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Effect of substrates of transplantation of the rare epiphytic orchid *Dendrobium farmeri* for conservation

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Abstract. *Nuammee A, Pingyot T, Foowan S, Pumikong S, Rujichaipimon W, Sornpood S, Panyadee P. 2024. Effect of substrates of transplantation of the rare epiphytic orchid* Dendrobium farmeri *for conservation. Biodiversitas 25: 708-715. Dendrobium farmeri* Paxton is an epiphytic orchid, one of the most celebrated for its beauty. However, its natural population is dwindling, and it faces the risk of extinction. Although tissue culture techniques are successful for orchid propagations, the transplantation of in vitro-derived plantlets to ex-vitro conditions is often restricted by high mortality, which is considered a challenging and crucial stage in orchid conservation. This study investigated the effect of substrate mixtures on the survival and growth of *D. farmeri* plantlets under greenhouse conditions. The substrates for acclimatization contained coconut husk, charcoal chips, brick chips, sphagnum moss, fern fiber, and leaf mold in various ratios. Survival percentage and vegetative growth characteristics of plantlets were the main observations recorded. The result showed that the substrate mixture containing brick chips, charcoal chips, and sphagnum moss in a 1:1:2 ratio was the most effective, yielding 100% at 30 days and 96.7% at 180 days post-transplantation, fostering improved development of plantlets under greenhouse conditions. The findings suggest that this optimized substrate mixture could be a standard approach for acclimating *D. farmeri* and potentially other epiphytic orchids, enhancing conservation efforts and commercial cultivation.

Keywords: Acclimatization, *Dendrobium farmeri*, growth, substrates, survival rate, transplantation

INTRODUCTION

The Orchidaceae comprises approximately 28,000 species worldwide (Christenhusz and Byng 2016; Zhang et al. 2023). Thailand's orchid flora is highly diverse, with nearly 1.300 species (Pedersen et al. 2011; Pedersen et al. 2014; Pingyot et al. 2021; Pedersen et al. 2022). *Dendrobium* Sw., the second-largest genus in Thailand after *Bulbophyllum*, consists of about 149 species (Chayamarit et al. 2014) known for their high ornamental, medicinal, and commercial value (Hinsley et al. 2018; Ramesh et al. 2019). However, several factors, such as the illegal collection of wild orchids, habitat loss, and climate change, have dramatically diminished the numbers and the abundance of wild orchids in Thailand, leading to 43 species of *Dendrobium* becoming rare and threatened (Chamchumroon et al. 2017).

Dendrobium farmeri Paxton is a sympodial epiphytic orchid and one of the most well-known for its beauty. It is native to India, Nepal, Bhutan, Myanmar, Thailand, Vietnam, and Peninsular Malaysia, and it grows in deciduous to evergreen forests (Rujichaipimon 2020). Its pseudobulbs are clavate to fusiform, abruptly widening from a stalk-like base and normally four longitudinal ridges, carrying 1-4 alternate lanceolate-oblong leaves. Its inflorescences are usually pendulous, comprising 9 to 28 flowers arranged in racemose inflorescences. Flowers are usually pale pinkish purple with yellow on labellum, 3-6 cm long. The labellum is described as broadly oblong, nearly square without side lobes, and not embracing a column (Figures 1A-B).

It has experienced declines in its natural population due to over-collection, habitat disturbance by anthropogenic activity, and a low fruit-set rate in nature. Consequently, *D. farmeri* has been listed as CITES Appendix II (CITES 2023) and is considered a rare species (Chamchumroon et al. 2017). Therefore, *D. farmeri* is included in the Queen Sirikit Botanic Garden conservation program.

The micropropagation or tissue culture technique is generally used for producing orchid plantlets, such as those of *Dendrobium* plantlets, derived from both asymbiotic and symbiotic germination seeds (Teixeira da Silva et al. 2015a; Teixeira da Silva et al. 2015b). Nonetheless, this technique is often restricted by the high mortality of plantlets during acclimatization (Mirani et al. 2017). The transition of in vitro plantlets to ex vitro conditions is challenging both the acclimatization in the greenhouse and subsequent field conditions. It is considered a difficult stage of orchid cultivation and conservation. Moreover, the post-acclimatization conditions such as light intensity, temperature, moisture of the greenhouse, substrate mix used, and the age and genotype of orchids are important factors for the survival and growth of plantlets (Teixeira da Silva et al. 2017). Although many previous studies have focused on tissue culture and in vitro propagation of *Dendrobium* (Teixeira da Silva et al. 2015a), there are few reports on the post-in vitro stage, especially the acclimatization of in vitro-derived plantlets to ex vitro conditions of *D. farmeri*.

Historically, most substrates used for the acclimatization of in vitro-raised *Dendrobium* plantlets are made up of brick or charcoal pieces, broken tiles, pine bark, cycad bark, cocopeat, coconut coir, sawdust, perlite, vermiculite, peat or sphagnum moss (Parthibhan et al. 2015; Teixeira da Silva et al. 2017; Bing et al. 2018; Faria et al. 2018; Barua et al. 2022). Generally, coconut husk was the most common substrate for commercial cultivation of *Dendrobium* (Faria et al. 2018). It was a beneficial substrate for the survival and growth of plantlets (Hariyanto et al. 2019; Lakshanthi and Seran 2019). However, the suitable substrate providing high survival and growth in different *Dendrobium* species was still different in the type and ratios of substrates.

Despite the recognized importance of substrate composition in the acclimatization process, there is a notable paucity of research focusing on the quantitative analysis of substrate effects on *D. farmeri* survival. This study aims to address this research gap by evaluating the influence of various substrate mixtures on plantlet acclimatization to establish an evidence-based protocol for the ex vitro establishment of this species.

Understanding the intricacies of this process can significantly enhance the conservation practices not only for *D. farmeri* but also for other epiphytic orchids facing similar threats. By improving survival rates during acclimatization, this research could contribute to the recovery of natural populations and the success of orchid reintroduction programs.

MATERIALS AND METHODS

Study site and environmental factors

The experiment was conducted from March until September 2023 at the Queen Sirikit Botanic Garden greenhouse, The Botanical Garden Organization, Chiang Mai, Thailand. The aim was to identify a suitable hardening substrate for ex vitro acclimatization of in vitro plantlets of *D. farmeri* (Figure 1) before reintroduction into the natural habitat. Environmental conditions in the greenhouse were controlled, maintaining temperatures at 23-25°C, relative humidity at 70-90% of relative humidity, and light intensity between 2,500-3,500 lux.

Plant material and preparation

In vitro plantlets of *D. farmeri* that germinated from seeds and exhibited 2-4 pseudobulbs with a height of 40-60 mm were taken from culture bottles. The plantlets were rinsed with sterilized water to eliminate any media adhering to the roots. The plantlets were immersed in a 0.01% captan solution (1 g/10 L) for 10 minutes before being transplanted into the pots to prevent fungal infection. Then, the plantlets were placed on sphagnum moss within a plastic tray inside a chamber to pre-acclimatize before being planted in the substrate mixtures.

Substrate used and experimental design

The hardening substrates were comprised of coconut husk, charcoal chips, brick chips, sphagnum moss, fern fiber, and leaf mold. Both coconut husk and charcoal were soaked in water for three days to leach out any acidity. All the substrates were sterilized and treated with a captan solution to reduce fungal contaminants before mixing. The mixtures were poured into small plastic pots measuring 9.5 cm in length and width. Five treatments with different mixtures were designed, as detailed in Table 1 (as modified from Vijayakumar et al. 2012; Teixeira da Silva et al. 2017; Barua et al. 2022; Ramasoot et al. 2022). A total of 150 plantlets were distributed across the five treatments, each with three replicates and each replicate containing 10 plantlets. No fertilization was applied during the treatments.

Table 1. The substrate mixtures and treatment codes for the experiments

Treatments code	Substrates
T1	Coconut husk
T2	Coconut husk:charcoal chips (1:1)
T3	Brick chips:charcoal chips (1:1)
T4	Brick chips:charcoal chips:sphagnum moss (1:1:2)
T5	Brick chips:charcoal chips:fern fiber:leaf mold: sphagnum moss (1:1:1:1:2)

Figure 1. *Dendrobium farmeri*. A. Plant in natural habitat; B. Inflorescence and flowers

The acclimatization protocol

The acclimatization was carried out under the greenhouse's 80% shade provided by black agro shade nets. Healthy and vigorous plantlets were selected for acclimatization in five different substrate mixtures. After potting, the plantlets were placed in a humid chamber and covered with a transparent polythene sheet to retain moisture and prevent desiccation. The plantlets were covered entirely for the first week post-transplantation to maintain high relative humidity.

In the second week, the cover was slightly opened for 1-2 hours daily to reduce relative humidity inside the humid chamber gradually. During the third week, the cover was opened for 5-6 hours daily, and by the end of the fourth week, it was completely removed, and the plantlets were also completely uncovered. Subsequently, watering was performed once or twice daily based on the substrate moisture level during summer but only once daily during the rainy season, with exceptions on rainy days. After 4 weeks of pre-acclimatization, only the surviving plantlets were kept for further growth assessment over 180 days, with 4 plants per replicate monitored.

Recorded measures and statistical analysis of data

Survival and mortality of plantlets were recorded after every 30 days post-transplantation to calculate the survival percentage. Vegetative growth parameters, including plant height, number of initial and dried pseudobulbs, number of initial leaves per plant, the length and width of initial leaves, number of new shoots, number of new leaves per plant, the length and width of new pseudobulb per plant, the length and width of new leaves per plant, were measured every 30 days. The collected data were analyzed using ANOVA, with the assumption of equal variances being tested. Given the significance of the ANOVA, a Duncan's Multiple Range (DMR) test was employed to discern specific group differences at a significance level of $P \leq 0.05$.

RESULTS AND DISCUSSION

Survival of plantlets

In this study, plantlets were transplanted into various substrate mixtures. The survival percentages recorded over 180 days post-transplantation did not show a significant difference)$P \leq 0.05$(among the substrate mixtures)Table 2(. However, during the study period (180 days), the highest survival percentage was found in treatment T1 and T4)96.7%(, followed by T2, T5)93.3%(, and T3)86.7%((Table 2).

The acclimatization for the initial days of transplantation is an important step for the successful transplantation process of in vitro plantlets because the orchid plantlets were grown under high humidity and stable temperature inside the culture bottles during the in vitro cultures. Maintaining maximum humidity and optimal temperatures around the plantlets during the initial days of transplantation is of utmost importance (Mirani et al. 2017; Lakshanthi and Seran 2019).

Growth performance of plantlets

Owing to the orchid plantlet's slow growth rate. Until one month after transplanting to ex vitro conditions in a greenhouse, the plantlet of *D. farmeri* was successful in survival rate, but the development was insignificant. Therefore, the plantlets were maintained for a further five months to investigate the vegetative growth of both initial organs of plantlets before growing in substrates and new organs produced during acclimatization. The results showed that the vegetative growth characteristics in treatments T4 and T5 were better than other treatments (Tables 3 and 4).

Although the survival percentage was highest during the acclimatization at 30 days of transplantation, many plantlets in treatment T3 produced wilting, charring, and falling leaves compared to other treatments (Figures 2A-K). However, they can produce new shoots and new roots later. These results indicated that the substrate mixtures may affect orchid plantlets' survival rate and growth performances.

Plant height, number of initial pseudobulbs, number of initial leaves, leaf length and width

Post-transplantation, the average height of *D. farmeri* plantlets showed a general increase across all treatments, with treatment T4 demonstrating a significant difference in the plant height ($P \leq 0.05$) compared to the others (Table 3). Although the average height of plantlets in the treatment T4 and T5 were higher than T1, T2, and T3 at the beginning of acclimatization, the height rate of the treatment T4 during transplantation within 180 days was highest, followed by T5, T2, T1, and T3 respectively.

The habit of *D. farmeri* is a sympodial epiphytic orchid; the number of pseudobulbs, including initial, new, and dried pseudobulbs, were monitored before and during acclimatization. The results showed that the number of initial pseudobulbs recorded from the beginning of acclimatization (at 0 days) ranging from 2.33 to 2.92 pseudobulbs gradually decreased in all treatments (Table 3). At the same time, the number of dried pseudobulbs was increased in all treatments (Table 3). At 180 days of transplantation, the initial pseudobulbs were dried, ranging from 0.42 to 1.25. However, there were no significant differences among the 5 treatments.

In addition, the number of initial leaves per plant was recorded from the beginning of acclimatization (at 0 days), ranging from 2.67 to 3.83 leaves per plant. It quickly decreased within 30 days during acclimatization because the plantlets were wilting, charring of leaves, and dropping leaves, especially the treatment T3, which had a higher rate of falling leaves than other treatments (Table 3). The number of initial leaves at 180 days of transplantation remained at 0.75 to 1.83 per plant. The treatment T4 was higher than T5, T2, T1 and T3 respectively. However, there were no significant differences among the 5 treatments.

Notably, the rate of falling leaves in treatment T4 was lower than other treatments. The remaining leaves continuously developed, and as a result, treatment T4 showed significant differences ($P \leq 0.05$) in the length and width of initial leaves among the 5 treatments (Tabel 3).

Table 2. Survival percentage of in vitro plantlets of *Dendrobium farmeri* at 0, 30, 60, 90, 120, 150 and 180 days to ex vitro conditions

Treatment	0 days	30 days	60 days	90 days	120 days	150 days	180 days
T1	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	96.7 ± 0.33a	96.7 ± 0.33a	96.7 ± 0.33a	96.7 ± 0.33a
T2	100.0 ± 0.00a	100.0 ± 0.00a	93.3 ± 0.67a	93.3 ± 0.67a	93.3 ± 0.67a	93.3 ± 0.67a	93.3 ± 0.67a
T3	100.0 ± 0.00a	96.7 ± 0.33a	96.7 ± 0.33a	90.0 ± 0.58a	86.7 ± 0.33a	86.7 ± 0.33a	86.7 ± 0.33a
T4	100.0 ± 0.00a	100.0 ± 0.00a	96.7 ± 0.33a	96.7 ± 0.33a	96.7 ± 0.33a	96.7 ± 0.33a	96.7 ± 0.33a
T5	100.0 ± 0.00a	100.0 ± 0.00a	100.0 ± 0.00a	96.7 ± 0.33a	93.3 ± 0.67a	93.3 ± 0.33a	93.3± 0.33a

Note: F test=P≤0.05. Data is based on the availability of the surviving plantlets. All the values are expressed as mean ± standard error of three replicates. According to Duncan's test, means followed by the same letter are not significantly different at a 5% significant level

Table 3. Vegetative growth characteristics of in vitro plantlets of *D. farmeri* in different substrate mixtures at 0, 30, and 180 days under ex-vitro conditions in terms of shoot length, initial pseudobulbs, and initial leaves

Days	Treatment	Vegetative growth characteristics					
		Plant height	No. of initial pseudobulbs	No. of dried pseudobulbs	No. of initial leaves per plant	Leaf length of initial leaves	Leaf width of initial leaves
0 days	T1	47.58 ± 3.47a	2.75 ± 0.37a	0.00 ± 0.00a	3.83 ± 0.52a	26.88 ± 2.34b	7.46 ± 0.38b
	T2	51.82 ± 3.83a	2.75 ± 0.35a	0.00 ± 0.00a	3.08 ± 0.19a	30.73 ± 3.46ab	8.00 ± 0.50b
	T3	47.51 ± 3.68a	2.33 ± 0.19a	0.00 ± 0.00a	2.67 ± 0.36a	31.41 ± 2.84ab	8.75 ± 0.59ab
	T4	56.47 ± 3.78a	2.33 ± 0.14a	0.00 ± 0.00a	2.75 ± 0.33a	38.30 ± 1.73a	10.26 ± 0.70ab
	T5	51.18 ± 4.02a	2.92 ± 0.42a	0.00 ± 0.00a	3.58 ± 0.50a	35.60 ± 2.36a	11.17 ± 1.80a
30 days	T1	39.32 ± 5.76b	2.58 ± 0.44a	0.08 ± 0.08a	1.58 ± 0.38ab	22.01 ± 4.51bc	6.95 ± 1.27b
	T2	41.97 ± 5.15b	2.25 ± 0.22a	0.50 ± 0.41a	1.75 ± 0.35ab	19.70 ± 3.99c	7.00 ± 1.26b
	T3	42.63 ± 5.44b	2.00 ± 0.17a	0.33 ± 0.19a	0.83 ± 0.24b	26.16 ± 6.30bc	7.05 ± 1.78b
	T4	63.30 ± 1.54a	2.25 ± 0.13a	0.08 ± 0.08a	2.41 ± 0.19a	39.63 ± 1.37a	12.68 ± 0.67a
	T5	59.85 ± 4.02a	2.67 ± 0.36a	0.25 ± 0.13a	2.50 ± 0.44a	33.38 ± 3.88ab	10.49 ± 1.11ab
180 days	T1	45.81 ± 6.01c	1.42 ± 0.36a	1.25 ± 0.25ab	0.83 ± 0.35b	18.75 ± 6.20b	4.79 ± 1.59b
	T2	52.96 ± 6.52bc	1.42 ± 0.26a	0.75 ± 0.28ab	1.25 ± 0.28ab	23.59 ± 5.08b	7.14 ± 1.35b
	T3	45.69 ± 5.60c	1.17 ± 0.21a	1.00 ± 0.28ab	0.75 ± 0.25b	18.88 ± 5.78b	5.24 ± 1.66b
	T4	72.40 ± 3.19a	1.92 ± 0.15a	0.42 ± 0.15b	1.83 ± 0.21a	42.93 ± 2.59a	12.25 ± 0.71a
	T5	65.82 ± 4.27ab	1.92 ± 0.31a	1.00 ± 0.28ab	1.33 ± 0.40ab	20.07 ± 5.71b	6.88 ± 1.82b

Note: F test=P<0.05. Data is based on the availability of the surviving plants. All the values are expressed as mean ± standard error of three replicates. According to Duncan's test, means followed by the same letter are not significantly different at a 5% significant level. Mean comparisons and Duncan's test were done among T1 to T5 for 0, 30 and 180 days

Table 4. Vegetative growth characteristics of in vitro plantlets of *D. farmeri* in different substrate mixtures at 0, 30, and 180 days under ex-vitro conditions in terms of new shoots, new pseudobulbs, and new leaves

Days	Treatment	Vegetative growth characteristics					
		No. of new shoots	The length of new pseudobulbs	The width of new pseudobulbs	No. of new leaves per plants	The length of new leaves per plant	The width of new leaves per plant
0 days	T1	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T2	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T3	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T4	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T5	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
30 days	T1	0.42 ± 0.19a	0.96 ± 0.42a	0.55 ± 0.24ab	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T2	0.25 ± 0.13a	1.28 ± 0.72a	0.40 ± 0.22ab	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T3	0.80 ± 0.83a	0.33 ± 0.33a	0.14 ± 0.14b	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
	T4	0.42 ± 0.15a	3.20 ± 1.52a	1.32 ± 0.53a	0.17 ± 0.11a	0.60 ± 0.60a	0.55 ± 0.37a
	T5	0.33 ± 0.19a	2.15 ± 1.36a	0.71 ± 0.43ab	0.83 ± 0.83a	1.63 ± 1.63a	0.39 ± 0.39a
180 days	T1	2.08 ± 0.29b	10.06 ± 1.64b	3.15 ± 0.38ab	2.67± 0.58a	24.18 ± 3.87b	7.69 ± 1.09b
	T2	1.33 ± 0.23b	9.68 ± 1.38b	3.15 ± 0.51ab	1.50 ± 0.29a	22.97 ± 4.53b	9.23 ± 1.14b
	T3	1.92 ± 0.26b	8.96 ± 1.14b	2.83 ± 0.34b	2.00 ± 0.30a	26.97 ± 3.48ab	8.27 ± 1.09b
	T4	2.17 ± 0.32ab	15.73 ± 1.41a	4.21 ± 0.36a	2.25 ± 0.33a	36.62 ± 3.23a	12.92 ± 1.20a
	T5	2.92 ± 0.26a	12.32 ± 1.00ab	3.54 ± 0.31ab	2.25 ± 0.41a	29.04 ± 4.49ab	9.42 ± 1.30b

Note: F test=P<0.05. Data is based on the availability of the surviving plants. All the values are expressed as mean ± standard error of three replicates. According to Duncan's test, means followed by the same letter are not significantly different at a 5% significant level. Mean comparisons and Duncan's test were done among T1 to T5 for 0, 30 and 180 days

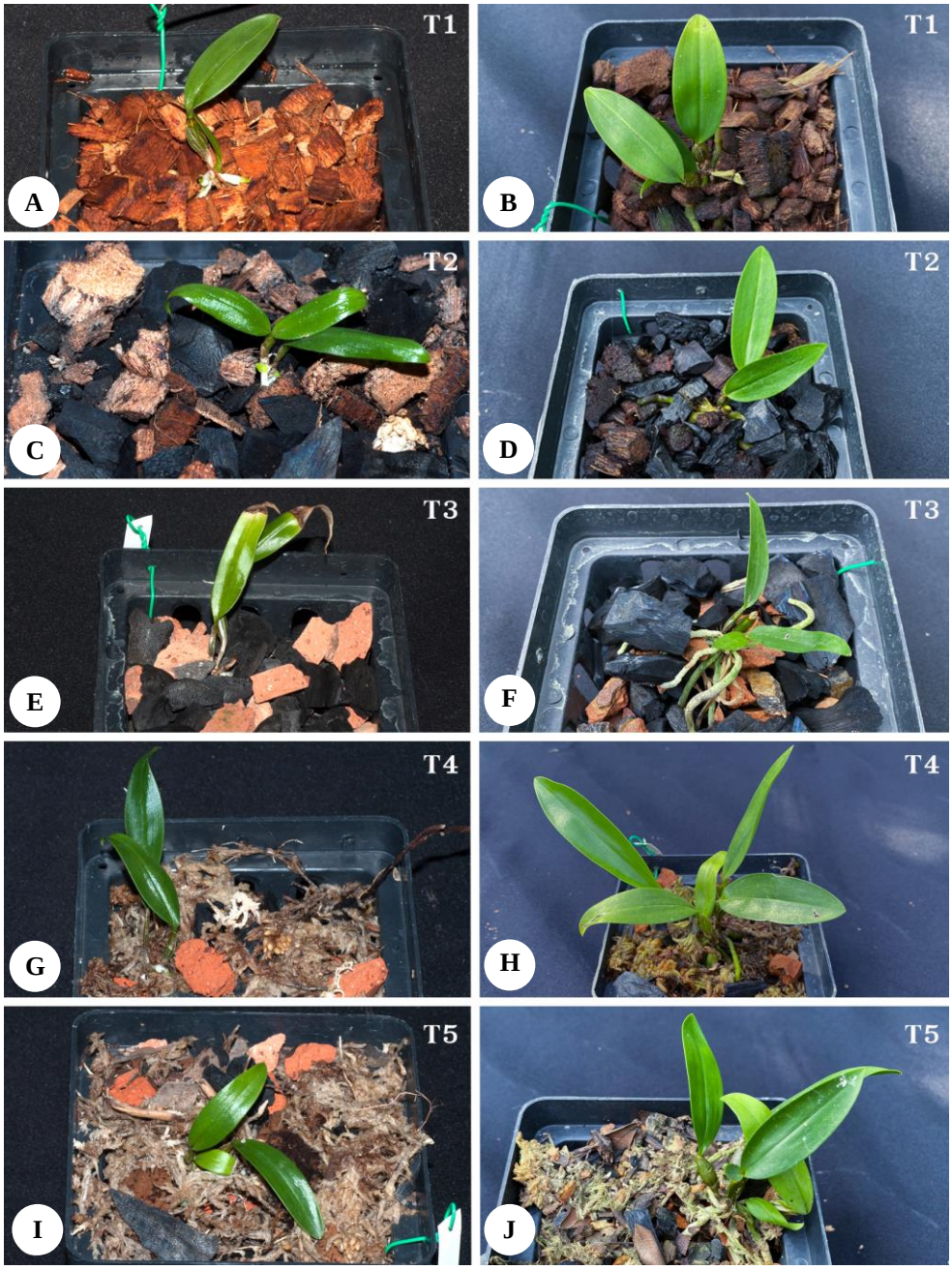

Figure 2. Acclimatized plantlets of *D. fameri* in different substrate mixtures at 30 days (A, C, E, G, J) and 180 days (B, D, F, H, K). A-B. The coconut husk substrate; C-D. The substrate mixtures of coconut husk and charcoal; E-F. The substrate mixtures of brick and charcoal; G-H. The substrate mixtures of brick, charcoal, and sphagnum moss; J-K. The substrate mixtures of brick, charcoal, fern fiber, leaf mold and sphagnum moss. Note that photos on the left column appear darker, but visually, there is no difference in leaf color between samples in 30 days and 180 days

New shoots, new pseudobulbs, and new leaves

The growth performances of new organs were monitored during and after the acclimatization phase. This result showed that producing new organs of *D. farmeri* plantlets in new shoots, new pseudobulbs, new leaves, and new roots were found at 30 days of transplanting to ex vitro conditions. The number of new shoots per plant in treatment T5, approximately 2.92 shoots per plant, was the highest and most significant difference ($P \leq 0.05$), followed by treatment T4 (approximately 2.17 shoots per plant) (Table 4, Figure 3A). In addition, the highest length and width of new pseudobulbs were found in treatment T4 and followed by T5 (Figures 3C-D). Particularly, the length of new pseudobulbs in treatment T4 was a significant difference ($P \leq 0.05$) from other treatments (Table 4).

The new leaves of *D. farmeri* plantlets were distinctly developed at 60 days of transplantation. The number of leaves per plant was the highest in treatment T1 (Figure 3B). Nevertheless, the length and width of new leaves in treatment T4 were better than other treatments (Table 4, Figures 3E-F).

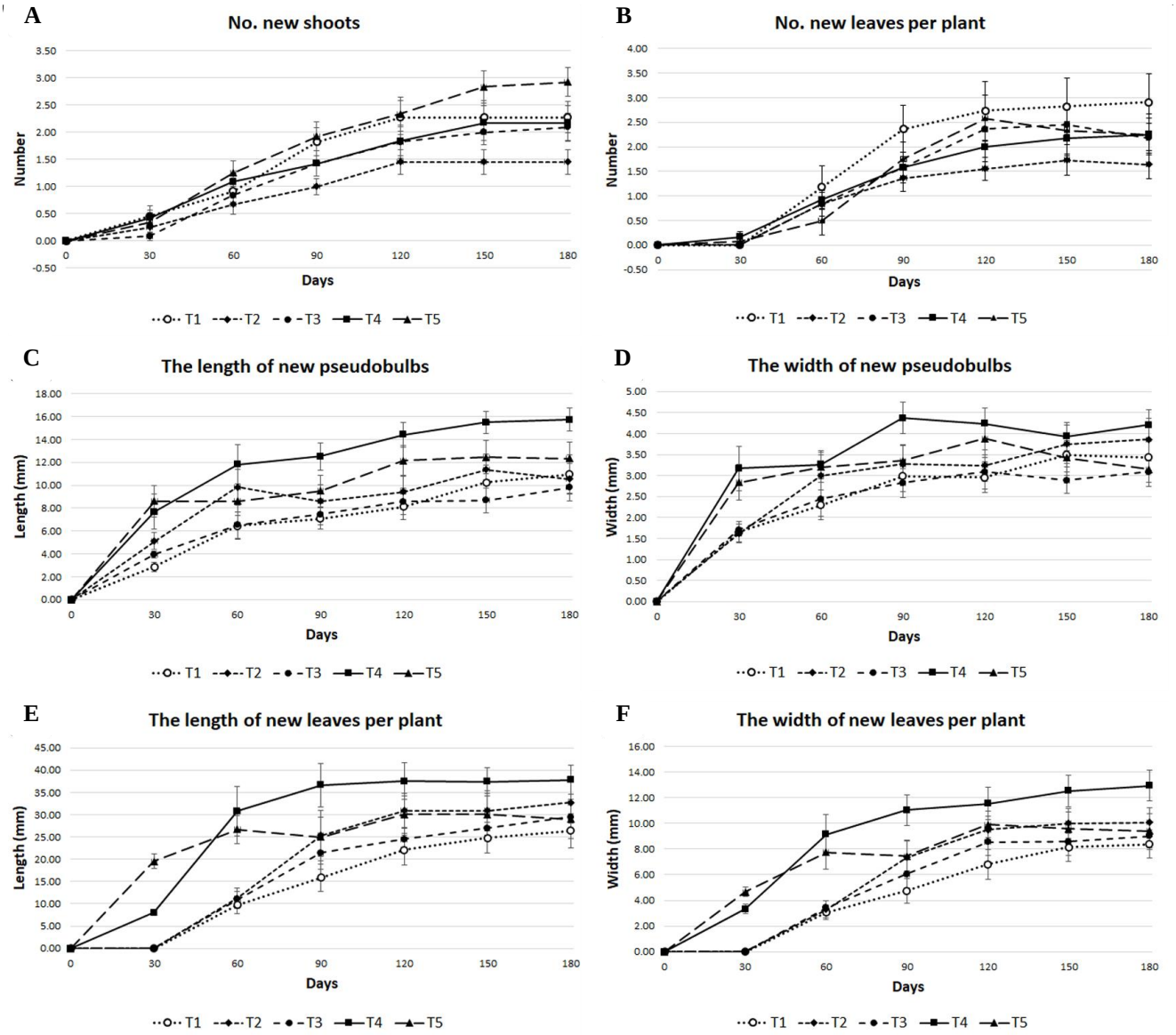

Figure 3. Effect of different substrate mixtures on in vitro plantlets of *D. farmeri*. A. The number of new shoots; B. The number of new leaves per plant; C. The length of new pseudobulbs; D. The width of new pseudobulbs; E. The length of new leaves per plant; F. The width of new leaves per plant

Discussion

In greenhouse conditions, humidity, temperature, and light intensity differ greatly from in vitro conditions. The sudden changes in humidity, temperature, and irradiance when transferring orchid plantlets to ex-vitro conditions increase plantlet mortality percentage (Mirani et al. 2017). Moreover, the stomata of plants grown in vitro show a characteristic inability to close with poor epicuticular wax formation when first removed from culture. This may cause excessive evapotranspiration after transplantation to ex vitro conditions. As a result, plantlets may rapidly wilt and die (Asayesh et al. 2017; Teixeira da Silva et al. 2017; Aliniaeifard et al. 2020).

In this study, relative humidity was maintained at 80-100% by keeping the plantlets inside the completely closed a humid chamber during the first week of transplantation. Then, the chamber was opened to gradually decrease the relative humidity and temperature in the second to the fourth weeks. This process can increase the adaptability of plantlets to ex vitro conditions and reduce fungal infection (Abul-Soad 2011). In addition, the closing of humid chamber for a long time would increase the temperature and maximize the relative humidity, which causes damage to the plantlets (Mirani et al. 2017; Lakshanthi and Seran 2019).

The results from growth performances indicated that substrate mixtures in treatments T4 and T5 were suitable for the transplantation of *D. farmeri* plantlets, whereas treatment T3 revealed very poor growth in most vegetative growth characteristics. These results indicated the crucial impact of the substrate mixtures on the development of plantlets. The substrate mixtures in treatment T4 and T5 were composed of sphagnum moss, which has a high water absorption capacity, low pH and absorbs large quantities of

mineral nutrients (Lichty et al. 2014; Aubé et al. 2015; Kämäräinen et al. 2018; Morandini et al. 2022). Therefore, sphagnum moss may be the best substrate for maintaining humidity during the acclimatization of *D. farmeri* plantlets, especially in more temperate climates where pots lose water rapidly (Teixeira da Silva et al. 2017), helping pseudobulb and leaves were vigorous.

Dendrobium orchids are mainly epiphytes. In nature, their exposed roots absorb moisture from humid air. Selecting suitable substrate mixtures for acclimatization must add one or two materials that can absorb water to improve plantlet survival and growth performances (Teixeira da Silva et al. 2017). The substrate having high water holding capacity, strong permeability, and moderate aeration was the best for acclimatization of in vitro-grown orchids (Lakshanthi and Seran 2019).

Although the previous reports indicated coconut husks are a suitable substrate for *Dendrobium* plantlets and suitable to improve water holding capacity of the substrate and nutrient contents at the initial stage (Lakshanthi and Seran 2019), the growth performances of *D. farmeri* plantlets revealed not well-developed. Therefore, coconut husk may not be suitable for acclimating *D. farmeri* plantlets. In addition, brick pieces and charcoal provide good aeration and mechanical support to plantlets (Lakshanthi and Seran 2019). This explains why the treatment T4 showed higher survival percentage and long-term growth performances after transplanting to ex vitro conditions. Nevertheless, sphagnum moss is rare and expensive, whereas coconut husk is a common and cheap substrate. So, coconut husk may be the alternative substrate for growing plantlets of *D. farmeri.*

In the present study, a protocol was developed to efficiently acclimate in vitro-grown plantlets to ensure subsequent growth of orchids under ex-vitro conditions. Substrate mixtures containing brick, charcoal, and sphagnum moss at a 1:1:2 ratio could be better to increase the survival and growth rate of *D. farmeri* plantlets during acclimatization to ex vitro conditions.

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