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Light Spectra and 6-Benzylaminopurine in the *In Vitro* Cultivation of Epidendrum lilas

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Abstract

In order to supply the growing market demand for orchids (Epidendrum Lilas), is usually recommended the micropropagation technique, mainly, for the production of quality seedlings in a short time and in large numbers. Since, the propagation of this species is slow through conventional means, factors such as the phytoregulators and the light spectrum that may interfere in the effectiveness of this technique. The objective of the work was to evaluate different concentrations of cytokinin and the use of different light spectra in the in vitro propagation of orchid Epidendrum lilas. Two factors were evaluated: concentrations of benzylaminopurine (BAP): (1.0; 1.5; 2.0; 2.5 and 3.0 mg L-1) and the light spectra: white, red, blue and green. After two months of establishment, the following characteristics were evaluated: plant height (H), number of leaves (NL), leaf length (LL) and shoots (S). The data obtained were submitted to analysis of variance and the means compared by the Tukey test ($p < 0.05$). There was a significant difference only for the light spectra. The red spectrum was more efficient for the development of the aerial part and leaf length. While the blue spectrum promoted a greater number of leaves. Higher shooting rates were observed using the white spectrum. It is concluded that during the orchid growth phase the red and blue spectra are more efficient, and in the multiplication phase the white spectrum is recommended.

Keywords: Orchid; Micropropagation; Light; Cytokinin

Introduction

The commercial production of flowers and ornamental plants has increased over the years, in all regions of Brazil [1]. Highlighting the orchid trade, which generates a commercialized amount of approximately US $ 20 billion per year [2]. Being highly appreciated by consumers, due to its exotic flowers of vibrant colors and floral longevity [3]. Despite the great demand in the market, the spread of orchid species is considered slow, requiring a long period to reach the reproductive stage, making the production of new seedlings time-consuming [4]. In natural conditions, the multiplication of these plants occurs through the natural propagation of the seeds, which do not have a functional endosperm. So that, under natural conditions, they depend on mycorrhizal fungi for symbiotic germination, which are necessary until adulthood for their survival [5]. In addition, seeds have relatively low germination rates, around 5% [6]. In view of the importance of orchids in the economic sector and due to the difficulty of their natural propagation, it is necessary to seek techniques that help the rapid propagation of this species. The micropropagation technique being the

most used, since it allows the use of all the seeds produced and the regeneration of adult plants from them [7].

In in vitro growth, the nutrient media are supplemented with growth regulators [8]. Highlighting the cytokinins, responsible for cell division, stretching and differentiation [9]. Among the most used cytokinis in in vitro cultivation, stands out the benzylaminopurine (BAP) [10]. However, the addition of these growth regulators is a factor that increases the costs of in vitro seedling production. An alternative would be manipulation of the cultivation environment through spectral quality [11]. In which it has proven action on plant development [12]. Spectral quality is essential in in vitro propagation, since light is essential for photomorphogenesis in plants [13]. With the variation in the light spectrum, the in vitro growth of several species can be manipulated, in an alternative way to the addition of phytoregulators to the culture medium [14]. In view of these aspects, the objective of the work was to evaluate the development and multiplication of the E. Lilas orchid under different light spectra associated to BAP doses.

Materials and Methods

Location of the Experiment

The experiment was conducted at the Plant Biotechnology Laboratory of the Agricultural Research Company of Minas Gerais-EPAMIG, EPAMIG Norte-Gorutuba Experimental Field, Nova Porteirinha-MG, during the years 2018/2019. The geographical location is defined by the coordinates 15º148'263" south latitude and 43º17'650" west longitude, the average altitude is 526 m.

Seed Establishment in Vitro

Orchid plants were previously established in vitro using seeds from their fruits (capsules). The collected capsules underwent a disinfection process and then brought to a laminar flow cabinet in which they were opened and the seeds removed to perform in vitro sowing. These seeds were placed in bottles with solid medium MS [15], supplemented with 0.5 mg L^{-1} of 6-benzylaminopurine (BAP), 30 g L^{-1} of sucrose, 0.1 g L^{-1} of inositol, with pH adjusted to 5.8 ± 0.1. After sowing, the flasks containing the seeds remained in a growth room for 60 days, or until sprouting, under irradiance of 40 µmol m^{-2} s^{-1} provided by cold white light, with 16 hours of photoperiod at 25 ± 2ºC. After 60 days, sprouts were used for the experiment.

In Vitro Cultivation Conditions

The obtained sprouts were individualized and transferred to 300 mL flasks containing 50 mL of MS medium supplemented with the following doses of BAP: 0.5, 1.0, 1.5, 2.0, 2.4 and 3.0 mg L^{-1}. Then the explants were taken to the growth room and subjected to different light spectra: white spectrum (cold white fluorescent light), red, blue and green spectrum. To obtain the colored spectra, the white lamps were wrapped with colored films of regenerated cellulose (cellophane), in the red (~ 625-440nm), blue (~ 440-485nm) and green (~ 500-565nm) colors. The material was kept in a growth room for 60 days at a temperature of 25 ± 2ºC and 16 hours of photoperiod (1,800 LUX). At 30 and 60 days after in vitro establishment, plant height (cm), leaf number, leaf length (cm) and shoots were evaluated. The experiment was conducted in a completely randomized design in a 6x5 factorial scheme (Doses of BAP x Colored spectra), with five replications.

Statistical Analysis

The variables were submitted to the Shapiro-Wilk normality test and the Bartlett test, both $p < 0.05$, to verify the normality of the data and homogeneity of the variances, respectively. All variables were transformed by the x + 1 root. After confirming the requirements for an analysis of variance, it was performed at a probability of 5% error. Significant differences were observed for the sources of variation involved in the experiment, the means of the variables were subjected to the Tukey test ($p > 0.05$) to detect the differences between treatments. All statistical analyzes were performed using the Genes software [16].

Results and Discussion

There was no significant interaction ($p < 0.05$) among the factors dose and light spectra for any of the characteristics evaluated at 30 and 60 days of cultivation. There was a significant difference only for single factor spectra for the following: plant height, number of leaves and shoots. For characteristic leaf length there was a significant difference only at 60 days of cultivation. There was no significant difference for BAP doses. This fact may be associated with the balance between endogenous and exogenous cytokinin, the endogenous being sufficient to supply the needs of the plants [17]. It can also be related to luminosity. According to Chee and Pool, [18] high light intensity leads to photoxidation causing degradation of cytokinins. The plants showed greater growth when submitted to the red spectrum, at 30 and 60 days of cultivation Table 1. Similar results were observed by Rocha, et al. [19] working with forage palm cv. Gigante (Opuntia ficus indica Mill.).

Espectra	30 days			60 days			
	H (cm)	NL	S	H (cm)	NL	LL (cm)	S
Blue	4.86 ab	1.77 ab	1.31 b	5.57 ab	2.36 a	3.77 ab	1.78 b
White	4.48 b	1.99 a	1.65 a	5.26 ab	2.58 a	3.33 b	2.13 a
Red	5.43 a	1.79 ab	1.22 bc	5.94 a	2.07 b	4.1 a	1.45 c
Green	4.43 b	1.70 b	1.04 c	4.78 b	1.81 b	3.52b	1.11 d

Table 1: Height (H), number of leaves (NL), leaf length (LL) and shoots (S) means of orchid Epidendrum Lilas, at 30 and 60 days of in vitro cultivation under different light spectra.

Means followed by the same letter do not differ statistically by Tukey's test ($p < 0.05$) for each variable. Some studies have concluded that the red spectrum promotes the growth of the aerial part of plants [20]. This is because the process of light absorption by plants (photomorphogenic route) is similar to the mechanism of action of hormones [13]. Rosa, et al. [21] working with Dendrobium phalaenopsis obtained plants with a length of 3.33 cm using 3.0 mg L^{-1} of BAP. Rodrigues, et al. [22] observed greater plant growth (2.36 cm) with 1.0 mg L^{-1}, BAP in Oncidium baueri. In this work, using only the red spectrum, the plants had a height of 5.43 and 5.94 cm at 30 and 60 days of cultivation, respectively. Based on the similarity of the responses, it appears that it is possible to manipulate the in vitro growth of orchids, in an alternative way to the addition of phytoregulators to the culture medium. Plants grown under white spectrum showed a higher number of leaves (1.99 cm), at 30 days of cultivation. And at 60 days, more leaves were obtained with the blue (2.36) and white (2.58) spectrum.

Similar results for these spectra were observed by Cunha, et al. [23], working with Mentha spicata and Santos, et al. [24] observed similar averages using 3.6 mg L^{-1} of BAP in Epidendrum ibaguense, confirming the efficiency of the spectra. These results may be related to the influence of these spectra on in vitro photosynthesis. Since leaves irradiated with white light absorb more blue, red and green wavelengths, which are necessary for energy gains through photosynthesis, as well as for other physiological processes [25]. Blue light is also essential in the processes of synthesis of pigments, enzymes, development of chloroplasts, stomatal opening and closing and several other photomorphogenic processes [26]. That may have favored the orchid leaf formation process. Plants exposed to the red spectrum showed greater leaf length (4.10 cm), at 60 days of cultivation. The red light generally emits a spectrum near to the maximum absorption of chlorophylls and phytochromes, being important for the development of the photosynthetic apparatus and for the accumulation of starch [27].

Shooting induction in orchids occurs more intensely when grown in white spectrum, at 30 (1.65 shoots / explant) and 60 days (2.13 shoots / explant). Similar results were observed by Ferrari, et al. [28] in Curcuma longa. Camargo, et al. [3] working with Oncidium baueri observed a close average (1.87 shoots / plant) using 2.0 mg L^{-1} of BAP. In general, plants grown in white light, an environment that is similar to the natural environment, present thicker leaves with longer and juxtaposed palisade parenchyma cells [26]. This may have favored in vitro photosynthesis and, consequently a higher shooting rate. Plants grown under green spectrum showed less development. Studies with Arabidopsis have reported that the green light causes similar effects when the plants are shaded, that is, they lead to etiolation and cause differences in the architecture of the plant such as lengthening of petioles, reorientation of leaves and reduction of leaf area [30]. Different results were observed by Rocha, et al. [31] working with three banana cultivars (Musa sp.), observed higher shooting rates using green light. However, the influence of spectral quality in relation to plant growth and development may be directly linked to the species, the stage of plant development and other environmental characteristics [32]. Which may justify the different results verified in this work. Since there was no influence of the phytoregulator used, the luminosity was the determining factor for the in vitro development of the orchid. These results indicate new possibilities for the micro propagation of E. Lilas, and can be considered as a starting point for further research on protocols, involving the cultivation environment, in order to improve the quality of the seedlings produced.

Conclusion

Under the conditions in which this experiment was carried out, the following conclusions were reached:

- The light spectra influences in the in vitro development and multiplication of orchids.
- The red spectrum provides greater aerial part development and leaf length. Whereas, plants exposed under the blue and white spectra have a higher number of leaves.
- Cultivation in a growth room under a white spectrum induces higher shooting rates.
- For economic matter, it is recommended to use the red and blue spectra in the growth phase and the white spectrum in the Epidendrum Lilas multiplication phase.

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