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IN VITRO PROPAGATION OF THE NEW ORCHID *DENDROBIUM TRANKIMIANUM* T. YUKAWA

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SUMMARY

Dendrobium trankimianum T. Yukawa is a beautiful, endemic orchid of Vietnam, a new species with a first - published description in 2004. It is very rare and expected to be added to the IUCN Red List status - CR. *In vitro* studies of orchid *D. trankimianum* T. Yukawa were conducted in order to conserve and increase the genetic pool of this precious wild orchid species. The results showed that full-strength MS medium supplemented with 2.0 mg/L BA and 0.5 mg/L NAA (10.24 PLBs/explant; 90.11% explants formed PLBs) or full-strength MS medium supplemented with 1.5 mg/L TDZ and 0.5 mg/L NAA (14.11 PLBs/explant; 92.06% explants formed PLBs) were the most suitable for protocorm formation. For subculture, suitable growth of shoots were obtained on full-strength MS medium supplemented 1.5 mg/L BA (22.35 shoots/explant; shoots length of 1.96 cm) and full-strength MS medium supplemented with 60 g ripe banana per liter (25.11 shoots/explant; shoots length of 2.12 cm). The shoots *in vitro* were transferred to half-strength MS supplemented with different concentrations of IAA, IBA and NAA to investigate root formation. The best rooting occurred at 0,5 mg/L NAA (7.91 roots/shoot; root length of 4.01 cm; 98.51% root formation). The plantlets with uniform growth were planted on different substrate: Eco clean soil, Coconut fiber, Fern fiber, 50% Rice husk in combination with 50% Eco clean soil for research the most suitable substrate. After 60 days of transplantion and acclimatization, the result showed that Fern fiber was suitable substrate for plantlet growth in a nursery garden (8.0 roots/ explant; root length of 5.5 cm; survival rate of 93.29%).

Keywords: *Conservation, Dendrobium trankimianum T. Yukawa, in vitro, substrate, PLBs, wild orchids*

INTRODUCTION

Orchidaceae is the largest and most diverse family of flowering plants, consisting of 30.000 - 35.000 species belonging to 600 - 800 genera (Freudenstein, Rasmussen, 1999; Singh *et al*., 2007; Bektas *et al*., 2013); orchids are outstanding in many ways, as they have diverse shapes, forms and colors. The genus *Dendrobium* is the second-largest orchid gennus in the world, after *Bulbophyllum* (Puchooa, 2004**)**. In Vietnam, there are 101 species and 1 genus belonging to the *Dendrobium*, distributed mainly in mountainous areas from North to South and on some coastal islands (Duong Duc Huyen, 2007). However, in the process of socio-economic development, due to different causes, such as wild orchid collection and illegal trade by the local people, many *Dendrobium* species in Vietnam have been or are threatened with extinction. Therefore, conservation of this orchid is now a matter of universal concern. *D. trankimianum* is a beautiful orchid with sepals and petals white; labellum white, disc and lateral lobes suffused and nerved with sanguine red and flowering in spring. It is in danger of becoming extinct; it is very rare and expected to gain IUCN Red List status - CR (Leonid V A *et al.,* 2016).

Conventional methods of propagation by division of offshoots is not suitable for large production of hight-quality planting material. Thus, nowadays micropropagation is mainly by the use of plant tissue culture techniques to generate high quality, genetically uniform plants. It is the only way to produce virus-free clones of infected plants. Orchids produce a large number of seeds that contain insufficient reserves for germination. But very few (<5%) of them germinate under natural conditions because the seeds are non - endospermic, minute and

require a mycorrhizal association (Rao, 1977). Generally, orchids are propagated through both vegetative and sexual means but the conventional processes are slow and uncertain. *In vitro* culture has proved particularly useful with groups of plants, which are difficult to propagate using conventional techniques; thousands of plants can be propagated within a short time. Tissue culture technique has been widely used for the *in vitro* mass propagation of several commercially important orchids (Malabadi *et al*., 2005) as well as to preserve many rare orchids. Therefore, to cater for the needs of conservation, several micropropagation protocols have been successfully developed for various important *Dendrobium* such as *D. transparens* L. (Sunitibala, Kishor, 2009); *D. draconis* Rchb.f. (Niramol, 2009); *D. chrysanthum* Lindl. (Koravisd, 2011); *D. aggregatum (*Vijayakumar *et al.,* 2012); *D. wangliangii* (Dake *at al.,* 2013); *D.Chrysanthum* Wall. Ex Lindl. (Rao, Barman, 2014), *D. nobile* Lindl. (Paromik B. *et al.,* 2014)… However, there is no report on propagation of *D. trankimianum* T. Yukawa. In order to conserve as well as rapidly multiply seedlings for this beautiful rare orchid, *in vitro* propagation of *D. trankimianum* T. Yukawa is an imperative and of great significance.

MATERIALS AND METHODS

Materials

The materials used for the present investigation were the latent bud of *D. trankimianum* T. Yukawa, collected from the Orchid conservation garden of Tay Nguyen Institute for Scientific Research.

Methods

Culture medium

Depending upon the experiment, full or half of MS medium (Murashige and Skoog, 1962) supplemented with or without plant growth regulators, activated charcoal and coconut water. The pH of the medium was adjusted at 5.8 before autoclaving at 121^{o}C for 25 min at 1 atm pressure. The culture tubes were kept at 25 ± 2^{o}C under 35 µ/m^{2}/s for 16/8h.

Surface sterilization of explants

The latent buds of *D. trankimianum* T. Yukawa were first cleaned with detergent and finally washed in running tap for 20 min till all the detergent was washed off clearly. After that, latent buds were surface sterilized sequentially with Streptomycin 2‰ (20 min), $HgCl_2$ 1‰ + few drops Tween 80 (8 min) and finally rinsed thoroughly three times with sterile distilled water. Cultured on ½ MS medium supplemented with 0.1 mg/L NAA; 1.0 mg/L BA (Dang Thi Tham *et al.,* 2018).

Ability to protocorm formation

Cultured on MS medium supplemented with BA 2.0 mg/L combination NAA (0; 0.2; 0.5; 1.0; 1.5 mg/L). Continue to experiment with MS medium supplemented with different concentrations of TDZ (0.05; 0.1; 0.5; 1.0; 1.5 mg/L) combination with NAA at 0.5 mg/L.

Regenerated shoot in vitro

Three bundle shoots of 6 mm height, each bundle shoot have three shoots were transferred in each culture vessel. They cultured on MS medium supplemented with BA (0; 0.5; 1.0; 1.5; 2.0; 2.5 mg/L) or mash (Carrot, Potato, ripe Banana).

The complete plants in vitro

For the root induction experiments, the shoots are equally high. They were transferred to ½ MS medium supplemented with different concentrations of IAA, IBA, NAA (0.3; 0.5; 1.0 mg/L) individually.

Hardening

Well-rooted plantlets were taken out from culture vessels; their roots were washed thoroughly under running tap water to remove the adhering agar medium and planted on different substrate: Coconut fiber, Fern fiber, Eco clean soil, 50% Rice husk combination with 50% Eco clean soil.

Experimental Design and Data Analysis

The experiments were designed following Complete Randomize Design (CRD). The experiments were repeated three times. At the *in vitro*, the significance of treatment effects was determined using analysis of variance (ANOVA, $p \leq 0.05$), and comparison between mean values of treatments were made by Duncan's test (Duncan, 1995). All statistical analyses were performed using the software SPSS 16.0. At the *ex vitro*, each substrate planted 45 plantlets, data was processed by software Microsorf Excel 2010.

RESULTS AND DISCUSSION

Effect of BA and NAA combination on

protocorm-like bodies (PLBs) formation of *Dendrobium trankimianum* T. Yukawa

The latent buds were cultured on medium approximately 30 days. Afterward, the sample fungal-free and un pathogen were transferred to MS medium supplemented with BA 2.0 mg/L and NAA (0; 0.2; 0.5; 1.0; 1.5 mg/L). Results are shown in table 1 below.

Table 1. Effect of BA (2.0 mg/l) and NAA combination on PLBs formation of *D. trankimianum* T. Yukawa after 45 days of culture.

NAA(mg/L)	Average No. of PLBs/ explant	% of explant with PLBs formation
0	6.67^{c*}	68.68
0.2	8.90^{b}	70.60
0.5	10.24^{a}	90.11
1.0	7.64^{c}	70.73
1.5	6.79^{c}	48.06

Note: *: *In each column, the mean values with different letter (a,b,c…) are significantly different with α = 0.05 in Ducan's test.*

After 45 days of culture, the combinations of these hormones induced multiple PLBs formation to a variable extent as shown in table 1. The highest percentage of forming PLBs (90.11%) and highest number of PLBs per explant (10.24) was found on modified MS medium supplemented with 2.0 mg/L BA and 0.5 mg/L NAA (Table1 and Figure 1c$_3$). On the contrary, the lowest response for forming PLBs (48.06%) was observed at 2.0 mg/L BA and 1.5 mg/L NAA (Table 1 and Figure 1c$_5$). Mean while, the lowest number of PLBs per explant (6.67) was found on modified MS medium supplemented with 2.0 mg/L BA and 0 mg/L NAA (Table 1 and Figure 1c$_1$). Thus, the ability to PLB formation depends on the concentration of the growth regulator contained in the medium culture. The combinations, concentrations and the ratio between them are usually critically important (Hosain *et al.,* 2010**)**. These finding agreeted with those reported by Sunitibala and Kishor (2009) who observed BA combined with NAA was better than BA combined with IBA or IAA in *D.transparens* L.. Niramol (2009) studied micropropagation of *D. draconis* Rchb.f reported the optimal growth regulator combination for maximal PLB development was 2.0 mg/L BA and 1.0 mg/L NAA, giving rise to 68% of responding explants with an average 11 PLBs per explant. This NAA concentration (1.0 mg/L) is higher than our optimal NAA (0.5 mg/L) on media culture. In such cases, difference may be due to the variation in the plant material and sample size.

Effect of TDZ and NAA combination on protocorm-like bodies (PLBs) formation

Continue to experiment with MS medium supplemented with different concentrations of TDZ (0.05; 0.1; 0.5; 1.0; 1.5 mg/L) combination with NAA at 0.5 mg/l. Results was summarised in table 2.

Table 2. Effect of TDZ and NAA combination on PLBs formation of *D. trankimianum* T. Yukawa after 45 days of culture.

TDZ (mg/L)	NAA (mg/L)	Average No. of PLBs/ explant	% of explant with PLBs formation
0	0	2.75^{e}*	29.84
0.5	0.5	8.55^{d}	60.89
1.0	0.5	10.86^{c}	72.11
1.5	0.5	14.11^{a}	92.06
2.0	0.5	13.20^{b}	89.46

Note: *: *In each column, the mean values with different letter (a,b,c…)are significantly different with α = 0.05 in Ducan's test.*

The results in table 2 show that, after 45 days of culture just 29.84 % explants of *D. trankimianum* T. Yukawa produced PLBs without exogenous hormone; when the medium supplemented with TDZ combined with NAA, the PLBs formation increased from 60.89% to 92.06%, number of PLBs/explant increased from 8.55 to 14.11. PLBs is spherical, greenish yellow. The optimal growth regulator combination for maximal PLBs formation and development was 1.5 mg/L TDZ and 0.5 mg/L NAA, giving rise to 92.06% of responding explants with an average 14.11 PLBs per explants (Table 2 and Figure 1d$_4$). It supported a higher rate percentage of PLBs formation and more higher number of PLBs/explant. However, when the concentration of TDZ increased to 2.0 mg/L with 0.5 mg/L NAA, the number of

PLBs/explant and percent of explant with PLBs formation decreased; the PLBs became succulent, yellowish white and died (Table 2 and Figure $1d_5$). The synergistic effect of TDZ, in the present study, has been observed for efficient PLBs formation so as to detect the immediate and long term effects of TDZ on the clonally propagated plants of this orchid species. Paromik *et al.,* (2014) also used TDZ in *in vitro* regenerated plants of *D. nobile* Lindl., the results showed that PLBs were induced from the pseudostem segments using Thidiazuron (1.5 mg/L TDZ).

In brief, MS medium supplemented with 2.0 mg/L BA and 0.5 mg/LNAA or MS medium supplemented with 1.0 mg/L TDZ and 0.5 mg/L NAA were the most suitable for protocorm formation of *D. trankimianum* T. Yukawa.

Regenerating shoot *in vitro* of *D. trankimianum* T. Yukawa. The effects of BA on growth and development of shoots

The type and concentration of growth regulators are an initial consideration for micropropagation of orchid species. In many studies, a number of treatments of BA ranging from 0 - 3.0 mg/L were employed for shoot proliferation of orchids. Hence, we conducted experiments on MS medium supplemented with BA (0; 0.5; 1.0; 1.5; 2.0; 2.5 mg/L). After 60 days, the ability to regenerate shoots of *D. trankimianum* T. Yukawa was shown in table 3.

Table 3. The effects of BA on growth and development of shoots.

BA (mg/L)	Average no.of shoots/explant	Average length of shoots (cm)
0	8.42^{d}	1.68^{d}*
0.5	15.33^{c}	1.77^{c}
1.0	19.04^{b}	1.87^{b}
1.5	22.35^{a}	1.96^{a}
2.0	20.02^{b}	1.75^{cd}
2.5	19.29^{b}	1.71^{cd}

Note: *:*In each column, the mean values with different letter (a,b,c…)are significantly different with α = 0.05 in Ducan's test.*

In the present study, concentration of BA influenced the average number of shoots produced per explant as well as mean length of the shoots. There was poor growth when explants were cultured in the media without BA (Table 3). The highest number of shoots/ explant was observed at the concentration of BA 1.5 mg/L, which was 22.35. Maximum shoot length (1.96 cm) was also found in this concentration (Table 3 and Figure $1e_4$). The concentration of BA increased from 0 - 1.5 mg/L, the shoot length increased 1.68 - 1.96 cm and number of shoots increased from 8.42 to 22.35, but when BA increased to 2.0 - 2.5 mg/L, the shoot length and number of shoots/explant were decreased (Table 3 and Figure $1e_{5,6}$). This indicates that concentrations of BA from 0 to 1.5 mg/L stimulate the protocorms to grow into shoots, in contrast, when BA increases up to 2.0 - 2.5 mg/L, it suppresses the protocorms that grow into shoots. BA is a cytokinin growth regulator. The addition of BA to the medium increased the number of shoots by stimulating quick cell division to induce large number of multiple shoots. Therefore, in plant tissue culture, BA is often used in the rapid multiplication phase. Results are also supported by findings of Roy *et al.,* (2002) who reported that BA enhances the shoot multiplication more actively than Kinetin. In addition, BA has been used for propagation in other *Dendrobium* such as: *D. nobile var.* Emma white (Sana Asghar *et al.,* 2011); *D. aggregatum* (Vijayakumar *et al*., 2012); *D. Chrysanthum* Wall. Ex Lindl. (Rao, Barman, 2014).

The effects of mash (carrot, potato, banana) on growth and development of shoots.

Three bundle shoots of 6 mm height, each bundle shoot have three shoots was transferred in each culture vessel containing a culture medium supplemented with mashes (potatoes, bananas, carrots). After 60 days, culture data on number of shoots and length of shoots were recorded in table 4.

Table 4. The effects of mashes (potatoes, bananas, carrots) on the growth and development of shoots after 60 days of culture.

	Average no.of shoots/explant	Average length of shoots (cm)
Control	8.42^{d}	1.68^{c}*
60 g potato/liter medium	22.62^{b}	1.85^{b}
60 g ripe banana /liter medium	25.11^{a}	2.12^{a}
60 g carrot/liter medium	20.82^{c}	1.71^{c}

Note: *: *In each column, the mean values with different letter(a,b,c…)are significantly different with α = 0.05 in Ducan's test.*

The table 4 shown that, in the control (free of mashes), the number and length of shoots were lower in comparison with that in mashes (potatoes, bananas, carrots) supplemented medium. The number of shoots was only 8.42 per explant in the control condition, whereas the numbers were 22.62, 25.11 and 20.82, respectively at 60 g potato/liter medium, 60 g ripe banana/liter medium and 60 g carrot/liter medium (Table 4). Free of mashes and different mashes enhanced the length of shoots differently. The highest number of shoots/explant of *D. trankimianum* T. Yukawa was observed at 60 g ripe banana/liter medium, which was 25.11. Maximum shoot length (2.12 cm) was also found in this medium (Figure $1f_3$). Nguyen Thi Son *et al.,* (2014) reported that among the experiments of shoot multiplication of *D. officinale* Kimura et Migo, the growth and development of shoots was maximum at 60 g ripe banana/liter medium, which is consistent with our results. Van Staden *et al.,*(1975) showed that the addition of blended banana to culture medium stimulates the orchid growth because it helps balance the pH medium. In addition, it contained the compound with cytokinin activities and some potent physiologic agents like serotonin, norepinephrine, dopamine and some unidentified catecholamine that make a significant impact on promoting the propagation of cultured cells (Van Staden *et al*., 1975).

In conclusion, MS medium supplemented with 60 g ripe banana/liter medium was the most suitable for growth and development of shoots of *D. trankimianum* T. Yukawa.

The effects of growth regulators (IAA, IBA, NAA) on root formation.

The shoots *in vitro* are equally high, obtained from the above experiments were transferred to ½ MS medium supplemented with different concentrations of IAA, IBA, NAA (0.3; 0.5; 1.0 mg/L individually) to investigate root formation. The regeneration root of *D. trankimianum* T. Yukawa after 60 days of culture was shown in table 5.

Table 5. The effects of growth regulators (IAA, IBA, NAA) on root formation.

IAA (mg/L)	IBA (mg/L)	NAA (mg/L)	Rooting percentage (%)	No. of roots/shoot	Root length (cm)
0	0	0	50.53	2.04^{f}	1.88^{g}
0.3	0	0	67.35	2.88^{e}	2.75^{e}
0.5	0	0	71.82	4.35^{d}	2.93^{de}
1.0	0	0	71.06	2.37^{ef}	2.15^{f}
0	0.3	0	72.67	4.44^{d}	2.87^{e}
0	0.5	0	81.15	5.62^{c}	3.22^{c}
0	1.0	0	80.53	3.93^{d}	3.15^{cd}
0	0	0.3	91.82	5.44^{c}	3.82^{ab}
0	0	0.5	98.51	7.91^{a}	4.01^{a}
0	0	1.0	93.18	6.46^{b}	3.63^{b}

Note:*: *In each column, the mean values with different letter (a,b,c…)are significantly different with α = 0.05 in Ducan's test.*

All the treatments produced root with varying root numbers and lengths. Supplementing Auxin (IAA, IBA, NAA) to culture medium positively affected the root formation, especially NAA. In control treatment where ½ MS medium was not supplemented with any auxin, all three parameters of root percentage, number of root and root length were lower than in medium added with auxin. The number of roots was only 2.04 roots/shoot, with the root length of 1.88 cm and 50.53 % of roots formation (Table 5). Auxin application to microshoots is said to intensify the number of adventitious roots by increasing the level of endogenous contents of enzymes. They are considered to have an increased effect on cell division, elongation and differentiation (Husen, Pal, 2007). Among IAA, IBA and NAA, the results showed that IAA produced the lowest number of roots, and roots length, as well as rooting rate at the same concentration (Figure 1g). Being supplemented with IBA at different concentrations (0.3; 0.5; 1.0 mg/L), the medium added with 0.5 mg/L IBA was the best response, reaching 5.62 roots per shoot, 3.22 cm of root length and 81.15% of roots formation, but the roots were fragile and weak (Figure $1g_6$).

These obtained results reviewed that NAA was a suitable growth regulator for the root formation of *D. trankimianum* T. Yukawa. In particular, the treatment of medium added with 0.5 mg/L NAA was the best response, which produced 7.91 roots/shoot with the root length of 4.01 cm and the rooting rate of 98.51% (Table 5 and Figure $1g_9$). The roots were strong with numerous stilt roots. Dake *et al.,* (2013) also reported that ½ MS medium supplemented with 0.5 mg/L NAA was appropriate for the *in vitro* root formation of *D. wangliangii*.. However, studies of another researchers like Sunitibala on the propagation of *D. transparens* indicated that the proper medium for root formation was ½ MS medium added with 1 mg/L IAA. Khawanduean (2017) studied the propagation of *D. signatum,* revealing that the best treatment for root formation was ½ MS medium supplemented with 0.5 mg/L NAA in combination with 2 mg/L BA. Thus, the suitable medium for root formation varies depending on the propagated plant. Thus, the results of the experiment showed that ½ MS medium supplemented with 0.5 mg/L NAA was suitable for root formation of *D. trankimianum* T. Yukawa.

The effects of substrate on survival and plantlets quality of *D. trankimianum* T. Yukawa.

The plantlets with uniform growth were planted on different substrates: Eco clean soil, Coconut fiber, Fern fiber, 50% Rice husk in combination with 50% Eco clean soil for research the most substrate. The survivability of the transferred plantlets was recorded after 60 days of transfer, the result was shown in table 6.

Table 6. The effects of substrates after 60 days planting in the nursery.

	No. of roots	Root length (cm)	Survival percentage (%)
Coconut fiber	4.0	3.5	62.09
Eco clean soil	5.0	4	81.62
Fern fiber	8.0	5.5	93.29
Rice husk combination with Eco clean soil (1:1)	3.5	3.7	78.23

When grown in a nursery garden under controlled conditions, based on the results in table 6, it can be seen that different substrates have various effects on the survival of plantlets. The lowest survival percentage was on the Coconut fiber substrate, illustrated by 62.09%, light green leaves and without new roots. Meanwhile, Rice husk combined with Eco clean soil had a higher survival percentage (78.23%), but plantlets were weak and also no roots were found. The formation of new roots was observed in Eco clean soil substrate with 81% survival percentage but the plantlets grow slowly. The maximum survival percentage (93.29%) with the highest number of roots (8 roots/ plantlet) and the longest root length (5.5 cm) were observed in Fern fiber substrate. The transfer of plantlets to nursery garden was very important for success in micropropagation. A variety of approaches, such as different *in vitro* culture media and concentration of agar and sucrose in growth medium, have been employes to reduce the loss of micropagated orchid plantlets at the acclimatization stage. Beside these approaches, the proper selection of soil substrates, correct shade management, irrigation and gradual lowering of humidity can solve the problems and reduce the losses during acclimatization (Ded , Imchen, 2010). Sunitibala and Rajkumar (2009) transferred *D. transparens* into potting mixture (brick and charcoal in 2:1 ratio) resulting in a 90% survival percentage. Nguyen Thanh Tung *et al.,* (2010) when planting *D. aduncum* in the potting mixture containing sphagnum moss and fern in 1:1 ratio resulting in 90% survival percentage and forming many new roots.

CONCLUSION

In the current study, the most suitable medium for PLB formation was MS medium supplemented with 2.0 mg/L BA and 0.5 mg/L NAA or MS medium supplemented with 1.0 mg/L TDZ and 0.5 mg/L NAA. For subculture, suitable growth of shoots was obtained on MS medium supplemented 1.5 mg/L BA and MS medium supplemented with 60 g ripe banana/liter medium. The medium used for root formation was ½ MS + 0,5 mg/L NAA. 60 days after of transplantion and acclimatization, Fern fiber substrate was suitable substrate for plantlet growth.

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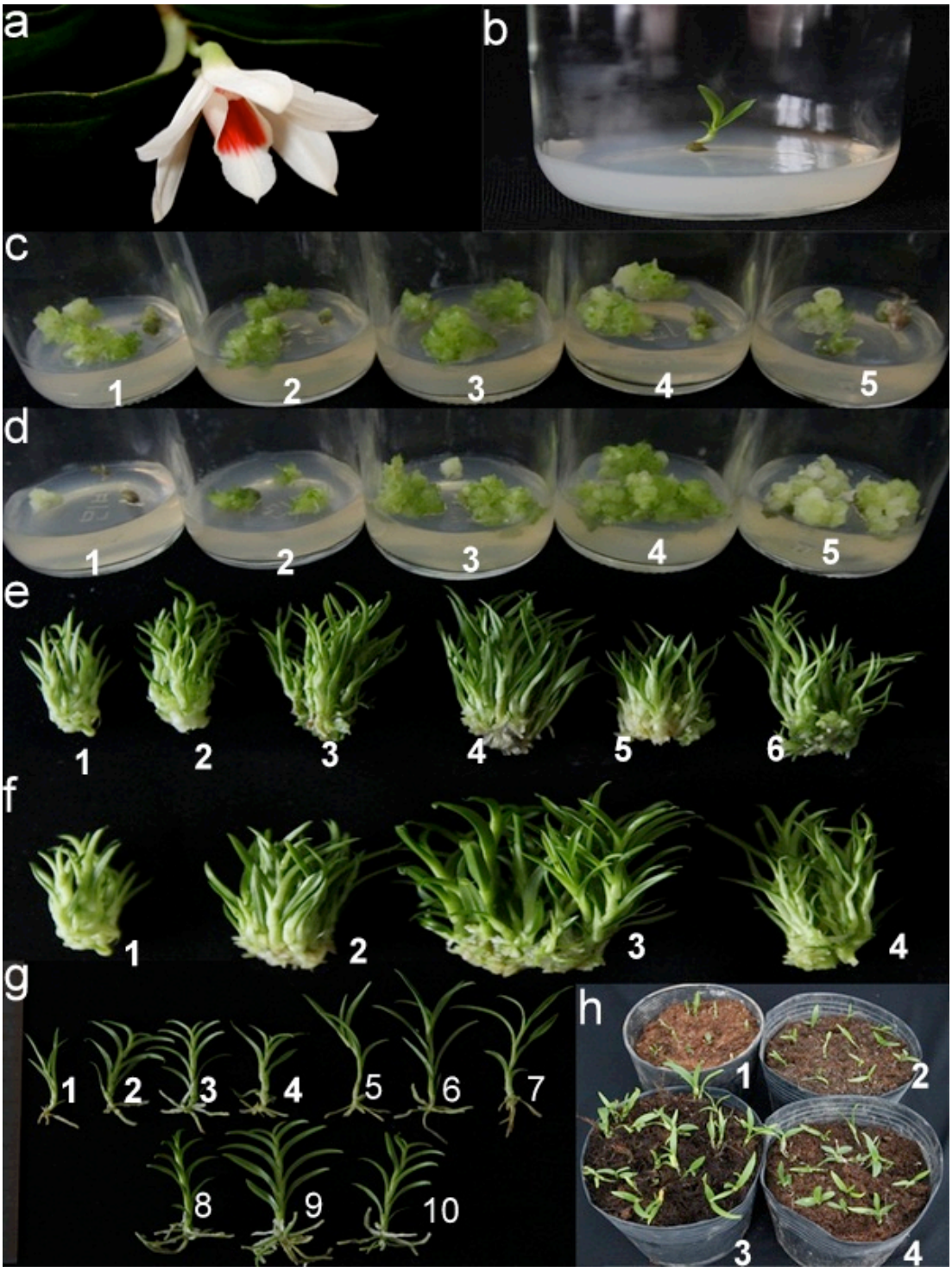

Figure 1. *In vitro* Propagation of *Dendrobium trankimianum* T. Yukawa: a. *Dendrobium trankimianum* T. Yukawa; b. Shoots after 30 days; c. Effect of BA and NAA combinations on PLBs formation; d. Effect of TDZ and NAA combinations on PLBs formation; e. The effects of BA on growth and development of shoots*;* f. The effects of mash (carrot, potato, banana) on growth and development of shoots*;* g. The effect of growth regulators (IAA, IBA, NAA) on root formation; h. Plantlets in different substrates.

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NHÂN GIỐNG *IN VITRO* LAN TRẦN KIM – *DENDROBIUM TRANKIMIANUM* T. YUKAWA

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TÓM TẮT

Lan Trần kim **(***Dendrobium trankimianum* T. Yukawa*)* là loài hoa tuyệt đẹp và đặc hữu của Việt Nam, được phát hiện lần đầu tiên vào năm 2004. *Dendrobium trankimianum* là loài lan quý hiếm, dự kiến sẽ trong tình trạng CR trong sách đỏ IUCN. Chúng tôi tiến hành nghiên cứu nhân giống *in vitro* nhằm mục đích bảo tồn và phát triển nguồn gen loài lan rừng quý này. Kết quả cho thấy môi trường nuôi cấy MS bổ sung 2,0 mg/L BA và 0,5 mg/L NAA (10,24 PLB/mẫu cấy, 90,11% mẫu tạo thành PLB) hoặc môi trường nuôi cấy MS bổ sung 1,5 mg/L TDZ và 0,5 mg/L NAA (14,11 PLB/mẫu cấy, 92,06% mẫu cấy tạo thành PLB) là thích hợp nhất cho sự hình thành protocorm. Môi trường nuôi cấy MS bổ sung thêm 1,5 mg/L BA (22,35 chồi/cụm, chiều cao chồi đạt 1,96 cm) và môi trường nuôi cấy MS bổ sung 60 g chuối chín/L (25,11 chồi/cụm, chiều cao chồi đạt 2,12 cm) đều là những môi trường thích hợp cho nhân nhanh cụm chồi. Chồi *in vitro* được cấy trên môi trường ½ MS bổ sung IAA, IBA và NAA để cảm ứng tạo rễ. Nồng độ NAA 0,5 mg/L là nồng độ tối ưu cho sự tạo rễ hình thành cây *in vitro* hoàn chỉnh (7,91 rễ/chồi, chiều dài rễ đạt 4,01 cm, 98,51% chồi ra rễ). Sau 60 ngày chuyển cây con *in vitro* vào vườn ươm, kết quả cho thấy giá thể Dớn là giá thể phù hợp nhất (8,0 rễ/mẫu, chiều dài rễ đạt 5,5 cm, tỷ lệ sống 92,29%).

Từ khóa: *Bảo tồn, Dendrobium trankimianum T. Yukawa, giá thể, in vitro, lan rừng, PLBs*