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SCIENTIFIC ARTICLE

In vitro propagation of alpinia cultivars in different light sources

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Abstract

The light source in tissue culture is often one of the punctual factors for the adequacy of plant production. For this, light emitting diodes (LEDs) have been used in order to increase the growth and development of the plants propagated *in vitro*. The objective was to evaluate the *in vitro* propagation of cultivars of *Alpinia purpurata* 'Red Ginger' and 'Pink Ginger' in two different light sources (fluorescent or LEDs tubes). The experiment was conducted in a completely randomized design, factorial scheme (2x2), totaling four treatments with ten replicates, and the experimental unit composed of one flask containing five explants. After 55 days, white LED and the 'Red Ginger' provided the highest number of shoots. For the number of leaves, the cultivar 'Red Ginger' and white LED lamps provided superior results. For shoot length (cm), the cultivar 'Red Ginger' kept under fluorescent lamps was superior, and 'Pink Ginger' presented superiority when cultivated under LED lights. Thus, even with differentiated responses among genotypes, the use of white LED lamps in the *in vitro* propagation of *Alpinia purpurata* 'Red Ginger' and 'Pink Ginger' cultivars is suggested.

Keywords: *Alpinia purpurata*, light emitting diodes (LEDs), tissue culture, Zingiberaceae.

Resumo

Propagação *in vitro* de cultivares de alpinia em diferentes fontes de luz

A fonte de luz na cultura de tecidos muitas vezes é um dos fatores pontuais para a adequação da produção de plantas, para isso, vêm se utilizando os diodos emissores de luz (LEDs) no intuito de aumentar o crescimento e desenvolvimento das plantas propagadas *in vitro*. O objetivo do trabalho foi avaliar a propagação *in vitro* de cultivares de *Alpinia purpurata* 'Red Ginger' e 'Pink Ginger' em duas diferentes fontes de luz (lâmpadas tubulares fluorescentes ou LED Branco). O experimento foi conduzido em delineamento inteiramente casualizado, esquema fatorial (2x2), totalizando quatro tratamentos com dez repetições, e a unidade experimental composta por um frasco contendo cinco explantes. Após 55 dias, foi possível observar que lâmpadas LED branco e a cultivar Red Ginger proporcionaram o maior número de brotos. Já para o número de folhas, a cultivar Red Ginger e lâmpadas LED branco proporcionaram resultados superiores. Para comprimento da parte aérea, a cultivar Red Ginger mantida sob lâmpadas fluorescentes foi superior, e a Pink Ginger apresentou superioridade quando cultivada sob luzes LED. Assim, mesmo com respostas diferenciadas entre genótipos, sugere-se o uso de lâmpadas LED branco na propagação *in vitro* das cultivares de *Alpinia purpurata* 'Red Ginger' e 'Pink Ginger'.

Palavras-chave: *Alpinia purpurata*, cultura de tecidos, diodos emissores de luz (LEDs), Zingiberaceae.

Introduction

The floriculture industry comprises the cultivation and marketing of cut flowers, foliage and potted plants; in the latter case, the plants can be sold for landscaping and urban planning purposes (Van Huylenbroeck, 2010; Chandler and Brugliera, 2011). With globalization, the world market for flowers previously dominated by European countries, has opened space for new production centers, especially Brazil and among the indicatives for the expansion of floriculture in Brazil are the continental dimensions and climatic conditions, which makes it possible to produce flowers and foliage during the whole year at reduced and competitive costs (França and Maia, 2008).

The Zingiberaceae family includes many species with applications in horticulture, particularly for use in cooking, medicinal, ornamentation and landscaping (Silva et al., 2017) and among these species the *Alpinia* genus stands out because it contains many attractive inflorescences species, which stand out due to their relevant productive potential

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(Souza et al., 2016) and great horticultural value. *Alpinia purpurata* (Viell.) K. Schum is a plant native to the Pacific islands and very popular in Brazil and India (Santos et al., 2012; Brito et al., 2017). Alpinia are not only known for their ornamental value, but also because of potential applications in biotechnology with the discovery of lectin with immunomodulatory effect on their inflorescences (Brito et al., 2017).

The use of biotechnology, especially the use of tissue culture techniques, allows the large-scale propagation of seedlings and plant biomass of high genetic and phytosanitary quality, ensuring rapid multiplication of the material and in a short period of time and physical area (Rescarolli and Zaffari, 2009; Pelizza et al., 2013) besides being important for the improvement and conservation of genetic resources However, to be successful, this technique is dependent on some factors related to the culture conditions such as temperature, composition of the nutrient medium, quantity and quality of light, among others.

Light is one of the most influential factors for plants grown *in vitro* (Pawłowska et al., 2018), because it is responsible for triggering a series of processes related to the growth and development of propagules. However, plant responses depend not only on the absence or presence of light, but also on the variation in luminous quality (Erig and Schuch, 2005).

Various light sources (incandescent, fluorescent, metal halide or high pressure lamps) are used in growth rooms, but with advances in lighting technology, there has been a progress in exploring LEDs in environmental controls and morphogenetic responses, as they have unique properties such as long service life, low energy consumption, low mass and volume, low radiant heat emission and the ability to emit specific spectral qualities (Batista et al., 2018; Pawłowska et al., 2018). The LEDs have specific wavelengths capable of stimulating the increase in the amount of chlorophyll and carotenoids in plant tissues, increasing the rate of multiplication and length of plants *in vitro* (Rocha et al., 2013).

It is essential to develop research using LED technology to evaluate the impact on morphogenesis of different plant species for protocol optimization (Batista et al., 2018), Unfortunately, the genus *Alpinia* did not receive sufficient attention in the application of tissue culture techniques, and information about the regeneration of the seedlings was limited. In this way, the objective of this work was to evaluate the *in vitro* propagation of *Alpinia purpurata* ('Red Ginger' and 'Pink Ginger') cultivars in two different light sources.

Materials and Methods

Plant material and growing conditions

Alpinia purpurata ('Red Ginger' and 'Pink Ginger') seedlings previously established in vitro and maintained on successive subcultures were used as explants in 350 mL glass bottle with 40 mL of MS culture medium (Murashige and Skoog, 1962), supplemented with 1.5 mg L^{-1} of 6-benzylaminopurine (BAP), 0.1 mg L^{-1} naphthalene acetic acid (ANA), 30 g L^{-1} sucrose, 100 mg L^{-1} myo-inositol and solidified with 6.0 g L^{-1} of agar, for multiplication of the seedlings. The pH was adjusted to 5.8 ± 0.1 before autoclaving at 120 °C, 108 KPa for 20 minutes. The material was maintained in a growth room with a temperature of 25 ± 2 °C, photoperiod of 16 hours and 36 µmol m^{-2} s^{-1} of light irradiance, from two fluorescent lamps ('Luz do Dia Especial', 40W, Osram, Brazil).

After *in vitro* multiplication of the seedlings for conducting the experiment, they were inoculated in flasks with the same culture medium and above mentioned conditions, the light sources (72 µmol m^{-2} s^{-1} of light irradiance, each) being from four fluorescent lamps or two tubular light-emitting diode lamps (white LEDs - PULSE LED®, 18W).

Parameters of growth and experimental design

The experiment was conducted in a completely randomized design (DIC), in a 2x2 factorial scheme, two cultivars ('Red Ginger' and 'Pink Ginger') and two light sources (Fluorescent and White LED), totaling four treatments with ten replicates and the experimental unit composed for a bottle containing five explants each. After 55 days, the seedlings were evaluated for number of shoots, number of leaves and length of shoot (cm).

The data were submitted to analysis of variance and the means were compared by the Tukey test ($p < 0.05$). Because they did not follow the normality assumptions of the errors, the values referring to the number of shoots and number of leaves were transformed to . Statistical analyzes were performed using the SISVAR software (Ferreira, 2011) and the figures made using Sigmaplot 12.5 (Systat Software, Inc, USA).

Results and Discussion

By the analysis of variance, there was a significant interaction between the cultivar factors x light sources for the number of shoots and height of plant, by the F test at 5% of probability. For the variable number of leaves, there was significant difference only for the cultivar factors and light source, separately ($p < 0.05$).

Our work describes the first report of the influence of the light source in *in vitro* multiplication of *Alpinia purpurata* cvs. 'Red Ginger' and 'Pink Ginger', which resulted in plants as evident differences in the evaluated parameters. In addition, the genotype factor also demonstrated a differentiated response, since the responses are often specific for each genotype under study, that is, the manifestation of the potential of each genotype depends on the genotype-environment interaction.

For the variable number of shoots, when comparing the cultivars within the light sources factor, there was superiority for the cultivar Red Ginger in white LED lamp (3.30) when compared to the fluorescent (2.09). For cultivar, regardless of the light source, Red Ginger was superior to Pink Ginger (Figure 1A). Rocha et al. (2017), working with three banana cultivars, observed that the "Grande Naine" presented 2.6 shoots, with differences of almost a shoot when compared to the other cultivars. The same authors also reinforce that the LEDs can exert positive effects in the increase of the number of shoots, which can vary between the cultivars of the same species.

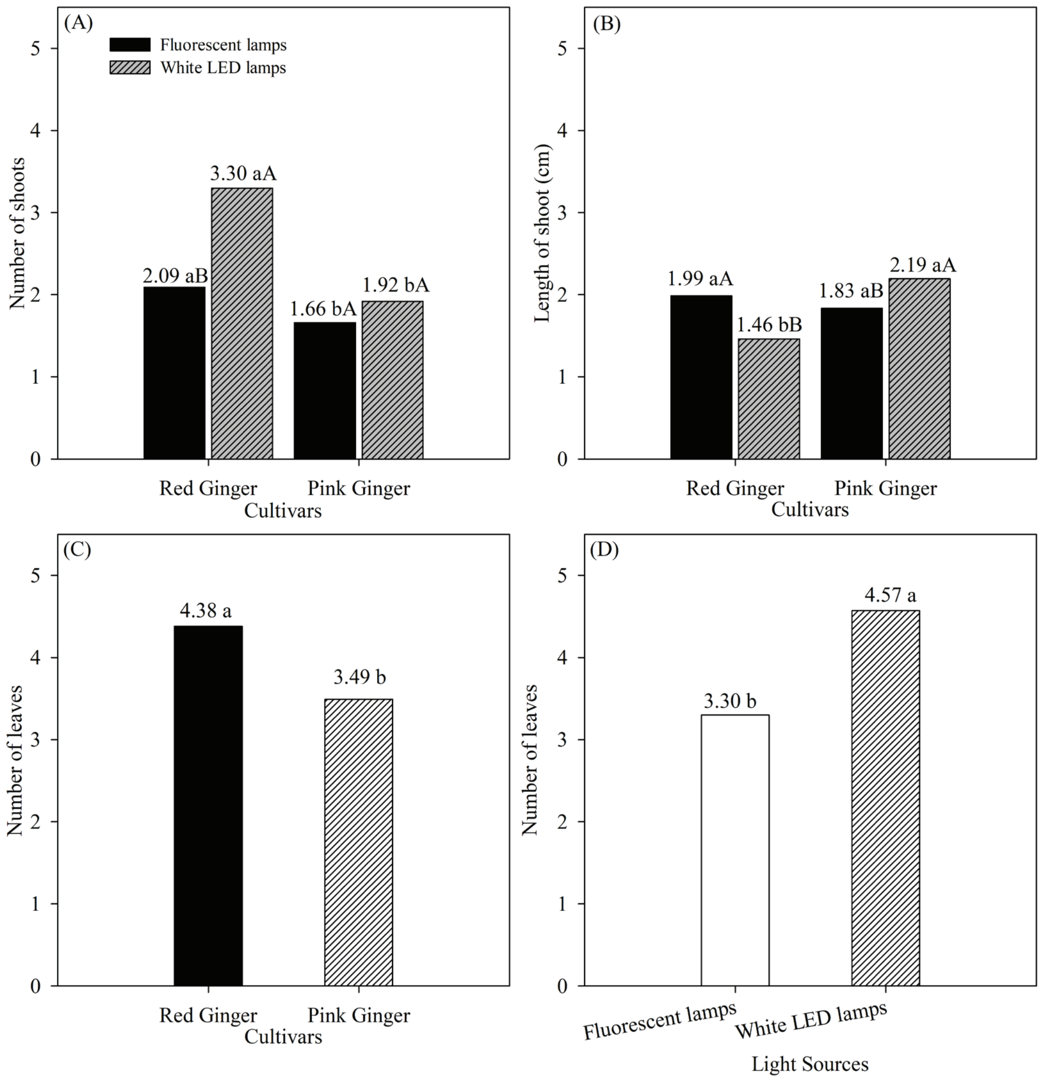

Figure 1. Number of shoots **(**A), shoot length (B) and number of leaves (C, D) of two cultivars of *Alpinia purpurata* (Red Ginger and Pink Ginger) *in vitro* submitted to two light sources (fluorescent or white LED lamps). Averages followed by the same lowercase letter do not differ the light sources and averages followed by the same capital letter do not differ the cultivars, by the test of Tukey, to 5% of probability (A-B); Equivalent lowercase letters do not differ the means by Tukey's test ($p < 0.05$) (C-D).

The light source contributed to the different *in vitro* growth responses of both cultivars of *Alpinia purpurata*; this is one of the factors that most influence the regulation of the photosynthetic and photomorphogenic responses necessary for the culture of each species *in vitro*, since there are active photoreceptors under certain wavelengths (Lee et al., 2007; Gupta and Jatothu, 2013), which influence the anatomical, physiological, morphological and biochemical processes of the plant (Chen et al., 2014). Plants respond to light stimuli, which may interfere with size, number of shoots per explant, length of shoots, habit of growth, flowering, fruiting, among others (Carvalho et al., 2011; Rocha et al., 2013).

To verify the reproducibility of anatomical and morphological changes, experiments should be performed comparing LED lights with fluorescent lamps (Macedo et al., 2011). Positive results with the use of white LED were obtained by Wilken et al. (2014) in *in vitro* development of *Musa* sp. When evaluating the behavior of the *Rubus idaeus* species cultivated under blue, red or green LED lamps; Growlux; or fluorescent lamps Rocha et al. (2013) observed smaller shoots mean when the plants were submitted to fluorescent lamps and superior results in the red LED. No presente trabalho, foi observado efeito diferenciado para cada cultivar, no qual a Red Ginger teve efeito diferenciado quando cultivado sob LED branco. These differences can be explained because the quality of light emitted stimulate changes in plant metabolism and act differently in each species cultivated in vitro (Gupta and Jatothu, 2013).

For the shoot length, comparing the light sources, the cultivar Red Ginger was superior when produced under fluorescent light (1.99 cm), being significantly superior to the white LED (1.46 cm). Differently from what was observed for Pink Ginger, in which the white LED (2.19 cm) was superior to fluorescent lamps (1.83 cm) ($p < 0.05$). When compared to light source, there was significant difference only for white LED, in which Pink Ginger (2.19 cm) was significantly higher than Red Ginger (1.46 cm) (Figure 1B). The apical elongation of the plants can be affected in different bands of the light spectrum, due to the alterations caused in the endogenous concentration of auxins present in the plants (Araújo et al., 2009). In the present study, the quality of the light spectrum may have been one of the factors responsible for the differences found in the shoot length variable among the cultivars, in which the cultivar Pink Ginger obtained higher averages. This indicates that there are differentiated responses between species and even between cultivars with respect to light absorption (Pasa et al., 2012)

The quality of fluorescent light is not considered ideal because it has wavelengths ranging from 350 to 750 nm (Rocha et al., 2007), acting in proportions below those required for the growth of plants using light quality. For Hung et al. (2015), the use of light emitting diodes has many advantages, among which the growth and *in vitro* morphogenesis of several species are strongly favored.

For the number of leaves variable, the cultivar Red Ginger (4.38) was superior to Pink Ginger (3.49) ($p < 0.05$) (Figure 1C). When analyzing the behavior of this variable for light qualities, the white LED (4.57) was significantly higher when compared to fluorescent (3.30), stimulating the performance of this variable (Figure 1D). Plant characteristics, such as growth rate, development of vegetative and reproductive organs, total biomass and distribution of photoassimilates are affected by the quality of light (Posada et al., 2011), due to the specific wavelengths contribute to the increase of the amount of chlorophylls and carotenoids in the plants (Rocha et al., 2013).

The results of this study offer potential to improve the efficiency of *in vitro* propagation of target species cultivars and reduce plant production costs. Thus, it is concluded that although there are differentiated responses between cultivars and light, the white LED is efficient for the *in vitro* multiplication of *Alpinia purpurata* cv. Red Ginger and Pink Ginger can be used as an additional source of light in *in vitro* propagation of this species, producing high quality plants with potential for large scale production to be applied as ornamental or in the production of phytochemical compounds and pharmacological factors.

Author Contribution

M.V.M.P. 0000-0002-5028-7818: conception of work, analysis and interpretation of data, elaboration and critical reviews of the article. D.S. 0000-0002-9963-4956: design work and critical reviews. M.I.D. 0000-0002-7905-2166: elaboration and critical revisions of the article. J.S. 0000-0003-1621-3556: design and conduct of work, analysis of data. L.A.T. 0000-0002-3439-842X: design work and article reviews. G.C.V.A. 0000-0002-8569-3031: design and conduct of work. E.H. 0000-0002-8018-6743: design and conduct of work.

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