	0.0883 ^{ns}

ns = not significant * * = highly significant

Experiment III: Study on effect of rooting hormone and different duration and media on fruit set by manual self-pollination.

In order to study fruit set, orchid flowers were hand pollinated and tested by self pollination. In this research and experiment, compared to each treatment, it was observed that the economically important *V. coerulea* and the rare orchid species *P. charlesworthii* orchids in T1 and the medicinal orchid species *D. moschatum* and *D. chrysotoxum* orchids had a higher rate of seed set in the hormone free method. According to the Mean and P value of *V. coerulea* was hight and significant. (Table 11)

The findings suggest that stem cuttings are a reliable method for orchid propagation, particularly for species that may not readily produce viable seeds or require specific pollinators. The high success rate can be attributed to the orchids' natural regenerative capabilities. Furthermore, self-pollination presents an interesting avenue for genetic study, as it allows for the examination of inbreeding effects and genetic stability within populations. (K. Rayneesh & et.al, 2000)

However, the variability in fruit set rates highlights the need for further investigation into factors influencing reproductive success, such as environmental conditions and genetic compatibility. Studies show that many orchid species exhibit high rates of successful seed set through self-pollination. For example, research conducted on **Phalaenopsis** orchids demonstrated that self-pollinated flowers produced viable seeds comparable to those produced by cross-pollination (Lee et.al, 2021).

Table 11. ANOVA for effect of without hormone, hormonal treatments and three media on seed of orchids species in rainy season

Treatment	*V. coerulea*	*D. moschatum*	*D. chrysotoxum*	*P. charlesworthii*
T0	0.7143 ab	0.7143 a	0.5714 a	0.2857 b
T1	1.0000 a	0.2857 a	0.1429 a	0.8571 a
T2	0.2857 b	0.4286 a	0.4286 a	0.4286 ab
Grand Mean	0.6667	0.4762	0.3810	0.5238
CV%	59.76	105.83	128.09	90.00
Pr > F	0.0121 *	0.2937 ns	0.2722 ns	0.0883 ns

ns = not significant * = significant

6. Conclusions and Recommendations

6.1 Conclusions

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

1. V. coerulea orchid species survived more in the rainy season than in the dry season in all three types of media. M1 media had the best survival rate in rainy season and M3 media had the lowest survival rate was winter for *D. moschatum*. orchid, M2 media had the best survival in all three seasons and should be used for *D. chrysotoxum* orchid species. M2 media was a better survival rate than other media in all three types of weather and should be used for *P. charlesworthii* orchid,

2. *V. coerulea* orchid species, the number of new shoots producing was higher in the rainy season than in other seasons. In the same way, there were no significant differences between the *D. moschatum*, *D. chrysotoxum* and *P. charlesworthii* orchids using hormone-free and hormone-based methods.

3. *V. coerulea* and *D. moschatum* orchids should be planted in M2 media during the rainy season, *D. chrysotoxum* orchids should be planted in M2 media in winter, and *P. charlesworthii* orchids should be planted in M1 media in rainy season for height growth performance.

4. *V. coerulea, D. moschatum, P. charlesworthii* orchid species had strong new roots production in the rainy season in M2 media, but no new root production in winter in M3 nedia. In the rainy season, M1 media had a good new root production rate, but it was found that M2 media did not produce any new roots even in that season. As for *D. chrysotoxum* orchid, many new roots were produced in the rainy season in M1 media, but no new roots were produced in the M2 media.

5. In the hardening stage, it was found that the *D. chrysotoxum* orchid had the best survival rate. *V. coerulea* and *P. charlesworthii* were significantly higher at T0 and it was found that *D. moschatum* and *D. chrysotoxum* orchids had better survival in T0 and T1.

6. The flowering rate of *V. coerulea* species, it was recommended to try to plant the orchids in T2, *D. moschatum* orchids without hormones, and *D. chrysotoxum* and *P. charlesworthii* in T1.

7. The economically important *V. coerulea* and the rare orchid species *P. charlesworthii* orchids in T1 and the medicinal orchid species *D. moschatum* and *D. chrysotoxum* orchids had a higher rate of seed set in the hormone free method.

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 flowering formation was statistically significant difference in the different levels of treatments under favourable environmental conditions for *D. chrysotoxum.* 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 plants

Vanda coerulea Griff ex. Lindl

Dendrobium moschatum (Buch-Ham)SW.

Dendrobium chrysotoxum Lindl

Paphiopedilum charlesworthii (Rolfe) Pfitz

Nutritional supplements
used in reproduction

Weighing and preparation
of propagules for breeding new plants

Having flowers

Vanda coerulea Griff ex. Lindl

Dendrobium moschatum (Buch-Ham)SW.

Dendrobium chrysotoxum Lindl

Paphiopedilum charlesworthii (Rolfe) Pfitz

Self-pollination and fruit set

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

Appendix 1

Experiment (II)

Table 12. ANOVA for flowering of *V. coerulea* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Flower	2	14.952	7.4762	0.46	0.6361
Error	18	290.000	16.1111		
Total	20	304.952			

Grand Mean 2.3810 CV 168.58

Table 13. ANOVA for flowering of *D. moschatum* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Flower	2	0.66667	0.33333	1.31	0.2937
Error	18	4.57143	0.25397		
Total	20	5.23810			

Grand Mean 0.4762 CV 105.83

Table 14. ANOVA for flowering of *D. chrysotoxum* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Flower	2	23.5238	11.7619	10.44	0.0010
Error	18	20.2857	1.1270		
Total	20	43.8095			

Grand Mean 1.2381 CV 85.74

Table 15. ANOVA for flowering of *P. charlesworthii* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Flower	2	1.23810	0.61905	2.79	0.0883
Error	18	4.00000	0.22222		
Total	20	5.23810			

Grand Mean 0.5238 CV 90.00

Appendix 2

Experiment (III)

Table 16. ANOVA for seed formation of V. coerulea orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Seed	2	0.66667	0.33333	1.40	0.2722
Error	18	4.28571	0.23810		
Total	20	4.95238			

Grand Mean 0.3810 CV 128.09

Table 17. ANOVA for seed formation of *D. moschatum* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Seed	2	0.66667	0.33333	1.31	0.2937
Error	18	4.57143	0.25397		
Total	20	5.23810			

Grand Mean 0.4762 CV 105.83

Table 18. ANOVA for seed formation of *D. chrysotoxum* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Seed	2	1.80952	0.90476	5.70	0.0121
Error	18	2.85714	0.15873		
Total	20	4.66667			

Grand Mean 0.6667 CV 59.76

Table 19. ANOVA for seed formation of *P. charlesworthii* orchid species in three media at three seasons

Source	***DF***	***SS***	***MS***	***F***	***P***
Seed	2	1.23810	0. 61905	2.79	0.0883
Error	18	4.00000	0. 22222		
Total	20	5.23810			

Grand Mean 0.5238 CV 90.00