Light sources on the germination and initial *in vitro* establishment of *Schomburgkia crispa* Lindl., a species of the Brazilian Cerrado

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ABSTRACT: *Light is one of the factors that influence the germination and initial establishment of orchids under in vitro cultivation. This study evaluated the effect of different light sources on these stages in in vitro cultivation of Schomburgkia crispa Lindl. After sowing in an aseptic environment, we stored the cultures in a screened greenhouse (natural light) or in a growth room with the following light sources: 3,000 K yellow LED; 6,500 K white LED [1]; 6,500 K white LED [2]; or 6,500 K white fluorescent lamp (control). We assessed germination percentage and initial seedling establishment at 45 and 90 days after sowing. Light did not influence the germination of S. crispa. However, the use of 3,000 K LED provided a faster initial establishment of S. crispa when compared to the other light sources, also presenting lower seedling mortality. Thus, the light source 3,000 K LED is a potential substitute for the 6,500 K fluorescent lamps and LEDs used in growth rooms in in vitro culture laboratories.*
Key words: *light-emitting diode, native species, ornamental horticulture, Orchidaceae.*

Fontes de luz na germinação e estabelecimento inicial *in vitro* de *Schomburgkia crispa* Lindl. uma espécie do Cerrado brasileiro

RESUMO: *A luz é um dos fatores que influenciam a germinação e o estabelecimento inicial no cultivo in vitro de orquídeas. Assim, objetivou-se avaliar o efeito de diferentes fontes de luz na germinação e no estabelecimento inicial in vitro de Schomburgkia crispa Lindl. Após semeadura em ambiente asséptico, as culturas foram acondicionadas em viveiro telado (luz natural) ou em sala de crescimento nas seguintes fontes luminosas: LEDs amarelo 3.000 K, branco 6.500 K [1], branco 6.500 K [2] ou lâmpada fluorescente branca 6.500 K (controle). Aos 45 e 90 dias após a semeadura foi avaliada a porcentagem de germinação e o estabelecimento inicial dos propágulos. A luz não influenciou a germinação das sementes de S. crispa, mas a utilização do LED 3.000 K proporcionou estabelecimento inicial de S. crispa em menor período e com menor mortalidade das plântulas, quando comparado com as demais fontes de luz utilizadas. A fonte de luz LED 3.000 K pode ser indicada como um substituto potencial para as lâmpadas fluorescentes e LEDs 6.500 K, utilizadas em salas de crescimento em laboratórios de cultivo in vitro.*
Palavras-chave: *diodo emissor de luz, espécie nativa, horticultura ornamental, Orchidaceae.*

INTRODUCTION

Schomburgkia crispa Lindl. is a species of the family Orchidaceae. It is an epiphyte that lives in gallery and dry forests of the Cerrado biome in Brazil (MENDONÇA et al., 2008; BARROS et al., 2018). In addition to their ornamental potential, Brazilian native orchids may also have some bioactive compounds with therapeutic applications. By means of phytochemical study in this species, the crispoic acid was isolated, which according to BELLOTO et al. (2017) has anticarcinogenic potential.

In vitro sowing of orchid seeds is a key tool in studies on biodiversity conservation because it maintains genetic variability, yielding a high germination percentage without symbiotic relationships (FAY, 2018). Orchid growth in natural environments is slow, which extends seedling production until the plants reach the reproductive stage. Using this type of cultivation is thus important to enable high-quality, large-scale production in a relatively short time, which contributes to reducing the risk of extinction (CARDOSO, 2014; HANUS-FAJERSKA & WOJCIECHOWSKA, 2017; FAY, 2018).

Several abiotic factors influence the germination and initial establishment of *in vitro* plants. Of these, we highlighted spectral composition and irradiance since light is fundamental for photosynthesis and photomorphogenesis in plants (SORGATO et al., 2015; HANUS-FAJERSKA & WOJCIECHOWSKA, 2017).

An alternative to this type of cultivation is plant production in natural light environments, replacing artificial light. In this sense, DIGNART et al. (2009) recommend cultivating *Catteya walkeriana* Gardn under natural light conditions and reducing sucrose in the culture medium. SILVA JÚNIOR et al. (2012) concluded that *Laelia purpurata* Lindl. var. carnea plants grown *in vitro* in a greenhouse adapt better for subsequent *ex vitro* cultivation than those grown in a growth room. Plants grown in the first condition showed higher epidermal thickness, leaf mesophyll thickness, root velamen thickness, and stomatal density. Moreover, EBURNEO et al. (2017) reported that *Catasetum x apolloi* Benelli & Grade stomata responded better to water stress when the orchid grew in a natural light environment.

Light-emitting diode (LED) offers possibilities in horticultural lighting since it can separate and mix different light spectra, allowing irradiance adjustments for plant photoreceptors (SINGH et al., 2015). Thus, this light source can regulate characteristics of *in vitro* grown plants, including morphological, anatomical variations, and physiological attributes like elongation, axillary bud formation, somatic embryo induction, rhizogenesis, leaf anatomy, and photosynthetic abilities (GUPTA & JATOTHU, 2013).

Notwithstanding, there are still few scientific studies using natural light or LED lamps as light sources in *in vitro* orchid culture. Furthermore, the effects of spectral quality and irradiance levels on the germination, growth, and initial establishment of plants are still unclear, mainly for native species. Given the above, this study evaluates the effect of different light sources on the *in vitro* germination and initial establishment of *Schomburgkia crispa* Lindl.

MATERIALS AND METHODS

The plant material consisted of seeds of *Schomburgkia crispa* Lindl. fruits produced from manual self-pollination. After harvesting, we took the fruits to the *in vitro* culture laboratory. Subsequently, we disinfected the fruits with 70% ethyl alcohol solution and opened them with a scalpel. We homogenized seeds of different fruits, further assessing the viability of 9,756 seeds by the tetrazolium test (SOARES et al., 2014). A total of 6,956 (71%) seeds were viable. We then discarded the seeds used in the viability test and stored the rest in a desiccator with silica gel (25 ± 2 °C; 75% RH) for 14 days.

For seed germination, we used ½ MS medium (MURASHIGE & SKOOG, 1962) solidified with 7.0 g L^{-1} bacteriological agar (Himedia®, India) and supplemented with 30 g L^{-1} sucrose. We adjusted the pH of the medium to 5.8 using 0.1M KOH. Then, we distributed the mixture in 50 mL screw-capped polypropylene vials (height and diameter = 5cm), considering a 20 mL culture medium volume per unit. Subsequently, we autoclaved the vials at 120 °C and 1 atm pressure for 20 minutes. After cooling (25 ± 2 ºC), we transferred the vials to a sterile environment.

We then weighed *S. crispa* seeds, further taking a 0.01 g portion to an aseptic environment and disinfecting it using 15 mL of 0.8% sodium hypochlorite solution for five minutes. After this period, we diluted the suspension to 100 mL using sterile distilled water. We washed the sample three times (80 mL per wash) and discarded the water after each wash. After this procedure, we completed the volume of the suspension to 100 mL with sterile distilled water for *in vitro* sowing, inoculating 1 mL of the seed suspension into each vial.

After inoculation, we took five vials to a greenhouse with two overlapping 50% shading screens (235 µmol m^{-2} s^{-1}) under average temperature and relative humidity of 22.6 ± 5 ºC and 73.9 ± 10%, respectively. We placed the other vials in a growth room with controlled temperature and photoperiod (25 ± 2 °C; 16 h), under the following light conditions: 3,000 K yellow LED (128 µmol m^{-2} s^{-1}); 6,500 K white LED [1] (58 µmol m^{-2} s^{-1}); 6,500 K white LED [2] (108 µmol m^{-2} s^{-1}); and 6,500 K white fluorescent lamp (23 µmol m^{-2} s^{-1}), which we used as control.

We assessed the germination percentage of the species at 45 days after sowing. For that, we washed the material contained in the vials with 3 mL of sterile distilled water; then, we placed it in 16-cell (0.5 x 0.5 cm) acrylic plates (2 x 2 x 0.5 cm). We repeated this procedure until there was no seed or seedling in the growing units. Then, we used a binocular stereoscopic microscope to count non germinated seeds (NS) and chlorophyll propagules (CP). We calculated germination percentage (G%) according to modified ROSA et al. (2013), using the following expression: $G\% = \dfrac{CP}{(NS + CP)} x100$.

We assessed the initial establishment at 45 and 90 days after sowing, following the methodology

described by SUZUKI et al. (2009). We also counted the number of nonchlorophyll propagules (NCP). After the evaluations, we photographed all treatments with a camera coupled to the stereoscopic microscope with the aid of AxionVision software version 3.1 (Zeiss®).

The experimental design was completely randomized with five treatments (natural light; 3,000 K LED; 6,500 K LED [1]; 6,500 K LED [2]; and 6,500 K fluorescent lamp) and five replicates consisting of a culture vial. We transformed the results to $\sqrt{(x+1)}$ and submitted them to analysis of variance and comparison by the Tukey test up to 5% probability with the aid of the SISVAR program version 5.3 (Statistical Analysis Program; Federal University of Lavras, MG).

RESULTS AND DISCUSSION

Light conditions affected all the characteristics (p<0.05) except germination percentage. All treatments provided high germination percentages, with an overall mean of 98% (Table 1).

The highest germination percentage occurred at 45 days with the use of 3,000 K LED, with no statistically significant difference between light sources, which is an important factor for *in vitro* cultured orchids (GUPTA & JATOTHU, 2013; HANUS-FAJERSKA & WOJCIECHOWSKA, 2017). However, these results suggested that light source is not a limiting factor for *S. crispa* seed germination, since all conditions led to low mortality, with an overall mean of chlorophyll propagules of 94%.

Regarding initial establishment, most of the propagules were in stage 1 (chlorophyll swollen protocorm) at 45 days. Natural light, 6,500 K LED [1], and 6,500 K LED [2] provided 100% of these

protocorms, and seedlings developed up to stage 2 (first-leaf seedlings) only when subjecting the cultures to 3,000 K LED (20%) or 6,500 K fluorescent lamp (1%). Thus, the results allowed us to infer that the use of 3,000 K LED in growth rooms can more rapidly enhance the initial establishment of *S. crispa* seedlings (Table 1).

Similarly to the results at 45 days after sowing, at 90 days of *in vitro* culture there was a significant effect (p<0.05) of the different light conditions only for the initial establishment of *S. crispa* propagules, whose overall mean of germination was 100%.

In this evaluation, the light condition 6,500 K LED [2] provided the highest number of propagules in stage 1 (99%), which represented the smallest differentiation between stages (protocorms and seedlings) among all experimental conditions. This has to do with the hypothesis of light stress: when in understory conditions, natural environment orchids receive, on average, 10 µmol m^{-2} s^{-1} (SOARES et al., 2020), a lower irradiance when compared to growth room plants subjected to 6,500 K LED [2] (Figure 1A).

Prolonged exposure of plants or organelles to excess light can lead to photodestruction of photosynthetic pigments, as discoloration (photooxidation) depends on oxygen and light. This phenomenon can lead to cell death, or organism death, and may relate to the high mortality of propagules in this light condition at 90 days (ARAÚJO & DEMINICIS, 2009).

The conditions of 6,500 K fluorescent lamp and 3,000 K LED accelerated the initial establishment of *S. crispa* seedlings, with 23 and 43% stage 4 seedlings, respectively. Using 3,000 K LED provided a 46% increase in the number of seedlings in this stage of development compared to 6,500 K

Table 1 - Percentages of germination (G%), chlorophyll propagules (%CP), nonchlorophyll propagules (%NCP), stage 1 protocorms (%P1), and stage 2 seedlings (%P2) of *Schomburgkia crispa* Lindl. under different light conditions at 45 days after sowing.

Light condition	%G	%CP	%NCP	%P1	%P2
Natural light	98 a	100 a	1 b	100 a	0 b
3,000 K LED	100 a	95 ab	5 ab	80 b	20 a
6,500 K LED [1]	98 a	93 ab	7 ab	100 a	0 b
6,500 K LED [2]	97 a	86 b	14 a	100 b	0 b
6,500 K fluorescent	98 a	96 ab	9 ab	96 a	1 b
Overall mean	98	94	7	95	4
C.V. (%)	1.28	2.85	48.19	2.09	17.46

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

Figure 1 - (A): Percentage of stage 1 protocorms (P1) and stage 2 (P2), 3 (P3), and 4 (P4) seedlings; and (B): percentage of chlorophyll propagules (CP) and nonchlorophyll propagules (NCP) of **Schomburgkia crispa** Lindl. under different light conditions at 90 days after sowing. Bars followed by the same letter in the x-axis do not differ by the Tukey test (p<0.05).
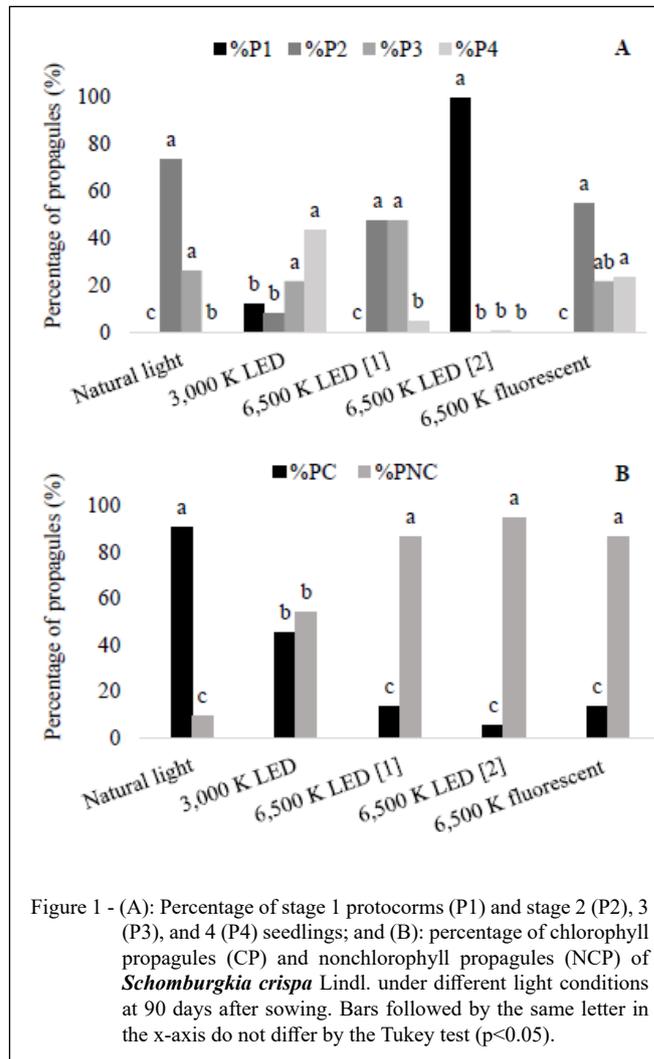
fluorescent lamp. Light-emitting diode (LED) lamps have several advantages over fluorescent lamps, such as: lower morphological and physiological variations in embryos; long shelf life; high efficiency in light generation; and low heat emission, requiring less energy in the growth room (GUPTA & JATOTHU, 2013; HANUS-FAJERSKA & WOJCIECHOWSKA, 2017). These results showed that, besides the possibility of cost reduction in seed propagation, the LED source accelerates the *in vitro* establishment of *S. crispa*.

The three physical quantities of light that most influence *in vitro* growth and morphogenesis are: duration of light exposure (photoperiod), flux density, and wavelength (HANUS-FAJERSKA & WOJCIECHOWSKA, 2017). Growth rooms usually use the cold white light emitted by fluorescent lamps.

However, the use of other spectral bands - for example, red, blue, green, yellow, or their combination - may improve or hinder the development of *in vitro* species (GUPTA & JATOTHU, 2013; HANUS-FAJERSKA & WOJCIECHOWSKA, 2017).

Although 3,000 K LED provides higher irradiance than 6,500 K LED [2], it has two winding peaks, at $\cong$ 460nm and $\cong$ 580nm, what probably contributes to the development of propagules (Figure 2). In addition, 3,000 K LED has more energy in the red region, which, from a physiological point of view, contributes more effectively to the excitation of photosynthetic pigments.

For plants cultured in screened greenhouses under natural light, 73% of seedlings were in stage 2 and 26% in stage 3. At 90 days of cultivation, only

Figure 2 - Spectral energy distribution of the LEDs and fluorescent lamp.
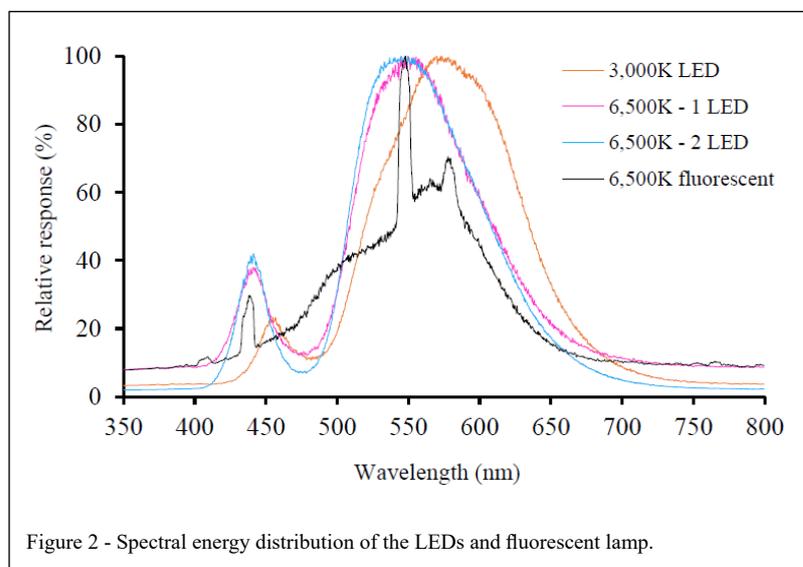
0.2% of the seedlings reached stage 4. Thus, the initial *in vitro* establishment of the species takes longer under these conditions compared to growth room conditions. The greenhouse environment is not controlled like the growth room environment, which results in a temperature oscillation inside the culture vial, slowing down the initial establishment of protocorms and seedlings (DIGNART et al., 2009).

Moreover, the light sources 6,500 K LED [1], 6,500 K LED [2], and 6,500 K fluorescent lamp led to a higher mortality of propagules, confirmed by the higher percentage of nonchlorophyll propagules: 87, 87, and 95%, respectively (Figure 1B).

Growth rooms often use white fluorescent lamps (6,500 K), which are losing ground for white LED lamps (6,500 K). Notwithstanding, it is crucial to adjust the irradiance and spectral composition of the light sources of these rooms according to the cultivated species (HANUS-FAJERSKA & WOJCIECHOWSKA, 2017). The results of this study for species *S. crispa* showed that 6,500 K white fluorescent lamps and 6,500 K LEDs provided a higher percentage of nonchlorophyll propagules. This raises the hypothesis of replacing these lamps by 3,000 K LED lamps aiming for fast initial establishment, with stage 4 seedlings in shorter time and low percentage of nonchlorophyll propagules.

CONCLUSION

Light is not a limiting factor for germination of *Schomburgkia crispa* Lindl seeds. Using 3,000 K LED provides faster initial establishment of *S. crispa*, with lower seedling mortality. The light source 3,000 K LED is a potential substitute for 6,500 K fluorescent lamps and LEDs used in growth rooms in *in vitro* culture laboratories.

DECLARATION OF CONFLICT OF INTERESTS

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

AUTHORS' CONTRIBUTIONS

All authors contributed equally to the manuscript.

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