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Original Paper

In vitro and *ex vitro* production of *Schomburgkia crispa*: effect of flask sealing systems and different light sources

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Abstract

The extraction of native orchids from natural habitats is relevant for the reduction of populations in the Cerrado biome, making it necessary to establish practices aiming their production both for reintroduction and commercialization. The objective here is to evaluate light sources and sealing systems on the *in vitro* and *ex vitro* growth of *Schomburgkia crispa*. Two flask sealing systems were tested: conventional (CSS) and with gas exchange (SSGE), and eight light sources: FL1- 100% white LED, FL2- 100% blue LED, FL3- 100% red LED, FL4- 50% white + 25% red + 25% blue LED, FL5- 50% red + 50% blue LED, FL6- 25% red + 75% blue LED, FL7- 75% red + 25% blue LED, and FL8- with fluorescent lamp, with five replications in each treatment. A completely randomized design was adopted with a 2x8 factorial scheme (vial sealing system x light sources). After 120 days of cultivation *in vitro* and 180 days *ex vitro*, the plants were evaluated as for number of leaves, roots and shoots, plant height, pseudobulb diameter, length of the largest root, largest leaf, and fresh mass. For the *in vitro* growth, the use of SSGE together with the light sources blue and red favors the cultivation of *S. crispa.* For the *ex vitro* growth, the cultivation *in vitro* in SSGE together with FL4 affects the acclimatization of plants.

Key words: acclimatization, light sources, micropropagation, native species, Orchidaceae.

Resumo

A extração de orquídeas nativas dos habitats naturais constitui um fator relevante para a diminuição das populações do bioma Cerrado, tornando-se necessário estabelecimento de práticas visando sua produção tanto para reintrodução quanto para comercialização. Objetivou-se avaliar as fontes de luz e o sistema de vedação no crescimento *in vitro* e *ex vitro* de *Shomburgkia crispa*. Foram testados dois sistemas de vedação dos frascos: convencional (CSS) ou com trocas gasosas (SSGE) e oito fontes de luz: FL1 - LED 100% branca; FL2- LED 100% azul; FL3- LED 100% vermelha; FL4- LED 50% branca + 25% vermelha + 25%azul; FL5- LED 50% vermelha + 50% azul; FL6- LED 25% vermelha + 75% azul; FL7- LED 75% vermelha + 25% azul e FL8- fluorescente branca, com cinco repetições, em cada tratamento. Foi adotado um delineamento inteiramente casualizado em esquema fatorial 2x8 (sistema de vedação dos frascos x fontes de luz). Após 120 dias de cultivo *in vitro* e 180 dias *ex vitro*, as plantas foram avaliadas quanto ao número de folhas, raízes e brotos, altura da planta, diâmetro do pseudobulbo, comprimento da maior raiz e da maior folha e massa fresca. Para o crescimento *in vitro,* a utilização do SSGE em conjunto com fontes de luz azul e vermelho, favoreceu o cultivo de *S. crispa.* Para o crescimento *ex vitro*, o cultivo *in vitro* em SSGE em conjunto com FL4, influenciou a aclimatização das plantas.

Palavras-chave: aclimatização, fontes de luz, micropropagação, espécies nativas, Orchidaceae.

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Introduction

Orchidaceae contains a large number of genera and species. It is the second largest family among angiosperms. They are distributed in all continents, with a greater concentration and diversity in tropical and subtropical regions (Ferreira *et al*. 2022). In Brazil, there are about 2,677 species of orchids, of which 1,484 are endemic to it (Flora & Funga of Brazil 2022, continuously updated). These plants stand out in the flower and ornamental plant sector due to genetic combinations and exuberance of flowers, in addition to their appeal for food and pharmacological purposes (Teixeira da Silva *et al*. 2015; Belloto *et al*. 2017; De Stefano *et al*. 2022).

The *Schomburgkia crispa* Lindl. is a species of epiphytic habit found both in gallery forests and dry forests of the Cerrado, including the state of Mato Grosso do Sul (Ostetto 2015; Barros *et al*. 2018). Morphologically, it presents bifoliate pseudobulbs with a length of 8 to 10 cm, leaf length from 24 to 26 cm, and leaf width from 5 to 7 cm. The inflorescences are, on average, 95 to 110 cm long, with brown flowers, petals and sepals, yellow borders, and a lilac lip (Sorgato *et al*. 2021b). In January 2017, this species was included in the Appendix II of the CITES (Cites 2017), which includes species not necessarily threatened with extinction but whose trade must be controlled in order to avoid uses incompatible with its survival.

As an alternative to a rapid and efficient multiplication of orchids and aiming conservation and production on a commercial scale, *in vitro* cultivation techniques are used (Ribeiro *et al*. 2019; Sorgato *et al*. 2020; Soares *et al*. 2020; Castilho-Pérez *et al*. 2021; Kumar *et al*. 2022). Among the techniques, the *in vitro* germination of orchid seeds stands out since this type of sowing leads to high percentages of germination compared to germination under natural conditions, which occurs only in the presence of mycorrhizal fungi (Zhang *et al*. 2018; Soares *et al*. 2020; Kumar *et al*. 2022). In this sense, the production of orchids by that method is important, as it allows a large number of seedlings to grow in a small space, in a short time, and with a high sanitary quality (Teixeira da Silva *et al*. 2015, 2017a; Ferreira *et al*. 2022; Pereira *et al*. 2022; Nowakowska *et al*. 2022).

Several factors affect the growth and development of the *in vitro* culture. Among them culture medium, flask sealing system, and light stand out. Culture media must meet the needs of plants in terms of mineral nutrition. Thus, there may be changes in formulations to meet the requirements of each species cultivated *in vitro* (Galdiano Júnior *et al*. 2013; Silva *et al*. 2015; Miler *et al*. 2019).

The conventional sealing system provides, inside *in vitro* cultivation flasks, a high relative humidity and low gas exchange. These factors may lead to anatomical and metabolic disorders in plants subjected to this system (Silva *et al*. 2016). Therefore, different strategies can be used for sealing, such as a natural ventilation of flasks (Silva *et al*. 2014, 2016; Ribeiro *et al*. 2019; Santos *et al*. 2020).

The light-emitting diode (LED) technology offers countless possibilities in horticultural lighting due to its ability to mix and separate different light spectra, allowing appropriate irradiance adjustments to the plant's photoreceptors, in addition to reducing energy consumption in growth rooms and causing less impacts on the environment (Singh *et al*. 2015; Loconsole *et al*. 2019). It is also possible to regulate parameters of plants cultivated *in vitro*: morphological and anatomical variations and physiological attributes, such as elongation, formation of axillary shoots, induction of somatic embryos, rhizogenesis, leaf anatomy, and photosynthetic abilities (Gupta & Jatothu 2013).

Cultivation protocols *in vitro* may also affect survival and establishment *ex vitro*. Thus, for the success of this cultivation, it is important to ensure the plant's adjustment to *ex vitro* conditions during acclimatization since these plants need to complete the autotrophism after transfer (Teixeira da Silva *et al*. 2017b; Santos *et al*. 2020).

However, there are still few scientific studies analyzing sealing systems and using LED lamps as a source of light energy in *in vitro* cultivation considering its influence on the acclimatization of native species, such as *S. crispa*. Thus, the objective here is to evaluate the effects of these two parameters in the *in vitro* and *ex vitro* growth of the orchid *Schomburgkia crispa* Lindl., native to the state of Mato Grosso do Sul, Brazil.

Materials and Methods

Experimental environment and biological material

This experiment was conducted at the *in vitro* Cultivation Laboratory of Flowers and Ornamental Plants (LabCFPO) of the Faculty of Agricultural Sciences (FCA) of the Federal University of Grande Dourados (UFGD).

The study material was ripe fruits of *Schomburgkia crispa* grown by hand pollination and matrices more than ten years old cultivated at the Orchid Garden of the Faculty of Agrarian Sciences (22°11'53.2"S; 54°56'02.3"W). The nursery is covered by an overlap of two 50% shading screens, providing a shading of 10% and an irradiance of 235 µmol m^{-2} s^{-1}, under average temperature and relative humidity of 22.6 ± 5 ºC and 73.9 ± 10%, respectively. Irrigation was performed by ballerina-type microsprinklers positioned one meter above the plants, totaling a water depth of 1 mm day^{-1}.

To meet the objective proposed here, the plants were submitted to *in vitro* cultivation in the LabCFPO and *ex vitro* cultivations in a nursery.

Cultivation *in vitro* – 120 days

A sample of 0.005 g of seeds was collected, and the tetrazolium test was performed according to the methodology of Soares *et al*. (2014). After confirming viability, another sample of 0.005 g of seeds was collected in an aseptic environment and disinfected according to the methodology of Soares *et al*. (2020) to obtain the seed solution. For *in vitro* sowing, 1.0 mL of the disinfested seed suspension was inoculated per culture flask. 60 ml of Murashige & Skoog (1962) culture medium were used at half the salt concentration (MS 1/2) per flask, which has a capacity of 600 ml. Then, the cultures were placed in a growth room with controlled temperature and photoperiod (25±2 °C; 16 h) and irradiance of 22 µmol m^{-2} s^{-1} provided by two white fluorescent lamps (6500K), remaining under these conditions for up to 120 days. Three subcultures were conducted.

After this period, the seedlings were standardized in terms of size (1.5 cm) and subcultured for the beginning of the experimental period. The MS culture medium solidified with 7.0 g L^{-1} bacteriological agar (Himedia®, India) and topped up with 30 g L^{-1} sucrose. The pH of the medium was measured and adjusted to 5.8 using KOH (0.1M) before sterilization in autoclave (121 °C and 1.1 atm pressure) for 20 minutes. 60 mL of the medium were distributed into flasks with a capacity of 600 mL. There were four seedlings per culture flask inoculated in an aseptic environment. Subsequently, half the flasks was hermetically sealed with polyvinyl chloride (PVC) film (conventional sealing system - CSS) and the other half with PVC with cotton filter (sealing system allowing gas exchange - SSGE).

The cultures were then placed in a growth room with controlled temperature and photoperiod (25 ± 2 °C; 16 h) under the following light sources: FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; and, as a control, FL8- white fluorescent lamp (Fig. 1).

Spectral distribution measurements were taken with an Ocean Optics portable spectrometer (Model MMO with fiber optics) at room temperature with an integration time of 10 ms.

After 120 days of cultivation, the flasks were removed from the growth room and opened. The seedlings were removed and washed under running water until the culture medium was completely removed. The following factors were evaluated with a digital caliper and a precision scale: survival (%SUR), number of leaves (NL), number of roots (NR), plant height (PHe) (mm), length of the largest leaf (LL) (mm), length of the largest root (LR) (mm), and fresh mass (TFM) (g). After the evaluations, the seedlings were photographed with a camera attached to a mini photographic studio.

Cultivation *ex vitro* – 180 days

For the evaluation of *ex vitro* growth, the plants were transferred to disposable transparent polypropylene containers with a capacity of 1,000 mL (20 × 10 × 5 cm). There were holes in the lid for gas exchange and holes in the base for substrate drainage, with 1/3 of its volume filled with pink sphagnum (Agrolink, Holambra, SP) plus coconut fiber (Golden-Mix Chips, Amafibra) (1:1, $v:v^{-1}$).

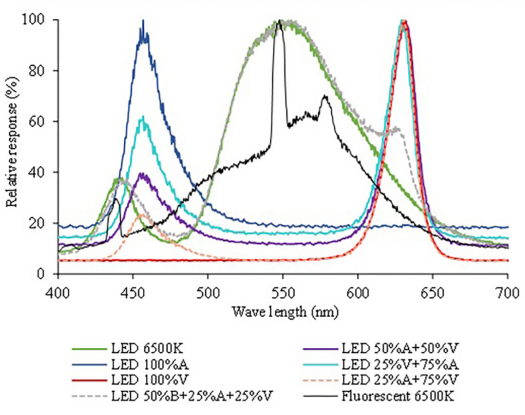

Figure 1 – Spectral energy distribution of LEDs and of the fluorescent lamp.

After transplantation, they were placed in a screened nursery and remained there for 180 days under the same conditions as those used for mother plants. In the first 15 days, the containers remained with the lids closed in order to minimize stress caused by the change in environment (*in vitro* to *ex vitro*). After this period, the lids were opened. Irrigation during the experimental period was performed by ballerina-type microsprinklers positioned one meter above the plants, totaling a water depth of 1 mm day^{-1}.

Fertilizations were carried out via leaf every 15 days with 2.0 mL L^{-1} of NPK 10-10-10, plus the following micronutrients: 0.025% magnesium, 0.02% boron, 0.05% copper, 0.10% iron, 0.05% manganese, 0.0005% molybdenum, and 0.05% zinc, with a maximum chlorine content of 0.025%. At 0, 30, and 60 days, the plants were preventively disinfected with O-S-dimethyl-N-acetyl-phosphoramidothioate (4 mg L^{-1}) and Mancozeb (4 mg L^{-1}). Both for leaf fertilization and disinfestation, a backpack sprayer with a capacity of 5 L was used.

After this, the plants were removed from the containers and washed under running water until the substrate was completely removed. Then, plants were evaluated as for the same initial characteristics (NL, PHe, NR, LR, LL, TFM, and %SUR).

In order to investigate the hypothesis of increased plant growth during the *ex vitro* phase according to the treatment to which plants were initially exposed, the increases in values (I) were calculated in relation to the initial values using the expression I = (FV - IV), where IV is the value of the characteristic before the plant is acclimatized and FV is the value of the same variable after the *ex vitro* period. The values were expressed in percentage and submitted to analysis of variance.

Experimental design and statistical analysis

The experimental design was completely randomized with a 2x8 factorial scheme (two sealing systems and eight light conditions). There were five replications. Each experimental unit consisted of a flask containing four seedlings. The data of the sealing system were submitted to analysis of variance, and the means were compared by F test. Light condition data were analyzed by Scott-Knott test at 5% probability using the SISVAR statistical program (Program of Statistical Analysis, v. 5.3, Federal University of Lavras, MG) (Ferreira 2011).

Results and Discussion

In vitro growth – 120 days

There was an isolated effect of light sources and sealing system on the number of leaves (NL) and number of roots (NR). There was also a significant effect of the interaction between light sources and sealing systems as for the characteristics plant height (PHe), length of the largest leaf (LL), length of the largest root (LR), total fresh mass (TFM), and percentage of survival (%SUR) after four months of *in vitro* cultivation (Fig. 2).

We noted that, for the isolated effect of light sources on the variable NL, plants of *S. crispa*, when cultivated in FL7, had a higher number of leaves (18.67) but no significant difference between FL8 and FL6, with means of 16.17 and 14.46 leaves, respectively. As for the sealing system, the plants, when cultivated under the CSS, had the highest NL: 14.16 leaves. The highest NR (8.71) occurred when plants were submitted to 100% white LED lamp, with a mean of 8.71

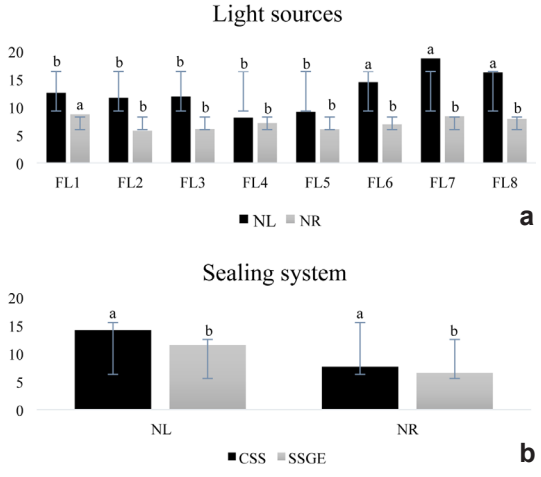

Figure 2 – a-b. Number of leaves (NL) and number of roots (NR) of *Schomburgkia crispa* in function after four months of *in vitro* cultivation – a. different light sources; b. sealing system. FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; FL8- white fluorescent lamp. CSS = conventional sealing system; SSGE = sealing system with gas exchange. Same letters differ by Scott-Knott test ($p < 0.05$) (a) and F test ($p < 0.05$) (b).

roots but no significant difference between FL8 and FL7: 7.88 and 8.33, respectively (Fig. 2a). The NR was higher in plants grown in a conventional sealing system, with a mean of 7.64 roots (Fig. 2b).

In this work, *S. crispa* showed an increase both in number of leaves and number of roots when grown under lamps that contain a red wavelength in a light composition of up to 75%. Hung *et al*. (2016) reported that the wavelength of the red light promotes leaf growth, anatomical changes, and carbohydrate accumulation in plant material. However, other authors pointed out that each cultivated species reacts differently to the light conditions provided and that this directly affects plant growth and development (Taiz *et al*. 2017).

Both the highest NR and NL occurred when CSS was used since the conditions established by this system are related to plant tillering, which is affected by the greater number of shoots (Ribeiro *et al*. 2019). Freitas *et al*. (2021) observed a similar result for *Cattleya nobilior* Rchb.f. cultivated *in vitro*. It presented a greater number of leaves and a greater fresh mass in plants cultivated under conventional system due to their tillering setting. This system interferes with gas exchange since the hermetic sealing of flasks results in an increase in CO_2 and ethylene gas. This may cause physiological changes in plants, tissue growth, and induction and differentiation of plant organs (Silva *et al*. 2014; Teixeira da Silva *et al*. 2017a).

For the interaction between light sources and sealing systems, plants grown in SSGE and submitted to FL6 showed a higher PHe (54.37 mm) and no significant difference from FL7 (47.01 mm). The plants of *S. crispa* showed a higher LL (40.51 mm) when cultivated in FL6 with SSGE but no significant difference to FL5 and FL7 (32.54 and 35.85, respectively). Regarding the LR (57.14 mm), the highest means occurred when plants were cultivated under FL4 using CSS (Tab. 1).

The highest FM values (0.999 g) occurred when plants were grown under FL1 using SSGE, although there was no significant difference to the FL6 treatment (0.939 g). Regarding the percentage of survival, all treatments showed satisfactory results, with 100% survival. However, the FL5 under SSGE resulted in 91.67% of live plants (Tab. 1).

When analyzing the results of this study, in general the SSGE and the FL6 light source are beneficial for the survival and *in vitro* growth of *S. crispa*.

This may be related to the use of SSGE because this system allows gas exchange between the internal and external environments to the flask, which results in a decrease in the accumulation of ethylene gas and CO_2, thus contributing to plant growth during *in vitro* cultivation (Silva *et al*. 2016).

Furthermore, according to Silva *et al*. (2014), the conditions of gas exchange in the *in vitro* cultivation of plants allow an approximate cultivation of photoautotrophy, promoting changes in tissues and favoring the growth of plant organs.

As for light sources, the best results observed in the combinations of blue and red may be related to the ability of these wavelengths to better excite photoreceptors (phytochromes, phototropins, and cryptochromes), thus increasing photosynthetic activity (Dou *et al*. 2017). Furthermore, the greater proportion of blue to red wavelengths (3:1) absorbed by phototropin and cryptochromes may be associated with leaf expansion since phototropin is related to this function (Macedo *et al*. 2011; Cunha *et al*. 2019).

Taiz *et al*. (2017) reported that the quality of light provided to plant material is considered important because it regulates plant morphogenesis and growth. However, white light is a mixture of low intensities of red and blue and other wavelengths of low-efficiency light (Cunha *et al*. 2019). This may be related to the higher values observed in white light treatments and in combinations of blue and red.

The results found in this study show that the light condition of the cultivation room for Orchidaceae species are, in addition to species-specific, also related to the stage of development of the plant. The results found in the literature differ according to the stage of seedlings used (Freitas *et al*. 2021; Sorgato *et al*. 2021a).

Figure 3 shows the variation in morphological aspects of plants as a function of sealing systems and irradiance. As can be visually assessed, plants grown in CSS appear to be smaller than those grown under SSGE. They showed a higher PHe, a higher LL, and a higher LR. Visually, it can be seen that plants grown in CSS show shoots with a higher number of leaves and roots compared to the growth of these vegetative structures under SSGE, which showed a greater growth, especially when plants were grown under the light sources FL2, FL4, FL6 and FL7.

Table 1 – Plant height (PHe mm), length of largest leaf (LL mm), length of largest root (LR mm), total fresh mass (TFM) (g), and percentage of survival (%SUR) of *Schomburgkia crispa* in function of sealing system (CSS = conventional sealing system; SSGE = sealing system with gas exchange) and different light sources after four months of *in vitro* cultivation.

Flask	PHe		LL		LR		TFM		%SUR	
	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE
FL1	29.48 bB	40.88 bA	19.63 bA	28.46 bA	34.35 bA	44.82 aA	0.488 bB	0.999 aA	100.00 aA	100.00 aA
FL2	35.93 bA	38.64 bA	23.89 bA	28.83 bA	28.38 bA	30.84 bA	0.381 bA	0.586 bA	100.00 aA	100.00 aA
FL3	33.57 bB	40.67 bA	18.91 bB	28.06 bA	31.78 bA	31.48 bA	0.733 aA	0.584 bA	100.00 aA	100.00 aA
FL4	50.45 aA	30.12 bB	39.58 aA	20.40 bB	57.14 aA	32.13 bB	0.960 aA	0.431 bB	100.00 aA	100.00 aA
FL5	28.88 bB	42. 95 bA	19.73 bB	32.54 aA	27.93 bB	50.38 aA	0.404 bA	0.681 bA	100.00 aA	91.67 bB
FL6	34.62 bB	54.37 aA	21.83 bB	40.51 aA	39.02 bB	52.48 aA	0.630 bB	0.939 aA	100.00 aA	100.00 aA
FL7	35.70 bB	47.01 aA	22.23 bB	35.85 aA	37.17 bA	47.64 aA	0.491 bA	0.704 bA	100.00 aA	100.00 aA
FL8	28.83 bA	34.05 bA	18.03 bA	26.42 bA	33.77 bA	41.27 aA	0.498 bA	0.718 bA	100.00 aA	100.00 aA
GM	34.68	41.09	22.98	30.13	36.19	41.38	0.573	0.705	100.00	98.96
CV (%)	16.64		20.19		17.65		14.47		0.18	

Means followed by the same lowercase letter in the column and uppercase in the row in the variables do not differ statistically from each other by Scott-Knott test and the F test, respectively ($p \geq 0.05$). CSS = conventional sealing system; SSGE = sealing system with gas exchange. FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; FL8- white fluorescent lamp.

Ex vitro growth - six months

For the 180 days of *ex vitro* cultivation of *S. crispa*, there was an interaction between light conditions and sealing systems previously used in *in vitro* cultivation ($p < 0.05$) for all characteristics evaluated. As for %SUR, the best results occurred when plants were previously cultivated under the SSGE system and the light sources FL1, FL3, FL4, FL5, FL6, and FL8, providing 100% of survival (Tab. 2).

At the end of the experimental period *ex vitro*, the highest PHe values occurred when the SSGE was previously used in conjunction with the light source FL4, leading to a 62.48% increase. For NL, higher increases occurred in SSGE under white LED (FL1) (6.17%), differing only from FL2 (0.17%) and FL7 (1.75%). Regarding the NR, the highest percentage of increase (6.50%) occurred when plants were previously submitted to CSS + FL2 (Tab. 2).

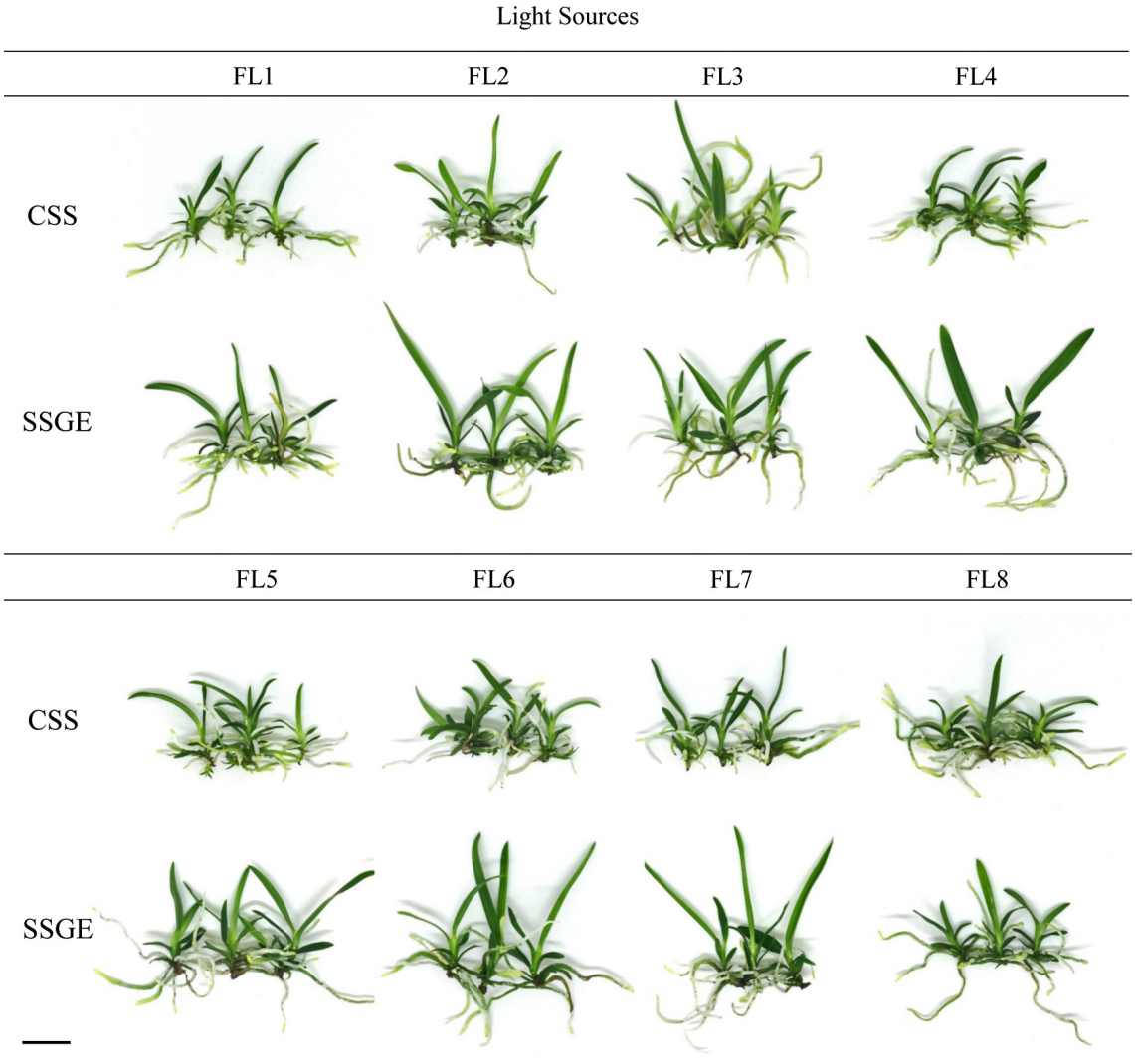

Figure 3 – *Schomburgkia crispa* plants at 120 days of *in vitro* cultivation as a function of light sources and sealing systems. FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; FL8- white fluorescent lamp. CSS = conventional sealing system; SSGE = sealing system with gas exchange.

Table 2 – Increases (%) in plant height (PHe, mm), number of leaves (NL), number of roots (NR), length of largest leaf (LL mm), length of largest root (LR mm), total fresh mass (TFM), and percent survival (%SUR) of *Schomburgkia crispa* in function of sealing system (CSS = conventional sealing system; SSGE = sealing system with gas exchange) and different light sources after six months of *ex vitro* cultivation.

Flask	PHe		NL		NR		LL		LR		TFM		%SUR	
	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE	CSS	SSGE
FL1	36.73 aA	27.77 cA	5.25 aA	6.17 aA	4.83 bA	5.83 aA	29.87 aA	22.42 cA	41.03 aA	27.90 bB	0.90 aA	0.73 bA	100.00 aA	100.00 aA
FL2	38.69 aA	2.28 dB	5.41 aA	0.17 bB	6.50 aA	0.33 cB	30.35 aA	0.70 dB	33.87 aA	1.67 dB	0.85 aA	0.02 cB	100.00 aA	8.33 cB
FL3	14.03 cB	43.96 bA	2.42 cB	5.25 aA	3.08 cB	3.74 aA	11.33 cB	38.90 bA	14.99 cB	35.22 aA	0.17 cB	0.64 bA	66.67 bB	100.00 aA
FL4	17.47 cB	62.48 aA	1.00 dB	6.08 aA	2.67 cB	5.25 aA	7.78 cB	49.74 aA	17.68 cB	45.23 aA	0.28 cB	1.11 aA	58.33 cB	100.00 aA
FL5	38.15 aB	49.12 bA	5.08 aA	4.25 aA	4.92 bA	4.92 aA	23.80 aB	34.00 bA	25.56 bB	38.72 aA	0.55 bA	0.75 bA	100.00 aA	100.00 aA
FL6	0.00 dB	39.43 bA	0.00 dB	5.42 aA	0.00 dB	5.83 aA	0.00 dB	36.32 bA	0.00 dB	39.21 aA	0.00 cB	1.06 aA	0.00 dB	100.00 aA
FL7	25.94 bA	30.61 cA	3.33 bA	1.75 bA	2.67 cA	2.25 bA	16.25 bA	19.81 cA	15.37 cA	17.71 cA	0.26 cA	0.19 cA	66.67 bB	83.33 bA
FL8	0.00 dB	28.10 cA	0.00 dB	5.50 aA	0.00 dB	4.50 aA	0.00 dB	23.89 cA	0.00 dB	28.58 bA	0.00 cB	0.54 bA	0.00 dB	100.00 aA
GM	21.4	35.47	2.81	4.32	3.08	4.08	14.92	28.22	18.56	29.28	0.38	0.63	61.46	86.46
CV (%)	26.38		22.28		22.20		27.90		29.79		11.01		1.08	

Means followed by the same lowercase letter in the column and uppercase in the row in the variables do not differ statistically from each other by Scott-Knott test and the F test, respectively ($p \geq 0.05$). CSS = conventional sealing system; SSGE = sealing system with gas exchange. FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; FL8- white fluorescent lamp.

As for LL, the highest *ex vitro* results occurred with a previous cultivation under SSGE + FL4 (49.74%). Regarding the LR, the highest values occurred after *in vitro* cultivation under SSGE and FL4 (45.23%), with no significant difference between FL3 (35.22%), FL5 (38.78%), and FL6 (39.21%) using the same sealing system. For TFM, the highest results occurred using SSGE + FL4 (1.11%), with no significant differences from FL6 using the same sealing system (1.06%) (Tab. 2).

The results for the *ex vitro* growth and establishment of *S. crispa*, in general, showed that when the SSGE and the light source FL4 were previously used, all characteristics obtained the best performance.

Such growth characteristics may be related to a greater aeration that SSGE allows. These results corroborate those of Silva *et al*. (2014) and those of Ribeiro *et al*. (2019). Plants of *Cattleya walkeriana* Gardner and Denphal, respectively, when cultivated with lids that allowed gas exchange, showed, when transferred to an *ex vitro* environment, growth attributes superior to those of plants cultivated in a sealed environment.

Furthermore, the results of this work suggest that the use of SSGE together with a mixture of white + blue + red LED light may have provided conditions that favored the *ex vitro* growth of *S. crispa*. These treatments may have affected plant physiology, so that it started its hardening while still in an *in vitro* cultivation, resulting in a better performance in *ex vitro* cultivation. Silva *et al*. (2016) explained that the benefits of cultivation in SSGE result from the decreases in *in vitro* humidity and from increased aeration, providing a later hardening in plants when transferred to *ex vitro* conditions.

Lazzarini *et al*. (2017) also report that, in plants, the absorption of blue and red lights emitted by LED lamps is around 90% of the light emitted. This indicates that the development of plants and plant physiology are strongly affected by these wavelengths. Thus, both red and blue lights may be effective in inducing photomorphogenic responses, as occurred with *S. crispa* plants.

In addition, plants need a broad spectrum of light to optimize photosynthetic processes. Such need varies according to plant species. For *S. crispa*, the highest results show that, in addition to the use of blue and red LEDs, the addition of a white LED contributed to the *ex vitro* growth of plants. The white LED (460–560 nm) has a higher proportion of blue and green in its spectrum and a lower ratio of red and extreme red compared to fluorescent lamps (Fraszczak *et al*. 2014). The use of this LED in crops may increase plant growth, as it allows light to penetrate the leaves better than monochromatic blue and red lights do, as observed for hydroponic *Lactuca sativa* (Lin *et al*. 2013).

Research with LED shows that plants need a broad spectrum of light to optimize photosynthetic processes. Such need may vary according to the species cultivated. Based on the results obtained for *S. crispa*, the combination of blue and red wavelengths is beneficial for an *in vitro* cultivation. However, for the *ex vitro* growth and establishment of these plants, it is necessary to cultivate them in white LED associated with red and blue LEDs. Therefore, further studies on ornamental species and the use of LEDs are needed since the use of this technology promotes benefits to several physiological aspects of cultivated plants.

This is even more relevant in studies on native species. The recent technologies used for the production of flowers and ornamental plants require the improvement of techniques and better cultivation conditions *in vitro*, as they are essential biotechnological tools in the production and propagation of plants. Ornamental horticulture seeks to obtain better quality seedlings and large-scale production in less time aiming both commercialization and species conservation.

Figure 4 shows that the *in vitro* cultivation conditions limited plant growth and survival in the *ex vitro* period. In general, the SSGE showed the highest results in all parameters visually observed, mainly under the light sources FL3, FL4, FL5 and FL6. This allows inferring that this sealing system, associated with light sources that use blue and red wavelength, may be appropriate for the *in vitro* cultivation of this species.

For *in vitro* growth, the use of a sealing system allowing gas exchange together with light sources combining blue and red wavelengths at different proportions favors the cultivation of *Schomburgkia crispa*.

For the *ex vitro* growth of this species, the previous *in vitro* cultivation in a system that allows gas exchange together with 50% white + 25% red + 25% blue LEDs positively affects the acclimatization of plants.

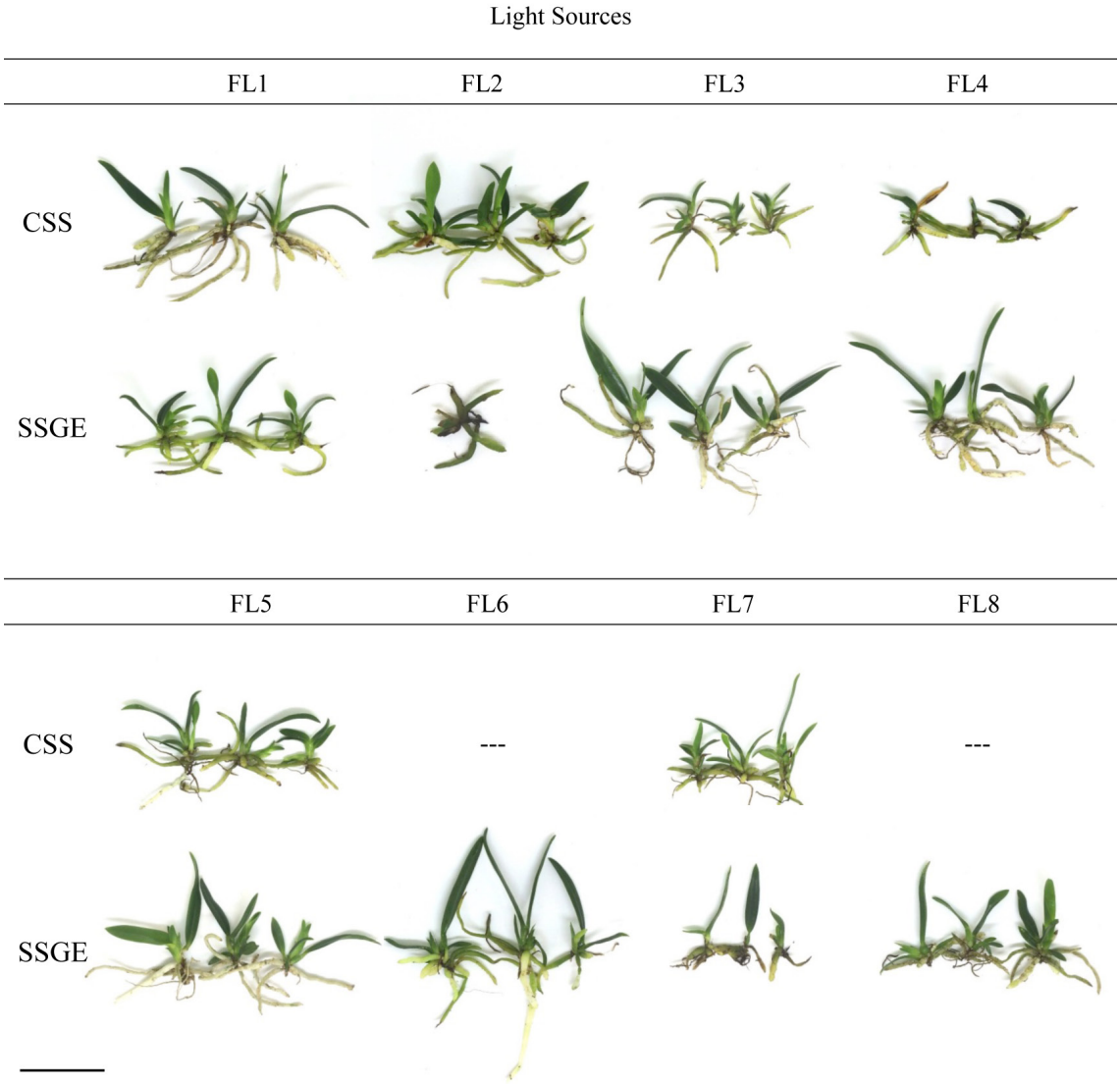

Figure 4 – *Schomburgkia crispa* plants at 180 days of *ex vitro* cultivation as a function of light sources and sealing systems. FL1- 100% white LED lamp; FL2- 100% blue LED lamp; FL3- 100% red LED lamp; FL4- 50% white + 25% red + 25% blue LED lamps; FL5- 50% red + 50% blue LED lamps; FL6- 25% red + 75% blue LED lamps; FL7- 75% red + 25% blue LED lamps; FL8- white fluorescent lamp. CSS = conventional sealing system; SSGE = sealing system with gas exchange.

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Data availability statement

In accordance with Open Science communication practices, the authors inform that there is no data sharing of this manuscript.

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