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Dendrobium anosmum Lindl. "TIM HUE' AND THE METHOD OF VEGETATIVE PROPAGATION FROM THE AXILLARY NODE SHOOTS OF THE STEM (KEIKIS)

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Abstract. *Dendrobium anosmum* Lindl. 'tim hue' has many outstanding features such as large stems and leaves, big-sized flowers, symmetrical flower structure, a characteristic purple color, and a gentle scent. The study found a suitable medium for propagating from keikis was sand with the highest keiki budding rate of 70.16%. Two growth stimulants BAP and TDZ were regarded as affecting the shooting ability of keiki, the best concentrations of BAP and TDZ were 200 mg/L and 250 mg/L respectively. The plant growth stimulant used to stimulate rooting was NAA, the most effective concentration was 250 mg/L.

Keywords: *Dendrobium anosmum* Lindl., morphological characteristics, microbial preparations, propagation from keiki.

1. INTRODUCTION

Dendrobium is a large genus in the Orchidaceae family including more than 1,200 species that widely distributed in South Asia, East Asia, and Southeast Asia (Givnish et al., 2015). *Dendrobium anosmum* Lindl. belongs to the genus *Dendrobium* (Ban, 2005) with attractive beauty and outstanding flower structure so that they was keep being favored.

Orchid seeds have no endosperm or a thin endosperm layer, therefore, their seeds need specific symbiotic fungi to be likely to germinate. Their natural budding ability was ultra-low (Kauth et al., 2008). Orchids propagating by conventional methods (propagating from stem cuttings, separating bushes, etc.) have the disadvantage of a low multiplication coefficient and disturbingly impact on the original plant (Martin & Madassery, 2006), however, this process is assessed as simple and easy to implement. In orchids, Keiki is often used for vegetative propagation. Keiki is referred to as a shoot that develops into a seedling from a dormant node in the stem instead of growing into a branch (De et al., 2015).

Recently, a several studies on vegetative propagation with high efficiency was published such as breeding *Dendrobium nobile* Lindl. orchids (Colombo et al., 2015; Venturieri & Pickscius, 2013), *Dendrobium johannis* Rchb.f. (Suryaningsih et al., 2018). *D. anosmum* Lindl., is a rather special orchid because the propagation via stimulating its keikes is more easily compared to other orchids. In this study, we specifically identified

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the orchid species *Dendrobium anosmum* Lindl. 'tim hue', and optimized substrate as well as growth stimulants to propagate *D. anosmum* Lindl. from keikis effectively.

2. MATERIALS AND METHODS

2.1. Plant materials

D. anosmum Lindl. 'tim hue' was discovered from a local origin, then transferred and grown at the orchid garden of the Institute of Biotechnology, Hue University, Thua Thien Hue province, Vietnam. The study used pseudobulb stems of 2 years old or older orchids. In August-September 2023 (after the flowering season), we cut theses stems into short sections of 4-5 cm with 1-3 axillary shoots per section.

2.2. METHODS

Describe morphological characteristics

Describe the morphological characteristics of *D. anosmum* Lindl. 'tim hue' orchid according to the method of orchid morphological characterization by De (2020).

Effect of substrate on the budding ability of Keiki

Qualified pseudobulb stems (large and strong, free of pests and diseases, and alive axillary shoots) were selected after removing the dry leaf sheaths. The pseudobulb stem was cut into short sections from 4-5 cm containing 2-3 axillary shoots. Short stem sections have a layer of healing glue applied at the cut to disinfect and reduce water loss. Then they were placed in different substrates (plastic tray, mix of coconut coir and rice husk charcoal (1:1, w/w), sand (Colombo et al., 2015), moss). The substrate ensures ventilation and moisture retention (Gurung & Gurung, 2014). Each experiment was repeated 3 times with 10 stem segments for each. The period of 60 days, observing budding time, budding rate, shoot length, shoot diameter, and number of leaves.

Effect of growth stimulants on the budding ability of Keikis

Using growth stimulants 6-Benzylaminopurine (BAP) and Thidiazuron (TDZ) (Tran et al., 2021) with concentrations ranging from 100-300 mg/L, sprayed directly on stem sections twice a week. The experiment was repeated 3 times, each time using 10 stem segments. During 60 days, observing budding time, budding rate, shoot length, number of leaves, and shoot diameter.

Effect of growth stimulants on the rooting ability of shoots from Keiki

Utilizing keiki which has shoots but no roots from the shoot creation experiment for this process. The growth stimulant used was naphthylacetic acid (NAA) (Tran et al., 2021) with a concentration from 100-300 mg/L. Sprayed directly NAA on the shoots twice a week. The experiment was repeated 3 times and used 10 shoots each time. During the 60-day phase, parameters of rooting time, rooting rate, root length, number of roots, and root characteristics were recorded.

Statistical analysis

Data were analyzed using Microsoft Excel 2019 and IBM SPSS Statistic 26 software. LSD standard (for 95% confidence) and ANOVA analysis (Ducan's test) for each formula were applied to evaluate differences.

3. RESULTS AND DISCUSSION

3.1. Morphological characteristics of D. anosmum Lindl 'tim hue' orchid

D. anosmum Lindl 'tim hue' was described in detail with its stem, root, leaf, and flower characteristics, these are morphologically identifiable characteristics of this species. The data on some characteristics are shown in Table 1, Fig 1.

Table 1. *Some characteristics of D. anosmum Lindl 'tim hue' orchid*

Characteristics	Mature tree (topped)	The tree is still growing
Height of trees (cm)	102.00^{a} ±2.78	58.53^{b} ±2.01
Diameter of tree (cm)	1.16^{a} ±0.06	0.83^{b} ±0.03
Length of the internode (cm)	3.67^{a} ±0.11	2.38^{b} ±0.07
Number of leaves	34.47^{a} ±0.69	24.67^{b} ±0.54
Leaf length (cm)	16.14^{a} ±0.58	10.38^{b} ±0.34
Maximum leaf width (cm)	3.42^{a} ±0.09	2.17^{b} ±0.09
Flower diameter (cm)	10.36±0.59	

Note: (Means ± standard errors; within a row followed by the same letter are not significantly different according to Duncan's multiple range test at p ≤0.05.)

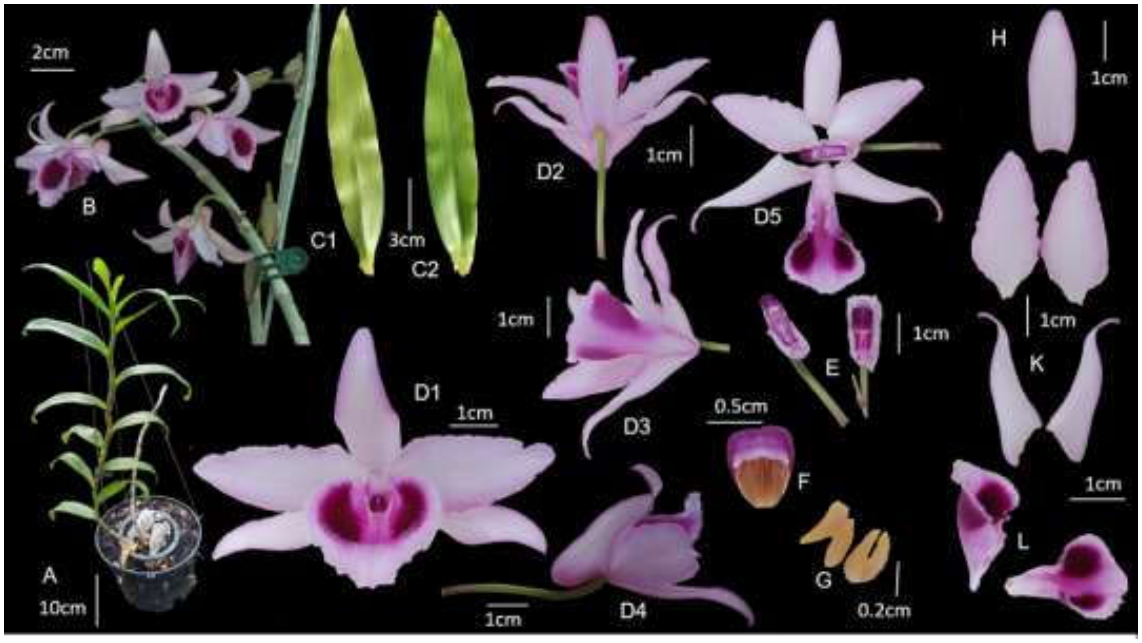

Fig 1. *Morphology of orchid D. anosmum 'tim hue'. A: Orchid; B. The location of the flower growing from the stem; C1,2: Upper and lower sides of leaves; D1,2,3,4,5: flower views (front, top, bottom, side and Spread flowers on a flat surface); E:* Column; *F: Anther and Pollinia; G: Pollinia; H: Upper sepal; I: Two Shoulders petals; K: Two lower sepals; L: Flower lips*

Flower: The flower has 5 petals including one upper sepal, two lower sepals, and two shoulder sepals (usually larger than the other wings flower), waxy petals with a pale pinkish-purple border, an oblong lance-shaped with a pointed apex, balanced flower structure, curled backward petals when blooming. There are flower lips, two flower eyes, throat lobes, a nose, and spurs. Flowers are fragrant (a bit like a lavender scent) and have a variety of colors but the most common are white purple, light pink, or dark purple. Flowers grow in clusters with 2-3 flowers at the location of nodes near the top and have many flower clusters.

Leaves: The leaves are oblong and green, the collar is a purple ring. On the surface of old leaves, the veins are clearly visible and vertical to both sides of the leaf blade; while in young leaves, the veins are faint and smooth. The leaves are brittle and unwatery if folded. The leaf sheath has a purple-black pigment of spots or streaks.

Stem: The stem is smooth and succulent, not hollow inside. It has pale green, pseudobulbs stem and some buds or flower buds. The leaf sheath is close to the stem, containing 4-5 white stripes running from the base of the internodes.

Morphological and structural characteristics of orchids include growth patterns, leaves, flowers, pseudobulbs, and roots. Flowers are the most important organs for species identification. The structure of orchids is mainly petals, sepals, pollen, flower pillars, and lip petals (Castro & Singer, 2019). In the morphological description of *D. anosmum* Lindl previously, some authors have described similar characteristics such as a balanced star-shaped flower pattern, scent, many color variations, and hairy petals (Burzacka-Hinz et al., 2022; Hartati et al., 2022; Nguyen et al., 2022).

3.2. Effect of substrate on the ability of keikis to sprout

The results of the substrate experiment affecting the ability of keikis to sprout are presented in Table 2.

***Table 2**: Effect of substrate on keiki's budding ability after 60 days of incubation.*

Treatment	Budding time (days)	Budding rate of keiki (%)	Shoot length (cm)	Shoot diameter (cm)	Number of leaves
CT1	31.47^{c}±0.57	46.62^{c}±1.03	1.65^{c} ±0.10	0.31^{b}±0.01	3.07^{a} ±0.21
CT2	25.80^{b}±0.43	66.02^{b}±0.89	2.34^{ab}±0.08	0.35^{b}±0.02	3.33^{a}±0.19
CT3	20.93^{a}±0.58	70.16^{a}±0.85	2.54^{a}±0.10	0.4^{a} ±0.02	3.67^{a}±0.19
CT4	26.40^{b}±0.57	66.77^{b}±0.51	2.27^{b}±0.06	0.33^{b}±0.01	3.07^{a}±0.21

Note: (Means ± standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at p ≤0.05. (CT1: Keiki is placed on a plastic tray; CT2: Keiki is placed on a mixed coconut coir and rice husk charcoal (1:1, w/w); CT3: Keiki is placed on sand; CT4: Keiki is placed on a moss).

The growing medium affects the ability of keikis to sprout, although the results are different between experimental formulas. Keiki grown on a sand substrate (CT3) given that the best results this result compared to the control CT1 is much higher. Inmixed coconut coir and rice husk charcoal (1:1, w/w) (CT2) and moss (CT4) showed different

results but were not statistically significant, these two types of substrates are effective are similar (Table 2, fig 2). Previous studies have also shown that the medium used to grow keiki has an impact on the ability of keiki to sprout. The publication of Venturieri & Pickscius, (2013), and Colombo et al., (2015) on propagating orchids *D. nobile* Lindl using keiki, shows that two types of substrate gravel, and sand are suitable for keiki to bud.

3.3. Effect of growth stimulants on Keiki's ability to sprout shoots

***Table 3**: Effects of growth* stimulants *on the budding ability of Keiki after 60 days of incubation*

CT	Growth stimulants (mg/L)		Budding time (days)	Budding rate (%)	Length of shoot (cm)	Shoot diameter (cm)	Number of leaves
	BAP	TDZ					
1	0	0	20.93^{ef}±0.58	70.16^{e}±0.85	2.54^{f}±0.10	0.44^{de}±0.02	3.67^{cd}±0.19
2	100	0	19.47^{def}±0.77	75.22^{d}±1.05	2.74^{ef}±0.10	0.4^{de}±0.01	3.80^{cd}±0.20
3	150	0	18.53^{cde}±0.96	81.22^{bc}±1.53	3.29^{cd}±0.08	0.48^{cd}±0.01	4.00^{abcd}±0.20
4	200	0	14.53^{ab}±0.87	86.56^{a}±1.90	3.63^{ab}±0.07	0.52^{bc}±0.01	4.60^{a}±0.19
5	250	0	16.53^{bc}±0.89	82.56^{b}±0.88	3.03^{de}±0.10	0.42^{e}±0.01	4.20^{abc}±0.20
6	300	0	21.60^{f}±0.64	77.22^{cd}±0.83	3.38^{bc}±0.09	0.44^{de}±0.01	3.93^{bcd}±0.21
7	0	100	20.27^{ef}±0.57	73.89^{de}±1.26	2.61^{f}±0.13	0.46^{de}±0.01	3.60^{cd}±0.21
8	0	150	19.20^{def}±0.92	77.36^{cd}±1.31	3.01^{de}±0.13	0.49^{cd}±0.01	3.87^{bcd}±0.22
9	0	200	17.33^{cd}±1.04	80.29^{bc}±1.39	3.37^{bc}±0.12	0.53^{b}±0.02	4.13^{abc}±0.19
10	0	250	13.87^{a}±0.74	87.22^{a}±1.79	3.89^{a}±0.08	0.62^{a}±0.02	4.47^{ab}±0.19
11	0	300	18.23^{cde}±1.21	73.49^{de}±1.22	2.81^{ef}±0.08	0.42^{e}±0.02	3.47^{d}±0.19

Note: (Means ±standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$.)

Without growth stimulants for keikis, the obtained results are not good (CT1; fig. 2B). This result is better improved with the influence of growth stimulants BAP or TDZ. The effectiveness of the growth stimulant on keiki increases gradually as its concentration also increases and reaches the best effect when using 200 mg/L for BAP (CT4) and 250 mg/L for TDZ (CT10) (Table 3, fig 2E, 2F). However, when the concentration exceeds the growth stimulant concentration threshold in CT4 and CT10, the effectiveness of keikis growth tended to reduce, this proves that when BAP and TDZ are too high, it will inhibit the budding process of keikis.

3.4 Effect of growth stimulants on the rooting ability of shoots from keikis

Growth stimulant NAA has a positive impact on the ability of plants to create roots. In this study, we used a concentration of 100-300 mg/L NAA sprayed directly on orchid shoots to investigate the rooting ability of the shoots. The research results are presented in Table 4.

D. anosmum 'tim hue' orchid shoots grown from keikis have strong rooting ability, 100% of experimental shoots rooted even without using growth stimulants (CT1, Fig 2G).

However, the quality of the roots of the shoots in each experiment was different. The rooting efficiency of orchid shoots was increased when using NAA. The most effective experiment was CT5 with an NAA concentration of 250 mg/L (Fig 2H). When increasing the NAA concentration to 300 mg/L, the rooting efficiency decreased (CT6), this proves that this NAA concentration is high causing the phenomenon of inhibition of the rooting process of *D. anosmum* 'tim hue' orchid shoots.

***Table 4**. Effects of growth stimulants on keiki's rooting ability after 60 days of experiment*

CT	NAA (mg/l)	Rooting time (days)	Rooting rate (%)	Number of roots	Root length (cm)
1	0	18.67^{d}±0.92	100	2.27^{e}±0.25	2.21^{d}±0.15
2	100	16.67^{c}±0.87	100	2.73^{de}±0.23	2.61^{d}±0.17
3	150	13.27^{d}±0.65	100	3.33^{cd}±0.19	3.12^{c}±0.16
4	200	11.93^{c}±0.65	100	4.13^{ab}±0.26	3.52^{bc}±0.15
5	250	7.33^{a}±0.55	100	4.60^{a}±0.25	4.12^{a}±0.20
6	300	9.60^{b}±0.77	100	3.80^{bc}±0.26	3.72^{ab}±0.13

Note: (Means ±standard errors within a column followed by the same letter are not significantly different according to Duncan's multiple range test at $p \leq 0.05$).

***Fig 2.** Propagating D. anosmum 'tim hue' orchids from keiki and the effects of microbial preparations on the growth of orchid seedlings; **A, B, C, D:** Effect of substrate on keiki's budding ability after 60 days. (A. keiki is placed on a plastic tray; B. keiki is grown on a sand substrate; C. the keiki are placed on a moss substrate; D. Keiki is placed on a mixed coconut coir and rice husk charcoal (1:1, w/w)); **E, F:** Effect of growth stimulants on keiki's budding ability after 60 days (E. 200 mg/LBAP; F. 250 mg/L TDZ); **G, H:** Effect of growth stimulant NAA on the rooting ability of orchid shoots after 60 days (G. Without growth stimulants; H. Use 250 mg/L NAA).*

4. CONCLUSION

D. anosmum Lindl. 'Tim hue' is an orchid species typical of Thua Thien Hue province - Vietnam, with many outstanding characteristics such as large leaf stems, balanced flower structure, large flowers, and solid purple color characteristics, and the flowers have a mild fragrance. *D. anosmum* Lindl. 'tim hue' is very suitable for propagation by keiki, with a Keiki budding rate of 70.16% in sand.

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LAN GIẢ HẠC TÍM HUẾ VÀ PHƯƠNG PHÁP NHÂN GIỐNG SINH DƯỠNG TỪ CHỒI NGỦ CỦA THÂN (KEIKIS)

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Abstract. Lan giả hạc tím huế (*Dendrobium anosmum* Lindl.) mang nhiều đặc điểm nổi bật như thân và lá có kích thước lớn, mặt hoa cân đối, hoa to, có màu tím đặc trưng và có mùi thơm dịu. Nghiên cứu đã lựa chọn được giá thể phù hợp cho nhân giống bằng keikis là cát với tỷ lệ nảy mầm của keiki 70,16%. Hai chất kích thích sinh trưởng BAP và TDZ có ảnh hưởng đến khả năng nảy chồi của keiki. Nồng độ tốt nhất cho kích thích nảy chồi keiki là 200 mg/L đối với BAP và 250 mg/L đối với TDZ. Chất kích thích sinh trưởng thực vật được sử dụng để kích thích ra rễ là NAA với nồng độ sử dụng hiệu quả nhất là 250 mg/L.

Từ khoá: *Dendrobium anosmum* Lindl., nhân giống từ keiki, đặc điểm hình thái, chế phẩm vi sinh.

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