Plant Cell Rep (2015) 34:1685–1706
DOI 10.1007/s00299-015-1829-2

REVIEW

Asymbiotic in vitro seed propagation of *Dendrobium*

Jaime A. Teixeira da Silva[1] · Elena A. Tsavkelova[2] · Tzi Bun Ng[3,4] · S. Parthibhan[5] · Judit Dobránszki[6] · Jean Carlos Cardoso[7] · M. V. Rao[5] · Songjun Zeng[8]

Received: 12 April 2015 / Revised: 12 June 2015 / Accepted: 19 June 2015 / Published online: 17 July 2015

Abstract The ability to germinate orchids from seeds in vitro presents a useful and viable method for the propagation of valuable germplasm, maintaining the genetic heterogeneity inherent in seeds. Given the ornamental and medicinal importance of many species within the genus *Dendrobium*, this review explores in vitro techniques for their asymbiotic seed germination. The influence of abiotic factors (such as temperature and light), methods of sterilization, composition of basal media, and supplementation with organic additives and plant growth regulators are discussed in context to achieve successful seed germination, protocorm formation, and further seedling growth and development. This review provides both a basis for the selection of optimal conditions, and a platform for the discovery of better ones, that would allow the development of new protocols and the exploration of new hypotheses for germination and conservation of *Dendrobium* seeds and seedlings.

Keywords Asymbiotic seed germination · *Dendrobium* propagation · Explant age · Lighting conditions · Organic and inorganic media components · Orchidaceae

Abbreviations

2-iP	2-Isopentenyladenine
AC	Activated charcoal
AH	Apple homogenate
BA	N^6-Benzyladenine

Communicated by N. Stewart.

✉ Jaime A. Teixeira da Silva
jaimetex@yahoo.com

✉ Elena A. Tsavkelova
tsavkelova@mail.ru

✉ Tzi Bun Ng
b021770@mailserv.cuhk.edu.hk

✉ S. Parthibhan
thibhan@gmail.com

✉ Judit Dobránszki
dobranszki@freemail.hu

✉ Jean Carlos Cardoso
jeancardosoctv@gmail.com

✉ M. V. Rao
mvrao_456@yahoo.co.in

✉ Songjun Zeng
zengsongjun@scib.ac.cn

[1] P. O. Box 7, Miki-cho Post Office, 3011-2, Ikenobe, Kagawa 761-0799, Japan

[2] Department of Microbiology, Faculty of Biology, Lomonosov Moscow State University, Leninskie gory 1-12, 119234 Moscow, Russia

[3] School of Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

[4] Shenzhen Research Institute, The Chinese University of Hong Kong, Shenzhen, China

[5] Department of Plant Science, Bharathidasan University, Tiruchirappalli 620 024, Tamil Nadu, India

[6] Research Institute of Nyíregyháza, University of Debrecen, P.O. Box 12, 4400 Nyíregyháza, Hungary

[7] Department of Rural Development, Centro de Ciências Agrárias, UFSCar, Via Anhanguera, km 174, CP 153, CEP 13.600-970 Araras City, Brazil

[8] Key Laboratory of South China Agricultural Plant Molecular Analysis and Gene Improvement, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

BP	Banana pulp/paste
CW	Coconut water
GA_3	Gibberellic acid
NAA	α-Naphthaleneacetic acid
IAA	Indole-3-acetic acid
IBA	Indole-3-butyric acid
Kin	Kinetin
OMF	Orchid mycorrhizal fungi
PGR	Plant growth regulator
PPFD	Photosynthetic photon flux density
TDZ	Thidiazuron (*N*-phenyl-*N*′-1,2,3-thiadiazol-5-ylurea)

The importance of medicinal and ornamental *Dendrobium*

The genus *Dendrobium* s.l. (Epidendroideae) has in excess of 1100 species of epiphytic orchids with a wide distribution from Central Asia throughout Australasia (Kamemoto et al. 1999; Kumar et al. 2011). This genus is one of the largest among the Orchidaceae, the largest family of angiosperms (Dressler 2005; Fay and Chase 2009). Species within the *Dendrobium* genus are highly prized ornamental assets, primarily as potted plants with showy flowers (Fig. 1a) that tend to have a long vase life (Vendrame et al. 2008). But the most important aspect of many orchid species, including *Dendrobium* species, is their medicinal and pharmaceutical value, particularly *Dendrobium nobile* Lindl., which is abundantly used in traditional Chinese medicine (Bulpitt et al. 2007; Singh and Duggal 2009; Ng et al. 2012).

To counter exploitation from wild resources, and to bolster production of clonal material, biotechnology—specifically micropropagation (Teixeira da Silva et al. 2015a), in vitro flowering (Teixeira da Silva et al. 2014a), cryopreservation and low-temperature preservation (Teixeira da Silva et al. 2014b)—serves as an important tool for propagation and preservation purposes (Roberts and Dixon 2008; Swarts and Dixon 2009). They also allow for the establishment of sterile in vitro cultures for the study of the genetics of flowering in orchids (Teixeira da Silva et al. 2014c). Another strategy is to use seeds aseptically germinated in vitro, under optimized conditions, modeling natural ones. The ease and high germination percentage of seeds of *Dendrobium* species (Table 1) under asymbiotic conditions relative to the more precise requirements of symbiotic germination, which needs to balance germination and pathogenesis (Teixeira da Silva et al. 2015b), makes this technique more practical and useful for mass in vitro propagation and/or germplasm conservation (Teixeira da Silva et al. 2014b).

Although many commercial *Dendrobium* hybrids are propagated using clonal procedures, asymbiotic seed propagation in *Dendrobium* has major importance for the conservation and propagation of wild species because of loss of habitats and overexploitation due to agriculture, urbanization, overcollection and medicinal uses. *Dendrobium* orchids are commonly used in traditional Chinese medicine and many wild populations, for example, of *D. catenatum* Lindl., have become drastically reduced due to overexploitation (Liu et al. 2011; Luo et al. 2013). The propagation of rare and wild species using asymbiotic germination for economic ornamental, as well as for conservation/restoration purposes, is another important market

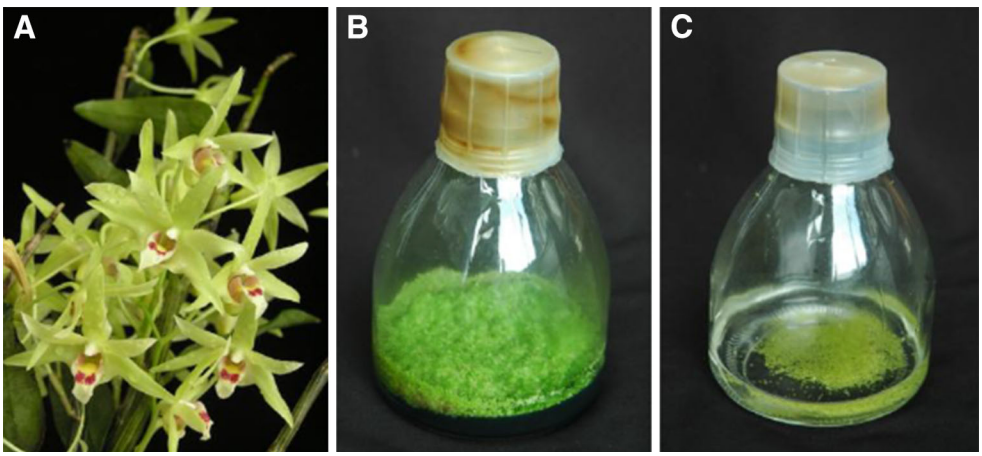

Fig. 1 In vitro development of *Dendrobium officinale* Kimura and Migo. **a** Flowering plant; **b** asymbiotic seed germination on N_6 solid medium containing 10 % coconut water (CW), 0.2 mg/l α-naphthaleneacetic acid (NAA), 1 g/l activated charcoal (AC), 0.65 g/l agar powder; **c** asymbiotic seed germination on N_6 liquid medium containing 0.2 mg/l NAA, 10 % CW. Unpublished photos (Songjun Zeng)

Table 1 Asymbiotic seed germination in vitro of *Dendrobium* species (listed chronologically, then alphabetically within each year)

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. tosaense	Mature or not fully mature seeds in undamaged capsules	70 % EtOH 30 s	Modified KC medium	White fluorescent tubes, 25 °C	The seed germination rate was 92 % on modified KC medium. KC medium supplemented with 2 % AC was favorable for rooting	Hu and He (1979)
D. tosaense	Mature or immature seeds	NR	Modified KC medium; MS	9–10-h PP, 2000 lux, 25 ± 5 °C	The mature seed germination rate was 95 % on modified KC medium, which was higher than that of immature seeds. Seed germinated better at 26–30 °C than at 24–26 °C; 24–26 °C was better than 18–21 °C	Xu and Yu (1984)
D. candidum (*D. officinale*)	Seeds (2–6 months old)	NR (reported in common procedure)	N_6 medium	25–28 °C, no other information	Seed germination of *D. candidum* was 6 % from 2-month-old seeds, 87 % from 3-month-old seeds, 95 % from 4-, 5-, or 6-month-old seeds	Ye et al. (1988)
D. huoshanense	Immature seeds	Capsules sterilized by common procedure	MS or KC media with 1.0 mg/l BA + 0.5 mg/l NAA	25–27 °C	Dark for 22 days then transfer to low light intensity (1700 lux) resulted in highest germination (91 %). High light intensity (27,000 lux) for 3 days then transfer to low light intensity resulted in lowest germination (10 %)	Yang and Wang (1989)
D. hancockii	Symbiotic germination of mature seeds	Sterilized tree leaves as a support of symbiotic germination	Fungal liquid extract	8-h PP; 25 °C	Seeds did not germinate without fungi. Seed germination exceeded 20 % when incubated with fungi. Seed germination varied from 40 to 70 % with liquid extract of the culture products of fungi	Guo and Xu (1990)
D. transparens	Undehisced mature capsule	1 % Teepol → 70 % EtOH 30 s	B_5 medium + 1 mg/l Kin +1 mg/l NAA	16-h PP, 3000 lux, 25 ± 1 °C	90 % of seeds germinated in 25 days, in which 53 protocorms formed leafy shoots and root	Hazarika and Sarma (1995)
D. candidum (*D. officinale*), *D. wangii*, *D. densiflorum*, *D. fimbriatum*, *D. loddigesii*, *D wilsonii*	Seeds of different ages	75 % EtOH 30 s → 0.1 % $HgCl_2$ 8 min → 5X SDW	MS, modified KC, VW, modified N_6 medium or PT medium [1.2 g/l Hyponex (N:P:K = 7:6:9) + 3 g/l peptone) + 20 g/l sucrose + 0.2 mg/l NAA + various additives (banana, tomato, green-bean seedling and PJ and CW)]	10–12-h PP, 1600-2000 lux, 25 ± 2 °C	Optimal medium for seed culture was modified N_6 or PT supplemented with 0.2 mg/l NAA. Optimal sucrose conc. was 2 %. Coconut juice was best for embryo sprouting. After sprouting, increasing PJ, green-bean seedling juice or banana juice improved growth of plantlets. Banana juice was best for rooting. 80 % survival for *D. candidum*; 85 % for *D. wangii*, *D. densiflorum*, and *D. fimbriatum*; 90 % for *D. loddigesii*	Zeng and Cheng (1996) and Zeng et al. (1998)
D. unicum	180-DAP seeds	70 % EtOH 30 s → 1.0 % NaOCl 15 min 2X → 3X SDW	VW or Hyponex medium (Hyponex I, N:P:K = 7:6:9; Hyponex II, N:P:K = 20:20:20) with CW, NAA, BA, AC or peptone	12-h PP, 1500–2000 lux, 25–28 °C	3 g/l Hyponex I medium + 10 % CW was suitable for seed germination and PLB proliferation, Hyponex medium (1 g/l Hyponex I and 1 g/l Hyponex II) with 2 g/l AC, 2 g/l peptone, 0.5 mg/l NAA, 0.2 mg/l BA was suitable for rooting	Chen et al. (2004)
D. devonianum	Seeds of different ages	75 % EtOH 30 s → 1.0 % NaOCl 10 min → 0.1 % $HgCl_2$ 8 min → 5X SDW	¼ MS, modified KC, VW, modified N_6 medium, with or without CW	12–16-h PP, 1500–3000 lux, 25 ± 2 °C	N_6 supplemented with 10 % CW was most suitable for seed germination. N6 supplemented with 10 % BP was most suitable for growth and rooting	Ding (2004)
D. tosaense	Seeds of 8–14 weeks of artificial pollination	70 % EtOH 30 s → 1.0 % NaOCl with 2 drops of Tween-20/ 100 ml → ultrasound 10 min → 5X SDW	MS or ½ MS + 30 g/l sucrose for seed germination, and MS + 1.5 % sucrose and various additives (banana, PJ and CW)	16-h PP, cool white fluorescent tubes, 40 μmol m^{-2} s^{-1}, 25 ± 1 °C	Maximum number of seeds germinated when seeds from 12-week-old capsules (hand-pollinated) were cultured on ½ MS basal medium + 30 g/l sucrose. Seedlings transferred to MS medium with 15 g/l sucrose + 8 % banana homogenate or PJ or CW and 20 weeks of incubation developed into healthy plantlets. Plantlets hardened in greenhouse on moss or moss and tree fern or tree fern as substrates in plastic trays	Lo et al. (2004a)
D. tosaense, *D. moniliforme*, *D. linawianum*	Seeds of 8–14 weeks of artificial pollination	70 % EtOH 30 s → 1.0 % NaOCl with 2 drops of Tween-20/ 100 ml → ultrasound 10 min → 5X SDW	MS or ½ MS + 30 g/l sucrose for seed germination; MS + 15 g/l sucrose and various additives (banana, PJ and CW)	16-h PP, cool white fluorescent tubes, 40 μmol m^{-2} s^{-1}, 25 ± 1 °C	Seeds of capsules derived after 12 weeks of hand pollination germinated asymbiotically (50–74 %) on ½ MS basal medium with 30 g/l sucrose and solidified with 0.9 % Difco agar. Active growth in germinated seedlings achieved by re-culturing on full-strength MS basal medium + 8 % banana homogenate, 8 % PH, 8 % CW, 1.5 % sucrose and 0.9 % Difco agar. Healthy plantlets transferred to plastic trays containing moss or moss and tree fern, successfully acclimatized (84–100 %) in the greenhouse	Lo et al. (2004b)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. nobile	Mature seeds	Undehisced capsules sterilized with 0.1 % $HgCl_2$ 10–20 min → 3X SDW. Seeds in dehisced capsules sterilized with 70 % EtOH 30 s → 1.0 % NaOCl 8–10 min	Modified KC medium, VW	10–12-h PP, 1000–1500 lux, 18–27 °C	Seed germinated better on modified KC medium than on VW; 2000 lux was better than 1000 lux or 3000 lux	Song et al. (2004)
D. candidum	Mature seeds	75 % EtOH 1 min → 1–2 X SDW → 0.5 % NaOCl 15 min → 5–6X SDW	½ MS medium + BA, NAA, PH, banana extract and activated carbon were used for seed germination, protocorm differentiation, plantlet propagation and plantlet rooting pH 5.0–5.3	18-h PP, 6000 lux, 24–26 °C	Maximum seed germination (79.37 %) on ½ MS + 20 % potato extract. ½ MS + 1.0 mg/l BA + 0.1 mg/l NAA was beneficial to protocorm differentiation and propagation. Highest root number and length in ½ MS + 0.5 mg/l NAA	Tang et al. (2005)
D. cochliodes	Mature seeds	70 % EtOH 30 s → 0.1 % $HgCl_2$ 15 min → 5X SDW	VW, ½ MS, N_6 or Hyponex medium (Hyponex I, N:P:K = 7:6:9; Hyponex II, N:P:K = 20:20:20) with CW, NAA, BA, AC or peptone	12-h PP, cool white fluorescent tubes, 30–40 µmol m^{-2} s^{-1}, 25 ± 2 °C	N_6 medium with 10 % CW suitable for seed germination, Hyponex medium 1 g/l Hyponex I and 1 g/l Hyponex II) with 2 g/l AC, 2 g/l peptone 0.2 mg/l NAA and N_6 medium with 10 % BP and 0.2 mg/l NAA were suitable for rooting	Yang et al. (2006)
D. secundum (Bl.) Lindl.	Seeds and seed-derived protocorms	NR	VW + 100 mg/l *myo*-inositol + 1 mg/l thiamine + 1 mg/l nicotinic acid + 1 mg/l pyridoxine + 4 mg/l glycine + 20 g/l sucrose + 15 % CW + 0.8 % agar; pH 5.4	16-h PP, 40 µmol m^{-2} s^{-1}, 25 ± 2 °C	After 3 m in culture, polyploid plantlets developed from protocorms treated with 0.05 % colchicine for 1 day. Sizes of leaves, stems, roots and flowers of polyploids were larger but chlorophyll content was lower than in diploids	Atichart et al. (2007)
D. candidum	Mature seeds	70 % EtOH 20 s → 0.1 % $HgCl_2$ 10 min → 4–5X SDW	VW with PGRs, additives and AC	NR	Optimal medium for seed germination and rooting: VW + 0.18 mg/l NAA + 0.53 mg/l BA + 0.28 mg/l GA_3 + 20 g/l sucrose + 100 g/l banana mud + 10 g/l AC	Du et al. (2007)
D. strongylanthum	Mature seeds	70 % EtOH 30 s → 3 % NaOCl with 2–3 drops of Tween-80/500 ml 20 min → 4–5X SDW	MS, ½ MS, ¼ MS, B_5 and N_6 media were compared; + 20 g/l sucrose + 0.7 % agar NAA (0.2 and 0.5 mg/l) + BA (0.1–0.8 mg/l) + testing the effect of mashed banana, PH and CW; pH 5.8	14-h PP, 1000–2000 lux, 26 °C	Protocorm induction on ½ MS + 0.2 mg/l NAA. ½ MS + 0.5 mg/l BA used for protocorm multiplication. Root development enhanced in ½ MS + 0.5 mg/l NAA and either 100 mg/l mashed banana or 20 % CW	Kong et al. (2007)
D. longicornu	Mature seeds	Sterilized with 0.5 % $KMnO_4$	½ MS supplemented with BA, NAA or their combination	12-h PP, 1000–1500 lux, 25 ± 1 °C	½ MS supplemented with 1.5 mg/l BA was most suitable for seed germination, ½ MS supplemented with 2.0 mg/l BA and 0.5 mg/l NAA was most suitable for PLB proliferation	Mo and Ling (2007)
D. thyrsiflorum	180-DAP seeds	75 % EtOH 30 s → 1.0 % NaOCl 20 min → 0.1 % $HgCl_2$ 15 min → 5X SDW	½ VW or VW + Kin, IBA, BP or their combination	12-h PP, 30–40 µmol m^{-2} s^{-1}, 25 ± 2 °C	95 % seed germination on ½ VW or VW + 100 g/l BP. 3–4 PLB proliferated on VW + 1.0 mg/l Kin + 0.02 mg/l IBA + 100 g/l BP. 90 % rooting on 3 g/l Hyponex I + 0.5 mg/l IBA and 0.2 % AC. Plantlets successfully (90 %) acclimatized on bark	Wang et al. (2007)
D. henryi	Mature seeds	75 % EtOH 30 s → 1.0 % NaOCl 20 min → 4–5X SDW	½ MS medium + 20 % BP or 20–30 % PH	12-h PP, 50 µmol m^{-2} s^{-1}, 25 ± 2 °C, pH 5.4	½ MS + 20 % PH suitable for seed germination and protocorm development. ½ MS + 30 % PH suitable for rooting	Liu (2008)
D. longicornu and *D. formosum*	Hand-pollinated 8-month-old capsule seeds	70 % EtOH 3 min → 3X flamed	MS, B_5, Mitra et al. KC used for germination. MS + IAA, IBA and GA_3 for seedling development	12-h PP, 50 µmol m^{-2} s^{-1}, 25 ± 2 °C	MS optimum for both species: 90–95 % seed germination for *D longicornu* and 80–85 % for *D. formosum*. Protocorm size improved on medium with 0.01 mg/l IAA (*D. longicornu*) and with 0.1 mg/l BA (*D. formosum*)	Dohling et al. (2008)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. devonianum	Mature seeds	Undehisced capsules sterilized with 75 % EtOH 10–15 s → 0.1 % $HgCl_2$ 5–20 min → 5X SDW	MS, ½ MS, KC, White's, VW, N_6 or ½ N_6 medium + NAA, BA, AC or CW	10–14-h PP, cool white fluorescent tubes, 35–42 µmol m^{-2} s^{-1}, 20–25 °C	N_6 most suitable for seed germination. N_6 + 0.5 mg/l NAA most suitable for growth and rooting	Sun et al. (2009)
D. lituiflorum	Seeds in green capsules	1 % Teepol 20 min → RTW → 0.1 % $HgCl_2$ → 3X SDW	Modified KC or MS + BE and BA.	12-h PP, cool white fluorescent tubes, 30 µmol m^{-2} s^{-1}, 25 ± 2 °C	High percentage germination achieved by culturing seeds on modified KC + 10 % (v/v) BE. Culture of shoots on KC + 12.5 % (v/v) BE led to multiplication, shoot elongation and vigorous rooting. Shoots cultured on MS + 10 mM BA showed maximum multiplication	Vyas et al. (2009)
D. chrysanthum Wall. ex Lindl.	Mature seeds from 8-month-old pods	RTW → dip in 70 % alcohol → 3X flamed	Testing 4 different basal media: MS, NN, B_5 and KC medium; pH: 5.8	12-h PP, 60 µmol m^{-2} s^{-1}, 25 ± 2 °C after culturing in dark for 2 w	In vitro asymbiotic seed germination used here for propagating plant germplasm. Plantlets with well-developed roots formed after 90 days of culture on MS medium (94 % of seeds germinated). Plantlets were hardened successfully (71 % survival) in a mixture of brick, charcoal and decaying litter (1:1:1) covered with moss layer	Hajong et al. (2010)
D. pendulum	Mature seeds	Undehisced capsules sterilized with 75 % EtOH 30 s → 1.0 % NaOCl 5–15 min → 5X SDW	MS, ½ MS, N_6, VW + NAA, BA or PH.	12-h PP, cool white fluorescent tubes, 20–40 µmol m^{-2} s^{-1}, 24 ± 2 °C	N_6 suitable for seed germination, MS + 0.2 mg/l BA + 0.2 mg/l NAA suitable for PLB proliferation. MS + 100 g/l PH suitable for rooting	Huo et al. (2010)
Dendrobium species	Seeds	INA	Weethong fertilizer (20–20–20), Pokon (21–21–21) and 2.5 g/l of Zingnong fertilizer (15–15–10) and MS	INA	1.35 g/l of Weethong fertilizer (20–20–20), MS are good. Addition of CW and BP results in promotional effects	Ngampanya and Homlaaor (2010)
D. bigibbum var. *compactum*, *D. formosum*	Mature seeds	Commercial soap → RTW → dip in 95 % EtOH → flamed	Modified VW + six chitosan types at 0, 10, 20, 40 or 80 mg/l	16-h PP, cool white fluorescent tubes, 35 µmol m^{-2} s^{-1}, 25 °C	Response of seed germination and protocorm development to chitosan dependent on species and developmental stage. All chitosan types and concentrations significantly enhanced the proportion of *D. formosum* seeds that germinated. Almost all chitosan types at 10 mg/l, except O90, significantly improved the growth of *D. bigibbum* var. *compactum* protocorms. 10 or 20 mg/l of P70 chitosan was best to enhance growth of *D. formosum* protocorms	Kananont et al. (2010)
D. chrysotoxum	180-DAP mature seeds	Undehisced capsules wiped with 75 % EtOH → 0.1 % $HgCl_2$ → 5X SDW	MS, ½ MS, B_5, N_6 + BH, IAA, NAA.	12-h PP, 2000 lux, 25 ± 2 °C	MS + 1 mg/l BA + 10 % BH + 1 g/l AC suitable for seed germination. N_6 + 2 mg/l NAA + 0.5 mg/l BA + 10 % BH + 0.5 g/l peptone + 0.5 g/l AC suitable for PLB differentiation. N_6 + 1.5 mg/l NAA + 10 % BH + 1.0 g/l AC suitable for rooting	Lan et al. (2010)
D. nobile Lindl.	Seeds after different DAP (96, 116, 129 and 158 days)	Dip in 100 % EtOH → flamed → SDW	Testing different media: P668 + 20 g/l sucrose, MS + 30 g/l sucrose, ½ MS + 0.6 % agar. pH 5.8	Darkness for 2 weeks, then 16-h PP, 50 µmol m^{-2} s^{-1}, white fluorescent lamps, 25 °C	Seed viability was highest when harvested at 116 DAP but was not correlated with in vitro germination response. In vitro germination was affected both by DAP and growth medium. When seeds were harvested at 129 DAP (globular-shaped embryos), maximum seed germination and advanced developmental stages of seedlings (from protocorms with rhizoids to enlargement of the first leaf) were observed. Germination was highest when seeds were cultured on P668 medium	Vasudevan and Van Staden (2010)
D. wilsonii	Mature seeds	Undehisced capsules wiped with 75 % EtOH → 1.0 % NaOCl 10-20 min → 2–3X SDW	¼ MS, ½ MS, MS + BH, NAA, BA, AC.	Seed germination at 12-h PP, 300–500 lux, 26 ± 1 °C. Protocorm differentiation and rooting at 14–16-h PP, 1000–1500 lux, 26 ± 1 °C	MS + 70 g/l BH and 3 g/l AC suitable for seed germination. ¼ MS + 70 g/l BH + 1 g/l AC suitable for PLB differentiation. ½ MS + 0.5 mg/l NAA + 70 g/l BH + 3 g/l AC suitable for seedling growth	Zhan et al. (2010)
D. aphyllum (Roxb.) CECE Fisher	PBLs from seeds (from 15-week-old capsules)[b]	Commercial detergent → RTW → 70 % EtOH 30 s → flamed 3X → 5X SDW	MS + 0.1 mg/l IAA, or + 0.15 mg/l Kin, or + 0.025 mg/l IAA + 0.075 mg/l Kin	16-h PP, 150 µmol m^{-2} s^{-1}, 22 ± 2 °C	90.6 % of seeds germinated and developed into PLBs after 3 weeks. Better germination (unquantified) and multiple shoot and root development observed on medium with IAA + Kin (average shoot length of 0.5 cm and root length of 0.78 cm) or with IAA alone (average shoot length of 0.38 cm and root length of 1.68 cm)	Dutta et al. (2011)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. parishii	Seeds from 8-month-old self-pollinated green capsule. Seedlings 2 cm in size with trimmed leaves used for in vitro experiments	95 % EtOH → flamed	VW for seed germination. 31 complex media + 20 g/l sucrose, 8 g/l agar and testing the effects of chemical fertilizer, 5 g/l pupa powder, 2 g/l AC, 5 g/l brown rice, 50 g/l BP, 150 ml/l CW alone and in combinations	12-h PP, fluorescent light, 60 µmol m^{-2} s^{-1}, 25 °C	Chemical fertilizer as scale (2 g/l of 21–21–21, NPK) + liquid (10 ml/l) + 5 g/l pupa powder + 50 g/l BP for maximum root and shoot formation. Equally good plantlet growth using chemical fertilizer as scale (2 g/l of 21–21–21, NPK) + liquid (10 ml/l) + 5 g/l pupa powder + 15 % (v/v) CW + 2 g/l AC. Most pseudobulbs formed on chemical fertilizer as scale (2 g/l of 21–21–21, NPK) + liquid (10 ml/l) + 5 g/l brown rice	Kaewduangta and Reamkatog (2011)
D. fimbriatum	Mature seeds	Undehisced capsules sterilized with 75 % EtOH 30 s → 1.0 % $HgCl_2$ 8-10 min → 2–3X SDW	KC + BA, NAA	12-h PP, 2000 lux, 15/30 °C or 25/30 °C	Seed germination was 98.3 % on KC at 25 °C. KC + 2.0 mg/l BA + 0.5 mg/l NAA was suitable for PLB induction and proliferation	Yu et al. (2011)
D. devonianum	Seeds of different ages	RTW 30 min then 4 sterilization procedures: (1) 70 % EtOH 20 s → 4X SDW → 1.0 % NaOCl 10 min → 6X SDW; (2) 75 % EtOH 30 s → 4X SDW → 1.0 % NaOCl 20 min → 6X SDW; (3) 70 % EtOH 20 s → 4X SDW → 0.1 % $HgCl_2$ 10 min → 6X SDW; (4) 70 % EtOH 30 s → 4X SDW → 0.1 % $HgCl_2$ 15 min → 6X SDW	MS, ½ MS, ⅓ MS + NAA	12-h PP, 2000 lux, 25 ± 1 °C	Sterilization procedure 4 was most effective (100 % seed germination). Embryos 10–11 months old were favorable for seed germination. MS + 0.3 mg/l NAA most suitable for seed germination and seedling growth	Cui et al. (2012)
D. aphyllum	Mature seeds	RTW → commercial detergent → 75 % EtOH 30 s → 0.1 % $HgCl_2$ 10 min → 5X SDW	MS + 1.0–4.0 mg/l BA + 0–1.0 mg/l NAA + 0–2.0 mg/l GA_3 (seed germination). ¼ MS + 0.5–2.0 mg/l BA + 0.5–2.0 mg/l NAA (root induction)	14-h PP, 2000 lux, 27 ± 2 °C	MS + 2.0 mg/l BA + 0.5 mg/l NAA + 1.0 mg/l GA_3 suitable for seed germination. ¼ MS + 0.5 mg/l BA + 2.0 mg/l NAA suitable for root induction	Du et al. (2012)
D. officinale	60–180 DAP seeds	RTW → 75 % EtOH 30 s → 1.0 % $HgCl_2$ 10 min → 5X SDW	MS or ½ MS + BA, NAA, BP or AC	12-h PP, 30-40 µmol m^{-2} s^{-1}, 25 ± 3 °C	Seeds at 150–180 DAP could germinate. MS + 1.0 mg/l BA, 0.1 mg/l NAA, 1.0 g/l AC and 200 g/l PH suitable for seed germination and PLB differentiation. MS + 1.5 mg/l BA + 0.1 mg/l NAA + 1.0 g/l AC + 100 g/l BP suitable for PLB proliferation. MS + 0.5 mg/l BA + 0.2 mg/l NAA + 1.0 g/l AC + 100 g/l BP suitable for seedling growth. ½ MS + 0.8 mg/l NAA + inorganic salt A + 1.0 g/l AC + 0.5 mg/l BP suitable for rooting (100 % rooting)	Fu et al. (2012)
D. shixingense	Seeds from intact capsules	RTW → 70 % EtOH 30 s → 0.1 % $HgCl_2$ 8–10 min → 5X SDW	MS, ½ MS, B_5, ½ B_5 + NAA, IBA, BA and AC.	Seed germination in dark. PLB proliferation in liquid medium in 110 rpm in 15 µmol m^{-2} s^{-1}. Differentiation and rooting in 20–40 µmol m^{-2} s^{-1}, 25 ± 2 °C	MS + 1.5 mg/l BA + 0.6 mg/l NAA + 0.1 % AC suitable for seed germination. MS + 0.8 mg/l BA + 0.3 mg/l NAA + 4.5 % sucrose suitable for PLB differentiation. MS + 2.5 mg/l BA + 0.5 mg/l NAA + 0.03 % AC suitable for PLB differentiation. ½ MS + 0.5 mg/l IBA + 0.05 % AC suitable for rooting	Meng et al. (2012)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. aqueum	Mature seeds	1–2 drops Teepol → RTW 15 min → dip in 70 % alcohol for a few seconds → flamed 3X	MS, mMS, ½ MS, ¼ MS, m½ MS, m¼ MS, KnC, KcO, Np, Th-GD, Pf, N3F, VW, RE, F, WS, Mitra et al. KP, Liddel, Curtis media without PGRs. Five PP also tested	16-h PP light, 30 µmol m^{-2} s^{-1}, 23 ± 2 °C. PP tested with 0/24, 8/18, 12/12, 16/8, 24/0 h (L/D)	Highest seed germination in ½ MS (93.41 %). 24/0 h (L/D) PP resulted in highest germination (97.75 %) on MS	Parthibhan et al. (2012)
D. hookerianum	Seeds from 8–9-month-old capsule	RTW → SDW → dip in 70 % alcohol → flamed 3–4X	MS, Mitra et al. KC and B_5 were tested	12-h PP, cool white fluorescent lamps, 60 µmol m^{-2} s^{-1}, 25 ± 2 °C, 70–75 % relative humidity	Seeds on MS germinated in 3–4 weeks compared with 7–8 weeks on B_5. Seedling development was also superior on MS. Highest seed germination (95.27 %) with seedling development on MS	Paul et al. (2012)
D. nobile	Seeds from mature opened pod	3 ml 2.5 % NaOCl + 6 ml SDW 15 min → diluted in SDW	Pre-germinative treatments with BA and GA_3 at 0.0, 1.0, 2.0 and 5.0 mg/l. For germination, modified Campos medium (2002) with 3 ml/l commercial fertilizer (10–10–10 NPK), 70 g/l ripe tomato, 15 % CW, 50 g/l mature dwarf banana	12-h PP, 28 µmol m^{-2} s^{-1}, 23 ± 2 °C	46.47 and 49.47 % germination obtained from PGR-untreated seeds. Lowest germination with BA and GA_3 (1.68 and 9.06 %, respectively)	Soares et al. (2012)
D. aggregatum	Immature seeds	Commercial detergent (Labolene) 10 min → 0.5 mg/l Bavistin 20 min → 70 % EtOH 30 s → 0.12 % $HgCl_2$ 10 min → 3–4X SDW	MS + several concentrations of BA alone or in combination with NAA and CW	14-h PP (light intensity NR), cool white fluorescent lights, 25 ± 2 °C	MS + 30 g/l sucrose + 1.5 mg/l BA and CW resulted in higher percentage germination, more protocorms and shoots, shoot elongation, and root formation	Vijayakumar et al. (2012)
D. aduncum	Immature seeds	Undehisced capsules wiped with EtOH → seeds sterilized in 75 % EtOH 30 s → 10 % NaOCl 10 min → 3–4X SDW	MS + different concentrations of BA and NAA for seed germination. MS + different concentrations of BA, Kin and NAA for PLB proliferation. ½ MS + different concentrations of IBA or NAA for rooting. pH 5.4–5.6	12-h PP, 2000 lux, 24–26 °C	MS + 0.5–1.0 mg/l BA + 0.2 mg/l NAA suitable for seed germination. Optimal medium for PLB proliferation was MS + 2.0 mg/l BA + 0.5 mg/l Kin + 0.5 mg/l NAA (propagation coefficient 12.4). MS + 0.5 mg/l BA + 0.5 mg/l Kin + 0.2 mg/l NAA suitable for PLB differentiation (97.5 %). ½ MS + 0.5 mg/l IBA + 0.5 mg/l NAA suitable for rooting (100 %)	Zhou et al. (2012)
D. aggregatum	3–4 months old hand-pollinated seeds	Commercial detergent Teepol 20 % → 0.2 % $HgCl_2$ 10 min → dip in 70 % EtOH 1 min → 2–3X SDW	MS and Phytamax at pH 5.8 and 5.4, respectively, used for germination. Protocorms proliferated with different PGRs supplemented in MS	14-h PP, 60 µmol m^{-2} s^{-1}, 25 ± 2 °C, 60 % RH	MS was more efficient in germination (95 %) compared to PM medium (72 %). 2–3 subcultures of secondary protocorms on the same media produced seedlings. MS with 2.0 mg/l BA and 1.0 mg/l NAA was most effective for secondary protocorm induction and subsequent seedling development	Hossain (2013)
D. aphyllum	Seed, then protocorms	RTW → 10 % Teepol → 0.04 % Bavistin and streptomycin sulfate solution 20 min → 3X SDW → 0.1 % $HgCl_2$ 10 min → SDW → dip in 70 % EtOH 30 s → flamed 2–3X	MS; Phytamax (Sigma) + 1 mg/l BA (seed germination). Phytamax + 1 mg/l BA + 1 mg/l NAA (PLB formation from protocorms). Mitra and KC were compared with two additives: peptone and AC	14-h PP, cool white fluorescent lamps, 60 µmol m^{-2} s^{-1}, 25 ± 2 °C	97 % germination which could be increased to 100 % following the inclusion of 2 g/l peptone on Phytamax. Maximum of 15.25 PLBs/protocorm section	Hossain (2013)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. fimbriatum	Mature seeds	0.2 % $HgCl_2$ 10 min → 3–4X double SDW	Phytamax, MS and modified VW + auxin, cytokinin and/or GA_3 in different concentrations and combinations used for germination. Liquid or solidified MS + different concentrations of NAA and KIN, BA and IBA, zeatin and IBA were used for shoot elongation	16-h PP, 2000–3000 lux (cool white fluorescent light), 25 ± 2 °C	Highest percentage of seed germination (100 %) obtained on Phytamax and lowest (86 %) on modified VW. Highest rate of shoot elongation on MS + 2.0 mg/l BA + 0.01 mg/l IBA	Kabir et al. (2013)
D. fimbriatum var. *oculatum*	Mature seeds	Undehisced capsules sterilized in 70 % EtOH 30–45 s → 3X SDW → 0.1 % $HgCl_2$ 5–8 min → 3–5X SDW	MS + several concentrations of BA, GA_3, Kin, NAA alone or in combination	12-h PP, 1200–2200 lux, 26 ± 2 °C	MS + 1.5 mg/l BA + 0.1 mg/l GA_3 suitable for protocorm induction (85 % induction rate). MS + 2.5 mg/l BA + 1.0 mg/l NAA + 0.5 mg/l Kin suitable for PLB proliferation and differentiation. MS + 0.1 mg/l NAA + 0.5 g/l AC suitable for rooting (100 % rooting)	Li et al. (2013)
D. huoshanense	Mature seeds	Undehisced capsules wiped with EtOH cotton → 75 % EtOH 30 s → 0.1 % $HgCl_2$ 15 min → 5X SDW	1.0 g/l Hyponex I + 1.0 g/l Hyponex II + 1.0 g/l peptone + 0.5 mg/l NAA + 27.8 mg/l $FeSO_4{\cdot}7H_2O$ + 37.3 mg/l Na_2EDTA + 15 g/l sucrose + 1.0 g/l AC + 50 g/l BP	12-h PP, 30-40 µmol m^{-2} s^{-1}, 25 ± 2 °C, pH 5.4-5.6	Germination percentage of seeds derived from cross-pollination and stored at 4 °C for 2.5 years exceeded 80 %, which was 20 % higher than self-pollinated seeds. Medium + juice of potato, banana and apple promoted seedling growth: banana juice was best. Optimum transplant matrix was 3/10 bark (bottom layer) + 7/10 sawdust (upper layer). Survival was 78 %	Qian et al. (2013)
D. nobile	Mature seeds	3 ml of 2.5 % NaOCl + 6 ml SDW 15 min → diluted in SDW	Modified Campos (2002) medium containing 70 g/l tomato paste without peel and seeds, 50 g/l banana without peel, 3 ml/l NPK fertilizer (10–10–10), 17 g/l bacteriological agar, 25 g/l sucrose, 3 g/l AC. Effect of CW was tested at 0, 5, 10, 15 and 20 %	12-h PP, 1000 lux, 23 ± 2 °C	20 % CW was optimal for longest and most pseudobulbs, most and longest leaves and roots, and highest plant fresh weight	Soares et al. (2013)
D. wangliangii	Seeds from hand-pollinated capsules collected at 180, 210, 240, and 270 DAP	75 % EtOH 45 s → 0.1 % $HgCl_2$ for 15 min → wash in SDW	Basal medium containing MS microelements, organics, *myo*-inositol, and ferric salt with 3 g/l Hyponex-1, 0.5 mg/l NAA, and 0.5 g/l AC used for seed germination. ½ MS + various PGRs and additives used for shoot differentiation from protocorms, shoot multiplication and in vitro flowering	Darkness and 16-h PP, 36 µmol m^{-2} s^{-1}, 22 ± 2 °C	Highest germination (92 %) obtained 240 days after hand pollination. Protocorm differentiation on medium with 2 mg/l BA + 0.1 mg/l NAA + 10 % CM or 2 mg/l BA + 0.1 mg/l NAA + 10 % CM after pre-treatment with 1 mg/l GA_3. Shoot multiplication efficient on medium with 2 mg/l BA + 0.1 mg/l NAA + 100 ml/l CM. 100 % inflorescences induced with 2 mg/l TDZ	Zhao et al. (2013a)
D. chrysotoxum	4-month-old unripe green capsules	RTW with 20 % Teepol 10–15 min → 0.4 % $HgCl_2$ 7–8 min → 4–5X SDDW → 70 % EtOH 8–10 min → flamed 2–3 s	Mitra medium supplemented with 0.4 % AC, BA, KN, IAA, IBA and NAA at various concentrations (0.5–4.5 mg/l)	12-h PP, 60 µmol m^{-2} s^{-1}, white fluorescent lamps, 25 ± 2 °C	Enhanced seed germination of 98.1 % was observed on medium containing 0.4 % AC, 2 mg/l BA and 2 mg/l IAA compared to PGR-free medium (82.4 %)	Nongdam and Tikendra (2014)

Table 1 continued

Species and/or cultivar	Explant used	Sterilization procedure	Culture medium, PGRs and additives	Culture conditions[a]	Remarks, experimental outcome, acclimatization and variation	References
D. nobile hybrids (*D.* Lucky Girl x *D.* Second Love 'Kirameli' and *D.* Lucky Girl x *D.* Hamana lake 'Kumi' and self-pollinated *D.* Second Love 'Kirameli')	Protocorms originated from immature seeds of 3–5-month-old capsules	70 % EtOH 3 min → 0.6 % NaOCl + a drop Tween-20, 10 min → 3X SDW → 95 % EtOH 15 s → flamed 2–3X 2–3 s	2 g/l Hyponex® (N:P:K = 7:6:19) + MS vitamins + 2 g/l AC + 1 g/l Bacto-tryptone + 50 g/l PH + 25 g/l BP + 5 g/l agar + 1 g/l Gelrite + sucrose at different concentrations: 0, 10, 20 and 40 g/l. pH 5.6–5.8	12-h PP, 40 ± 10 µmol m^{-2} s^{-1}, cool white fluorescent lamps, 25 ± 2 °C	Germination highest (80–90 %) when seeds originated from 4-month-old capsules. Seed germination percentage not modified by sucrose concentration in all stages. Protocorm development, however, was affected by sucrose concentration: as concentration increased, number of seedlings at stage 6 (2 or more leaves and roots in seedlings) decreased. With 10 g/l sucrose, all seedlings developed to stage 6 and in the least time	Udomdee et al. (2014)
D. pendulum, *D. primulinum*, *D. wardianum*	Seeds of different maturity (90, 150, 270, 360, 390 DAP)	75 % EtOH surface wipe → 2.0 % $HgCl_2$ 5 min → 5–6X SDW	MS, ½ MS, Hyponex I, Hyponex II, N_6 basal media supplemented with different concentrations of BA, NAA and organic amendments	10–12 h PP, 2000–3000 lux, 24 ± 1 °C	Germination highest (80–90 %) from mature seeds (over 150 DAP) cultured on MS supplemented with 0.05–0.40 mg/l BA and 10 % CW	Gong et al. (2015)

B_5 medium or Gamborg medium (Gamborg et al. 1968), *BA* N^6-benzyladenine [BA is used throughout even though BAP (6-benzylamino purine) may have been used in the original, according to Teixeira da Silva 2012], *BE* banana extract, *BP* banana pulp, *BPA* *N*-benzyl-tetrahydropyranyladenine, *CM* coconut milk (as a percentage, v/v), *CW* coconut water (as a percentage, v/v), *DAP* days after pollination, *DW* distilled water, *EtOH* ethanol (as a percentage, v/v), GA_3 gibberellic acid, $HgCl_2$ mercuric chloride, *IAA* indole-3-acetic acid, *IBA* indole-3-butyric acid, *INA* information not available, *KC* Knudson's C medium (Knudson 1921), *Kin* kinetin, *Mitra* Mitra et al. (1976) medium, *MS* Murashige and Skoog (1962) medium, *NaOCl* sodium hypochlorite, *N6 medium* Chu's medium (Chu et al. 1975), *NAA* α-naphthaleneacetic acid, *NR* not reported, *PGR* plant growth regulator, *PH* potato homogenate (also used for potato paste and potato extract), *PJ* potato juice, *PLB* protocorm-like body, *PP* photoperiod, *PT medium* (Zeng et al. 1998), *RTW* running tap water, *SDW* sterile distilled water, *TDZ* thidiazuron (*N*-phenyl-*N*′-1,2,3-thiadiazol-5-ylurea), *VW* Vacin and Went (1949) medium, *Zea* zeatin

[a] The original light intensity reported in each study has been reported but can be converted from lux to µmol m^{-2} s^{-1} based on conversion factors for different illumination sources (main ones represented): for fluorescent lamps, 1 µmol m^{-2} s^{-1} = 80 lux; the sun, 1 µmol m^{-2} s^{-1} = 55.6 lux; high voltage sodium lamp, 1 µmol m^{-2} s^{-1} = 71.4 lux (Thimijan and Heins 1983)

[b] The authors referred to the structures as PLBs, but in fact they were protocorms

for *Dendrobium*, mainly because each mature capsule contains 2–3 million seeds with a high percentage of viable seeds that can germinate under in vitro asymbiotic conditions (Paul et al. 2012). Another commercially important application of asymbiotic germination is in breeding programmes aimed at accelerating the speed at which new cultivars are obtained (Cardoso 2012).

This review aims to examine the available literature (53 studies; Table 1) related to asymbiotic in vitro *Dendrobium* seed germination (covering 37 species and multiple hybrids; Table 2) in a bid to identify those trends that could assist researchers better formulate a study or conservation programme for commercial or for rare species and hybrids. All nomenclature has been verified on The Plant List (http://www.theplantlist.org/).

Seed germination in vitro

Seed germination is defined as the emergence of radicle through the seed coat and involves a sequence of previous steps, as imbibition of water followed by embryo expansion (Figs. 1, 2a, b), resulting in initial seedling development under favorable conditions (Manz et al. 2005). Nowadays, germination of orchid seeds is easy, though a labor-intensive procedure (Kauth et al. 2008), but several advances have taken place, as outlined in Table 1. The first investigators, L. Knudson and N. Bernard, reported difficulty in germinating orchid seeds due to environmental conditions and small size of embryos (Knudson 1922) and, as described by Bernard (1909), strong dependence on mycorrhizal fungi that inhabit the roots of orchids. Knudson (1922) was able to germinate an epiphytic orchid (*Laelia–Cattleya* hybrid) in aseptic mineral media supplemented with diverse types of carbohydrates such as glucose and sucrose.

Pod or seed sterilization

The sterilization of *Dendrobium* pods, as recorded in the literature (Table 3), shows several patterns and trends (Teixeira da Silva et al. 2015b). The sterilization of undehisced *Dendrobium* pods, having immature and mature seeds, starts with a wash in running tap water containing Teepol, a detergent (1–20 %; 20 min) or a household or commercial bleach (12 %; 20 min). Surface-

Table 2 Species and seed stages or ages used for in vitro asymbiotic germination of *Dendrobium* species

Species used in papers	Seed stage, days (d), weeks (w) or months (m) after pollination (AP)								Species used	% papers used[b]
	Different ages	Immature	Mature	8–15 weeks AP	3–5 months AP	180 days AP	8–9 m AP	NR		
D. aggregatum		1			1				2	3.8
D. aphyllum			1	1				1	3	5.7
D. candidum (*D. officinale*)	4		2						6	11.3
D. chrysotoxum					1	1			2	3.8
D. devonianum	2		1						3	5.7
D. fimbriatum	1		3						4	7.6
D. formosum			1				1		2	3.8
D. huoshanense		1	1						2	3.8
D. loddigesii	2								2	3.8
D. longicornu			1				1		2	3.8
D. nobile	1		3						4	7.6
D. pendulum	1		1						2	3.8
D. tosaense	2			2					4	7.6
D. wangii	2								2	3.8
D. wilsonii	1		1						2	3.8
D. hybrids or *D.* sp.					3			1	4	7.6
Other species[a]	4	1	8	2		2	2	3	21	39.6
Total seed ages used	20	3	23	5	5	3	4	5		
% papers used[b]	37.7	5.7	43.4	9.4	9.4	5.7	7.6	9.4		

[a] Other species (one of each): *D. aduncum*, *D. aqueum*, *D. bigibbum*, *D. chrysanthum*, *D. cochliodes*, *D. densiflorum*, *D. hancockii*, *D. henryi*, *D. hookerianum*, *D. linawianum*, *D. lituiflorum*, *D. moniliforme*, *D. parishii*, *D. primulinum*, *D. secundum*, *D. shixingense*, *D. strongylanthum*, *D. thyrsiflorum*, *D. transparens*, *D. unicum*, *D. wangliangii* and *D. wardianum*

[b] The sum of values in percentage may exceed 100 % because some papers used more than one species and/or seed stages and/or ages, and because a single decimal point was assigned

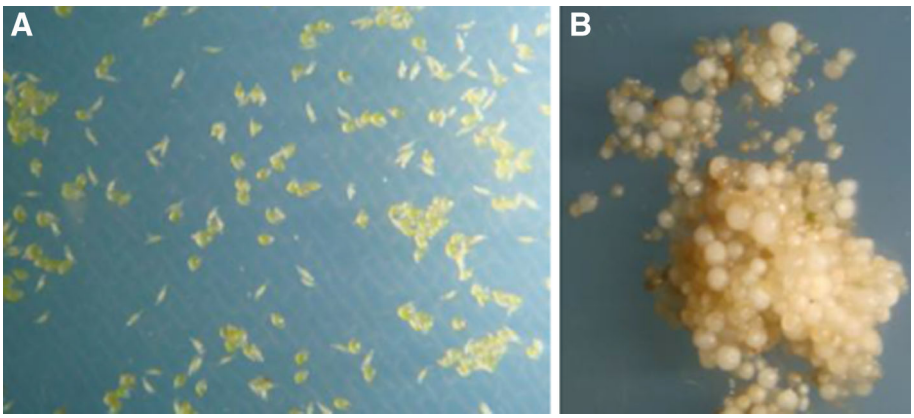

Fig. 2 Imbibed seeds on solidified agar medium (pH 5.7), without sugar, nutrients and additives in light (16-h photoperiod) (**a**). Swollen seeds of *Dendrobium aqueum* on basal medium with half-strength MS macronutrients in the dark after 4 months (**b**). Unpublished photos (Parthibhan et al.)

Table 3 Sterilization agents used for different types of organs used in papers related to in vitro asymbiotic seed germination of *Dendrobium* species

Sterilization procedures[a]	Plant organ used for sterilization						Papers used	% papers used[c]
	Different ages pods or seeds	Immature pods	Mature pods	Immature seeds	Mature seeds	Seeds or capsules ANR		
EtOH	1		1				2	3.8
EtOH + $HgCl_2$	5		5	1[A]	3	1	15	28.3
EtOH + NaOCl			2	1	5		8	15.1
EtOH + NaOCl + $HgCl_2$	1				1		2	3.8
EtOH + NaOCl + ultrasound	2						2	3.8
EtOH + flaming	1	1[B]	4		2		8	15.1
$HgCl_2$	1		1		1	1	4	7.6
NaOCl					2		2	3.8
Others[b]	1	1		1	1	1	5	9.4
NR	2			1	1	2	6	11.3
Papers used	14	2	13	4	16	5		
% papers used[c]	26.4	3.8	24.5	7.6	30.2	9.4		

ANR age not reported, *EtOH* ethanol, *$HgCl_2$* mercuric chloride, *NaOCl* sodium hypochlorite, *NR* not reported

[a] When different procedures were tested, only the most effective was used for calculations

[b] Others, including the use of fungicides, antibiotics, $KMnO_4$

[c] The sum of values in percentage could exceed 100 % because some papers used more than one type of explants and/or sterilization procedure, and because a single decimal point was assigned

[A] 3–4-month-old age (considered as immature)

[B] 15-week-old capsules (considered as immature); 180-DAP old seeds and 8-month-old were considered as mature

disinfected pods are first transferred to a laminar air hood and surface sterilized with ethanol (EtOH; rubbed or dipped, 70–90 %; 20–30 s or 1–15 min). Some *Dendrobium* pods (*D. aphyllum* (Roxb.) C.E.C.Fisch., *D. aqueum* Lindl., *D. bigibbum* Lindl. var. *compactum*, *D. chrysanthum* Wall. ex Lindl., *D. chrysotoxum* Lindl., *D. formosum* Roxb. ex Lindl., *D. hookerianum* Lindl., *D. longicornu* Lindl., *D. nobile*, *D. parishii* Rchb.f.) are also flamed (Dohling et al. 2008; Hajong et al. 2010; Kananont et al. 2010; Vasudevan and Van Staden 2010; Dutta et al. 2011; Kaewduangta and Reamkatog 2011; Parthibhan et al. 2012; Paul et al. 2012; Hossain 2013; Nongdam and Tikendra 2014). Additional treatment, either alone or in combination, involves $HgCl_2$ (0.1–1.0 %; 8–12 min) and NaOCl (1.0–10 %; 5–15 min), which were also reported to be effective. The reported type, concentration, and duration of exposure of sterilants differ considerably, and thus need to be standardized for any species. EtOH and $HgCl_2$ are most frequently used for seed sterilization, and have a protein-denaturing property that exterminates bacteria while NaOCl (the second most used

sterilant for seeds), an alkali, loses chloride causing the active oxidizing ion to capture oxygen, thus killing aerobic microorganisms and fungal spores that are mainly responsible for most of the contaminations (Alvarez-Pardo et al. 2006). EtOH is usually used in asymbiotic seed germination with an additional source of chlorine for complete sterilization, usually derived from $HgCl_2$ (in 28.3 % of papers) or NaOCl (in 15.1 % of papers). EtOH followed by flaming is also applied for the sterilization of mature and green capsules (Tables 1, 3; Teixeira da Silva et al. 2015b). In some instances, $KMnO_4$, a commercial fungicide (Bavistin), a bactericide (streptomycin sulfate), and even sonication were also applied for better sterilization. To start *Dendrobium* in vitro culture, mature seeds and pods are the main explants (30.2 and 24.5 %, respectively) (Table 3).

Media composition

Orchid plants produce numerous minute seeds and each seed contains insufficient nutrient reserves for germination. The establishment of a symbiosis with an appropriate fungus is indispensable for germination under natural conditions. In vitro, orchid seed germination can be achieved using either asymbiotic or symbiotic methods (Rasmussen 1995; Yam and Arditti 2009; Fig. 1), which are strongly influenced by several abiotic factors, including medium composition and culture conditions. This is likely a genotype-specific response, as was observed in *Cypripedium* spp. (Zeng et al. 2015). MS and ½ MS are the most commonly used basal culture medium for *Dendrobium* seed germination, while KC or modified KC and N6 are the second most used formulations (Fig. 3).

OMF

Several studies on in vitro symbiotic *Dendrobium* seed germination with cultures of OMF (Swangmaneecharern et al. 2012; Wu et al. 2012) and asymbiotic germination using liquid extract of OMF (Guo and Xu 1990) support the favorable role of mycorrhizae (Teixeira da Silva et al. 2015b). Swangmaneecharern et al. (2012) reported the effectiveness of five isolates of the OMF *Epulorhiza* sp. (isolated from *Paphiopedilum*, *Dendrobium*, and *Cymbidium*) in promoting seed germination and protocorm development of four *Dendrobium* species (*D. pulchellum* Roxb. ex Lindl., *D. crepidatum* Lindl. and Paxton, *D. findlayanum* E.C. Parish and Rchb. f. and *D. crystallinum* Rchb. f.). However, the promoting effects of different fungal isolates on seed germination of each orchid species were not equal: the isolates Da-KP-0-1 (from *Dendrobium*) and Ps-KT-0-1 (from *Paphiopedilum*) were effective in *D. pulchellum* and *D. crepidatum*, whereas the isolates Pv-PC-1-1 (from *Paphiopedilum*) and Da-KP-0-1 (from *Dendrobium*) were reported to be the best for *D. findlayanum*.

Basal media

Although PGR-free basal media usually (54.7 % of papers) support the in vitro germination of *Dendrobium* seeds

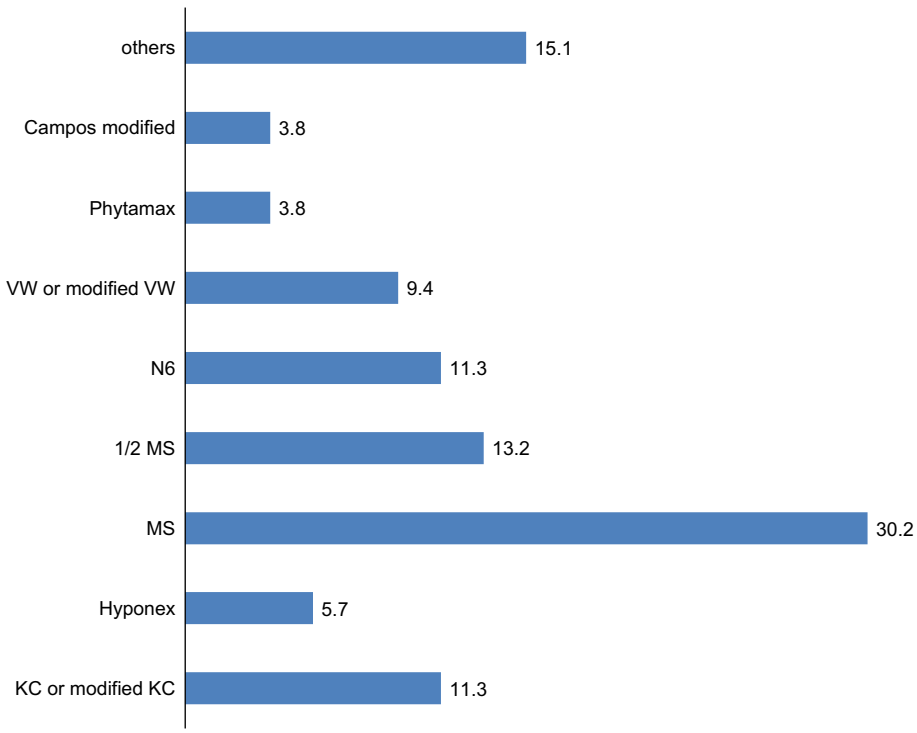

Fig. 3 The percentage of culture media used in papers on asymbiotic germination of *Dendrobium*. Campos modified (Soares et al. 2013), *KC* Knudson's C medium (Knudson 1921), *MS* Murashige and Skoog (1962) medium, *N_6 medium* Chu's medium (Chu et al. 1975), *PT medium* (Zeng et al. 1998), *VW* Vacin and Went (1949) medium. The sum of percentages may exceed 100 % because some authors used or recommended more than one culture medium in the same paper. In the case that authors used several culture media, only the best media were used to calculate the percentage values

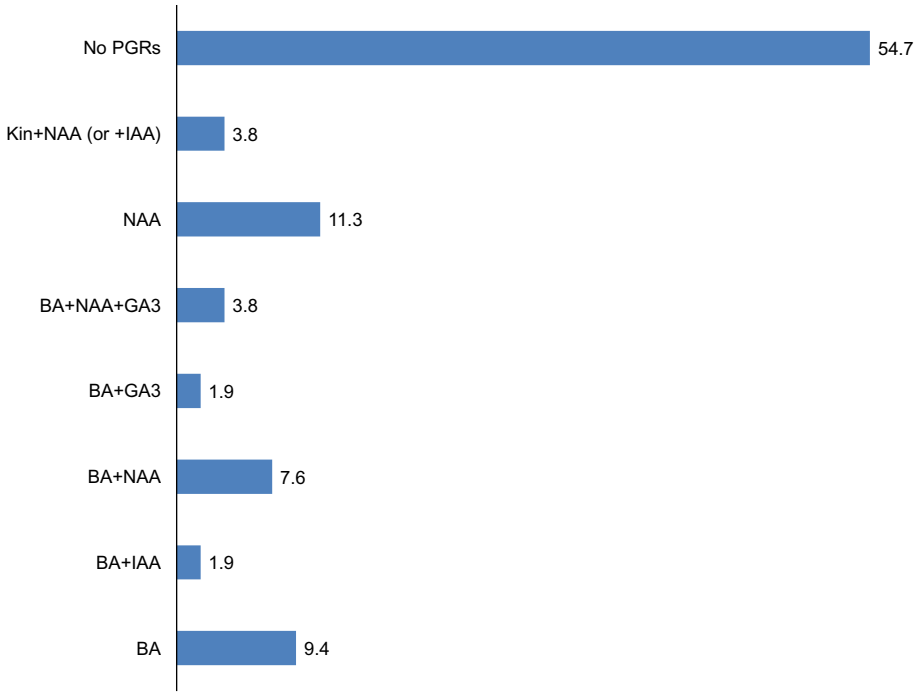

Fig. 4 The percentage of studies that used plant growth regulators, alone or in combination, in basal culture media for asymbiotic germination of *Dendrobium* species. *BA* N^6-benzyladenine (BA is used throughout even though BAP (6-benzylamino purine) may have been used in the original, according to Teixeira da Silva (2012), GA_3 gibberellic acid, *IAA* indole-3-acetic acid, *Kin* kinetin, *NAA* α-naphthaleneacetic acid, *PGR* plant growth regulator

(Fig. 4), and have been used for many orchids (Parthibhan et al. 2012; Paul et al. 2012; Hossain 2013; Teixeira da Silva 2013), several PGRs have also been effectively used. The most frequently used PGRs for the asymbiotic germination of *Dendrobium* seeds are α-naphthaleneacetic acid (NAA) and 6-benzyladenine (BA), or a combination of both (Fig. 4). Even though in most studies (50.9 %) no special additives were used (Fig. 5), diverse organic compounds (malt, yeast, casein, peptone, beef and tryptone extracts at 0.01 to 0.1 g/l) and natural supplements (banana pulp, coconut water (CW), potato juice, sugarcane juice and tomato juice at 5 to 15 %) can greatly affect (improve or inhibit) *Dendrobium* germination (Fig. 4; Teixeira da Silva 2013; Parthibhan et al. unpublished). CW is the main natural supplement used for this aim (13.2 % of papers) (Fig. 5). Orchid seeds prefer and require external nutrients or growth substances for effective germination and/or seedling growth in nature and in vitro. Unlike epiphytic orchids, terrestrial orchid seeds will not grow beyond the protocorm stage unless infected by a suitable mycorrhizal fungus, since they are subterranean in nature. Even after a suitable symbiotic relationship has been established, non-green protocorms can take many weeks, months or even years to grow and produce leaves and roots, depending upon the species, to maintain a mutualistic symbiosis (McKendrick 2000). Thus, in general, as a result of this relationship, terrestrial orchids only require a low concentration of nutrients or more diluted media such as ½ MS, ¼ MS and ⅛ MS for some *Paphiopedilum* species (Hossain et al. 2013a; Teixeira da Silva 2013; Zeng et al. 2012, 2014, 2015), when germinated symbiotically in vitro, because of mycotrophy (Rasmussen 1995). Epiphytes prefer high nutrition or concentrated media and a carbon source for germination and seedling development (Arditti 1979). Despite this, highly viable *D. aqueum* seeds remain ungerminated even after imbibition for 5 months on nutrient- and sugar-free solidified agar (0.7 %) medium (Parthibhan et al. unpublished).

McKendrick (2000) suggested that when attempting to germinate a new species, it is important to test media at both full and half strength to determine the best nutrient base. Since *Dendrobium* species and hybrids have commercial, ornamental and medicinal value, conservation strategies are also required (Bulpitt 2005; Bulpitt et al. 2007; Kuehnle 2007; Teixeira da Silva et al. 2014b). A combination of asymbiotic seed germination and vegetative propagation forms the basis of economic horticultural production of orchid plants (Smith and Read 2008).

Asymbiotic seed germination is used to accelerate and increase the efficiency of germination of *Dendrobium* orchids. However, the relatively ease with which they can be micropropagated using clonal techniques, such as shoot tip or axillary bud culture, rather than seed propagation, presents several advantages for commercial production, especially for the flower market. Despite this, in many countries, most micropropagation research was developed using seeds as initial explants (Table 1). Orchids, including the genus *Dendrobium*, present high heterozygosity and seed propagation results in high genetic variation of progeny (Gu et al. 2007; Chattopadhyay et al. 2012). Faria

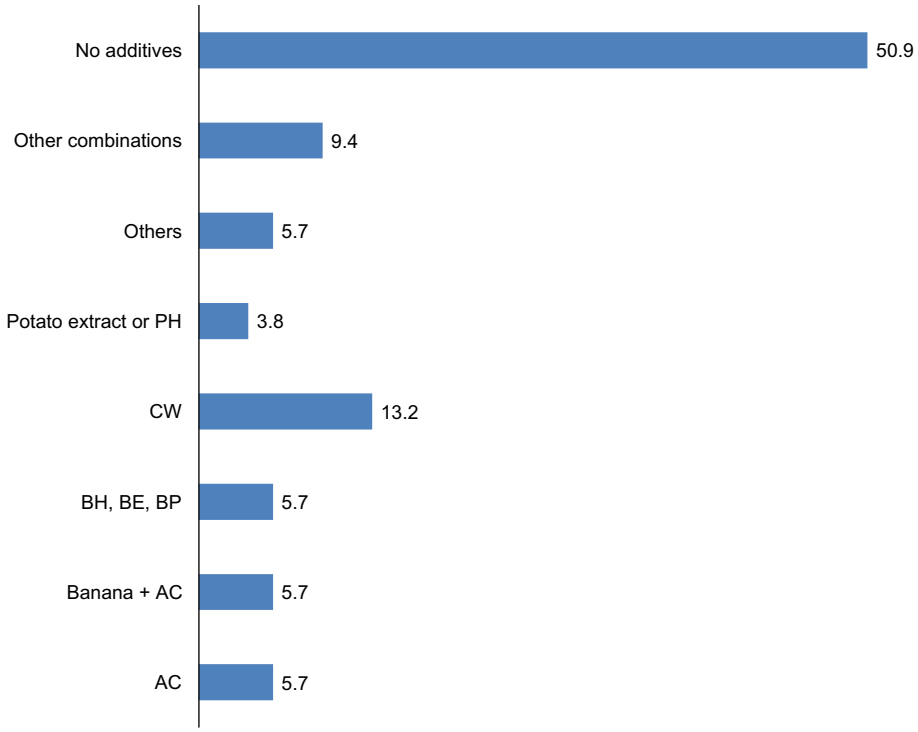

Fig. 5 The percentage of studies on asymbiotic germination of *Dendrobium* species that used additives, either alone, or in combination. *AC* activated charcoal, *BE* banana extract, *BH* banana homogenate, *BP* banana pulp, *CW* coconut water, *NR* not reported, *PH* potato homogenate

et al. (2004a) observed significant variation in morphological characteristics, such as height (2.38 to 6.10 cm), number of roots (2.8 to 8.7), fresh (0.41 to 1.95 g) and dry (0.03 to 0.12 g) weight from 20 different crosses and self-pollination obtained from different cultivars of in vitro cultivated *D. nobile* plantlets. Lone et al. (2008) also observed a variation of 4.23–6.89 cm in plant height, 5.2–12.87 roots/plantlet, and 0.23–0.63 g of total fresh weight/plantlet after 109 crosses using *D. phalaenopsis*. Seed propagation is commonly used for conservation of endangered species, and for breeding purposes (Faria et al. 2004b; Lone et al. 2008; Cardoso 2012). In these cases, genetic variation is a positive factor expected for conservation or development of new cultivars.

A large number of media types were tested in *Dendrobium* seed germination and seedling development (Table 1). These media include MS, half- or a quarter-strength (the latter not being recommended) of MS macro- and microelements (½ MS or ¼ MS, respectively), Chu's N_6 medium (Chu et al. 1975), B_5 (Gamborg et al. 1968), Vacin and Went (1949; VW), KC, or Hyponex (Chen et al. 2004; Yang et al. 2006) media (Fig. 3). The ideal medium for germination of each *Dendrobium* species differs. KC or modified KC media are usually used for *Dendrobium* seed germination (Table 1; Hu and He 1979; Xu and Yu 1984; Yu et al. 2011). Parthibhan et al. (2012) studied 20 basal media devoid of PGRs and additives for *D. aqueum*. Half-strength MS medium macronutrients, to which 2 % sucrose was added, resulted in highest seed germination (93.41 %) and seedling development. Zeng et al. (1998) reported that the optimal medium for embryo culture of five *Dendrobium* species was N_6, to which 0.2 mg/l NAA was added. Song et al. (2004) reported that *D. nobile* seed germinated better on modified KC medium than on VW medium. For seed germination, de Moraes et al. (2010) used 30 ml of MS medium with 7 g/l of agar and pH adjusted to 6.0. MS medium resulted in highest germination (90–95 % and 80–85 %) of *D. longicornu* and *D. formosum*, respectively (Dohling et al. 2008). Hajong et al. (2010) reported the best germination (94 %) on PGR-free MS medium in *D. chrysanthum* under a 12-h photoperiod. Similar results were obtained by Paul et al. (2012) with *D. hookerianum* in which maximum germination percentage (95.27 ± 0.68 %) was observed using MS medium, when compared with Mitra (87.85 ± 0.81 %), KC (73.00 ± 1.23 %) and B_5 (51.38 ± 1.31 %) media. The same authors also observed more rapid germination (about 2 weeks after inoculation) and the best development of seedlings from protocorms on MS medium.

Carbohydrates

Sucrose has served as the main source of carbohydrate used for micropropagation of *Dendrobium* orchids, and only in 2.2 % of *Dendrobium* micropropagation studies have other carbohydrates improved the effect of sucrose (Teixeira da Silva at el. 2015a). Luo et al. (2009) tested the effect of sucrose, maltose, glucose and fructose at 5–40 g/l and

found that the best shoot development of *D. huoshanense* from PLBs was achieved on medium with 10 g/l maltose. Glucose and fructose were best for PLB proliferation of *D.* 'Alya Pink' among six carbohydrates tested (mannitol, galactose, sorbitol, glucose, sucrose or fructose, all applied at 2 %) (Nambiar et al. 2012). Faria et al. (2004a) tested five sucrose concentrations (0, 5, 10, 20, 30, and 60 g/l) in ½ MS at pH 5.8 for in vitro cultivation of *D. nobile* seedlings. The best plant height (4.21 cm), fresh weight (0.17 g), root length (4.75 cm) and number of shoots (4.4) resulted after 120 days of cultivation using 60 g/l sucrose. Similarly, sucrose was the carbohydrate source when culture medium contained an artificial carbohydrate (21.2 % of the studies shown in Table 1) during asymbiotic seed germination of *Dendrobium*. The seed germination percentage of *D. nobile* hybrids was not modified by a sucrose concentration between 10 and 40 g/l in all stages (Udomdee et al. 2014; Table 1). However, as sucrose concentration increased, the number of seedlings with two or more leaves and roots (stage 6) decreased, and seedling development was most rapid, developing to stage 6, when 10 g/l sucrose was used.

Plant growth regulators

Seed germination of *Dendrobium* germplasm is usually enhanced by the inclusion of PGRs in medium. BA, kinetin (Kin), NAA, and GA_3 have different effects on seed germination and seedling growth of different *Dendrobium* species (Mo and Ling 2007; Cui et al. 2012; Fu et al. 2012; Li et al. 2013). In *D. transparens* Lindl., 90 % germination was achieved on B_5 medium supplemented with 1.0 mg/l Kin and 1.0 mg/l NAA (Hazarika and Sarma 1995). Soares et al. (2012) observed better germination of *D. nobile* in PGR-free medium (46.47–49.47 % germination): when BA or GA_3 was added, germination percentage decreased to 1.68 and 9.06 %, respectively. The optimum medium composition for germination of *D. candidum* was VW basal medium containing 0.18 mg/l NAA, 0.53 mg/l BA, 0.28 mg/l GA_3, 20 g/l sucrose, 100 g/l banana homogenate (BH) and 10 g/l AC (Du et al. 2007). *D. chrysotoxum* seeds germinated on Mitra medium supplemented with AC (0.4 %), BA (2 mg/l) and IAA (2 mg/l) showed enhanced germination (as much as 98.10 %) compared to PGR-free medium (82.4 %) (Nongdam and Tikendra 2014). Hossain (2013) noted that MS medium was more efficient for germination of *D. aggregatum* seeds than Phytamax™ (PM; Sigma Chemical Co., USA) medium and the addition of 2.0 mg/l BA and 1.0 mg/l NAA caused the profuse development of secondary protocorms from primary protocorms; the most effective medium for plantlet formation was MS containing 0.5 mg/l IAA. In contrast to these results, Kabir et al. (2013) noted that in 100 % of flasks, *D. fimbriatum* Hook. seeds germinated using PM culture medium (more effective than MS or modified VW), although further seedling elongation was achieved when liquid MS medium was supplemented with 2.0 mg/l BA and 0.01 mg/l IBA. Shoots, on the other hand, were induced in the presence of 1.0 mg/l BA and 0.5 mg/l picloram, while ½ MS supplemented with 1.0 mg/l IAA was most suitable for effective induction and growth of adventitious roots.

Organic and inorganic additives

Dendrobium seed germination and protocorm development are stimulated or inhibited by organic amendments including coconut water (CW; Fig. 2a, b), apple homogenate (AH), BH, or potato homogenate (PH) (Lo et al. 2004a; Zhan et al. 2010; Gong et al. 2015). Su et al. (2012) observed that a simple culture medium using 3 g/l of commercial fertilizer (8 % nitrogen, 9 % P_2O_5, 9 % K_2O) and banana paste/pulp (BP) (6 % w/v) used for in vitro *D. nobile* cultivation resulted in taller plantlets (8.06 vs 6.65 cm in the control), but with fewer leaves (6.84 vs 7.10 in the control) and roots (8.86 vs 7.10 in the control), greater dry weight (0.81 vs 0.58 g in the control) and a longer main root (5.48 vs 4.22 cm in the control), when compared to ½ MS culture medium with BP (6 % w/v). Song et al. (1999) also observed that 6 % BP increased the in vitro growth of *D. nobile* plantlets. Ngampanya and Homlaaor (2010) also reported that the addition of BP and CW promoted seed germination of *Dendrobium* sp.

A popular complex mixture used for *Dendrobium* orchid cultivation in vitro is CW (Fig. 5). Soares et al. (2013) used a complex culture medium consisting of a mixture of 70 g/l tomato, 50 g/l BP, 3 ml of commercial fertilizer (containing 10 % N, 10 % P_2O_5, and 10 % K_2O), 25 g/l commercial sugar, 3 g/l AC and 17 g/l of agar, at pH 5.0. To that medium, 0, 50, 100, 150 or 200 ml/l of CW was added. Highest number of pseudobulbs (2.5/plantlet), leaves (6.9/plantlet) and roots (5.6/plantlet), height of plantlets (2.2 cm), shoot fresh weight (0.5 g), and root length (5.4 cm) was observed when 20 % (v/v) of CW was used compared to the control (1.87/plantlet, 4.74/plantlet and 4.10/plantlet; 1.37 cm, 0.24 g and 4.57 cm). Half MS medium supplemented with 20 % potato extract resulted in highest germination of *D. candidum* (Tang et al. 2005). Zeng et al. (1998) reported 10 % CW (v/v) to be the best for seed germination of *D. wangliangii* G. W. Hu, C. L. Long and X. H. Jin, *D. candidum*, *D. densiflorum* Lindl., *D. fimbriatum* and *D. loddigesii* Rolfe. Similar conclusions were made by Soares et al. (2013) using 20 % CW (v/v) for *D. nobile* and by Vijayakumar et al. (2012) using 15 % CW (v/v) for *D. aggregatum*. After shoot emergence, adding potato juice, green-bean seedling juice or banana juice can improve the growth of plantlets, although banana juice was

observed to be the best for rooting (Ye et al. 1988; Ding 2004; Lo et al. 2004b; Yang et al. 2006; Kong et al. 2007; Sun et al. 2009; Vyas et al. 2009; Qian et al. 2013). Lo et al. (2004a, b) reported that full-strength MS basal medium supplemented with 8 % BH, 8 % PH, or 8 % CW improved seedling growth of *D. tosaense*, *D. moniliforme* (L.) Sw., and *D. linawianum* Rchb.f. than when ½ MS medium with 3 % sucrose was used.

Kananont et al. (2010) reported that the responses of seed germination and protocorm formation to chitosan were dependent on the species and developmental stage. All six tested types of chitosan polymers or oligomers formed with 70, 80 or 90 % deacetylation (P70, P80, P90, O70, O80 and O90), and five concentrations (0, 10, 20, 40 and 80 mg/l) significantly enhanced the proportion of *D. formosum* seeds (90.6 and 91.2 %) that germinated compared with the control (67.8 %). In contrast, only 10 mg/l of O70 or P80 chitosan resulted in enhanced seed germination (15.0 % and 13.7 %) compared to the control (10.5 %). Further protocorm growth of *D. bigibbum* var. *compactum* was significantly improved on chitosan at 10 mg/l, except for O90, whilst 10 or 20 mg/l of P70 chitosan enhanced the growth of *D. formosum* protocorms the most.

Agar can be replaced in part by other types of gelling agents. Soares et al. (2014) observed that the use of 7 g/l agar + 7 g/l corn starch resulted in better development of *D. nobile* shoots and roots, resulting in a 188, 156 and 177 % increase in the number of leaves, roots and fresh weight, respectively, compared with 14 g/l of agar for control seedlings.

Results regarding the use of activated charcoal (AC) are contradictory. Galdiano-Júnior et al. (2011) used MS basal medium with half the concentration of macronutrients (½ MS), 2 % sucrose, 7 g/l agar and pH 5.7, and tested 0, 1 and 2 g/l of AC applied to the in vitro culture (from seed germination to the first subculture of seedlings) of *D. nobile* seedlings for 180 days. They observed that AC, independent of the concentration, reduced the number of roots (29.7 %), length of the main root (47.0 %), number of leaves (13.6 %), total fresh (27.8 %) and dry weight (33.3 %) when compared with culture medium without AC. However, many protocols used AC. For example, Faria et al. (2004a) used ½ MS supplemented with 1 g/l AC and 7 g/l agar for germination of 20 crosses and self-pollinated seed. The same culture medium was used by Lone et al. (2008) for the germination of seeds resulting from several *D. phalaenopsis* crosses.

Abiotic factors

The quality, quantity and periodicity of light are another set of factors that can significantly influence seed germination (Lin et al. 2011; Zeng et al. 2012; Parthibhan et al. 2012). In most studies, *Dendrobium* seed germination was possible at a temperature ranging from 22 to 28 °C (Fig. 6; Faria et al. 2004a; Lone et al. 2008; de Moraes et al. 2010; Galdiano-Júnior et al. 2011; Su et al. 2012; Soares et al. 2013) with a 9–16-h photoperiod under cool white fluorescent tubes (30.77 % of papers) in which the photosynthetic photon flux density (PPFD) was 10–60 μmol m^{-2} s^{-1} (Fig. 6; Xu and Yu 1984; Kong et al. 2007; Kananont et al. 2010; Table 1). Sometimes seed germinated in the dark (Meng et al. 2012), or at different intensities and quality of illumination [fluorescent white light; red light-emitting diodes (LEDs); blue LEDs; half red plus half blue (R:B = 1:1) LEDs; 67 % red plus 33 % blue (R:B = 2:1) LEDs; and 33 % red plus 67 % blue (R:B = 1:2) LEDs] at different developmental stages (Lin et al. 2011), or high PPFD (60 μmol m^{-2} s^{-1} by Kaewduangta and Reamkatog 2011 and Hossain et al. 2013b, or 150 μmol m^{-2} s^{-1} by Dutta et al. 2011). Ali et al. (2011) observed that continuous light resulted in highest seed germination of *D. tetrachromum* Rchb.f. (100 vs 48.9 % for a 16-h photoperiod) and *D. hamaticalcar* (97.5 vs 29.3 % for a 16-h photoperiod). de Moraes et al. (2010) used a 12-h photoperiod with a PPFD of 40 μmol m^{-2} s^{-1} for 180 days for seed germination of *D. nobile*. In fact, 43.4 % of papers used a 12-h photoperiod for the germination stage of *Dendrobium* species (Table 4). Early stages of germination were uniform from 0- to 24-h photoperiod in *D. aqueum*, but final germination and seedling production differed depending on photoperiod: increasing photoperiod from 8 to 24 h improved germination and seedling development from 48.5 to 96.0 % on ½ MS medium, but seeds germinated under continuous darkness showed lower germination (31.4 %), and seedlings failed to grow (Parthibhan et al. 2012). Soares et al. (2013) also used a 12-h photoperiod to germinate *D. nobile* seeds, but a low PPFD (13.5 μmol m^{-2} s^{-1}). Galdiano-Júnior et al. (2011) used 75 μmol m^{-2} s^{-1} and a 16-h photoperiod for 90 days, obtaining *D. nobile* plants approximately 0.5 cm long and with two leaves after the subculture of seedlings germinated from seeds. Su et al. (2012) used a 16-h photoperiod with a PPFD of 17.55 μmol m^{-2} s^{-1} for 180 days during in vitro cultivation of *Dendrobium* (species not defined). Faria et al. (2004a) also used a 16-h photoperiod for *D. nobile* but PPFD was undefined, while Lone et al. (2008) used a PPFD of 27 μmol m^{-2} s^{-1} for *D. phalaenopsis*. Zhao et al. (2013a) observed that 92 % of *D. wangliangii* seeds germinated under a 16-h photoperiod and a PPFD of 36 μmol m^{-2} s^{-1} but seeds cultured under continuous darkness died, even after transfer from dark to light. Initial incubation of *D. huoshanense* seeds in the dark for 22 days, followed by transfer to low light intensity (1700 lux = 23 μmol m^{-2} s^{-1}), resulted in 91 % of germinated seeds (Yang and Wang 1989).

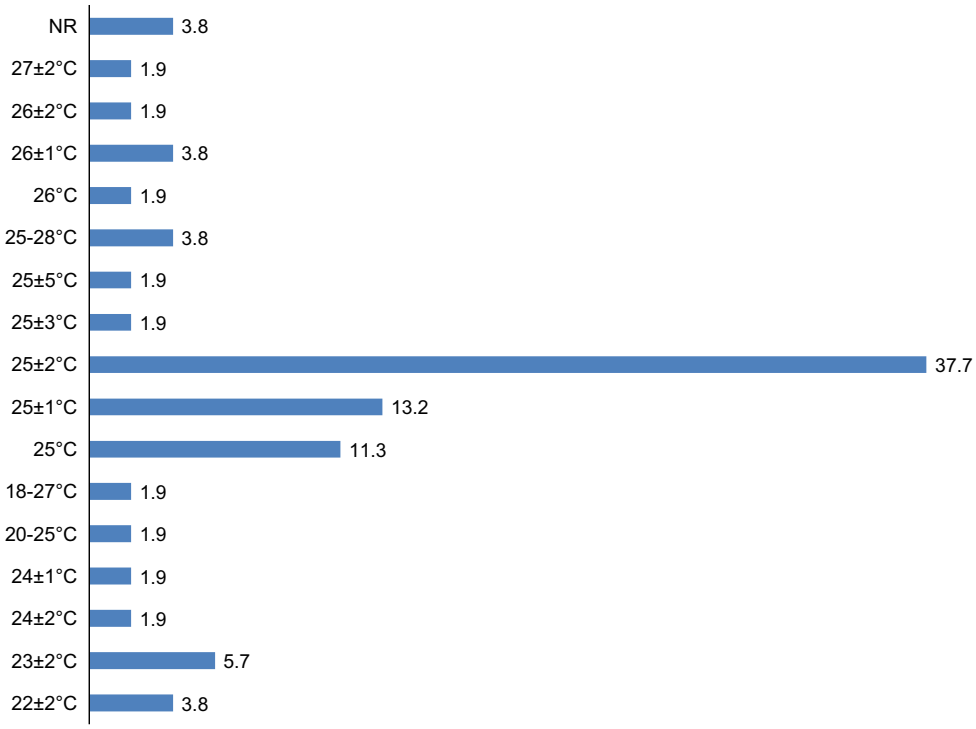

Fig. 6 Temperatures or range of temperatures used in papers on the asymbiotic germination of *Dendrobium* species, reported as percentages. Some temperature classes were joined as they lie within a defined range. *NR* not reported

Table 4 Light intensity and photoperiod used in papers related to in vitro asymbiotic seed germination of *Dendrobium* species

Light intensity	Photoperiod (h)											Papers used	% papers used[a]
	Darkness	Darkness + 16 h	<12	10–14	12	14	12–16	16	18	24	NR		
Darkness	1											1	1.9
300–500 lux					1							1	1.9
1000 lux					1							1	1.9
1000–2200 lux			3		3	1						7	13.2
1500–3000 lux							1					1	1.9
2000–3000 lux			1					1				2	3.8
2000 lux			1		4	1						6	11.3
3000 lux								1				1	1.9
6000 lux									1			1	1.9
30 µmol m^{-2} s^{-1}					1					1		2	3.8
20–40 µmol m^{-2} s^{-1}					2							2	3.8
30–42 µmol m^{-2} s^{-1}		1		1	4			1				7	13.2
40 µmol m^{-2} s^{-1}								3				3	5.7
40 ± 10 µmol m^{-2} s^{-1}					1							1	1.9
50 µmol m^{-2} s^{-1}		1			2							3	5.7
60 µmol m^{-2} s^{-1}					4	2						6	11.3
150 µmol m^{-2} s^{-1}								1				1	1.9
NR			1			1					5	7	13.2
Papers used	1	2	6	1	23	5	1	7	1	1	5		
% papers used[a]	1.9	3.8	11.3	1.9	43.4	9.4	1.9	13.2	1.9	1.9	9.4		

[a] 30.2 % of papers used white fluorescent lamps and 69.8 % did not report the source of light; the values of light intensity were presented in µmol m^{-2} s^{-1} (photosynthetic photon flux density) and in lux separately because in most cases the source of light was not reported (NR)

Other factors

Culture vessels

Orchid seeds germinate better in flasks than in culture tubes due to a greater volume of air (6- to 25-fold more) and thereby CO_2 diffusion is greater when compared to culture tubes (Knudson 1922). Research on the influence of culture vessels or flask size with respect to orchid seed germination in vitro is still scant (Buffa Filho et al. 2002). de Moraes et al. (2010) tested the effect of flask size (100, 200 and 400 ml) on seedling growth. They observed that greatest height (2.24 cm), number of roots (2.41) and leaves (1.95), fresh (0.36 g) and dry (0.11 g) weight were obtained using 100-ml flasks, but seed germination *per se* was not tested, or quantified. Galdiano-Júnior et al. (2011) used 220-ml plastic flasks with 30 ml of culture medium. Some authors used 250-ml borosilicate flasks with 50 ml of culture medium for *D. nobile* (Faria et al. 2004a, b; Su et al. 2012) and *D. phalaenopsis* (Lone et al. 2008). Soares et al. (2013) used 600-ml flasks containing 80 ml of culture medium. These culture vessels were used for both seed germination and seedling growth.

Explant age

Normally, after pollination, about 100–140 days are required for *Dendrobium* seed to mature (Nimoto and Sagawa 1961). However, green pod culture or immature seed germination involves the use of green, undehisced capsules, which are harvested 70 days after pollination, opened and seeds are inoculated on culture media (Soundararajan 2009; Table 1). The time required for seed germination ranges from 30 to 59 days (Alam et al. 2002; Sunitibala and Kishor 2009; Parthibhan et al. 2012). Green pod seeds grown on Robert Ernst medium (Ernst 1982), KP medium (Knop 1865), KC medium, Curtis medium (Curtis 1936) and Pfeffer (Harvais 1972) medium became pale green or whitish green and eventually produced weak seedlings but on VW medium, KC medium, Thomale GD medium (Thomale 1954), Mitra et al. medium (Mitra et al. 1976) and Wolter and Skoog medium (WS; Wolter 1968) the seeds became brown and could not germinate. However, the addition of MS vitamins in WS medium resulted in comparatively better germination and seedling growth (e.g., Thomale GD and RE medium resulted in 67.7 and 48.4 % germination of *D. aqueum* seeds, whereas higher germination (71.9 and 58.0 %) and seedling growth was observed on the same medium when supplemented with MS vitamins) (Parthibhan et al. 2012; unpublished). Sharma et al. (2005) used VW medium to germinate 80–90 % of immature *D. fimbriatum* seeds from 70-day-old green, undehisced fruits which developed protocorms and later shoots, but only when 15 % CW and 0.1 mg/l NAA were added to the medium. Vijayakumar et al. (2012) also used immature seed germination via green pod culture for *D. aggregatum*, and observed that MS culture medium with 3 % sucrose, 1.5 mg/l BA and 15 % CW resulted in best germination, protocorm and shoot production (75 shoots/flask). Kumar et al. (2006) used KC green pod culture 70 days after pollination of *D. chrysanthum* and obtained 80–90 % seed germination, but that study was invalidated following a retraction.

Conclusions and future perspectives

Experiments and reviews on various factors that influence seed germination and seedling development in orchids, especially in *Dendrobium*, have been reported (Arditti 1967; Lo et al. 2004a, b; Saiprasad et al. 2004; Kauth et al. 2008; Mweetwa et al. 2008; Chugh et al. 2009; Ferreira et al. 2011). Moreover, nutritional and environmental conditions are species-specific. The environmental factors such as light exposure (photoperiod, light vs dark) and temperature will also influence seed germination both in nature and in vitro (Rasmussen 1995).

Asymbiotic germination of *Dendrobium* species is possible because of the large quantity of seeds produced per pod and the high rate of seed germinated in vitro. Nevertheless, abiotic conditions that are required for symbiotic *Dendrobium* seed germination are not unified and are often species-specific (Zhao et al. 2013b) and the use of established in vitro conditions provides a better environment for controlled asymbiotic seed growth and development. This would also allow for the identification of diverse factors and compounds that define successful germination and subsequent propagation of seedlings. Moreover, germinating seeds together with a species-specific mycorrhizal fungus could improve the success of seed-based conservation programs (Teixeira da Silva et al. 2015b), both in in situ germplasm conservation and in reintroduction efforts (Keel et al. 2011).

A meta-analysis of the *Dendrobium* asymbiotic seed germination literature reveals that 37 *Dendrobium* genotypes (including species and hybrids) were used in studies of asymbiotic germination, the most frequent being *D. candidum* (*D. officinale*) (Table 2). Mature seeds are usually used to start *Dendrobium* in vitro germination (Table 2). EtOH and $HgCl_2$, EtOH and NaOCl, and EtOH and flaming were the most frequently used procedures for seeds and fruit sterilization (Table 3). MS and ½ MS were the most common basal culture medium (Fig. 3). Most papers used no PGRs or additives in basal media (Figs. 4, 5). As abiotic factors, 25 ± 2 °C as temperature (Fig. 6), a 12-h photoperiod, 1000–2200 lux, and 30–42 μmol m^{-2} s^{-1} light

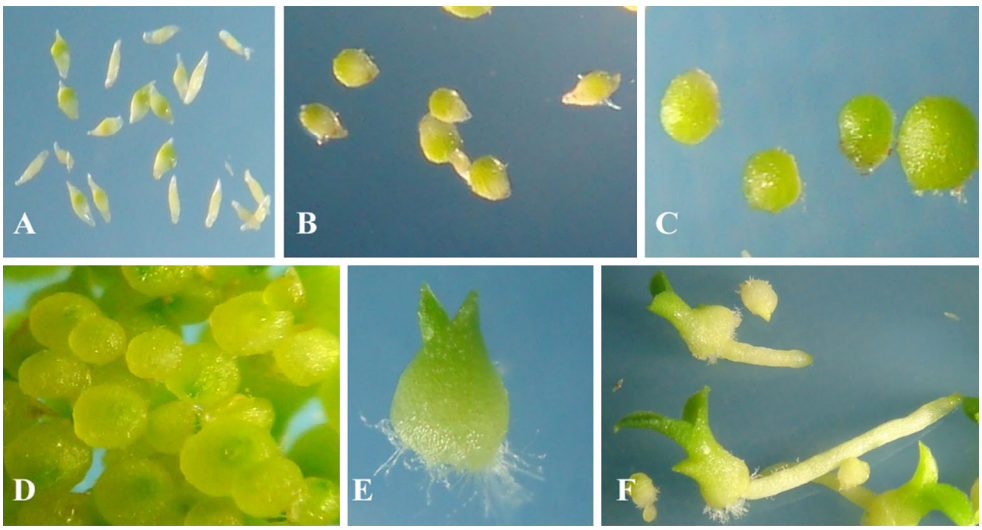

Fig. 7 Asymbiotic seed germination stages in *Dendrobium aqueum* (Lindley) cultured on half-strength (macronutrients) MS medium (devoid of PGRs) under 16-h photoperiod at 23 ± 2 °C (more details in Parthibhan et al. 2012). **a** Imbibed, enlarged seeds (zygotic embryos) with testa, turned into *green* (photosynthetic) ×20 after 15 days of culture. **b** Enlarged seeds (zygotic embryos) with half-ruptured testa after 35 days of culture. **c** Enlarged seeds (zygotic embryos) without a testa after 57 days after culture. **d** Protocorms with *green pointed* shoots 75 days after culture. **e** Protocorms with developed shoots and rhizoids after 107 days after culture. **f** Seedlings with shoots and developed roots after 126 days after culture. Unpublished photos (Parthibhan et al.)

intensity (white fluorescent lamps) were reported as the most favorable conditions (Table 4). The application of optimized conditions leads to the successful development of seeds through an established set of phases (Fig. 7).

This review may serve as a detailed handbook, summarizing the studies on *Dendrobium* asymbiotic germination; thus being useful for both experienced researches and *Dendrobium* amateurs, since there are no ideal parameters for germination of diverse species and hybrids of the genus *Dendrobium*.

Author contribution statement All authors contributed equally to all aspects related to the manuscript.

Acknowledgments The authors thank Dr. Meesawat Upatham (Prince of Songkla University, Thailand) for comments and opinions on an earlier version of the manuscript.

Conflict of interest The authors declare that they have no conflicts of interest.

References

Alam MK, Rashid MH, Hossain MS, Salam MA, Rouf MA (2002) *In vitro* seed propagation of dendrobium (*Dendrobium transparens*) orchid as influenced by different media. Biotechnol 1:111–115

Ali MJ, Murdad R, Latip MA (2011) *In vitro* seed germination of Bornean endemic orchids *Dendrobium tetrachromum* and *Dendrobium hamaticalcar*. Empower Sci Tech Innov: Towards Better Tomorrow, Proceedings of UMTAS 2011. Universiti Malaysia Sabah, Malaysia, pp 770–778

Alvarez-Pardo VM, Ferreira AG, Nunes VF (2006) Seed disinfestation methods for *in vitro* cultivation of epiphyte orchids from Southern Brazil. Hortic Bras 24:217–220

Arditti J (1967) Factors affecting the germination of orchid seeds. Bot Rev 33:1–97

Arditti J (1979) Aspects of the physiology of orchids. Adv Bot Res 7:421–655

Atichart P, Bunnag S, Theerakulpisut P (2007) *Agrobacterium*-mediated transformation of *Dendrobium secundum* (Bl.) Lindl. with antisense ACC oxidase. Asian J Plant Sci 6:1065–1071

Bernard N (1909) L'evolution dans la symbiose, les Orchidées et leur champignons commensaux. Ann Sci Nat Bot 9:1–196

Buffa Filho W, Pereira AMS, França SC, Furla-ni M (2002) Indução de metabólitos bioativos em culturas de células de *Maytenus ilicifolia*. Eclética Química 27:403–416 **(in Portuguese with English abstract)**

Bulpitt CJ (2005) The uses and misuses of orchids in medicine. Q J Med 98:625–631

Bulpitt CJ, Li Y, Bulpitt PF, Wang J (2007) The use of orchids in Chinese medicine. J R Soc Med 100:558–563

Campos DM (2002) *Orquídeas*: manual prático de cultura. 3rd edn. Expressão e Cultura, Rio de Janeiro, p 143 **(in Portuguese)**

Cardoso JC (2012) *Dendrobium* 'Brazilian Fire 101'—new option of color of flowers for the orchid market. Hortic Bras 30:561–564

Chattopadhyay P, Banerjee N, Chaudhary B (2012) Genetic characterization of selected medicinal *Dendrobium* (Orchidaceae) species using molecular markers. Res J Biol 2:117–125

Chen ZL, Zeng SJ, Duan J (2004) *In vitro* propagation of *Dendrobium unicum*. Plant Physiol Commun 40:342 **(in Chinese with English abstract)**

Chu CC, Wang CC, Sun CS, Hsu C, Yin KC, Chu CY (1975) Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci Sin 18:659–668

Chugh S, Guha S, Rao IU (2009) Micropropagation of orchids: a review on the potential of different explants. Sci Hortic 122:507–520

Cui QH, Sun YY, Duan ML, Li K, Huang CX, Yan H (2012) Optimization of sterile germination condition for *Dendrobium devonianum*. J Northwest Forest Univ 27:106–110 **(in Chinese with English abstract)**

Curtis J (1936) For native orchids in culture. Am Orchid Soc Bull 5:42–47

de Moraes CP, Diogo JA, Canabrava RI, Pedro NP, Furtado ALFF, Marteline MA (2010) Desenvolvimento *in vitro* de *Dendrobium nobile* Lind. (Orchidaceae) em recipientes de diferentes volumes. R Bras Bioci Porto Alegre 8(2):225–228 **(in Portuguese with English abstract)**

Ding CC (2004) Study on tissue culture and rapid propagation technic of *Dendrobium devonianum*. Trop Agric Sci Technol 27:10–11 **(in Chinese with English abstract)**

Dohling S, Kumaria S, Tandon P (2008) Optimization of nutrient requirement for asymbiotic seed germination of *Dendrobium longicornu* Lindl. and *D. formosum* Roxb. Proc Indian Natl Sci Acad 74(4):167–171

Dressler RL (2005) How many orchid species? Selbyana 26:155–158

Du G, Yang HY, Zhu SL, Zhang GQ (2007) Experiment on seeding induction from seed of *Dendrobium candidum*. J Chin Med Mater 30:1207–1208 **(in Chinese with English abstract)**

Du G, Lai TC, Yang HY (2012) Study on the tissue culture of *Dendrobium aphyllum* (Roxb) C. E. C. Fisch. North Hort 8:140–141 **(in Chinese with English abstract)**

Dutta S, Chowdhurry A, Bhattacharjee B, Nath PK, Dutta BK (2011) *In vitro* multiplication and protocorm development of *Dendrobium aphyllum* (Roxb.) CEC Fisher. Assam Univ J Sci Technol Biol Env Sci 7:57–62

Ernst R (1982) *Paphiopedilum*. In: Arditti J (ed) Orchid Biology: Reviews and Perspectives, vol II., Cornell University Press-Ithaca, New York, pp 350–353

Faria RT, Rodrigues FN, Oliveira LVR, Müller C (2004a) *In vitro Dendrobium nobile* plant growth and rooting in different sucrose concentrations. Hortic Bras 22:780–783 **(in Portuguese with English abstract)**

Faria RT, Vicente APRM, Costa TMM, Fonseca ICB, Silva GL, Takahashi LSA (2004b) Seleção de genótipos de *Dendrobium* (Orchidaceae) na fase de propagação *in vitro*. Semina: Ciências Agrárias 25:309–314 **(in Portuguese with English abstract)**

Fay MF, Chase MW (2009) Orchid biology: from Linnaeus via Darwin to the 21st century. Ann Bot 104:359–364

Ferreira WM, Suzuki RM, Pescador R, Figueiredo-Ribeiro RCL, Kerbauy G (2011) Propagation, growth, and carbohydrates of *Dendrobium* Second Love (Orchidaceae) *in vitro* as affected by sucrose, light, and dark. In Vitro Cell Dev Biol Plant 47:420–427

Fu CM, Zhao ZG, Huang NZ, He JX, Tang FL, Shi YP (2012) Study on technology of aseptic sowing and rapid propagation of *Dendrobium officinale*. Guihaia 32:238–242

Galdiano-Júnior RF, Neto PC, Mantovani C (2011) Crescimento *in vitro* de *Dendrobium nobile* Lindley com adição de carvão ativado. Científica 40:28–34 **(in Portuguese with English abstract)**

Gamborg OL, Miller RA, Ojima K (1968) Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50:151–158

Gong JY, Wang HX, Chen BL, Chen E, Li YX, Wang ZN (2015) Asymbiotic in vitro seed germination of three *Dendrobium* species and capsule characteristics observation. Jiangsu Agric Sci 43(1):184–186 **(in Chinese)**

Gu S, Ding XY, Wang Y, Zhou Q, Ding G, Li XX, Qian L (2007) Isolation and characterization of microsatellite of markers in *Dendrobium officinale*, an endangered herb endemic to China. Mol Ecol Notes 7:1166–1168

Guo S, Xu J (1990) Effects of fungi and its liquid extract on seed germination of *Dendrobium hancockii* Rolf. J Chin Mat Med 15(7397–7399):445 **(in Chinese with English abstract)**

Hajong S, Kumaria S, Tandon P (2010) *In vitro* propagation of the medicinal orchid *Dendrobium chrysanthum*. Proc Indian Natl Sci Acad 76:1–6

Harvais G (1972) The development and growth requirements of *Dactylorhiza purpurella* in asymbiotic culture. Can J Bot 50:1223

Hazarika RB, Sarma CM (1995) *In vitro* germination and regeneration of *Dendrobium transparens* Lindl. J Ochid Soc India 9:51–54

Hossain MM (2013) *In vitro* embryo morphogenesis and micropropagation of *Dendrobium aggregatum* Roxb. Plant Tissue Cult Biotech 23:241–249

Hossain MM, Kant R, Van PT, Winarto B, Zeng SJ, Teixeira da Silva JA (2013a) The application of biotechnology to orchids. Crit Rev Plant Sci 32:69–139

Hossain MM, Sharma M, Pathak P (2013b) In vitro propagation of *Dendrobium aphyllum* (Orchidaceae)—seed germination to flowering. J Plant Biochem Biotechnol 22:157–167

Hu Z, He JB (1979) Sterile germination and *in vitro* rapid propagation of *Dendrobium tosaense*. Plant J 3:6–7 **(in Chinese)**

Huo LL, Pan HT, Zhang QX (2010) Asepsis sowing and rapid propagation of *Dendrobium pendulum* Roxb. Plant Physiol Commun 46:1269–1270 **(in Chinese)**

Kabir MF, Rahman MS, Jamal A, Rahman M, Khalekuzzaman M (2013) Multiple shoot regeneration in *Dendrobium fimbriatum* hook an ornamental orchid. J Anim Plant Sci 23:1140–1145

Kaewduangta W, Reamkatog P (2011) Effect of modification medium on growth development of *Dendrobium parishii in vitro*. Am Eurasian J Agric Environ Sci 11:117–121

Kamemoto H, Kuehnle AR, Amore TD (1999) Breeding *Dendrobium* orchids in Hawaii. University of Hawaii Press, Honolulu

Kananont N, Pichyangkura R, Chanpramed S, Chadchawan S, Limpanavech P (2010) Chitosan specificity for the *in vitro* seed germination of two *Dendrobium* orchids (Asparagales: Orchidaceae). Sci Hortic 124:239–247

Kauth PJ, Dutra D, Johnson TR, Stewart SL, Kane ME, Vendrame W (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. 1st edn. vol V., Global Science Books, Ltd., Isleworth, Chapter 45, pp 375–391

Keel BG, Zettler LW, Kaplin BA (2011) Seed germination of *Habenaria repens* (Orchidaceae) *in situ* beyond its range, and its potential for assisted migration imposed by climate change. Castanea 76:43–54

Knop W (1865) Quantitative Untersuchungen uber die Ernahrungsprozesse der Pflanzen. Landwirtsch Vers Stn 7:93–107

Knudson L (1921) La germinación simbiótica de las semillas de orquideas. Bol Real Soc Española Hist Nat 21:250–260

Knudson L (1922) Nonsymbiotic germination of orchid seeds. Bot Gazette 73:1–25

Kong Q, Yuan SY, Gy Végvári (2007) Micropropogation of an orchid *Dendrobium strongylanthum* Rchb.f. Int J Hortic Sci 13:61–64

Kuehnle AR (2007) Orchids: *Dendrobium*. In: Anderson NO (ed) Flower breeding and genetics. Springer, pp. 539–560

Kumar K, Majumdar S, Sharma R, Sharma B (2006) Green pod culture and rapid micropropagation of *Dendrobium chrysanthum* Wall—a horticultural and medicinal orchid. Folia Hortic 18:81–90 (Retracted; http://www.degruyter.com/view/j/fhort.2013.25.issue-2/fhort-2013-0019/fhort-2013-0019.xml?format=INT)

Kumar P, Rawat GS, Wood HP (2011) Diversity and ecology of Dendrobiums (Orchidaceae) in Chotanagpur plateau, India. Taiwania 56:23–36

Lan YT, Liu SY, Luo YT, Huang L, Li ZL, Wei XL (2010) Sterile germination of *Dendrobium chrysotoxum* seeds and rapid propagation of its plantlets. Agric Sci Technol 11:89–91 **(in Chinese with English abstract)**

Li C, Zhang ZJ, Wei Y, Li LX, Ling ZZ, Wei KH (2013) Study on tissue culture and rapid propagation of *Dendrobium fimbriatum* Hook. var. *oculatum* Hook. North Hortic 6:105–107 **(in Chinese with English abstract)**

Lin Y, Li J, Li B, He T, Chun Z (2011) Effects of light quality on growth and development of protocorm-like bodies of *Dendrobium officinale* in vitro. Plant Cell Tiss Organ Cult 105:329–335

Liu SQ (2008) Tissue culture and rapid propagation of *Dendrobium henryi*. Subtrop Plant Sci 37(4):57–58 **(in Chinese without English abstract)**

Liu ZJ, Zhang YT, Wang Y, Huang QH, Chen XQ, Chen LQ (2011) Recent developments in the study of rapid propagation of *Dendrobium catenatum* Lindl. with a discussion on its scientific and Chinese names. Plant Sci J 29:763–772 **(in Chinese with English abstract)**

Lo SF, Nalawade SM, Kuo CL, Chen CL, Tsay HS (2004a) Asymbiotic germination of immature seeds, plantlet development and *ex vitro* establishment of plants of *Dendrobium tosaense* Makino—a medicinally important orchid. In Vitro Cell Dev Biol Plant 40:528–535

Lo SF, Nalawade SM, Mulabagal V, Mathew S, Chen CL, Kuo CL, Tsay HS (2004b) *In vitro* propagation by asymbiotic seed germination and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity studies of tissue culture raised plants of three medicinally important species of *Dendrobium*. Biol Pharm Bull 27:731–735

Lone AB, Barbosa CM, Faria RT, Takahashi LSA, Fonseca ICB (2008) Seleção de genótipos de *Dendrobium phalaenopsis* (Orchidaceae) nas fases de propagação in vitro e aclimatização. Semina: Ciências Agrárias 29:755–760 **(in Portuguese with English abstract)**

Luo JP, Wawrosch C, Kopp B (2009) Enhanced micropropagation of *Dendrobium huoshanense* C.Z. Tang et S.J. Cheng through protocorm-like bodies: the effects of cytokinins, carbohydrate sources and cold pretreatment. Sci Hortic 123:258–262

Luo XQ, Wu MK, Shen G, Zhang XB (2013) Guizhou Karst areas *Dendrobium officinale* re-introduction conservation and sustainable utilization. Chin Wild Plant Resour 32(6):47–50 **(in Chinese with an English abstract)**

Manz B, Müller K, Kucera B, Volke F, Leubner-Metzger G (2005) Water uptake and distribution in germinating tobacco seeds investigated in vivo by nuclear magnetic resonance imaging. Plant Physiol 138:1538–1551

McKendrick S (2000) *In vitro* germination of orchids: a manual. Ceiba Foundation for Tropical Conservation. www.ceiba.org/documents/CFTCpropman.doc. Accessed 12 June 2015

Meng Y, Liu CH, Kong LJ, Zhang XY, Yang BY (2012) Sterile seeding and rapid propagation of *Dendrobium shixingense*. J Nanchang Univ (Nat Sci) 36(4):385–388 **(in Chinese with English abstract)**

Mitra GC, Prasad RN, Choudhury RA (1976) Inorganic salts and differentiation of protocorms in seed callus of orchid correlative changes in its free amino acid content. Indian J Exp Biol 14:350–351

Mo ZZ, Ling ZH (2007) Effects of exogenous hormones on seed germination and protocorm multiplication of *Dendrobium longicornu* Lind. J Anhui Agri Sci 35:9568–9570 **(in Chinese with English abstract)**

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15:473–497

Mweetwa AM, Welbaum GE, Tay D (2008) Effects of development, temperature, and calcium hypochlorite treatment on *in vitro* germinability of *Phalaenopsis* seeds. Sci Hortic 117:257–262

Nambiar N, Tee CS, Maziah M (2012) Effects of organic additivies [sic] and different carbohydrate sources on proliferation of protocorm-like bodies in *Dendrobium* Alya Pink. Plant Omics J 5:10–18

Ng TB, Liu JY, Wong JH, Ye XJ, Sze SCW, Tong Y, Zhang KY (2012) Review of research on *Dendrobium*, a prized folk medicine. Appl Microbiol Biotechnol 93:1795–1803

Ngampanya B, Homlaaor W (2010) Simple media for *Dendrobium* orchid seed germination and protocorm development. Acta Hortic 878:219–224

Nimoto DH, Sagawa Y (1961) Ovule development in *Dendrobium*. Amer Orchid Soc Bull 30:813–819

Nongdam P, Tikendra L (2014) Establishment of an efficient in vitro regeneration protocol for rapid and mass propagation of *Dendrobium chrysotoxum* Lindl. using seed culture. Sci World J 2014:740150

Parthibhan S, Franklin BJH, Muthukumar M, Sherif NA, Senthil Kumar T, Rao MV (2012) Influence of nutritional media and photoperiods on *in vitro* asymbiotic seed germination and seedling development of *Dendrobium aqueum* Lindley. African J Plant Sci 6:383–393

Paul S, Kumaria S, Tandon P (2012) An effective nutrient medium for asymbiotic seed germination and large-scale *in vitro* regeneration of *Dendrobium hookerianum*, a threatened orchid of northeast India. AoB Plants 2012:plr032

Qian WL, Zhang JX, Wu KL, Zeng SJ (2013) Study on propagation and cultivation technique of *Dendrobium huoshanense* seedlings. J Trop Subtrop Bot 21:240–246 **(in Chinese with English abstract)**

Rasmussen HN (1995) Terrestrial Orchids: From Seed to Mycotrophic Plant. Cambridge University Press, Cambridge, p 460

Roberts DL, Dixon KW (2008) Orchids. Curr Biol 18:R325–R329

Saiprasad GVS, Raghuveer P, Khetarpal S, Chandra R (2004) Effect of various polyamines on production of protocorm-like bodies in orchid—*Dendrobium* 'Sonia'. Sci Hortic 100:161–168

Sharma KKR, Sharma B, Majumdar S (2005) Micropropagation of *Dendrobium fimbriatum* Hook. by green pod culture. J Plant Biol 48:253–257

Singh A, Duggal S (2009) Medicinal orchids: an overview. Ethnobot Leaflets 13:351–363

Smith SE, Read DJ (2008) Mycorrhizal symbiosis, 3 edn. Academic Press/Elsevier, London

Soares JS, Rosa YBCJ, Suzuki RM, Scalon SPQ, Rosa Junior EJ (2012) Asymbiotic germination and development of *Dendrobium nobile* Lindl. under the effect of plant growth regulators in pre-germinative treatment. Rev Bras Plant Med 14:617–623 **(in Portuguese with English abstract)**

Soares JS, Rosa YBCJ, Suzuki RM, Scalon SPQ, Rosa Junior EJ (2013) Cultivo in vitro de *Dendrobium nobile* com uso de água de coco no meio de cultura. Hortic Bras 31:63–67 **(in Portuguese with English abstract)**

Soares JS, Rosa YBCJ, Sorgato JC, Rosa DBCJ, Pereira STS (2014) Utilização de agentes geleificantes alternativos no cultivo *in vitro* de *Dendrobium nobile* Lindl. Enciclopédia Biosfera 10:1832–1838 **(in Portuguese with English abstract)**

Song MKR, Silva GL, Faria RT, Takahashi LSA (1999) Análise do crescimento e enraizamento *in vitro* de híbridos de *Dendrobium nobile* Lindl. (Orchidaceae) semeados em diferentes meios de cultura. In: Congresso Brasileiro de Floricultura e Plantas Ornamentais, vol 12. *Anais* do Congresso Brasileiro de Floricultura e Plantas Ornamentais, Brasil, p 110 (**abstract**)

Song XQ, Guo N, Zhan XC, Nie L, Song QQ (2004) Research of non-symbiotic sproution of *Dendrobium nobile* Lindl and germplasm

storage. J Guizhou Normal Univ (Nat Sci) 22:13–16 **(in Chinese with English abstract)**

Soundararajan T (2009) Micropropagation studies on *Bacopa monnieri*, *Nerium oleander* and Bioreactor studies on micropropagation of *Dendrobium*, an orchid. PhD thesis, Bharath University, India, p 191. http://shodhganga.inflibnet.ac.in/handle/10603/51. Accessed 12 June 2015

Su MJ, Schnitzer JA, Faria RT (2012) Polpa de banana e fertilizantes comerciais no cultivo *in vitro* de orquídea. Científica 40:28–34 **(in Portuguese with English abstract)**

Sun YY, Li HA, Yan H, Li K (2009) Asepsis sowing and tissue culture of *Dendrobium devonianum* Paxt. Plant Physiol Commun 45:1017–1018 **(in Chinese)**

Sunitibala H, Kishor R (2009) Micropropagation of *Dendrobium transparens* L. from axenic pseudobulb segments. Indian J Biotech 8:448–452

Swangmaneecharern P, Serivichyaswat P, Nontachaiyapoom S (2012) Promoting effect of orchid mycorrhizal fungi *Epulorhiza* isolates on seed germination of *Dendrobium* orchids. Sci Hortic 148:55–58

Swarts ND, Dixon KW (2009) Terrestrial orchid conservation in the age of extinction. Ann Bot 104:543–556

Tang GX, Wang FD, Zhou WJ (2005) Studies on the seed embryo germination and propagation of *Dendrobium candidum* in vitro. China J Chinese Materia Medica 30:1583–1586 **(in Chinese with English abstract)**

Teixeira da Silva JA (2012) Is BA (6-benzyladenine) BAP (6-benzylaminopurine)? Asian Australas J Plant Sci Biotech 6:121–124

Teixeira da Silva JA (2013) Orchids: advances in tissue culture, genetics, phytochemistry and transgenic biotechnology. Floric Ornam Biotech 7:1–52

Teixeira da Silva JA, Aceto S, Liu W, Yu H, Kanno A (2014a) Genetic control of flower development, color and senescence of *Dendrobium* orchids. Sci Hortic 175:74–86

Teixeira da Silva JA, Zeng SJ, Dobránszki J, Cardoso JC, Kerbauy GB (2014b) In vitro flowering of *Dendrobium*. Plant Cell Tissue Organ Cult 119:447–456

Teixeira da Silva JA, Zeng SJ, Dobránszki J, Galdiano R Jr, Cardoso JC, Vendrame WA (2014c) Preservation of *Dendrobium* germplasm. Plant Cell Rep 33:1413–1423

Teixeira da Silva JA, Dobránszki J, Cardoso JC, Zeng SJ (2015a) Micropropagation of *Dendrobium*: a review. Plant Cell Rep 34:671–704

Teixeira da Silva JA, Tsavkelova E, Zeng SJ, Ng TB, Dobránszki J, Parthibhan S, Cardoso JC, Rao MV (2015b) Symbiotic in vitro seed propagation of *Dendrobium*: fungal and bacterial partners and their influence on plant growth and development. Planta 242:1–22

Thimijan RW, Heins RD (1983) Photometric, radiometric, and quantum light units of measure: a review of procedures for interconversion. HortScience 18:818–822

Thomale H (1954) *Die Orchideen*. Verlag Eugen Ulmer, Stuttgart

Udomdee W, Wen PJ, Lee CY, Chin SW, Chen FC (2014) Effect of sucrose concentration and seed maturity on in vitro germination of *Dendrobium nobile* hybrids. Plant Growth Regul 72:249–255

Vacin E, Went FW (1949) Some pH changes in nutrient solutions. Bot Gaz 110:605–613

Vasudevan R, Van Staden J (2010) Fruit harvesting time and corresponding morphological changes of seed integuments influence in vitro seed germination of *Dendrobium nobile* Lindl. Plant Growth Regul 60:237–246

Vendrame WA, Carvalho VS, Dias JMM, Maguire I (2008) Pollination of *Dendrobium* hybrids using cryopreserved pollen. HortScience 43:264–267

Vijayakumar S, Rajalkshmi G, Kalimuthu K (2012) Propagation of *Dendrobium aggregatum* through the culture of immature seeds from green capsules. Lankesteriana 12:131–135

Vyas S, Guha S, Bhattacharya M, Usha Rao I (2009) Rapid regeneration of plants of *Dendrobium lituiflorum* Lindl. (Orchidaceae) by using banana extract. Sci Hortic 121:32–37

Wang LH, Zhu YQ, Jiang YL, Feng YC (2007) Tissue culture and rapid propagation of *Dendrobium thyrsiflorum* Rchb. F. Plant Physiol Commun 43(5):894 **(in Chinese)**

Wolter KE (1968) Root and shoot initiation in aspen callus cultures. Nature 219:509–510

Wu HF, Song XQ, Liu HX (2012) *Ex-situ* symbiotic seed germination of *Dendrobium catenatum*. Acta Ecol Sin 32:2491–2497 **(in Chinese with English abstract)**

Xu YZ, Yu LW (1984) Sterile germination and *in vitro* propagation of *Dendrobium tosaense*. Plant Physiol Commun 4:35–36 **(in Chinese with English abstract)**

Yam TW, Arditti J (2009) History of orchid propagation: a mirror of the history of biotechnology. Plant Biotechnol Rep 3:1–56

Yang QG, Wang LA, Wang LZ (1989) Studies on in vitro culture of premature seeds of *Dendrobium huoshanense* Tang et Cheng. China J Chin Mater Med 14(3):19–20, 62 **(in Chinese with English abstract)**

Yang N, Chen ZL, Duan J, Zeng SJ (2006) *In vitro* propagation of *Dendrobium cochliodes* Schltr. Plant Physiol Commun 42(2):248 **(in Chinese with English abstract)**

Ye XL, Cheng SJ, Wang FX, Qian NF (1988) Morphology of immature seeds and development in *in vitro* of *Dendrobium candidum*. Acta Bot Yunnan 10:285–290 **(in Chinese with English abstract)**

Yu L, Lan QY, Tang GG (2011) Study on nonsymbiotic sproution of *Dendrobium fimbriatum* Hook. J Fujian Coll For 31:346–348 **(in Chinese with English abstract)**

Zeng SJ, Cheng SJ (1996) Tissue culture and rapid propagation of *Dendrobium*. J Chin Med Mater 19:490–491 **(in Chinese with English abstract)**

Zeng SJ, Cheng SJ, Zhang JL, Zhao FP (1998) Embryo culture and propagation of *Dendrobium in vitro*. Acta Horti Sin 25:75–80 **(in Chinese with English abstract)**

Zeng S, Wu K, Teixeira da Silva JA, Zhang J, Chen Z, Xia N, Duan J (2012) Asymbiotic seed germination, seedling development and reintroduction of *Paphiopedilum wardii* Sumerh., an endangered terrestrial orchid. Sci Hortic 138:198–209

Zeng SJ, Teixeira da Silva JA, Wu KL, Zhang JX, Duan J (2014) Seed biology and in vitro seed germination of *Cypripedium*. Crit Rev Biotechnol 34:358–371

Zeng SJ, Huang WC, Wu KL, Zhang JX, Teixeira da Silva JA, Duan J (2015) *In vitro* propagation of *Paphiopedilum* orchids. Crit Rev Biotechnol. doi:10.3109/07388551.2014.993585

Zhan QC, Li X, Qi Y, Yang SX, Ye QM, Jian LG (2010) The rapid propagation of *Dendrobium wilsonii* Rolfe and its plantlet. North Hortic 16:137–139 **(in Chinese with English abstract)**

Zhao D, Hu G, Chen Z, Shi Y, Zheng L, Tang A, Long C (2013a) Micropropagation and *in vitro* flowering of *Dendrobium wangliangii*: a critically endangered medicinal orchid. J Med Plants Res 7:2098–2110

Zhao MM, Zhang G, Zhang DW, Hsiao YY, Guo SX (2013b) ESTs analysis reveals putative genes involved in symbiotic seed germination in *Dendrobium officinale*. PLoS One 8:e72705

Zhou Y, Yin LQ, Tang GJ, Huang WC, Hu YH, Li XF, Jiang JP, Liu Z, Hu HF (2012) Establishment of technological system for *in vitro* propagation of *Dendrobium aduncum*. J Shanghai Jiaotong Univ (Agric Sci) 30(6):62–66 **(in Chinese with English abstract)**