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ASYMBIOTIC SEED GERMINATION AND SEEDLING DEVELOPMENT OF *Vanda dearei*

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ABSTRACT

The effects of basal media, complex additives, plant growth regulators and carbon sources on *in vitro* seed germination and seedling development of *Vanda dearei* are reported. Immature seeds from four months old capsule were used as plant materials. All cultures were grown under 24h light at 25±2ºC. Results showed that seeds cultured on Knudson C (KC) basal medium germinated after 25 days with 63.0±3.2% germination rate followed by half-strength Murashige & Skoog (½MS) (45.4±10.4%) and Vacin and Went (VW) (41.8±4.0%). Addition of 0.5% (w/v) yeast extract significantly enhanced (85.9±0.7%) seed germination and shortened germination time to 23 days. A NAA at 0.1mg/l had similar performance (80.2±20.5%), however, this treatment delayed seed germination and induced necrosis to protocorm development. Sucrose at 1% (w/v) also enhanced seed germination (98.3±2.3%), while glucose and fructose treatments showed moderate effects. For growth and development of protocorms, KC basal media recorded the highest percentage of protocorm with root (37.0±4.3%), mean number of leaf (4.50±1.00) and mean number of roots produced (2.0±0.6) with largest leaf area (3.7x2.3mm) and longest root length (11.7±8.4mm). Addition of 20% (v/v) coconut water significantly improved protocorm development and shoot growth.

Key words: Orchidaceae, basal media, complex additives, plant growth regulators, carbon source.

INTRODUCTION

Vanda is one of the best-known and attractive Asian orchid genera. *Vanda* can mostly be found in tropical lowlands and foothills particularly in Thailand and Philippines, whilst, six species have been recorded from Borneo (Wood *et al.,* 2011). *Vanda dearei* is one of the endemic orchid species in Borneo distributed around Kinabatangan and Tenom in Sabah; Kuching in Sarawak; Sekayan River in West Kalimantan and Kutai in East Kalimantan (Chan *et al.,* 1994). This species has a large beautiful collar and strongly scented flowers that are produced all over the year.

These characteristics make this species rank among the preferred and ecstatic orchid species and have been extensively used as a progenitor of many merited hybrids such as *Vanda* Clare Leong (*Vanda Charles Good fellow* x *Vanda dearei*) (Royal Horticultural Society, 2001), *Vanda* Tan Chay Yan (*Vanda* Josephine van Brero x *Vanda dearei*) (Chan *et al*., 1994) and *Aranda* Nancy (*Arachnis hookeriana var. luteola* x *Vanda dearei*) (Loh *et al.,* 1978). Conventionally, stem cutting and seed germinated in the presence of suitable mycorrhiza are used to propagate this species. However, propagation through these methods is extremely slow with low success rate (Rangsayatorn, 2009). Thus, asymbiotic seed germination has emerged as an important tool for propagating a large number of orchid species and hybrids (Arditti, 1967; Nishimura, 1982).

The breakthrough of *in vitro* seed germination ensures better germination frequency and favors the production of virus-free seedlings at a faster rate. However, the medium used for asymbiotic germination and seedling development is more complex than that for symbiotic germination, as all organic and inorganic nutrients and carbon sources must be in a form readily available (Temjensangba & Deb, 2005; Naha *et al.,* 2013; Prakash *et al.,* 2013). In *Vanda*, protocols for *in vitro* seed germination and seedling development have been extensively described (Roy & Banerjee, 2002; Kishor *et al.,* 2006; Johnson & Kane, 2007; Manners *et al.,* 2011; Roy *et al.,* 2011; Prakash *et al.,* 2013; Naha *et al.,* 2013). In addition, those requirements are too specific and depend on the orchid variety,

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species or genera. Therefore, the present study examined the influence of medium composition such as basal media, complex additives, plant growth regulators and carbon source sources on *in vitro* seed germination and seedling development of *V. dearei*.

MATERIALS AND METHODS

Seed sources

Four months-old of hand pollinated immature seed capsules of *V. dearei* (Fig. 1) were obtained from Sabah Agriculture Park, Lagud Sebrang, Tenom. The capsules were wiped with 70% (v/v) ethanol and packed in plastic wrap before storage in refrigerator at 4ºC for 24h. The capsules and seeds were characterized in term of size, weight and morphology. Measurement of seed size (length x width) was conducted using Image Analyzer (Dino Lite Digital Microscope, Taiwan).

Seed disinfection and preparation

The capsule of *V. dearei* was gently washed and cleaned under running tap water using soft brush and soap. Subsequently, the capsule was surface sterilized by soaking in 80% (v/v) ethanol for 2 seconds, rinsed 3 times with sterile distilled water and then immersed in 30% (v/v) Clorox® with addition of 2 drops of Tween 20 for 20 min. After that the capsule was thoroughly washed with sterile distilled water and aseptically transferred into sterile glass Petri dishes for culture preparation.

Seed germination

The seed pot was dissected and seeds were sprinkled on solidified media in sterile plastic Petri dishes. Three basal media were tested namely Knudson C (KC) (Knudson, 1946), half-strength MS nutrients (½MS) (Murashige & Skoog, 1962), and Vacin and Went (VW) (Vacin &Went, 1945). Subsequently, the KC medium was selected for further experiments. The medium was supplemented with various types and concentrations of complex additives i.e., coconut water (10-20%, v/v), tomato juice (10-20%, v/v), banana homogenate (2.5-12.5%, w/v), peptone (0.10-0.50%, w/v) and yeast extract (0.10-0.50%, w/v); plant growth regulators i.e., NAA or BAP at concentration of 0.1-1.0mg/l; and carbon sources i.e., sucrose, glucose and fructose at concentrations of 1-4% (w/v). In addition, 0.50% (w/v) yeast extract was added into carbon sources experiments and basal KC medium was used as negative control in the respective experiments. The medium was adjusted to pH 5.3 before solidification with 0.9% (w/v) agar and autoclaved at 121ºC (15p.s.i) for 20 min. All cultures were grown in 25±2ºC under continuous illumination (20-50 $\mu molm^{-2}s^{-1}$) provided by cool white fluorescent tubes (Philips, Malaysia). As the seed coat is transparent, changes during culture were directly observed under the stereoscopic microscope. Germination time and percentage of seed germination were observed every week over 150 days. Seeds were considered germinated when protocorms were formed.

Growth and development of protocorms

Three months old mature protocorms (Fig. 3(A) were cultured on KC, ½MS and VW media. The best basal medium was then supplemented with various types and concentrations of complex additives such as coconut water (10-20%, v/v), tomato juice (10-20%, v/v) and banana homogenate (2.5-12.5%, w/v). Growth development of protocorm to seedling was measured after 240 days of culture.

Acclimatization

The seedlings were first isolated from the culture media and rinsed under running tap water before treatment with 0.5% (w/v) Imas-Thiram 80 solution for 15 min (Kishor *et al.,* 2006). Seedlings were planted in pots containing moss and charcoal in ratio of 50:50. The space on the upper part of pot was covered by plastic with holes and put under temperature of 25ºC with dim light condition.

Experimental design and data analysis

Experiments were performed in a completely randomized design (CRD). Each treatment was conducted in 10 replicates. Data were analyzed by SPSS (Statistical Package for Social Science) Ver. 12 and the means difference were compared with Duncan Multiple Test at $p<0.05$.

RESULTS AND DISCUSSION

Seed characterization

Capsule and seed characterization of *V. dearei* are given in Fig. 1(A-D) and Table 1. The four-month old green capsule was approximately 36.74g and 15.50cm (length) x 3.00cm (width) with six lines/curves at the outside layer. Seeds were 0.128±0.003mm (length) x 0.048±0.003mm (width), yellowish color, transparent, tubular with oval embryo, and attached in fiber structure inside the capsule.

Seed germination

Seed germination stages are given in Fig. 1(D-G). The seed germination was began from pre-germination stage (embryo swells to the width of the seed coat) (Fig. 1(E)). At this stage the embryo started to absorb chlorophyll and turn green. Subsequently, the embryos enlarged and emerged

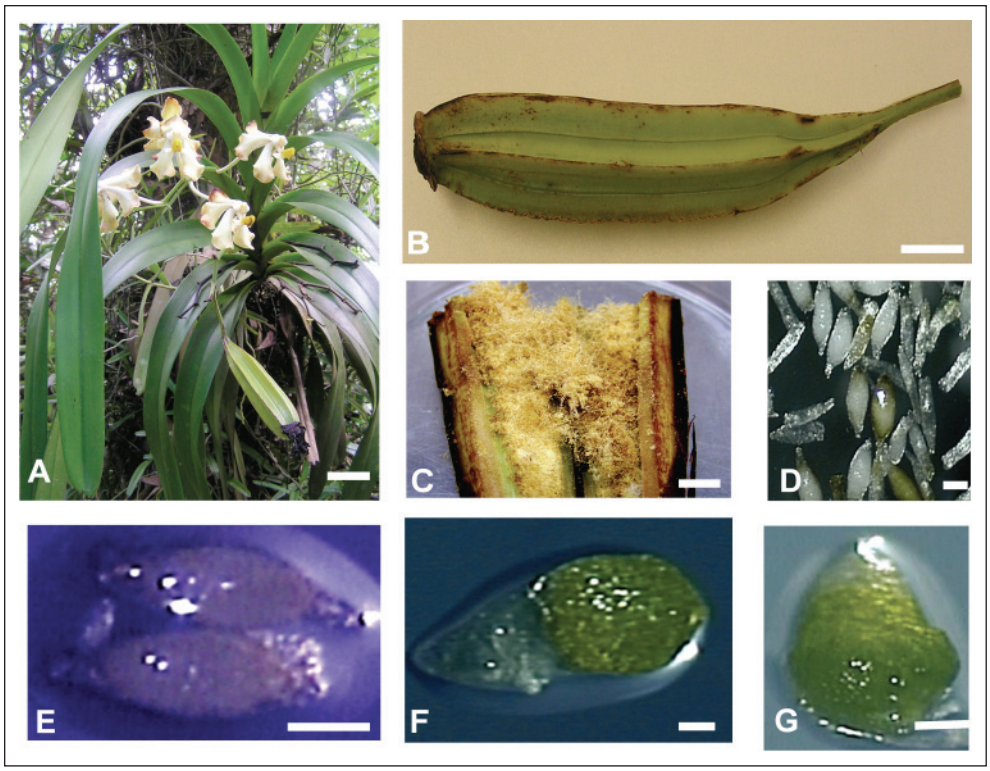

Fig. 1. *Vanda dearei* plant and seed germination. (A) Plant; (B) Capsule; (C) Seeds (inside the capsule); (D) Fresh seeds; (E) Swollen seeds after 7 days after culture; (F) Embryo emergence from seed coat after 20 days of culture; (E) Protocorm formed after 30 days of culture (Scale bars, A=8cm; B=2cm; C=1cm; D-E=100µm; F-G=0.5mm).

Table 1. Characterization of capsule and seeds of *V. dearei*

Parameter	Explants	
	Capsule	Seeds
Colour	Green	Creamy yellow
Weight	36.7g	–
Length	15.5cm	0.128 mm
Width	3.0cm	0.048 mm
Others	The four month old after hand pollination shows a 6 line/curve at the outside.	The seed hastubular in shape with an oval embryo.

from the seed coat (Fig. 1(F)) and later developed to mature protocorm (Fig. 1(G)). Seed germination to form a protocorm is considered to be a peculiarity of postseminal development in orchids and its shape is taxon specific (Arditii, 1982). Common form of protocorm are round, oval, elongated, branched, spherical, disk-, thorn-, or spindle-shaped (Batygina *et al*., 2003). In the early development of a protocorm, a growth appendix of meristematic tissues appeared at the upper part of the spherule, which looked like a closed ridge (Fig. 1(F-G)). This meristematic zone indicated the location of development of future foliar organs.

Effect of basal media on seed germination

Effects of different basal media on seed germination are shown in Fig. 2(A-B) and Table 2. Seed germination in KC medium was significantly higher (63.0±3.2%) compared to VW (41.8±4.0%) and ½MS medium (45.4±10.4%) observed after 150 days of culture. This result was in line with optimum basal media for seed germination of *V. tessellata* (Roy & Banerjee, 2002). However, Prakash *et al.* (2013) reported on the same species where MS medium showed higher germination percentage compared to KC medium (65%). KC medium was first discovered to assist better early development of orchids seeds (Curtis & Spoerl, 1948; Raghavan & Torrey, 1964) and frequently used for tropical epiphyte orchids (Arditti, 1982; Hew *et al.,* 2002). KC medium has a simple formulation with a few macro and micro salts and without vitamins. Hence, become a priority target for nutrient modification in orchid propagation.

Effect of complex additives on seed germination

Effect of supplemented complex additives on seed germination are shown in Fig. 2(C-D) and Table 3. Seeds were able to germinate with and without complex additives. However, 0.50% (w/v) yeast extract recorded the fastest seed to germinate

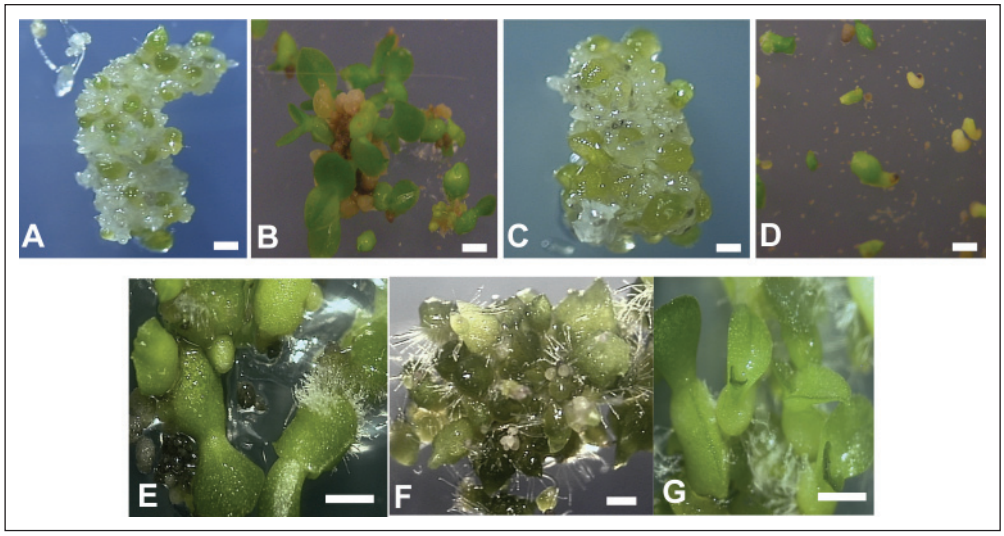

Fig. 2. Germination of *Vanda dearei* seed on various tested media. (A) KC basal medium after 30 days of culture; (B) KC basal medium after 150 days of culture; (C) 0.5% (w/v) yeast extract after 30 days of culture; (D) 0.5% (w/v) yeast extract after 150 days of culture; (E) 0.1mg/l NAA after 150 days of culture; (F) 1% (w/v) sucrose after 30 days of culture; (G) 1% (w/v) sucrose after 150 days of culture (Scale bar=1mm).

Table 2. Effect of basal media on germination of *V. dearei* seeds

Basal media	Time response (Days)	Seeds germination (%)		
		Time (Days)		
		30	90	150
KC	25	35.9±9.7^{a}	51.6±3.2^{a}	63.0±3.2^{a}
VW	28	30.6±7.6^{a}	38.8±1.5^{b}	41.8±4.0^{b}
½MS	29	8.8±2.3^{b}	44.7±10.4^{b}	45.4±10.4^{b}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at $p<0.05$. KC-Knudson, 1946; VW-Vacin & Went, 1949; MS-Murashige & Skoog, 1962.

Table 3. Effect of complex additives on seed germination of *V. dearei*

Complex additives		Time response (Days)	Seeds germination (%)		
			Time (Days)		
			30	90	150
Control (KC medium)		25	35.8±3.2^{ab}	51.3±12.7^{a}	63.7±3.3^{bc}
Peptone (%, w/v)	0.15	23	30.4±7.7^{bcde}	42.4±10.1^{ab}	54.9±7.1^{cdef}
	0.20	23	34.1±5.6^{bc}	42.8±17.1^{ab}	59.4±5.5^{cde}
	0.25	23	32.9±3.6^{bcd}	52.9±20.5^{a}	63.1±15.9^{bcd}
	0.50	23	37.1±6.5^{ab}	51.4±14.9^{a}	65.1±2.5^{bc}
Yeast extract (%, w/v)	0.15	23	25.9±3.1^{def}	27.6±4.3^{bcd}	35.8±8.9^{ghi}
	0.20	23	25.1±2.4^{def}	36.4±7.5^{abc}	44.1±2.3^{fgh}
	0.25	23	42.7±5.5^{a}	43.7±6.7^{ab}	51.1±5.4^{def}
	0.50	23	43.3±1.7^{a}	52.3±8.5^{a}	85.9±0.7^{a}
Coconut water (%, v/v)	10	30	21.1±2.2^{fgh}	52.6±14.5^{a}	71.8±12.2^{b}
	15	30	23.5±2.5^{efg}	37.2±9.4^{abc}	47.6±7.8^{efg}
	20	31	28.1±9.2^{bcdef}	42.1±7.1^{ab}	57.0±6.1^{cde}
Tomato juice (%, v/v)	10	30	14.0±2.0^{hi}	22.8±3.4^{cd}	37.7±7.2^{ghi}
	15	33	12.9±0.6^{i}	23.2±9.5^{cd}	32.3±3.3^{hij}
	20	33	14.6±2.2^{hi}	17.3±2.6^{d}	26.1±2.2^{ij}
Banana homogenate(%, w/v)	2.5	33	27.2±3.8^{bcdef}	49.2±10.4^{a}	58.5±4.2^{cde}
	7.5	35	27.3±4.7^{ghi}	19.6±6.8^{cd}	26.7±3.1^{ij}
	12.5	35	12.4±0.1^{i}	14.1±1.2^{d}	20.9±2.6^{j}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at $p<0.05$.

and with higher germination percentage (85.9± 0.7%). High nitrogen content in complex additives was reported to stimulate initial stages of seedling growth and differentiation (Paul *et al*., 2012) in many orchids species including *V. tessellata* (Roy & Banerjee, 2002), *Dendrobium parishii* (Buyun *et al*., 2004), and *V. teres* (Sinha & Roy, 2004). Yeast extract contains about 9.8% total nitrogen comprising primarily 5.1% amino nitrogen as amino acids (Arditti & Ernst, 1993). Supplementation of organic extracts to the orchid culture medium is simple and practical method to improve culture media used for commercial production.

Effect of plant growth regulators on seed germination

The seeds of *V. dearei* showed approximately between 47 to 81% germination on both hormone free KC and KC supplemented with different concentration of NAA and BAP (Fig. 2(E) and Table 4). KC supplemented with 0.1 mg/l NAA favoured was better germination (80.2±20.5%) in comparison to other concentration but with no significant difference with 0.5mg/L BAP (70.8±7.4%). In this culture condition, germination was delayed 3 to 9 days as compared to 25 days in hormone free medium. This result was in accord with the findings that seed germination was greatly influenced by external plant growth regulators (Mathews & Rao, 1980; Kishor *et al.,* 2006). Similarly, Nongdam & Chongtham (2012) and Naha *et al.* (2013) have reported higher germination percentage of *Cymbidium dayanum* and *Vanda testacea* seeds, respectively, in the presence of NAA. The production of auxin-like symbiotic fungus such as *Mycorrhizal hizopogon roseolus* (Hadley & Harvais, 1968) facilitate seed germination. This fact can explain the roles of exogenous plant growth regulators in asymbiotic seed germination (De Pauw *et al.,* 1995; Lo *et al.,* 2004). Although plant growth regulators significantly increased germination percentage, this study also indicated that 65% of germinated seeds (protocorms) tend to necrosis (data not shown) indicating that PGRs may not be essential for growth and development for *V. dearei* protocorm culture.

Effect of carbon sources on seed germination

The response of different sources and concentrations of carbon are shown in Fig. 2 (F-G) and Table 5. Results indicated that lower concentration of sugar significantly improved germination of *V. dearei* seeds. Treatment with 1% (w/v) sucrose promoted up to 98.3±2.3% germination followed by 95.3±2.0% and 74.1±4.7% on 1% (w/v) glucose and 1% (w/v) fructose, respectively. Increased concentration of carbon significantly decreased the germination percentages and also promoted necrosis. This finding in contrast to other orchid species such as *V. tessellata* (Roy & Banerjee, 2002), *Ascocenda* Kangla (Kishor *et al.,*

Table 4. Effect of NAA and BAP on germination of *V. dearei* seeds after 150 days of culture

Treatment (mg/l)		Time response (Days)	Seed germination (%)
Control (KC medium)		25	63.4±10.4^{ab}
NAA	0.1	28	80.2±20.5^{a}
	0.5	31	47.5±6.8^{c}
	1.0	33	48.4±4.2^{c}
BAP	0.1	32	66.2±9.6^{ab}
	0.5	34	70.8±7.4^{a}
	1.0	34	51.7±3.6^{bc}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at $p<0.05$.

Table 5. Effect of type of sugar on seed germination of *V. dearei*

Carbon source	Concentration (%, w/v)	Time response (Days)	Seeds germination (%)		
			Time (Day)		
			30	90	150
Control (KC added with 0.5% (w/v) yeast extract)		23	43.1±20.0^{cde}	52.4±5.6^{de}	86.4±10.6^{ab}
Sucrose	1	23	79.1±7.7^{a}	90.7±2.5^{ab}	98.3±2.3^{a}
	2	23	56.0±8.3^{bc}	66.2±9.0^{c}	68.4±10.3^{bc}
	4	25	29.9±11.6^{de}	50.5±10.9^{e}	52.1±2.3^{de}
Glucose	1	25	63.5±3.9^{b}	87.7±2.7^{b}	95.3±2.0^{a}
	2	26	44.0±7.3^{cd}	63.3±6.5^{cd}	64.4±5.5^{c}
	4	28	27.2±6.2^{e}	40.7±1.1^{e}	46.1±1.2^{e}
Fructose	1	25	42.4±8.2^{cde}	70.8±4.9^{c}	74.1±4.7^{b}
	2	26	39.7±3.9^{de}	55.1±2.3^{de}	56.2±2.3^{d}
	4	29	9.3±11.0^{f}	20.3±1.7^{f}	22.0±2.6^{f}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at $p<0.05$.

2006) and *Calopogon tuberosus* (Kauth *et al.,* 2006); which required 2% (w/v) sucrose to enhance seed germination. Osmotic tolerance is the main reason on the differences and suggests that exogenous sugar for seed germination is specific to variety, species or genera (Arditti, 1967). It is also known that soluble carbohydrates serve as signal imbibed seeds for fungal infection in orchids, but this only happened for symbiotic germination. In asymbiotic germination, universal source of energy and carbon in living cells such as sucrose and its monomer glucose and fructose; has been shown to slow or stop seed germination of wild-type *Arabidopsis* and seeds of other species by slowing the breakdown of ABA (Zhao *et al*., 2009; Zhu *et al*., 2009; Johnson *et al*., 2011. These may become a preference for higher doses of sucrose, glucose or fructose to decrease germination or development but not at lower concentrations.

Growth and development of protocorms

Effect of basal media and complex additives on growth and development of protocorms are shown in Table 6, Table 7 and Fig. 3(A-G). As the protocorm continued to grow, an opening developed at the appendix and foliar organs emerged (Fig. 3(1-E)). These organs in the majority of orchids appear during postseminal development or also known as protocorm stage and it is inappropriate to refer them as cotyledons or leaf-like organs (Batygina *et al*., 2003). This means that a protocorm will produce proper shoot leaves from the meristematic tissue although the embryo itself has no cotyledons. In this study, although KC media contained fairly low amount of both macro and micro nutrients and lack of vitamins (Hossain *et al.,* 2009), results indicated that growth and protocorm development was superior compared to VW and ½MS basal media. This suggests that growth and development of *V. dearei* protocorms preferred lower concentration and simple nutrients complexity. KC medium promoted highest percentage of protocorm with root (37.0±4.3%), mean number of leaf (4.5±1.0) and mean number of roots produced (2.0±0.6) with largest leaf area (3.7x2.3mm) and longest root length (11.7±8.4mm). This finding was in agreement with Geetha & Shetty (2000) for *Vanilla.*

It was also observed that 20% (v/v) coconut water promoted higher percentage of protocorm with leaf (90.5±5.9%) or rooting (89.1±3.8%) and mean number of leaves (6.0±0.5); with largest leaf area

Table 6. Development of *V. dearei* protocorm on various media after 240 days of culture

Basal Media	Percentage of protocorm with leaf	Mean number of leaf per explant	Percentage of protocorm with root	Mean number of root per explant	Leaf		Root
					Length (mm)	Width (mm)	Length (mm)
KC	46.2±14.9^{b}	4.5±1.0^{a}	37.0±4.3^{a}	2.0±0.6^{a}	3.7±1.3^{a}	2.3±1.2^{a}	11.7±8.4^{a}
VW	20.2±10.2^{c}	2.3±1.0^{b}	12.5±6.9^{b}	1.3±0.5^{a}	3.6±1.5^{a}	2.2±1.0^{a}	0.8±0.3^{b}
½MS	73.3±26.6^{a}	3.2±1.4^{ab}	35.0±25.7^{a}	1.5±0.5^{a}	2.8±1.1^{a}	2.0±0.8^{a}	1.9±0.9^{b}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at p<0.05. KC-Knudson, 1946; VW-Vacin & Went, 1949; MS-Murashige & Skoog, 1962

Table 7. Effect of complex additives on protocorm development of *V. dearei* after 240 days of culture

Treatment		Percentage of protocorm with leaf	Mean number of leaf per explant	Percentage of protocorm with root	Mean number of roots per explant	Leaf		Root
						Length (mm)	Width (mm)	Length (mm)
Control		46.2±14.9^{e}	4.5±1.0^{bc}	37.0±4.3^{e}	2.0±0.6^{bcd}	3.7±1.3^{bcd}	2.3±1.2^{bcd}	11.7±8.4^{ab}
CW	10	56.6±24.6^{cd}	4.2±1.6^{bc}	51.6±19.9^{cd}	2.7±0.4^{ab}	4.4±1.4^{abc}	3.0±1.4^{abcd}	8.5±3.5^{bcd}
	15	88.0±5.5^{ab}	5.3±0.7^{ab}	84.0±7.6^{a}	3.1±0.7^{a}	4.6±0.2^{ab}	3.3±0.2^{abc}	13.4±2.2^{ab}
	20	90.5±5.9^{a}	6.0±0.5^{a}	89.1±3.8^{a}	2.9±0.3^{a}	5.5±0.8^{a}	3.6±0.4^{a}	16.1±2.0^{a}
TJ	10	68.6±20.3^{bc}	4.3±1.2^{bc}	52.5±9.5^{cd}	1.9±0.3^{bcde}	3.7±1.1^{bcd}	3.1±0.7^{abcd}	9.2±1.6^{bc}
	15	88.3±11.3^{ab}	4.7±1.2^{abc}	86.6±9.4^{a}	2.4±1.2^{abc}	4.6±0.3^{ab}	3.3±0.4^{ab}	9.1±4.1^{bc}
	20	57.6±8.3^{cd}	4.1±0.2^{bc}	50.8±6.3^{cde}	1.8±0.2^{bcde}	3.8±0.8^{bcd}	3.1±0.1^{abcd}	8.3±4.4^{bcd}
BH	2.5	84.0±11.1^{ab}	3.4±0.6^{cd}	68.0±8.6^{b}	1.4±0.3^{de}	3.0±0.8^{cd}	2.1±0.4^{bcd}	3.2±1.1^{de}
	7.5	70.6±7.6^{abc}	2.4±1.0^{d}	48.0±8.6^{de}	1.0±0.7^{e}	2.6±1.6^{d}	2.0±1.3^{d}	1.6±1.0^{e}
	12.5	69.3±16.0^{bc}	3.3±0.6^{cd}	64.0±11.1^{bc}	1.8±0.2^{cde}	4.2±0.6^{abc}	2.1±0.4^{cd}	4.6±1.0^{cde}

Note: Data obtained from ten replicates. Data followed by the same letters are not significantly different at p<0.05. CW – Coconut water (%, v/v); TJ – Tomato juice (%, v/v); BH – Banana homogenate (%, w/v).

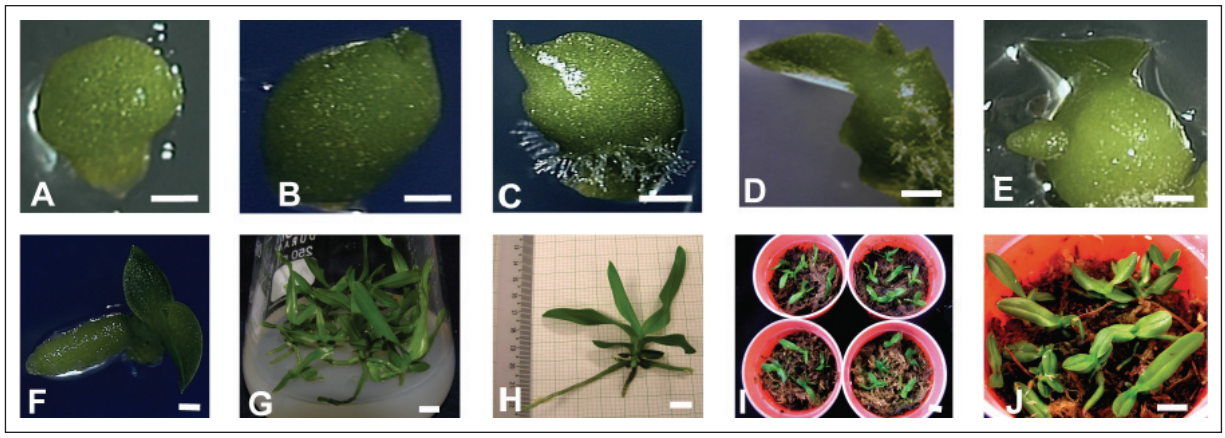

Fig. 3. Developmental stages of *V. dearei* from protocorms to seedling. (A) Three months old protocorm; (B) Curve formation from the upper site of the protocorms (white circle) after 2 week; (C) Shoot initiation and papille (white hair) formed after 3 week; (D-F) Second leaf formation and rooting after 2 months; (G) Seedlings development after 8 months; (H) Seedling used for acclimatization; (I) Seedling planted on pot containing moss and charcoal with ratio of 50:50; (J) Seedling after 180 days of transfer (Scale bars, A-E=0.5mm; G=1.0cm; H-I=2.5cm).

(5.5x3.6mm) and longest root length (16.1±2.0mm). Other complex additives such as tomato juice and banana homogenate also significantly enhanced protocorm growth and development especially at lower concentration (10-15%). This was supported by other studies where coconut water was effective for protocorm development of *Paphiopedilum wardii* (Zeng *et al.,* 2012) and *V. teres* (Sinha & Roy, 2004). Coconut water is usually contains carbohydrates, vitamins, amino and organic acids, organic ions and enzymes which are important for plant cell development (Ngampanya and Homla-aor, 2010).

Acclimatization

Preliminary study indicated that protocorm takes at least 8 to 10 months to achieve a suitable size prior to acclimatization (data not shown). At this stage, the seedlings are approximately 6.5 cm height, contain 10 leaves (length of 8.0mm and width of 5.0mm) and 5 roots (56.0mm length) (Fig. 3(G-H). Seedlings were well adapted to the nursery environment and continued to grow and developed into healthy seedlings (Fig. 3(I-J).

CONCLUSIONS

The reliable protocols for germination of seed and growth development of *V. dearei*, an endemic orchid to Borneo was successfully established. The seeds easily germinated in KC medium added with 0.50% (w/v) yeast extract and 1% (w/v) sucrose. Replacement of yeast extract with 20% (v/v) coconut water enhanced the growth and development of protocorms.

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