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Effects of light, agar, activated charcoal, and culture medium on the germination and early development of *Dendrobium* seedlings

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Abstract

The objectives of this study were to determine the optimal light conditions, agar concentration, and quantity of activated charcoal in asymbiotic media to improve the *in vitro* seed germination rate and early seedling development of the epiphytic orchids *Dendrobium nobile* Lindl. and *Dendrobium phalaenopsis* Fitzg. Two independent experiments with complete randomized designs were conducted. (1) Treatments were arranged in a split-plot scheme. Seeds in each sub-plot were exposed to one of four light conditions (dark, white fluorescent, red fluorescent + white fluorescent, or red fluorescent) and grown in one of four types of culture media (MS: Murashige and Skoog, ½ MS: half strength MS, K: Knudson C, and VW: Vacin and Went media). (2) Treatments were arranged in a 4 × 5 × 5 factorial scheme (four types of culture media: MS, ½ MS, K, and VW; five concentrations of agar: 0.0, 2.0, 4.0, 6.0, or 8.0 g L^{-1}; and five concentrations of activated charcoal: 0.0, 1.5, 3.0, 4.5, or 6.0 g L^{-1}). The highest germination rates and early seedling development were observed 45 days after seeding in the presence of white light for *D. nobile* and red + white light for *D. phalaenopsis* in MS and ½ MS culture media. Based on the findings of the present study, the use of MS and ½ MS culture media solidified with 4.0–8.0 g L^{-1} of agar and without activated charcoal is recommended for the optimal propagation of seeds and seedlings of these *Dendrobium* species.

Keywords: orchids, ornamental species, asymbiotic germination, PAR, gelling agent.
Abbreviations: D_continuous darkness; CP_chlorophyll propagules; K_ Knudson C culture medium (1946); MS_Murashige and Skoog (1962) culture medium; ½ MS_Murashige and Skoog (1962) culture medium at half the salt concentration; VW_Vacin and Went (1949) culture medium; NS_non-germinated seeds; %G_germination rate (%); NTW_no triple-wash (type of suspension washing); TW_triple-wash (type of suspension washing); R_red fluorescent lamp; WF_white fluorescent lamp; RW_red fluorescent lamp + white fluorescent lamp.

Introduction

In vitro culture techniques have been widely used to propagate orchids (Suzuki et al., 2010). The asymbiotic germination of orchid seeds has been performed since the early 20th century, when Knudson (1922) reported the successful germination of orchids using aseptic culture medium. Seed germination varies according to the specific nutritional requirements of each species and may differ at the different stages of development (Stewart and Kane, 2006; Paul et al., 2012; Silva et al., 2015). The proper choice of medium for *in vitro* seeding is, therefore, one of the main factors ensuring the success of asymbiotic culture techniques. Regarding the *in vitro* seeding of the genus *Dendrobium*, Silva et al. (2015) reported that the four types of culture media most commonly used are Murashige and Skoog (MS; Murashige and Skoog, 1962), ½ MS (half strength MS), Knudson C (K; Knudson, 1946), and Vacin and Went (VW; Vacin and Went, 1949). Aside from the effects of the type of culture medium on germination, it is also important to consider the physical state of the medium, as the diffusion of nutrients to the propagated plant material increases or decreases according to the concentration of the gelling agent used (Faria et al., 2012). In addition, activated charcoal can also be used to further enrich the culture media as it has the capacity to adsorb toxic substances that are present in the medium or formed through the oxidation of phenolic compounds during the *in vitro* culturing process (Faria et al., 2012). However, in addition to the uptake of toxic substances, activated charcoal can also unfavorably absorb and retain vitamins, nutrients, and growth regulators, depending on the concentration of the charcoal used (Guson et al., 2012; Cid and Teixeira, 2014).

Another important factor in asymbiotic seed germination is the presence of light (Silva et al., 2015). Orchid seed germination is differentially influenced by light (Arditti and Ernst, 1984; Silva et al., 2015), and it is universally accepted that the light requirements of orchids vary under natural conditions and according to the habitat of each species. Thus, the seeds of epiphytic orchids are theoretically expected to require the presence of light, while those of terrestrial species are expected to need darker conditions to germinate. The different responses to growing conditions, however, are often species-specific, regardless of the growth habit (Kauth et al., 2008). Some authors have reported that seed germination in several species of terrestrial orchids is inhibited by the presence of light (Stoutamire, 1974; Stewart and Kane, 2006; Godo et al., 2010). Thus, it is generally accepted that the symbiotic and asymbiotic germination of terrestrial orchid seeds occurs most commonly under continuous darkness (Kitsaki et al., 2004; Yamazaki and Miyoshi, 2006; Lee et al., 2007). However, as several authors have observed, seeds from some epiphytic species are able to germinate in both the presence and absence of light *in vitro* (Arditti and Ernst, 1984; Dutra et al., 2009; Tsutsumi et al., 2011).

Moreover, in addition to the effects of photoperiod on germination, some studies have analyzed the effects of different wavelengths on the germination, growth, and development of plants cultured *in vitro.* However, relatively few studies to date have reported on the morphophysiological effects of different light conditions on orchid plants.

Therefore, the objectives of the present study were to determine the optimal conditions (light, agar concentration, and activated charcoal concentration) for the *in vitro* culture of the epiphytic orchid species *Dendrobium nobile* Lindl. and *Dendrobium phalaenopsis* Fitzg in asymbiotic media for increased germination and early seedling development.

Results and discussion

Effects of light and culture media on the germination and early development of Dendrobium seedlings.

The percentage germination rates (%Gs) of *D. nobile* and *D. phalaenopsis* were affected by both the light and culture media, as individual factors ($p < 0.01$) and as combined factors ($p < 0.01$). The seeds of both species germinated under all light conditions and using all four types of culture media. The overall mean %G for *D. nobile* and *D. phalaenopsis* was 50.6% and 82.7%, respectively (Table 1), supporting the notion that the seeds of these species are neutral photoblastic (Taiz and Zeiger, 2013). Epiphytic orchids are known to be tolerant of dark environments, despite a shortage of water and the scarcity of minerals, which may explain the germination of *D. nobile* and *D. phalaenopsis* seeds in the absence of light (Benzing et al., 1982).

In both orchid species, higher germination rates were observed in the presence of light (16 h), regardless of the culture medium. The average %G observed in response to the different types of culture media and in the presence of light was 55.5% for *D. nobile* and 84.3% for *D. phalaenopsis*; i.e., respectively 20.0% (35.5%) and 6.5% (77.8%) higher than those recorded for the same species maintained in continuous darkness (0 h) (Table 1). Similar results were confirmed by Parthibhan et al. (2012), who studied the effects of different photoperiods on the germination of *Dendrobium aqueum* Lindl., and found that 46 days after inoculation, the %G was only 50.8% in seeds maintained in continuous darkness but ranged from 67.9% to 93.9% as the light exposure increased from 8 to 24 h, respectively.

In the present study, the %G of the two species varied in response to the different types of culture media and depending on the light conditions. For *D. nobile* seeds, the %G was statistically similar in all culture media when seeds were exposed to WF light, with an average germination rate of 57.3%. When seeds were exposed to R and RW light or kept in the dark, the %G was higher in MS and ½ MS media (Table 1). When the seeds of *D. phalaenopsis* were exposed to WF light, the %G was statistically higher for MS medium than for the other media. Under R light and continuous darkness (D), the %G was higher for seeds cultured in MS and ½ MS media, whereas under RW light conditions, the %G was higher when MS, ½ MS, and VW media were used (Table 1).

While some orchid species have been shown to germinate better in culture media that contains high concentrations of nutrients, others require nutrient-poor media for germination (Stewart, 1989). In the present study, %G was generally higher in both species when cultured with MS and ½ MS media (58.4% and 60.9% for *D. nobile* and 90.2% and 87.9% for *D. phalaenopsis*, respectively), than when cultured with VW and K media. Thus, the data obtained in this study corroborate the findings of Lo et al. (2004), who reported that K and VW media are less effective than MS and ½ MS media in promoting germination in *Dendrobium tosaense* Makino. Regarding the effect of light conditions, the %G was higher when seeds were cultured under WF and RW light conditions (57.3 % and 57.4% for *D. nobile* and 84.9 and 86.5% for *D. phalaenopsis*, respectively) (Table 1).

Forty-five days after inoculation, the seedlings and protocorms were larger when cultured in MS and ½ MS media. However, we found seedlings at different stages of development in all media (Figure 1). Seedlings were classified as described by Suzuki et al. (2009), into stage 2 (seedlings with the first leaf formed), stage 3 (seedlings with two leaves), and stage 4 (seedlings with leaves and one or more roots). Under D, stage 1 protocorms (protocorm-like bodies) were observed to have germinated 45 days after inoculation. A visual assessment of these protocorms revealed that they were predominantly elongated in shape and that their color was notably white (Figure 1). This latter observation is consistent with the findings that the differentiation of proplastids into chloroplasts and chlorophyll synthesis are light-dependent processes (Taiz and Zeiger, 2013). These results confirm that light is essential for the early stages of growth and development of the two orchid species.

Red light is known to play an important role in the elongation of buds and stems, the promotion of phytochrome responses during photomorphogenesis, and changes in plant anatomy (Shin et al., 2008; Taiz and Zeiger, 2013). In the present study, red light wavelengths promoted the development of seedlings, which reached more advanced stages of early seedling development (stages 3 and 4; Figure 1). These findings indicate that red light is effective

for the promotion of the growth and development of these two orchid species.

Effects of agar, charcoal, and culture media on the germination and early development of Dendrobium seedlings.

Dendrobium species vary in their nutritional requirements for germination (Silva et al., 2015). The %G of both *D. nobile* and *D. phalaenopsis* varied according to the type of culture medium and as a function of agar and charcoal concentrations (Figures 2 and 3). However, their mean values were, in some cases, statistically similar (Table 2). Agar, charcoal, and the type of culture medium significantly affected the %G of both *D. nobile* and *D. phalaenopsis*, as individual factors ($p < 0.01$) and as combined factors ($p < 0.01$) (with the exception of the effect of charcoal on *D. phalaenopsis*). The mean germination rate of *D. nobile* was 71.3%, while that of *D. phalaenopsis* was 92.6%. The highest germination rates were obtained using MS, ½ MS, and K media for *D. nobile* (statistically similar) and MS, ½ MS, and VW media for *D. phalaenopsis* (statistically similar) (Table 2). For *D. nobile*, the highest values for %G were obtained in MS and VW media supplemented with 6.0 g L^{-1} charcoal and were lower for charcoal concentrations ranging between 0.0 g L^{-1} and 0.5 g L^{-1} in MS, VW, and K media (Figure 2). In *D. phalaenopsis*, the values for %G were higher in agar concentrations of up to 0.5 g L^{-1} in VW, MS, and ½ MS media, and lower in agar concentrations above 5.0 g L^{-1} in all culture media (Figure 3).

The results of the present study confirmed the successful germination of *D. nobile* and *D. phalaenopsis* in all compositions of culture media. In contrast, Soares et al. (2012) reported the asymbiotic germination of the orchid *Brassavola tuberculata* Hook. only in culture medium supplemented with activated charcoal.

Similar to the findings of Hossain et al. (2008), where activated charcoal increased germination in the epiphytic orchid *Epidendrum ibaguense* Kunth, the germination of *D. nobile* increased following the addition of 6.0 g L^{-1} activated charcoal to the culture media (Figure 2). These authors surmised that the high adsorption affinity of activated charcoal for excessive and inhibitory compounds, as well as the darkening of the culture media, promoted the germination process.

In the present study, the highest values of %G were generally obtained for both species when a liquid medium was used. According to Faria et al. (2012), liquid media lead to higher %G by promoting the diffusion of nutrients to the seeds to a greater extent than solidified media. Furthermore, it is important to highlight that the use of MS and ½ MS liquid media (supplemented with 0.0 g L^{-1} charcoal and up to 2.0 g L^{-1} agar) appeared to delay seedling development in the present study. Under these conditions, we observed protocorms (protocorm-like bodies) in stage 1 and seedlings (the first leaf formed) in stage 2 according to the classification proposed by Suzuki et al. (2009). In contrast, in MS and ½ MS supplemented with more than 4.0 g L^{-1} agar (semisolid medium), the seedlings reached a more advanced stage of development, usually stage 3 (two leaves) (Figures 4 and 5).

These findings demonstrated that solidified media was essential for the initial culture of the study species, as media with low concentrations of agar might hinder the establishment of the seedlings (Faria et al., 2012), which could in turn delay their growth and development.

Although the use of 6.0 g L^{-1} of charcoal yielded higher %G for both *D. nobile* and *D. phalaenopsis* in the MS medium, the addition of charcoal delayed the growth and development of protocorms and seedlings, which were visibly smaller (i.e., did not surpass stages 1 and 2 of development). In contrast, the same medium without charcoal favored larger and more developed seedlings, which were classified as having reached stages 2 and 3 (Figures 4 and 5). In general, activated charcoal is added to culture media at concentrations of 0.2–3.0% (Faria et al., 2012; Cid and Teixeira, 2014). According to Pasqual et al. (1997), although charcoal itself is not a growth regulator, its presence modifies the compositions of culture media, and thus promotes or inhibits *in vitro* growth in a species- and tissue-specific manner.

According to Thomas (2008) and Guson et al. (2012), activated charcoal can also affect the absorption of vitamins, metal ions, and growth regulators. In addition, activated charcoal has the ability to retain both growth-inhibiting and growth-promoting substances, slow down their release, and thus ultimately have a positive or negative effect on development, depending on the species. The adsorption promoted by charcoal may also lead to the retention of ions, such as Cu and Zn, present in the medium (Cid and Teixeira, 2014). As Zn plays an important role in the metabolism of plant growth, some authors suggest that the increased availability of this nutrient in the culture medium is necessary to improve the growth of different species (Figueiredo et al., 2007; Villa et al., 2014).

Villa et al. (2014) observed that increased concentrations of activated charcoal in K medium had a negative effect on the leaf morphogenesis of *Brassocattleya* Pastoral × *Laeliocattleya* Amber Glow. These authors attributed this effect to the combination of a solid medium (which inhibits the diffusion of phosphorous, potassium, and zinc) with high concentrations of activated charcoal. This association might have inhibited leaf formation owing to a lack of these elements, which are required for organ formation. Galdiano Júnior et al. (2012) also reported the negative effects of adding charcoal to a culture medium (e.g., the removal of organic nutrients and phytohormones from the culture medium, and the inhibition of the growth and morphogenesis of the cultured species). Moreover, Araújo et al. (2006) stated that the non-selective effect of activated charcoal could negatively affect micropropagation. In summary, the variation in the findings obtained with regard to the effect of the use of activated charcoal when culturing different species of the family Orchidaceae appears to be mostly attributed to genotype, the adsorption of some chemical substances and organic compounds, and the release of toxic metabolites in the *in vitro* culture (Galdiano Júnior et al., 2012; Prizão et al., 2012; Villa et al., 2014).

The results of the two experiments in the present study highlight the importance of research on orchid germination, as it not only broadens our understanding of the two species studied here but also offers insights into how orchids interact with the culture medium. In addition, the present study sheds light on the poorly understood behavioral patterns in this exotic and commercially important family of plants. The influences of light and the formulation and

Table 1. Germination rates of *Dendrobium nobile* and *Dendrobium phalaenopsis* seeds grown in different culture media and under different light treatments.

	D. nobile seed germination (%)				
Light	MS	½ MS	K	VW	Mean*[1]
WF	58.5 bA	64.7 aA	53.5 aA	52.7 aA	57.3
R	59.2 bA	64.2 aA	43.3 bB	40.8 bB	51.9
RW	69.5 aA	60.3 aA	50.2 aB	49.9 aB	57.4
D	46.5 cA	54.2 bA	22.5 cB	18.8 cB	35.5
Mean*[2]	58.4	60.9	42.4	40.6	50.6*[3]
	D. phalaenopsis seed germination (%)				
Light	MS	½ MS	K	VW	Mean*[1]
WF	94.6 aA	89.1 aB	74.6 aD	81.2 bC	84.9
R	89.4 bA	88.3 aA	74.0 aB	74.7 cB	81.6
RW	90.1 bA	89.6 aA	76.0 aB	90.3 aA	86.5
D	86.5 bA	84.7 bA	69.4 bB	70.6 dB	77.8
Mean*[2]	90.2	87.9	73.5	79.2	82.7*[3]

Lowercase letters indicate significantly different means in the column (Tukey's test at 5% probability).
Uppercase letters indicate significantly different means in the row (Tukey's test at 5% probability).
WF: white light, R: red light, RW: red light + white light, D: continuous darkness, MS: Murashige and Skoog, ½ MS: half strength MS, K: Knudson C, VW: Vacin and Went media.
*[1] means of rows; *[2] means of columns; *[3] overall means.

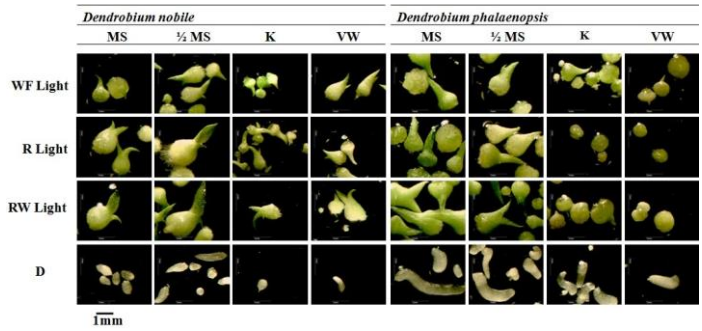

Fig 1. Seeds, protocorms, and seedlings of *Dendrobium nobile* and *Dendrobium phalaenopsis* at 45 days post inoculation in response to different types of culture media and light conditions. WF: white light, R: red light, RW: red light + white light, D: continuous darkness, MS: Murashige and Skoog, ½ MS: half strength MS, K: Knudson C, VW: Vacin and Went media.

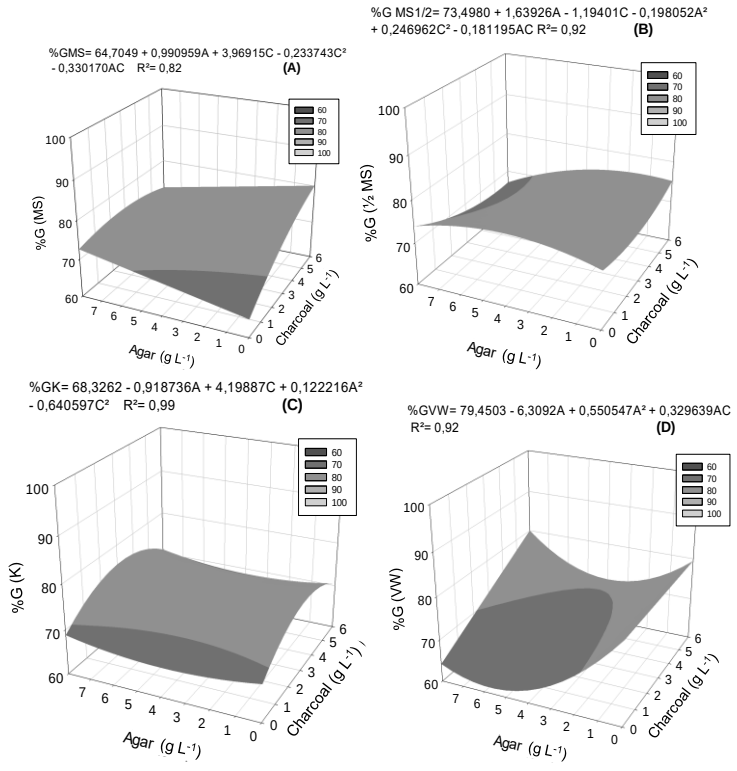

Fig 2. Germination rates (%G) of *Dendrobium nobile* seeds in culture media: (A) Murashige and Skoog (MS), (B) half strength MS (½ MS), (C) Knudson C (K), and (D) Vacin and Went (VW), according to the different concentrations of agar and activated charcoal.

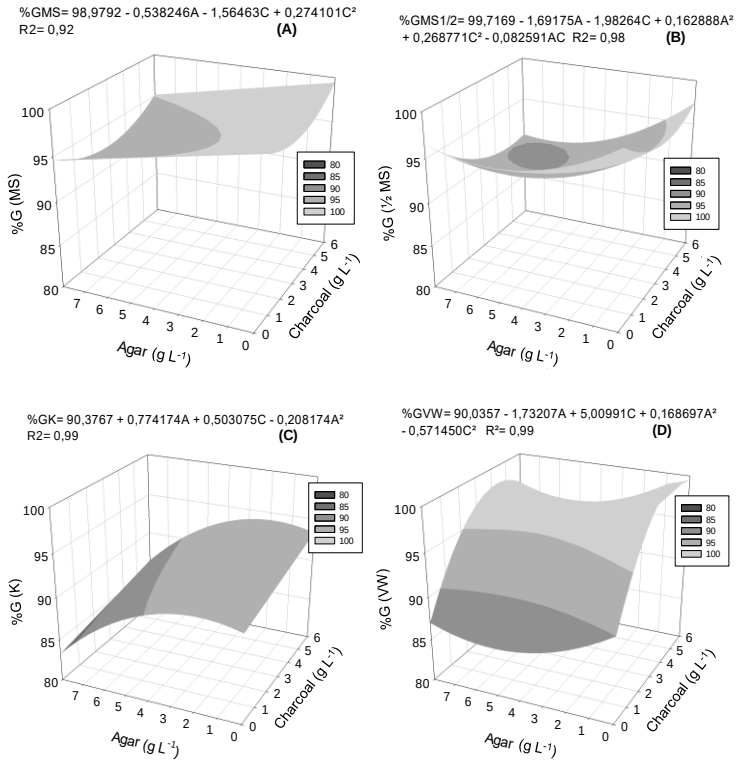

Fig 3. Germination rates (%G) of *Dendrobium phalaenopsis* seeds in the culture media: (A) Murashige and Skoog (MS), (B) half strength MS (½ MS), (C) Knudson C (K), and (D) Vacin and Went (VW), according to the different concentrations of agar and activated charcoal.

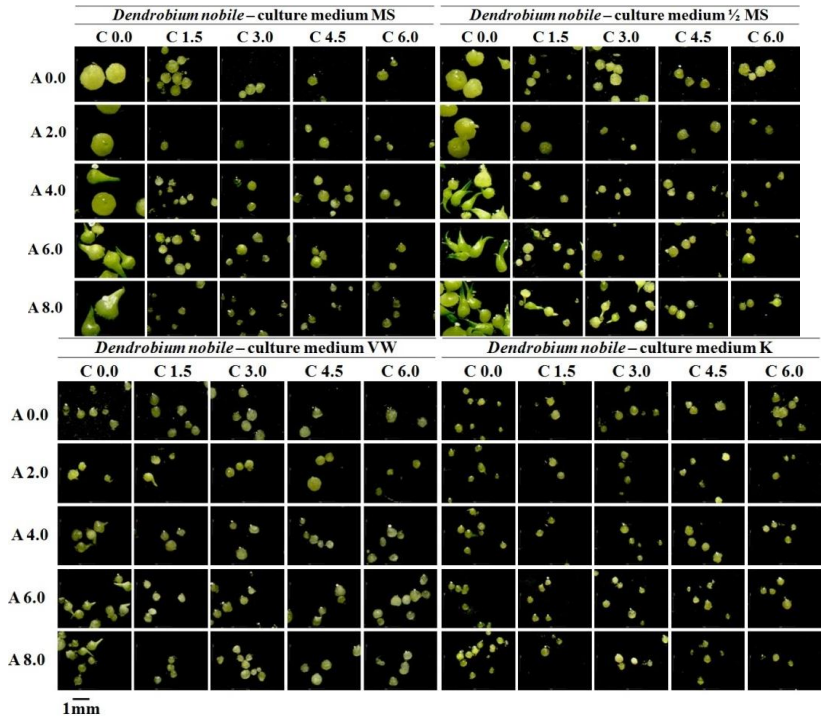

Fig 4. Seeds, protocorms, and seedlings of *Dendrobium nobile* at 45 days post inoculation in response to different types of culture media, concentrations of agar (A), and activated charcoal (C). MS: Murashige and Skoog, ½ MS: half strength MS, K: Knudson C, VW: Vacin and Went media.

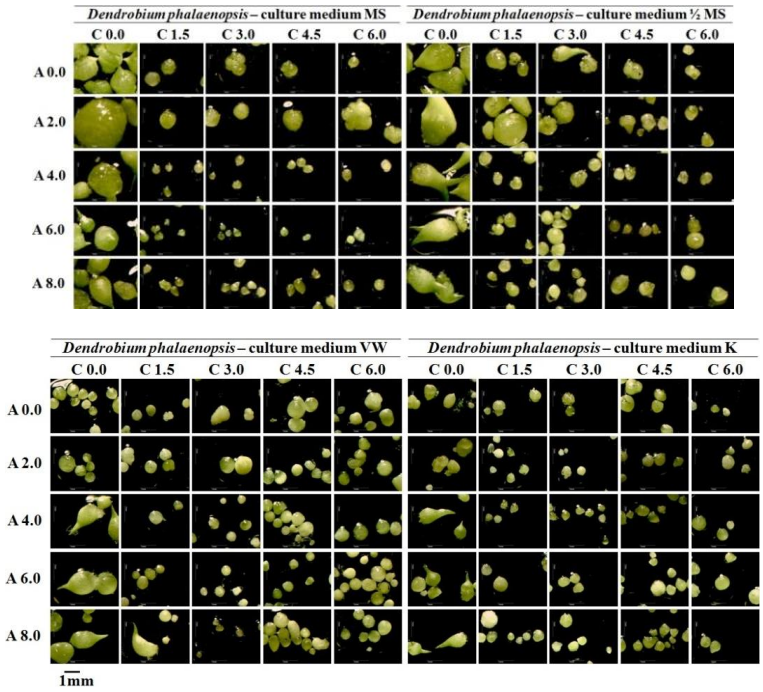

Fig 5. Seeds, protocorms, and seedlings of *Dendrobium phalaenopsis* at 45 days post inoculation in response to different types of culture media, concentrations of agar (A), and activated charcoal (C). MS: Murashige and Skoog, ½ MS: half strength MS, K: Knudson C, VW: Vacin and Went media.

enrichment of culture media at each stage of orchid development should be further elucidated to improve current knowledge and to help establish new protocols in orchid culture.

Materials and methods

Collection and disinfestation of capsules

The mature capsules used in this study were collected from hand-pollinated *D. nobile* and *D. phalaenopsis*, which were more than 8 years old. The orchids were cultured in a greenhouse covered by two 50% shade screens that ensured a daily mean photosynthetically active radiation of 160 µmol m^{-2} s^{-1}, a mean temperature of 22.6 ± 5°C, and a relative humidity of 73.9 ± 10%. Irrigation was performed using micro-sprinklers.

Capsules were removed from mother plants using pruning shears and transported to the *in vitro* culture laboratory at the Faculty of Agrarian Science, Federal University of Grande Dourados, Mato Grosso do Sul, Brazil. The capsules were disinfected with 70% ethanol, and two capsules from each species were opened using a scalpel. The seeds were removed, combined and thoroughly mixed for each species, and then placed in a desiccator with silica gel (25 ± 2°C; 75% relative humidity) for 14 days. After desiccation, the seeds of each species were wrapped in aluminum foil and stored at 5 ± 2°C in opaque polypropylene bottles with lids.

Seed viability test

A tetrazolium test was performed for both species according to the methods of Soares et al. (2014). Once seed viability was confirmed, 35 mg of seeds were weighed for each species. The seeds were placed in an aseptic environment and disinfected by immersion in 105 mL of 0.8% sodium hypochlorite for 5 min. Seed suspensions were subsequently diluted in sterile distilled water to a total volume of 350 mL and then rinsed three times with sterile distilled water (280 mL per wash). After washing, seeds were suspended again in 350 mL of sterile distilled water for *in vitro* seeding. The inoculation was performed by adding 1000 µL of seed suspension to each flask using an automatic pipette.

To achieve the objectives of this study, two independent experiments were designed: (1) light + culture media and (2) culture media + agar concentration + activated charcoal concentration, as follows:

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In vitro seeding

The culture media used for seed germination were MS, ½ MS, VW, and K. Culture media were solidified with 4.0 g L^{-1} of bacteriological agar (HiMedia Laboratories, Mumbai, India) and supplemented with 30 g L^{-1} of sucrose. The pH of each medium was adjusted to 5.8 using HCl (0.1M) or KOH (0.1M). Twenty milliliters of each culture medium were poured into 50 mL polypropylene bottles with screw caps (height = 5 cm, mouth diameter = 5 cm), and bottles were sterilized in an autoclave at 120°C at a pressure of 1 atm for 20 min. After cooling, the bottles were transferred to a sterile environment.

Experimental treatment conditions

After inoculation, the cultures were placed in a growth room with a photoperiod of 16-hour light (presence of light) or 0-hour light (D = 0.0 µmol m^{-2} s^{-1}) and controlled temperature

(25 ± 2°C). The cultures that were subjected to a 16-hour photoperiod were exposed to the following light conditions: WF light (18.9 µmol m^{-2} s^{-1} irradiance provided by two white fluorescent lamps of 40 W each), RW light (14.85 µmol m^{-2} s^{-1} irradiance provided by a white fluorescent lamp of 40 W and a red fluorescent lamp of 30 W; Gro-lux, Sylvania, Brazil), and R light (9.45 µmol m^{-2} s^{-1} irradiance provided by two red fluorescent lamps of 30 W; Gro-lux, Sylvania, Brazil).

Experimental evaluation

The %G and early seedling development for each species were determined 45 days after seeding. The seedlings contained in the bottles were rinsed with 3 mL of sterile distilled water and placed on acrylic plates (2.0 × 2.0 × 0.5 cm) with a 0.5 × 0.5 cm square grid. The numbers of non-germinated seeds (NS) and chlorophyll propagules (CP) were determined using a binocular stereomicroscope with transmitted and reflected lights (Bel Photonics, Brazil). The %G was calculated using the following equation:

$$\%G = \left[\frac{CP}{(NS + CP)}\right] \times 100.$$

Early seedling development was evaluated according to the staging scheme of Suzuki et al. (2009). After counting, the treatments were photographed using a digital camera coupled to a stereomicroscope and analyzed using the AxionVision software, version 3.1 (Carl Zeiss, Jena, Germany).

Experimental design and statistical analyses

A complete randomized experimental design was implemented, with treatments arranged in a split-plot scheme. The plots were each treated with one of four light conditions, and one of the four types of culture media were assigned to each of the sub-plots. Four replications, each represented by a single culture bottle, were prepared per treatment. The results of the %G were transformed to $\sqrt{(X+1)}$, subjected to an analysis of variance, and compared using Tukey's test at 5% probability. The statistical analyses were conducted using SISVAR v.5.3. (Federal University of Lavras, MG, Brazil).

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Experimental treatment conditions and in vitro seeding

The four types of culture media: MS, ½ MS, VW, and K, were prepared following the afore mentioned protocols with the exception that, after adding sucrose, the media were supplemented with 0.0, 1.5, 3.0, 4.5, or 6.0 g L^{-1} activated charcoal and solidified by adding 0.0, 2.0, 4.0, 6.0, or 8.0 g L^{-1} agar. After the polypropylene bottles containing the various media were sterilized as previously described, they were cooled and transferred to a sterile environment for *in vitro* seeding.

After inoculation, the cultures were placed in a growth room with controlled temperature (25 ± 2°C) and photoperiod (16 h light). The ideal light regimes of the two species were designated according to the results obtained in the first experiment, and thus *D. nobile* seeds and seedlings were cultured under WF light and *D. phalaenopsis* seeds and seedlings were cultured under RW light. The %G and early seedling development were determined following the same protocols outlined in the first experiment.

Experimental design and statistical analyses

A complete randomized design was used, and treatments were arranged in 4 × 5 × 5 factorial scheme (four culture media, five concentrations of agar, and five concentrations of activated charcoal). Each treatment was replicated three times and each replicate consisted of a single culture bottle. The results of %G were transformed to $\sqrt{(X+1)}$ and subsequently subjected to an analysis of variance. The qualitative parameters were compared using Tukey's test at 5% probability and the quantitative parameters were compared via regression analyses using SAEG 9.1 (Arthur Bernardes Foundation, Federal University of Viçosa, MG, Brazil).

Conclusion

The presence of light and the type of culture medium used in the present study were not the limiting factors in the *in vitro* germination of the two study orchid species. The highest %G and the optimum early seedling development were observed in the presence of WF light for *D. nobile* and RW light for *D. phalaenopsis* in MS and ½ MS culture media. MS and ½ MS culture media were the most effective in promoting the *in vitro* germination of *D. nobile* and *D. phalaenopsis*. The use of MS and ½ MS culture media solidified with 4.0–8.0 g L^{-1} of agar without activated charcoal is recommended for the optimal development of *D. nobile* and *D. phalaenopsis* seedlings.

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