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Scientific Article

Light condition, flask sealing, and cultivation time on the germination and initial *in vitro* development of *Dendrobium nobile* Lindl.

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Abstract

One of the obstacles for the production of Orchidaceae plants is the seed propagation. Thus, in *in vitro* cultivation, luminosity and the type of sealing of the bottles are factors that influence germination and initial development of orchids. Therefore, the objective was to evaluate the light condition, the type of sealing of the bottles and the evaluation time in germination and initial *in vitro* development of *Dendrobium nobile* Lindl. After sowing, cultures were subjected to two irradiance conditions according to the sealing of the flasks: 7.0 µmol m^{-2} s^{-1} (threaded cap) and 19.0 µmol m^{-2} s^{-1} (plastic film) and two light conditions: white fluorescent lamp and red fluorescent lamp. At 30, 60 and 90 days, the percentage of germination, survival, of protocorms in stage 1, stage 2, stage 3 and stage were evaluated 4. DIC was used, in a sub-divided plot scheme for 30 and 60 days after sowing and in a 2 x 2 factorial scheme at 90 days. The highest germination percentage was observed when using white fluorescent lamp with a plastic film cover (70.33%) and at 30 days (70.13%). The highest survival percentage (100%) was observed when using plastic film cover and white fluorescent lamp at 90 days. The greatest development of propagules, reaching stage 4 was verified in the red fluorescent lamp with threaded cap (9.55%).

Keywords: plant propagation, Orchidaceae, ornamental horticulture.

Resumo

Condição de luz, vedação dos frascos e tempo de cultivo na germinação e desenvolvimento inicial *in vitro* de *Dendrobium nobile* lindl.

Um dos obstáculos para produção de plantas de Orchidaceae é a propagação por sementes. Dessa forma, no cultivo *in vitro* a luminosidade e o tipo de vedação dos frascos são fatores que influenciam a germinação e o desenvolvimento inicial de orquídeas. Sendo assim, objetivou-se avaliar a condição de luz, o tipo de vedação dos frascos e o tempo de avaliação na germinação e desenvolvimento inicial *in vitro* de *Dendrobium nobile* Lindl. Após semeadura, as culturas foram submetidas à duas condições de irradiâncias conforme a vedação dos frascos: 7,0 µmol m^{-2} s^{-1} (tampa rosqueável) e 19,0 µmol m^{-2} s^{-1} (filme plástico) e duas condições de luz: lâmpada fluorescente branca e lâmpada fluorescente vermelha. Aos 30, 60 e 90 dias foi avaliado a porcentagem de germinação, de sobrevivência, de protocormos em estádio 1, estádio 2, estádio 3 e estádio 4. Foi utilizado DIC, em esquema de parcelas sub-subdivididas para 30 e 60 dias após semeadura e em esquema fatorial 2 x 2 aos 90 dias. A maior porcentagem de germinação foi observada quando se utilizou lâmpada fluorescente branca com tampa de filme plástico (70,33%) e aos 30 dias (70,13%). A maior porcentagem de sobrevivência (100%) foi observada quando se utilizou tampa de filme plástico e lâmpada fluorescente branca aos 90 dias. O maior desenvolvimento de propágulos, chegando ao estádio 4 foi verificado na lâmpada fluorescente vermelha com tampa rosqueável (9,55%).

Palavras chave: horticultura ornamental, propagação de plantas, Orchidaceae.

Introduction

The flower and ornamental plants sector have the potential to increase production, with consequent economic growth. It stands out as a relevant sector in Brazilian agribusiness, with a turnover of approximately R$ 10.9 billion in 2021 (IBRAFLOR, 2022).

Orchids are among the evidence plants in this segment due to their good capacity for genetic combination and ornamental potential (Zahara et al., 2017). Junqueira and Peetz (2018) state that producers have responded attentively to the continuous growth of this sector, annually introducing new orchid hybrids resulting from improvements. Noteworthy, 2,345 cultivars are currently

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registered. Among the most commercialized orchids in the segment of potted flowers stand out *Cattleya* sp., *Dendrobium* sp., and *Phalaenopsis* sp.

The genus *Dendrobium* has a large number of species and hybrids that adapt to all climates, in addition to being used as a cut flower (Araújo, 2017; Junqueira and Peetz, 2017). Among its species, one of the most cultivated is *Dendrobium nobile*, an epiphyte, herbaceous, perennial, clustered plant native to China and the Himalayas (Hou et al., 2017). Reports in the scientific literature on its pharmacological properties include its use as antioxidant, anti-inflammatory, immunomodulatory, antitumor, and antimutagenic (Teixeira da Silva et al., 2015; Zhang et al., 2021).

Plants of this species can be propagated vegetatively. However, they show slow growth, requiring a longer growing period before commercialization, which can increase unit value (Alves et al., 2017; Ferreira et al., 2022). On the other hand, *in vitro* culture techniques provide an alternative for orchid multiplication. This type of cultivation can produce a large number of seedlings with high phytosanitary and physiological quality in a shorter period, being important for both research and preservation of species, as well as for commercial scale production (Silva et al., 2017; Ferreira et al., 2022; Cavallaro et al., 2022). Asymbiotic germination stands out in *in vitro* culture of orchids, leading to high germination percentages and thus being a viable and relevant technique for the production of this genus (Silva et al., 2015; Sorgato et al., 2020a; Soares et al., 2021).

One of the factors that influence the germination and development of propagated seedlings is the microenvironment inside culture flasks. Variations in this microenvironment may depend on flask type and size and on the type of sealing (Ribeiro et al., 2019). The material used to seal flasks may thus influence the development of some cultures (Moraes et al., 2009).

Light also acts in several metabolic processes in plants. In this way, studies use different wavelengths, photoperiods, and irradiance as a way to optimize the propagation of plants grown *in vitro* (Silva et al., 2015; Gupta and Agarwal, 2017; Sorgato et al., 2021; Cavallaro et al., 2022).

There are still few studies reporting the morphophysiological effects that vary in the microenvironment inside *in vitro* culture flasks and the different light conditions on plants (Hashim et al., 2021). Given the above, this study assessed the germination and initial development of *Dendrobium nobile* Lindl. as a function of light condition, flask sealing, and cultivation time.

Materials and Methods

The experiment was carried out in the Laboratory of *in vitro* Culture of the Faculty of Agricultural Sciences (FCA) of the Federal University of Grande Dourados, in Dourados city, Mato Grosso do Sul State, Brazil. The study material included seeds of ripe fruits, obtained from manual pollination of the orchid *Dendrobium nobile* Lindl. These seeds originated from mother plants older than 15 years, grown in a nursery covered by the overlap of two 50% shading screens. Irradiance in the environment was 235 µmol m^2 s^{-1}, and average temperature and relative humidity were 22.6 ± 5 ºC and 73.9% ± 10%, respectively.

Seed samples weighing 0.005 g were submitted to the tetrazolium test according to the methodology of Soares et al. (2021). After confirmation of viability, 0.005 g samples were transferred to an aseptic environment and disinfected with 0.8% sodium hypochlorite solution for five minutes, according to the methodology proposed by Sorgato et al. (2020b). Subsequently, the volume of the suspension was made up to 50 ml with sterile distilled water for *in vitro* sowing, inoculating 1 mL of the seed suspension per culture flask. Twenty ml of Murashige and Skoog (1962) culture medium were used with half the normal salt concentration (½ MS) per flask. These flasks were made of transparent polypropylene, having a capacity of 50 ml. Subsequently, half of the flasks were sealed with a transparent polyvinyl chloride (PVC) film, and the other half with an opaque polypropylene screw cap (SC).

The cultures were then placed in a growth room with controlled temperature and photoperiod (25 ± 2 °C; 16 h) under two light conditions: white fluorescent lamp (W light) and red fluorescent lamp (R light) (Gro-lux®). For screw cap-sealed flasks, the irradiance provided was 7.0 µmol m^{-2} s^{-1}; for PVC-sealed flasks, irradiance was 19.0 µmol m^{-2} s^{-1}.

At 30 and 60 days after sowing, the propagules were assessed for germination percentage (%G = [Chlorophyllous protocorms / (Number of seeds + Chlorophyllous protocorms)] x 100). At 90 days, the initial development of protocorms was assessed by means of the survival percentage (%SUR) and the percentages of protocorms and seedlings at stage 1 (%P1), stage 2 (%P2), stage 3 (%P3), and stage 4 (%P4). Classification was as follows: stage 1 (swollen, chlorophyllous protocorm), stage 2 (seedling with formation of the first leaf), stage 3 (seedling with two leaves), and stage 4 (seedling with leaves and one or more roots) (Suzuki et al., 2009).

The materials contained in the flasks were washed with 3 ml of sterile distilled water and placed in acrylic plates (2 x 2 x 0.5 cm and 0.5 cm squares). This procedure was repeated until no seed or propagule remained in the culture flask. After the evaluations, the treatments were photographed with a camera attached to a stereoscopic microscopic with the aid of the computer program AxionVision version 3.1 (Zeiss®).

The experimental design used was completely randomized (CRD). Germination percentage (%G) was arranged in subdivided plots, in which light condition corresponded to the plot, type of sealing to the subplot, and cultivation time to the subsubplot. Regarding the initial development of protocorms, the CRD was arranged in a 2x2 factorial scheme, corresponding to two light conditions and two types of sealing. For both analyses, four replicates of one culture flask were used. The data were submitted to analysis of variance, being compared using the Tukey test ($p < 0.05$) with the aid of the SISVAR program (Statistical Analysis Program v.5.3. - Federal University of Lavras, MG).

Results and Discussion

Germination at 30 and 60 days after sowing

There was a joint effect between light condition and flask sealing ($p < 0.05$) and an isolated effect of cultivation time ($p < 0.05$) on the germination percentage (%G) of *D. nobile* at 30 and 60 days after sowing.

The highest germination values occurred with the use of W light in PVC-sealed flasks (%G = 70.33), although without statistical difference neither from the treatment using the same light and SC-sealed flasks (%G = 67.14), nor from the treatment using R light in PVC-sealed flasks (%G = 70.27) (Table 1).

Table 1. Germination percentage (%G) depending on light condition (W light = white light; R Light = red light) and type of sealing (SC: screw cap; PVC: polyvinyl chloride film) in *Dendrobium nobile* Lindl.

Light condition	%G	
	SC	PVC
W light	67.14 aA	70.33 aA
R light	64.51 aB	70.27 aA
Mean	65.83	70.30
C.V. (%)	0.71	

Means followed by lowercase letter in the column and uppercase letter in the row do not differ by the Tukey test ($p < 0.05$).

Germination responses may vary considerably between different genera and even between species of the same genus. This indicates the need to study the ideal germination conditions for each species so as to improve plant propagation (Ferreira et al., 2017; Ferreira et al., 2022).

According to Hashim et al. (2021) that fluorescent light elicitation (blue, red, green, and white) has a positive influence in the plants and that different light regime can aid in optimizing plant growth and developmental changes for the propagation of commercially significant species in vitro.

D. nobile showed higher values of %G at 30 days (%G = 70.13) than at 60 days (%G = 66.00), differing statistically (Table 2). These values may correlate with the random choice of flasks for the evaluation. Orchid germination responses can be determined both by the media used and cultivation conditions - including light quality, quantity, and temperature - and by the genetic material under study (Yeung et al., 2018; Ferreira et al., 2022).

Table 2. Germination percentage (%G) depending on cultivation time in *Dendrobium nobile* Lindl.

Time (Days)	%G
30	70.13 a
60	66.00 b
Mean	68.06
C.V. (%)	2.87

Means followed by the same letter do not differ by the Tukey test ($p < 0.05$).

Schneiders et al. (2012) obtained similar results when studying *Cattleya forbesii* Lindl. The authors observed higher germination at thirty days after seed inoculation, regardless of culture medium. In contrast, when studying the germination of *Cyrtopodium glutiniferum* Raddi under different light conditions, Vogel and Macedo (2011) found the first signs of germination from 50 to 90 days after sowing, a period longer than that of the present study.

Germination responses may vary within the same family, genus, or even species. These responses depend on the conditions in which the fruits are produced, their maturation stage, and the processes used in storage (Juras et al., 2019; Sousa et al., 2020; Ferreira et al., 2022) (Figure 1), shows that, in addition to presenting high %G, the treatment with W light and SC sealing (W light - SC) was the only cultivation condition that led to root formation at 60 days after sowing.

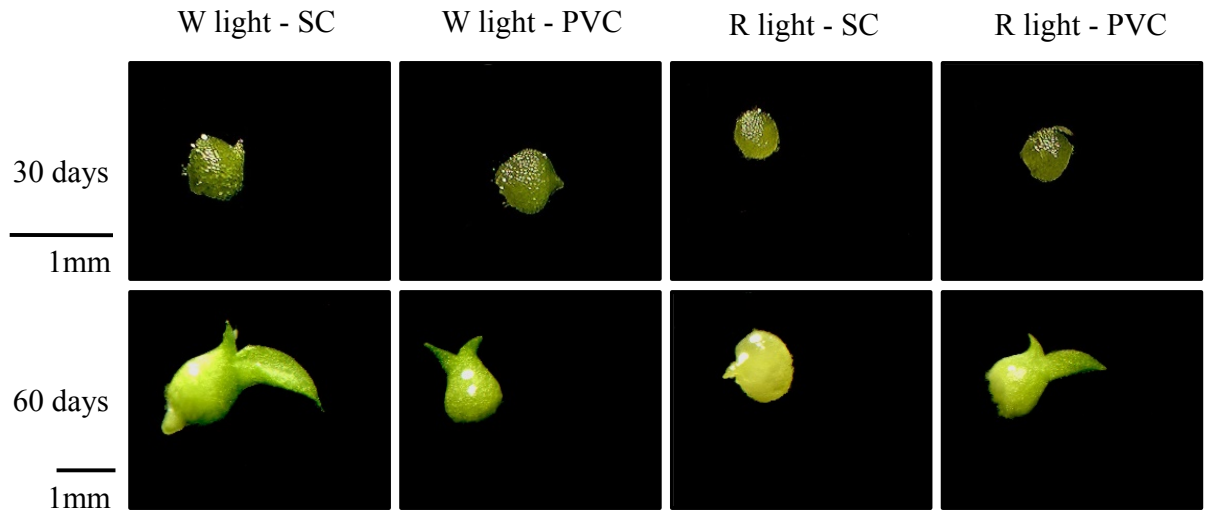

Figure 1. *Dendrobium nobile* Lindl. propagules at 30 and 60 days after sowing, under white light / screw cap (W light - SC), white light / PVC film (W light - PVC), red light / screw cap (R light - SC), and red light / PVC film (R light - PVC).

Initial development of protocorms 90 days after sowing

At 90 days after sowing, there was a joint effect between light condition and flask sealing on the percentages of survival (%SUR), stage 1 protocorms (%P1), stage 2 protocorms (%P2), stage 3 seedlings (%P3), and stage 4 seedlings (%P4).

The highest survival values occurred with the use of SC-sealed flasks and R light (%SUR = 100) (Table 3). Godo et al. (2011) states that light is an important but not limiting factor in orchid germination. Moreover, as observed by Sorgato et al. (2020a) when studying *Dendrobium* species and by Sorgato et al. (2021) when studying *Schomburgkia crispa* Lindl., species of the Orchidaceae family can germinate under different light conditions.

Table 3. Survival percentage (%SUR) depending on light condition (W light = white light; R light = red light) and type of sealing (SC: screw cap; PVC: polyvinyl chloride film) in *Dendrobium nobile* Lindl.

Light condition	%SUR	
	SC	PVC
W light	97.02 bA	95.38 aB
R light	100.00 aA	94.71 bB
Mean	98.51	95.05
C.V. (%)	0.32	

Means followed by lowercase letter in the column and uppercase letter in the row do not differ by the Tukey test ($p < 0.05$).

Regarding the initial development of protocorms, there was a significant difference for %P1 and %P2 under W light and SC sealing, presenting similar values (15.59%). In turn, %P3 was higher under R light and PVC sealing (32.70%), however without statistical difference in relation to W light with the same type of sealing (27.70%) (Table 4).

Table 4. Percentage of protocorms and seedlings in stage 1 (%P1), 2 (%P2), 3 (%P3), and 4 (%P4) depending on light condition (W light = white light; R light = red light) and type of sealing (SC: screw cap; PVC: polyvinyl chloride film) in *Dendrobium nobile* Lindl.

Light condition	%P1		%P2		%P3		%P4	
	SC	PVC	SC	PVC	SC	PVC	SC	PVC
W light	15.59 aA	0.00 aB	15.59 aA	10.82 aB	15.59 aB	27.70 aA	53.24 bB	61.47 aA
R light	0.00 bA	0.00 aA	0.00 bB	9.20 aA	0.00 bB	32.70 aA	100.00 aA	58.09 aB
Mean	7.80	0.00	7.80	10.01	7.80	30.20	76.62	59.78
C.V. (%)	7.67		7.99		7.63		3.11	

Means followed by lowercase letter in the column and uppercase letter in the row do not differ by the Tukey test ($p < 0.05$).

Considering that the aim of *in vitro* sowing is to produce the largest number of seedlings possible in the shortest time, the use of R light and SC sealing led to the best results. This treatment accounted for the highest number of stage 4 *D. nobile* seedlings at 90 days after sowing (100.00%) (Table 4). According to Taiz et al. (2017), the quality of the light provided to the propagated material is important to regulate the biochemical pathways that control plant growth and morphogenesis.

Hashim et al. (2021) shows in their review that for cultivation in a controlled environment, it is vital to reduce costs with electrical equipment and materials. Thus, buying fluorescent lamps is still more affordable than light-emitting diode (LED) lamps. In addition, they can be used in *in vitro* cultivation due to their high efficiency, excellent color reproduction and long shelf life.

Godo et al. (2009; 2011) and Gupta and Jatothu (2013) explain that the white light emitted by fluorescent lamps is the most used in growth rooms. Notwithstanding, the authors state that other qualities of light such as red (or combinations between them) can promote or inhibit plant growth and morphogenesis, being species-specific. Red light wavelength is 650 - 680 nm, being effective in the absorption of chlorophyll and resulting in an excellent photosynthetic activity (Hung et al., 2016). Thus, this quality of light contributed to the greater initial development of stage 4 *D. nobile* propagules (Table 4).

Regarding the type of sealing, SC does not allow the light to reach the explants with full intensity. This feature possibly avoids photooxidation, which is the prolonged exposure of plants or organelles to excess light, causing photodestruction, once excessive light can alter physiological processes that are essential to plant survival and photorespiration induction and consequently damage photosynthetic structures (Silva et al., 2017; Oliveira et al., 2021).

This may explain the higher percentage of stage 4 seedlings in this type of sealing. When studying different containers for *in vitro* culture of *Dendrobium nobile* Lindl., Moraes et al. (2009) observed that factors such as flask size and types of lid used to close containers can influence development in certain cultures. The authors also point out that the *in vitro* multiplication of a given plant species depends on several factors, and that the appropriate combination of all of them is what makes propagation successful.

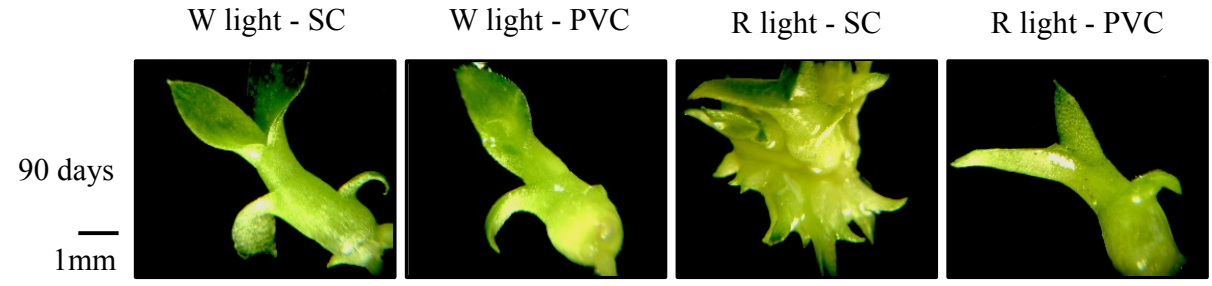

Figure 2. *Dendrobium nobile* Lindl. propagules at 90 days after sowing, under white light / screw cap (W light - SC), white light / PVC film (W light - PVC), red light / screw cap (R light - SC), and red light / PVC film (R light - PVC).

Figure 2 shows that seedlings little changed in size at 90 days after sowing; however, those submitted to R light and SC showed greater tillering than the others. Thus, light condition and flask sealing were not limiting either in germination or in *in vitro* growth of the plants.

Conclusions

For the germination of *Dendrobium nobile* Lindl. seeds, it is recommended to use white fluorescent light in conjunction with flask sealing with PVC film for 30 days of *in vitro* cultivation. For the initial development, red light in flasks with screw cap promotes better results for survival and a higher percentage of stage 4 protocorms.

Author Contribution

ISR: experimental execution, literature review and writing. **LMR**: planning, experimental execution and evaluation. **JSS**: planning, experimental execution, literature review and writing. **JCMR**: interpretation and discussion of results and writing. **JCS**: planning, experimental execution and evaluation, interpretation and discussion of results and writing.

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