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Efficient propagation with in vitro seed germination of *Vanda falcata**

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Vanda falcata (Thunb.) Beer (Orchidaceae), a famous native orchid of China, Japan, and Korea, is known as one of the most beautiful and charming orchid species in the world (Ohwi, 1965; Lawler, 1984; Arditti, 2008). *V. falcata* is widely cultivated and delights the world with its compact plant shape, elegant white blooms, and sweet coconut-like scent. However, vegetative propagation by division has limited the development of *V. falcata* because of its inefficiency (Mitsukuri et al., 2009a, 2009b).

Previous findings have shown that both in vitro micropropagation and asymbiotic germination of orchids could have the advantage of ensuring a large supply of clonal plantlets throughout the year (Teixeira da Silva et al., 2015). Thus, several studies have been focused on the establishment of a micropropagation system for *V. falcata*. Different kinds of explants have been studied for their ability to establish aseptic cultures, such as shoot apexes, florets, new stocks, scapes, leaves, and root tips (Shimasaki, 1993; Mitsukuri et al., 2009a). These scientists found that shoot apexes of *V. falcata* were the most suitable explant materials, although they exuded browning compounds into the medium (Mitsukuri et al., 2009a). Generally, two to three weeks of dark-preconditioning treatment or treatment with L-2-aminooxy-3-phenylpropionic acid (AOPP) must be carried out on shoot apexes or leaf tissue cultures to control browning (Mitsukuri et al., 2009a, 2009b, 2010).

Asymbiotic germination involves the in vitro inoculation and germination of seeds with the aid of a sucrose-containing culture medium, and represents the most efficient method of native epiphytic orchid propagation for conservation purposes (Arditti et al., 1981; Cardoso et al., 2020). These studies showed that in vitro germination rates could rise higher than 70%, while these rates barely exceed 5% in ex vitro (natural environmental) conditions (Rao, 1997; Kunakhonnuruk et al., 2018). However, no efficient in vitro propagation method has been reported for *V. falcata*. Here, we describe a simple and feasible protocol for propagation using immature seeds.

Mature capsules were collected from five-year pot-grown mother plants in the Orchid Garden in Hangzhou Flower Garden, Hangzhou, China, after self-pollination for 4–5 months. First, the capsules were washed with tap water for 1 h and immersed in 70% alcohol for 30 s. Subsequently, the capsules were soaked in a solution of 10% sodium hypochlorite and agitated for 10 min. Finally, they were washed thoroughly in sterile water for five times after pouring the disinfectant out. The sterilized capsules were cut longitudinally using a sharp sterilized surgical blade to facilitate seed extraction.

Seeds from these capsules were sown on modified MS (Murashige and Skoog, 1962) basal medium containing half-strength macro- and micro-elements (Figs. 1a and 1b). To test the effect of some plant

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growth regulators (PGRs) and additives, which are commonly used in orchid tissue culture, we added the following to the basal MS medium, either individually or in combination: sucrose (30 g/L), activated charcoal (3 g/L), coconut water (100 mL/L), coconut meat (100 g/L), peptone (PEP, 3 g/L), lactoalbumin hydrolysate (3 g/L), α-naphthalene acetic acid (NAA; 0.1, 1.0, 3.0, or 5.0 mg/L), 6-benzyladenine (BA; 0.1, 1.0, 3.0, or 5.0 mg/L), and/or kinetin (KT; 1 mg/L); details are in Table 1. The coconut water (liquid endosperm) and coconut meat (solid endosperm) were obtained from commercial coconuts. Our objective was not to test all permutations exhaustively, which would have resulted in hundreds of different treatments, but to test whether several key factors that were previously found to be effective in orchid seed germination (Kauth et al., 2008) would also be successful for *V. falcata* seed germination. Each treatment was repeated twice with six replicates. All media were solidified with a 0.7% agar strip (Sigma, America). Media were adjusted to pH 5.6–5.8 with 1 mol/L NaOH or HCl before autoclaving at 121 °C 105 kPa for 15 min. The average number of seeds per capsule inoculated was more than 1000. The germination status was recorded after four weeks of culture.

Table 1 Growth media

Code name	Medium composition
CW	1/2 MS+S 20 g/L+AC 3 g/L+CW 100 mL/L
CM	1/2 MS+S 20 g/L+AC 3 g/L+CM 100 g/L
PEP	1/2 MS+S 20 g/L+AC 3 g/L+PEP 3 g/L
N-1	1/2 MS+NAA 1.0 mg/L+BA 0.1 mg/L+AC 3 g/L+CW 100 mL/L
N-3	1/2 MS+NAA 3.0 mg/L+BA 0.1 mg/L+AC 3 g/L+CW 100 mL/L
N-5	1/2 MS+NAA 5.0 mg/L+BA 0.1 mg/L+AC 3 g/L+CW 100 mL/L
NP-3	1/2 MS+NAA 3.0 mg/L+BA 0.1 mg/L+AC 3 g/L+PEP 3 g/L
B-1	1/2 MS+NAA 0.1 mg/L+BA 1.0 mg/L+AC 3 g/L+CW 100 mL/L
B-3	1/2 MS+NAA 0.1 mg/L+BA 3.0 mg/L+AC 3 g/L+CW 100 mL/L
B-5	1/2 MS+NAA 0.1 mg/L+BA 5.0 mg/L+AC 3 g/L+CW 100 mL/L
BP-1	1/2 MS+NAA 0.1 mg/L+BA 1.0 mg/L+AC 3 g/L+PEP 3 g/L
K-1	1/2 MS+NAA 0.1 mg/L+KT 1.0 mg/L+AC 3 g/L+CW 100 mL/L
LH	1/2 MS+S 20 g/L+AC 3 g/L+LH 3 g/L

AC, activated charcoal; BA, 6-benzyladenine; CM, coconut meat; CW, coconut water; KT, kinetin; LH, lactoalbumin hydrolysate; MS, Murashige and Skoog; NAA, α-naphthalene acetic acid; PEP, peptone; S, sucrose

We used the orthogonal matrix L9(34) method to measure the effects of the PGRs on multiple-shoot induction in MS medium. After a preliminary experiment to test the effects of various PGRs on multiple-shoot induction by one-factor-at-a-time, we selected four PGRs (NAA, BA, indole-3-butyric acid (IBA), and KT) with different concentrations. Three different combinations were used, including 0.2 mg/L NAA+0.5 mg/L BA, 0.5 mg/L NAA+1.0 mg/L BA+0.5 mg/L IBA+0.5 mg/L KT, and 1.0 mg/L NAA+2.0 mg/L BA+1.0 mg/L IBA+1.0 mg/L KT. The number of shoots (length of ≥2 mm) was investigated after 30 d of culture.

Four influential factors in rooting, namely NAA, BA, sucrose, and PEP to induce roots, were optimized by orthogonal experiment. The combinations included 0.2 mg/L NAA+0.2 mg/L BA+10 g/L sucrose, 0.5 mg/L NAA+0.5 mg/L BA+20 g/L sucrose+1 g/L PEP, and 1.0 mg/L NAA+1.0 mg/L BA+40 g/L sucrose+3 g/L PEP. Each treatment was repeated twice with four replicates. We found that the best medium for rooting was the combination of solid MS supplemented with 1.0 mg/L NAA, 0.5 mg/L BA, and 40 g/L sucrose. We counted the number of roots (length of ≥2 mm) of eight-week-old plantlets. The 4–5-cm-high rooted seedlings were then transferred to plastic pots (12 cm diameter×18 cm height) containing sphagnum moss, wood charcoal, and gravel (1:1:1, volume ratio), and covered with polythene bags to maintain a high level of humidity. Plantlets were acclimated in the culture room for 3–4 weeks and then transferred to the greenhouse, where the climate conditions were the same (temperature range 20–25 °C, photoperiod 13 h/11 h (light/dark), and relative humidity 60%–70%).

To evaluate the effectiveness of the culture medium on germination, we used Ridit analysis, which is designed to facilitate the data analysis of variables that are more than dichotomous classifications. These variables are ordered but do not reach the standards of refined measurement systems, such as those meeting the criteria for equal-interval or ratio scales (Bradburn, 1969).

The multiple-shoot induction and rooting data obtained using the orthogonal test were analyzed using SPSS software (Version 20, IBM, USA), and range analysis was applied to determine the impact of various factors on the experimental index. Statistical significance was determined using the least-significant-difference test. This statistical analysis used P=0.05.

This is the first complete protocol that has been developed for mass propagation of *V. falcata*, an epiphytic orchid species. The main morphogenetic pathway is summarized in Fig. 1.

Although seeds germinated on all the media tested, the germination rate varied among them. On CM medium (code names of media are listed in Table 1, the same for the following media), the embryos enlarged to occupy the entire seed coat (Figs. 1c and 1d) and developed into the protocorm stage, whereas the embryos remained swollen on other media. The highest seed germination rate (R=0.9487) was observed on CM medium, followed by CW (R=0.6538), B-3 (R=0.6538), and B-5 (R=0.6729) media (Fig. 2). LH, NP-3, and BP-1 media were not as effective as CM, CW, PEP, N-1, N-3, B-1, B-3, B-5, and K-1 media for seed germination. However, when PEP was applied in combination with NAA or BA, seed germination was poor.

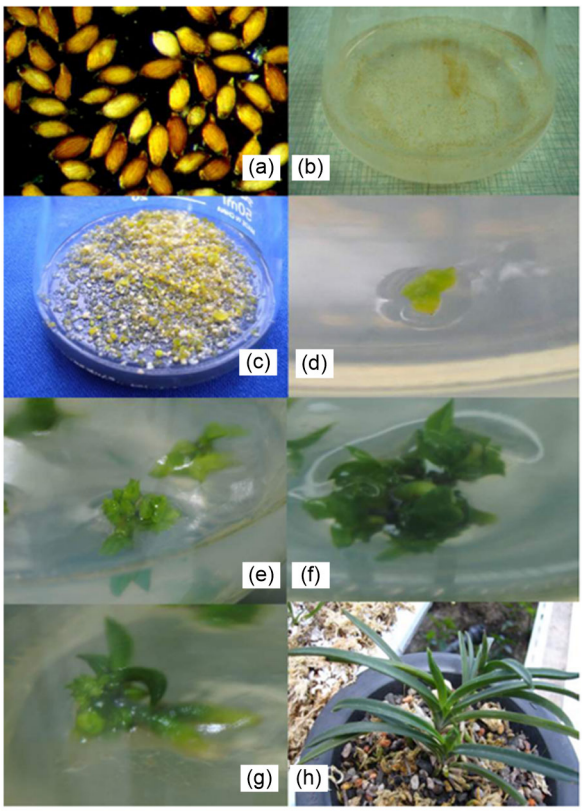

Fig. 1 In vitro seed germination and seedling development in *Vanda falcata*

(a) Mature seeds (×100); (b) Aseptic seedling; (c) Protocorm multiplying; (d) Development of the protocorms; (e) Initiation of multiple shoots; (f) Development of multiple shoots; (g) Development of a stout root system in a plantlet; (h) A hardened plant growing in a pot in the greenhouse

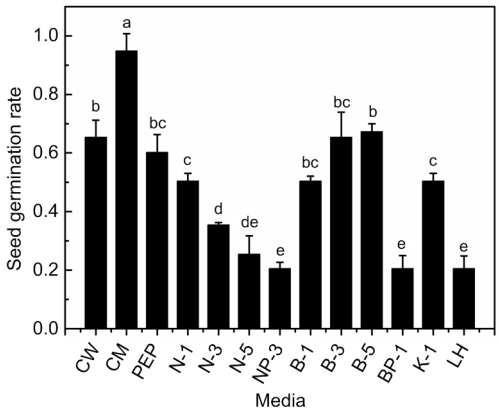

Fig. 2 Effects of different culture media on seed germination of *Vanda falcata*

Code names of media are listed in Table 1. Data are presented as the means of three replicates, and error bars indicate the standard deviation. The differences (with different lowercase letters) were tested at 0.05 levels of significance

According to the average value in Table 2, NAA exerted the most significant effect in multiplying shoots (Figs. 1e and 1f), and the order of importance that influenced multiplication was found to be NAA>KT>IBA>BA.

NAA showed the most significant effects on rooting (Fig. 1g), followed by PEP (P<0.05, Table 2). The best medium for rooting was the combination of solid MS supplemented with 1.0 mg/L NAA, 0.5 mg/L BA, and 40 g/L sucrose. Each rooted seedling survived successfully after acclimatization (Fig. 1h).

The effects of various PGRs or additives on seed germination and plant regeneration in *V. falcata* have been thoroughly studied. The simple and efficient method of regenerating a large number of plantlets from a single capsule containing coconut meat, NAA, and BA described here could be useful for large-scale propagation and ex situ conservation of the commercially important epiphytic orchid species.

In vitro seed germination has been suggested as a suitable propagation method for conservation of orchids (Arditti et al., 1981; Ballard, 1990; Thornhill and Koopowitz, 1992; Zettler and McInnis, 1993; Kauth et al., 2006; Stewart and Kane, 2006; Mahendran and Narmatha Bai, 2009; Hossain et al., 2010). It has been documented that coconut water is a useful and potent germination enhancer and that it aids the germination of orchids in vitro (Withner, 1959; Minea et al., 2004). This finding is also supported by the results obtained in the present experiment, albeit at

Table 2 Shoot multiplication and rooting test results

Composition (average)	Number of shoots/roots		
	Level 1	Level 2	Level 3
Shoot multiplication			
NAA (7.92)	21.08[a]	14.50[b]	13.17[b]
BA (6.58)	20.17[a]	13.58[b]	15.00[b]
IBA (7.17)	19.92[a]	12.75[b]	16.08[ab]
KT (7.25)	20.83[a]	14.33[b]	13.58[b]
Rooting			
NAA (4.63)	3.61[b]	2.91[b]	7.54[a]
BA (1.06)	4.17[a]	5.22[a]	4.67[a]
Sucrose (0.91)	4.52[a]	4.32[a]	5.22[a]
PEP (1.91)	5.37[a]	3.46[b]	5.22[a]

A value followed by the same letter within the same row (shoot multiplication or rooting) indicates insignificance at P=0.05. BA, 6-benzyladenine; IBA, indole-3-butyric acid; KT, kinetin; NAA, α-naphthalene acetic acid; PEP, peptone

different concentrations of coconut water. However, coconut meat was shown to possess stronger stimulatory properties compared to those of coconut water, although the mechanism by which this occurs remains to be elucidated by further study. Initially, the protocorms were achlorophyllous and subsequently acquired chlorophyll, which agrees with the view of McKendrick (2000). Much research has been focused on determining the nutritional and culture requirements for optimal germination and early seedling development in vitro. Beneficial modifications in the present study include reducing the concentration of inorganic salt (Fast, 1982; Mitchell, 1989; Anderson, 1991) and adding half-strength macro- and microelements to the MS medium.

The success of rapid and direct shoot and root regeneration from protocorm explants reveals another efficient way to mass-propagate *V. falcata*. Generally, auxins stimulate root formation, cytokinins enhance the development of shoots and cell division, and sugar stimulates the development of seedlings (Wotavová-Novotná et al., 2007; Palama et al., 2010). In the present study, protocorms developed multiple shoots and roots directly on medium supplemented with NAA and BA. In most orchids, including *Dendrobium* (Yasugi and Shinto, 1994), *Coelogyne stricta* (Basker and Narmatha Bai, 2006), *Geodorum densiflorum* (Sheelavantmath et al., 2000), *Oncidium varicosum* (Kerbauy, 1984), and *Cymbidium pendulum* (Nongdam et al., 2006), the combination of NAA and BA (with a concentration range from 0.1 to 2.0 mg/L for NAA and 0.1 to 5.0 mg/L for BA) promoted optimal shoot proliferation and rooting. Moreover, our results indicate that a higher concentration of sucrose (40 g/L) promotes orchid seedling growth (expressed by the growth of shoots and roots), a finding which is consistent with the observations of Wotavová-Novotná et al. (2007).

Based on our results, the optimal medium for germinating seeds of *V. falcata* (Thunb.) Beer was MS medium with half-strength macro- and microelements, full-strength vitamins and inositol, 20 g/L sucrose, 3 g/L activated charcoal, and 100 g/L coconut meat. This medium was made even more effective by adding NAA 0.2 mg/L and BA 0.5 mg/L; this combination showed the strongest effect on multiple shoot induction from the protocorm. Furthermore, the best formula for the rooting induction medium was NAA 1.0 mg/L, BA 0.5 mg/L, and sucrose 40 g/L.

Contributors

Xiao-fen LIU and Li-li XIANG summarized the experimental reports and wrote the manuscript. Yan HUANG carried out the tissue culture experiment. Xiao-fen LIU, Ya-jing LI, and Fang LI analyzed the data. Fang LI has received research grants and designed this experiment. All authors have read and approved the final manuscript and, therefore, have full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Xiao-fen LIU, Li-li XIANG, Yan HUANG, Ya-jing LI, and Fang LI declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

Anderson AB, 1991. Symbiotic and asymbiotic germination and growth of *Spiranthes magnicamporum* (Orchidaceae). *Lindleyana*, 6(4):183-186.

Arditti J, 2008. Micropropagation of Orchids, 2nd Ed. Blackwell Publishing Ltd., Malden, USA, p.778-781.

Arditti J, Michaud JD, Oliva AP, 1981. Seed germination of North American orchids. I. Native California and related species of *Calypso*, *Epipactis*, *Goodyera*, *Piperia*, and *Platanthera*. *Bot Gaz*, 142(4):442-453. https://doi.org/10.1086/337245

Ballard WW, 1990. Further notes on *Cypripedium* germination. *In*: Sawyers CE (Ed.), North American Native Terrestrial Orchid Propagation and Production. Chadds Ford Press, Pennsylvania, USA.

Basker S, Narmatha Bai V, 2006. Micropropagation of *Coelogyne stricta* (D. Don) Schltr. via pseudobulb segment cultures. *Trop Subtr Agroecosys*, 6:31-35.

Bradburn NM, 1969. The Structure of Psychological Well-being. Aldine Pub. Co., Chicago, USA.

Cardoso JC, Zanello CA, Chen JT, 2020. An overview of orchid protocorm-like bodies: mass propagation, biotechnology, molecular aspects, and breeding. *Int J Mol Sci*, 21(3):985. https://doi.org/10.3390/ijms21030985

Fast G, 1982. European Terrestrial Orchids (Symbiotic and Asymbiotic Methods). Cornell University Press, New York, USA.

Hossain MM, Sharma M, Teixeira da Silva JA, et al., 2010. Seed germination and tissue culture of *Cymbidium giganteum* Wall. ex Lindl. *Sci Hortic*, 123(4):479-487. https://doi.org/10.1016/j.scienta.2009.10.009

Kauth PJ, Vendrame WA, Kane ME, 2006. In vitro seed culture and seedling development of *Calopogon tuberosus*. *Plant Cell Tissue Organ Cult*, 85(1):91-102. https://doi.org/10.1007/s11240-005-9055-1

Kauth PJ, Dutra D, Johnson TR, et al., 2008. Techniques and applications of in vitro orchid seed germination. *In*: Teixeira da Silva JA (Ed.), Floriculture, Ornamental and Plant Biotechnology: Advances and Topical Issues. Global Science Books, Ltd., Isleworth, UK, p.375-391.

Kerbauy GB, 1984. Plant regeneration of *Oncidium varicosum* (Orchidaceae) by means of root tip culture. *Plant Cell Rep*, 3(1):27-29. https://doi.org/10.1007/BF00270224

Kunakhonnuruk B, Inthima P, Kongbangkerd A, 2018. In vitro propagation of *Epipactis flava* Seidenf., an endangered rheophytic orchid: a first study on factors affecting asymbiotic seed germination, seedling development and greenhouse acclimatization. *Plant Cell Tissue Organ Cult*, 135(3):419-432. https://doi.org/10.1007/s11240-018-1475-9

Lawler LJ, 1984. Ethnobotany of the Orchidaceae. *In*: Arditti J (Ed.), Orchid Biology: Reviews and Perspectives. Cornell University Press, Ithaca.

Mahendran G, Narmatha Bai V, 2009. Mass propagation of *Satyrium nepalense* D. Don.—a medicinal orchid via seed culture. *Sci Hortic*, 119(2):203-207. https://doi.org/10.1016/j.scienta.2008.07.029

McKendrick S, 2000. In Vitro Germination of Orchids: A Manual. Ceiba Foundation for Tropical Conservation, p.3-17.

Minea M, Piluek C, Menakanit A, et al., 2004. A study on seed germination and seedling development of *Spathoglottis* Bl. orchids. *Kasetsart J Nat Sci*, 38(2):141-156.

Mitchell RB, 1989. Growing hardy orchids from seeds at Kew. *Plantsman*, 11(3):152-169.

Mitsukuri K, Johkan M, Yamasaki S, et al., 2009a. Effects of anti-browning conditioning on adventitious bud formation in *Neofinetia falcata* Hu. in vitro. *Hortic Environ Biotechnol*, 50(4):295-299.

Mitsukuri K, Mori G, Johkan M, et al., 2009b. Effects of explant source and dark-preconditioning on adventitious bud formation in *Neofinetia falcata* H. H. Hu in vitro. *J Jpn Soc Hortic Sci*, 78(2):252-256. https://doi.org/10.2503/jjshs1.78.252

Mitsukuri K, Johkan M, Yamasaki S, et al., 2010. L-2-aminooxy-3-phenylpropionic acid (AOPP) controls browning and promotes adventitious bud formation in *Neofinetia falcata* in vitro. *J Jpn Soc Hortic Sci*, 79(4):367-371. https://doi.org/10.2503/jjshs1.79.367

Murashige T, Skoog F, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiol Plant*, 15(3):473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Nongdam P, Chongtham N, Tewari R, 2006. In vitro multiplication of *Cymbidium pendulum* orchids via embryo culture. *Plant Cell Biotechnol Mol Biol*, 7(3):145-150.

Ohwi J, 1965. Flora of Japan. Smithsonian Institution, Washington, USA.

Palama TL, Menard P, Fock I, et al., 2010. Shoot differentiation from protocorm callus cultures of *Vanilla planifolia* (Orchidaceae): proteomic and metabolic responses at early stage. *BMC Plant Biol*, 10:82. https://doi.org/10.1186/1471-2229-10-82

Rao AN, 1997. Tissue culture in the orchid industry. *In*: Reinert J, Bajaj YPS (Eds.), Applied and Fundamental Aspects of Plant Cell Tissue and Organ Culture. Springer, New York, USA, p.44-49.

Sheelavantmath SS, Murthy HN, Pyati AN, et al., 2000. In vitro propagation of the endangered orchid, *Geodorum densiflorum* (Lam.) Schltr. through rhizome section culture. *Plant Cell Tissue Organ Cult*, 60(2):151-154. https://doi.org/10.1023/A:1006426905052

Shimasaki K, 1993. Induction of Protocorm-like Body (PLB) and subsequent plantlet formation from floret culture of *Neofinetia falcata* Hu. *Plant Tissue Cult Lett*, 10(1): 75-77. https://doi.org/10.5511/plantbiotechnology1984.10.75

Stewart SL, Kane MK, 2006. Asymbiotic seed germination and in vitro seedling development of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. *Plant Cell Tissue Organ Cult*, 86(2):147-158. https://doi.org/10.1007/s11240-006-9098-y

Teixeira da Silva JA, Cardoso JC, Dobránszki J, et al., 2015. *Dendrobium* micropropagation: a review. *Plant Cell Rep*, 34(5):671-704. https://doi.org/10.1007/s00299-015-1754-4

Thornhill A, Koopowitz H, 1992. Viability of *Disa uniflora* Berg (Orchidaceae) seeds under variable storage conditions: is orchid gene-banking possible? *Biol Conserv*, 62(1):21-27. https://doi.org/10.1016/0006-3207(92)91148-L

Withner CL, 1959. The Orchid: A Scientific Survey. Ronald Press Company, New York, USA, p.648.

Wotavová-Novotná K, Vejsadová H, Kindlmann P, 2007. Effects of sugars and growth regulators on in vitro growth

of *Dactylorhiza* species. *Biol Plant*, 51(1):198-200. https://doi.org/10.1007/s10535-007-0040-x

Yasugi S, Shinto H, 1994. Formation of multiple shoots and regenerated plantlets by culture of pseudobulb segment in nobile type *Dendrobium*. *Plant Tissue Cult Lett*, 11(2): 153-156. https://doi.org/10.5511/plantbiotechnology1984.11.153

Zettler LW, McInnis TM, 1993. Symbiotic seed germination and development of *Spiranthes cernua* and *Goodyera pubescens* (Orchidaceae: Spiranthoideae). *Lindleyana*, 8(3): 155-162.

中文概要

题　目：基于种子萌发的高效风兰（*Vanda falcata*）繁殖体系

目　的：鉴于风兰（*Vanda falcata* (Thunb.) Beer）传统繁殖能力弱，本文拟利用植物组织培养技术建立一种高效的繁殖体系，以满足市场对风兰数量增多的需要。

创新点：建立了适用于风兰的高效组培繁殖体系，包括离体种子萌发、原球茎的形成，以及芽和根的生成。

方　法：本研究首先以 5 年生母株自花授粉 4~5 个月后生成的种子为试材，依次经 70%乙醇和 10%次氯酸钠进行表面消毒后，分别接种于含不同成分的 13 种培养基中，统计分析种子萌发情况。进而将形成原球茎的试材，接种于含不同植物生长调节剂组合的培养基，统计分析芽和根的生长情况。

结　论：评估的 13 种不同配方的培养基中，1/2 MS+维生素+肌醇+20 g/L 蔗糖+3 g/L 活性炭+100 g/L 椰肉组合可达到 100%种子萌发率并形成较大原球茎；0.2 mg/L α-萘乙酸+0.5 mg/L 6-苄基腺嘌呤组合具有最高的芽诱导效率；1 mg/L α-萘乙酸+0.5 mg/L 6-苄基腺嘌呤+40 g/L 蔗糖组合具有最高的根诱导效率。长势良好的植株经炼苗后可适应露天栽培。

关键词：风兰；种子萌发；原球茎；无菌播种；植株再生