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The effect of spectral light quality on *in vitro* culture of sugarcane

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ABSTRACT. The effects of different light quality treatments on *in vitro* growth and multiplication of sugarcane (RB867515) were investigated. The plantlets were cultivated on MS medium containing 1.3 µM 6-benzylaminopurine (BAP), and exposed to five light treatments: three combinations of blue/red LED (70:30, 50:50, 30:70), white-LED and fluorescent lamps, during 24 days. Among the LED light treatments, blue/red combination in 50:50 proportions proved to have the best results for stem length, fresh mass, leaf number and shoot multiplication. Higher content of photosynthetic pigments was also obtained with LEDs. Results suggested that the light quality emitted by LEDs was suitable for plant growth and development and it may be used as alternative and economic light source for micropropagation of sugarcane variety under analysis.

Keywords: *in vitro* propagation, light wavelength, *Saccharum* spp.

Efeito da qualidade espectral da luz no cultivo *in vitro* de cana-de-açúcar

RESUMO. Foram investigados os efeitos de diferentes tratamentos de qualidade da luz sobre o crescimento e multiplicação *in vitro* de cana-de-açúcar (RB867515). As plantas foram cultivadas em meio MS contendo 1,3 µM de 6-benzilaminopurina (BAP) e expostas a cinco tratamentos de luz: três combinações de LED azul/vermelho (70:30, 50:50, 30:70), LED branco e lâmpadas fluorescentes, durante 24 dias. Entre os tratamentos de luz de LED, a combinação azul/vermelho na proporção 50:50 apresentou os melhores resultados para altura, massa fresca, número de folhas e multiplicação de brotos. Maior teor de pigmentos fotossintéticos também foi obtido com a utilização de LEDs. Os resultados sugerem que a qualidade da luz emitida pelos LEDs foi adequada para o crescimento e desenvolvimento das plantas e pode ser utilizada como fonte de luz alternativa e econômica para a micropropagação da variedade de cana-de-açúcar estudada.

Palavras-chave: propagação *in vitro*, comprimento de onda da luz, *Saccharum* spp.

Introduction

Sugarcane (*Saccharum* spp., Poaceae) is an important agricultural crop which is capable of producing valuable by-products such as sugar, biofuel, biofibers, wax and bioplastic (Singh, Kumar, Tiwari, Rastogi, & Singh, 2013). Favorable soil and climate conditions rank Brazil as the world's major sugarcane producer, followed by India and China (Monteiro, & Sentelhas, 2014). Among the several commercially cultivated varieties of sugarcane developed by the RIDESA (Brazilian Interuniversity Network for the Development of Sugarcane Industry) breeding program, RB867515 is one of the most frequently planted varieties in Brazil, featuring drought tolerance, high sucrose content, rapid growth and high productivity (Daros, Oliveira, Zambon, & Bespalhok Filho, 2010).

In vitro multiplication of sugarcane has recently received considerable research attention owing to its economic importance. Micropropagation techniques have facilitated the rapid multiplication of newly developed varieties and assured the production of high quality and disease-free plantlets (Snyman, Meyer, Koch, Banasiak, & Watt, 2011). Although sugarcane is one of the main species propagated in Brazilian plant biofactories (Gerald, & Lee, 2011), several limitations exist concerning the feasibility of sugarcane micropropagation. In fact, high production cost is a very important issue and the development of new technologies is required to such an end. Innovations, such as the use of light emitting diodes (LEDs) instead of white fluorescent lamps, have given satisfactory results for a variety of plants cultivated *in vitro* like cotton, anthurium, rapeseed and banana (Budiarto, 2010; Li, Xu, & Tang, 2010; Li, Tang, & Xu, 2013; Vieira et al., 2015).

LED irradiation system has several advantages over conventional light systems. These include

low energy consumption, longer bulb life and maximum PAR efficiency between 80 and 100%, while their fluorescent lamps counterpart provides only 20-30% (Darko, Heydarizadeh, Schoefs, & Sabzalian, 2014). Due to their high luminous efficiency, LED bulbs produce less heat and, consequently, an indirect reduction in refrigeration costs. Another advantage provided by LEDs is the versatility with controlled spectrum components that plants need for various morphogenic responses (Gupta & Jatothu, 2013; Darko et al., 2014).

Since different spectral light quality influences morphological and/or physiological aspects of plants in *in vitro* culture conditions, the effect of alterations in the spectral profile of light on growth and multiplication of sugarcane is evaluated.

Material and methods

Plant material and growth conditions

Sugarcane plantlets (variety RB867515), previously established *in vitro*, were provided by Biofactory Governador Miguel Arraes (Recife, Brazil) and transferred to test tubes with 10 mL of MS (Murashige & Skoog, 1962) liquid medium supplemented with 3% sucrose, 100 mg L^{-1} myo-inositol and 1.3 µM BAP. The pH of the medium was adjusted to 5.8 prior to sterilization and cultures were maintained during 24 days in a growth room at 25 ± 2ºC, under 16 h photoperiod and one of the five light treatments.

Light treatments

There are two parts in each light-emitting diode set: an easily detachable electric circuit for emitting light and one direct current power supply used to control the light intensity by electricity adjustment. The illumination system consisted of RGB LED-bars (IHS, New York), each containing 15 triple-LED-chips. LED-bars were assembled and fixed in panels (40 × 30 cm) which were placed approximately 10 cm above the test tubes. Experimental plants were randomly assigned to each light treatment and their corresponding photosynthetic photon fluxes (PPF) were as follows: (1) B:R= 70:30, 70% blue light and 30% red light (72 µmol m^{-2} s^{-1}); (2) B:R= 50:50, 50% blue light and 50% red light (60 µmol m^{-2} s^{-1}); (3) B:R= 30:70, 30% blue light and 70% red light (53 µmol m^{-2} s^{-1}); (4) WL= 100% white-LED (77 µmol m^{-2} s^{-1}) and (5) FL= fluorescent lamp (46 µmol m^{-2} s^{-1}).

The typical light spectrum emanating from the LED-bar system and fluorescent lamps was recorded by a fiber integrated spectrograph Ocean Optics model USB2000 (Figure 1).

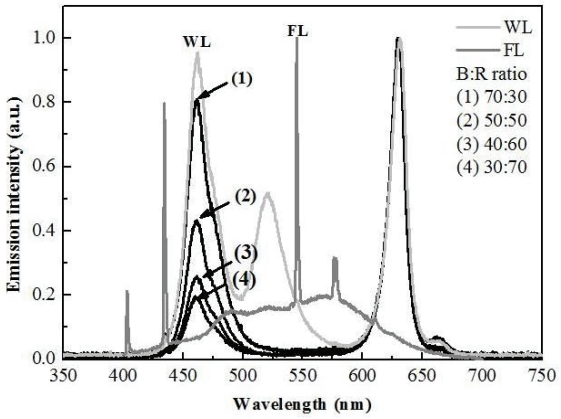

Figure 1. Spectral distributions in relative energy of the LEDs and fluorescent lamps.
Source: the authors

Plantlets growth and multiplication

The effect of each light treatment was evaluated according to the following growth parameters: stem length, fresh mass, number of tillers and number of leaves per plant. The stem length was measured, with a ruler, from the base of stalk to the last expanded leaf. So that fresh mass could be weighed, the material was removed from the culture test tubes and immediately weighed in an analytical balance to prevent dehydration. The number of leaves (green and senescent) and shoot multiplication of plantlets was also examined and counted.

Photosynthetic pigment contents

For chlorophyll and carotenoid extraction 0.2 g of fresh leaf tissue was used, and samples were ground in a mortar. The extraction was performed with 20 mL (V) acetone 80%, and the optical density was measured with a UV-VIS spectrophotometer (SP-220, Biospectro, São Paulo) at 663 nm for chlorophyll *a* (Chl-*a*), at 645 nm for chlorophyll *b* (Chl-*b*) and at 470 nm for carotenoid. Chlorophyll and carotenoid contents were determined using equations of Lichtenthaler (1987).

Statistical analysis

The experiment consisted of a completely randomized design, with five light treatments and 25 replications. The experimental unity consisted of one test tube containing a single plantlet. Data were processed by ANOVA and the mean differences were compared by Scott-Knott test, using the software Assistat ver. 7.6 beta (UFCG, Campina Grande).

Results

Effect of different lighting spectral quality on growth and multiplication

Light treatments promoted different responses on growth parameters (Table 1). When compared to other treatments, the longest stem occurred in plantlets cultivated under B:R=50:50 LED light. The highest total fresh mass occurred in B:R=70:30 and 50:50 LED combinations, and in the FL. Concerning total number of leaves, FL exhibited the highest means when compared to others. Average number of green leaves was higher in B:R=70:30, B:R=50:50 and FL, while foliar senescence was favored by B:R LEDs with 50:50 and 30:70 ratios and FL. The number of tillers was smaller in B:R=30:70 LED light, and in the other treatments did not occur difference statistical. However, plantlets grown under fluorescent spectrum presented numerically the highest multiplication rate (1:3.33), followed by B:R=50:50 LED light (1:3).

Effect of lighting spectral quality on contents of pigments

Chlorophyll and carotenoid concentrations of sugarcane plantlet leaf varied in response to different spectral light qualities (Figure 2). Chl-*a* contents were highest in WL, followed by blue/red LED treatments. The lowest concentrations of Chl-*a*, Chl-*b* and Chl (a+b) were found in plantlets cultivated in FL and B:R=70:30 LED light. Chl-*b* and Chl (a+b) content in the other spectra studied did not differ statistically. Exposure to WL was beneficial for the accumulation of carotenoids pigment.

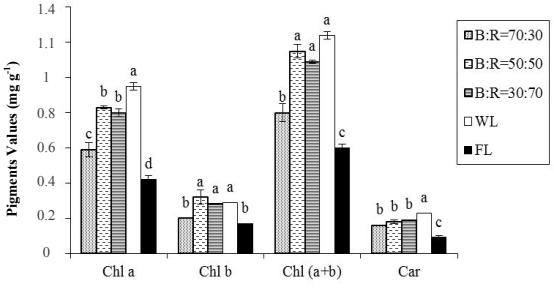

Figure 2. Chlorophyll and carotenoid contents of sugarcane cultured *in vitro* under different light qualities. Different letters indicate significant differences at $p < 0.05$ according to Scott-Knott test (n = 5). Bars represent the SE.
Source: the authors.

Discussion

Plant growth and development are strongly influenced by spectrum light quality, or rather, the wavelengths reaching the plant's surface (Chen et al., 2014) influence several anatomical, physiological, morphological, and biochemical parameters. Current results showed that the evaluated growth parameters were affected by the light treatments applied (Table 1). Among the LED light treatments, B:R=50:50 promoted the highest stem length, fresh mass production and tillering of the sugarcane plantlets. Similar results with blue/red LED in equal proportion were obtained in cotton (Li et al., 2010) and in sugarcane variety RB92579 (Silva et al., 2014). A mixture of blue and red light sources may compound the advantages of monochromic red and monochromic blue light (Li et al., 2013), and the best proportion needs to be studied for each species. Plantlets cultivated under B:R=70:30 LED treatment also provided high fresh mass, the lowest number of senescent leaves and the highest percentage of green leaves, 71.4%.

Fluorescent lamps promoted the highest leaves which, consequently, increased the plants fresh weight. However, the number of senescent leaves was also higher in this treatment, with nearly half of the total number of leaves. The most remarkable events in leaf senescence are the loss of chlorophyll and the disassembly of the photosynthetic apparatus, resulting in a decrease in the photosynthetic energy conversion capacity and efficiency (Falqueto, Cassol, Magalhães Júnior, Oliveira, & Bacarin, 2009). This fact may reduce the survival rate of plants during acclimatization. Plantlets of the sugarcane variety RB862552, grown under red LEDs, had higher surviving percentages when compared to plants grown under fluorescent lamps (Rocha, Oliveira, & Scivittaro, 2013).

Tillering was present for all light sources in the experiment, and multiplication rates between 2.22 and 3.33 were readily obtained. In *Anthurium*, a larger number of shoots was observed when exposed to a higher amount of blue LEDs rather than red (Budiarto, 2010), and in *Brassica napus* the best proliferation rate was obtained with 100% blue LED (Li et al., 2013).

Table 1. Effects of different light qualities on sugarcane plantlets growth and multiplication *in vitro*.

Light treatment	Stem length (cm)	Fresh mass (g)	Number of tillers	Number of leaves		
				Green	Senescent	Total
B:R=70:30	10.70 b	1.35 a	2.88 a	5.0 a	2.0 b	7.0 b
B:R=50:50	12.71 a	1.38 a	3.00 a	4.3 a	3.1 a	7.4 b
B:R=30:70	10.41 b	1.04 b	2.22 b	3.8 b	3.0 a	6.8 b
WL	9.45 b	1.09 b	2.77 a	3.8 b	2.6 b	6.5 b
FL	8.88 b	1.34 a	3.33 a	4.5 a	3.6 a	8.2 a

Different letters within the column indicate significant differences at $p < 0.05$ according to Scott-Knott test (n = 16).

As observed in the variety CTC-07, the highest rates were obtained when sugarcane plantlets were cultivated under FL. The effects of LED light in growth, morphology and tillering have been studied in several sugarcane varieties (Maluta, Bordignon, Rossi, Ambrosano, & Rodrigues, 2013; Rocha et al., 2013; Silva et al., 2014), suggesting that LEDs are an interesting light source for micropropagation. However, results suggest that the role of spectral light quality in controlling *in vitro* morphogenesis is not yet fully understood and may differ according to species and/or cultivars.

Light quality is a significant environmental factor that influences the biosynthesis of photosynthetic pigments. Plantlets of the sugarcane var. RB867515 cultivated under WL presented the highest Chl-*a* content (Figure 2), probably due to the spectral profile of white lighting, as has been reported in other sugarcane varieties (Silva et al., 2014). Among the blue/red LED spectra, only the treatment with higher proportion of blue LED (B:R=70:30) differed from the others, with a reduction of Chl-*a*, Chl-*b*, and Chl (a+b) contents. This behavior also occurred in FL and indicated that maximum and minimum light intensity applied (B:R=70:30 and FL, respectively) failed to benefit the synthesis of pigments. The highest Chl-*a* content in sugarcane variety CTC-07 was produced under 100% blue LED light (Maluta et al., 2013). However, the chlorophyll content of *in vitro* plantlets grown under different light qualities may be correlated with species or cultivars (Li et al., 2013).

Carotenoid is the auxiliary pigment of antenna complexes in chloroplasts. They may help in the photo-protection of chlorophyll molecules, determining the dissipation of excess energy and the protection against toxic reactive oxygen species (Ramel, Mialoundama, & Havaux, 2013). In current study, the carotenoid content of sugarcane leaves under white and B:R LED treatments was higher when compared to fluorescent light. In fact, it is one of the advantages presented by LED spectrum.

Conclusion

This study has shown that LED-based lighting is potentially advantageous for *in vitro* culture of sugarcane var. RB867515. The spectral quality, provided by the combination of blue/red LEDs in equal proportion, was an effective lighting source to the development of plants. Further, it also improved the physiological quality of sugarcane plantlets produced. Current results should be confirmed by assays with other varieties of sugarcane.

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References

Budiarto, K. (2010). Spectral quality affects morphogenesis on *Anthurium* plantlet during *in vitro* culture. *Journal of Agricultural Science, 32*(3), 234-240.

Chen, C., Huang, M., Lin, K., Wong, S., Huang, W., & Yang, C. (2014). Effects of light quality on the growth, development and metabolism of rice seedlings (*Oryza sativa* L.). *Research Journal of Biotechnology, 9*(4), 15-24.

Darko, E., Heydarizadeh, P., Schoefs, B., & Sabzalian, M. R. (2014). Photosynthesis under artificial light: the shift in primary and secondary metabolism. *Philosophical Transactions of the Royal Society B, 369*, 1-7.

Daros, E., Oliveira, R. A., Zambon, J. L. C., & Bespalhok Filho, J. C. (2010). *Catálogo nacional de variedades "RB" de cana-de-açúcar*. Curitiba-PR: Ridesa.

Falqueto, A. R., Cassol, D., Magalhães Júnior, A. M., Oliveira, A. C., & Bacarin, M. A. (2009). Physiological analysis of leaf senescence of two rice cultivars with different yield potential. *Pesquisa Agropecuária Brasileira, 44*(7), 695-700.

Gerald, L. T. S., & Lee, L. L. (2011). Biofábrica de plantas: por que biorreator? In L. T. S. Gerald (Ed.). *Biofábrica de plantas: produção industrial de plantas in vitro* (p. 14-31). São Paulo-SP: Antiqua.

Gupta, S. D., & Jatothu, B. (2013). Fundamentals and applications of light-emitting diodes (LEDs) in *in vitro* plant growth and morphogenesis. *Plant Biotechnology Reports, 7*(3), 211-220.

Li, H. M., Tang, C., & Xu, Z. (2013). The effects of different light qualities on rapeseed (*Brassica napus* L.) plantlet growth and morphogenesis *in vitro*. *Scientia Horticulturae, 150*(1), 117-124.

Li, H. M., Xu, Z., & Tang, C. (2010). Effect of light-emitting diodes on growth and morphogenesis of upland cotton (*Gossypium hirsutum* L.) plantlets *in vitro*. *Plant Cell, Tissue and Organ Culture, 103*(2), 155-163.

Lichtenthaler, H. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Methods in Enzymology, 148*(1), 350-382.

Maluta, F. A., Bordignon, S. T., Rossi, M. L., Ambrosano, G. M. B., & Rodrigues, P. H. V. (2013). Cultivo *in vitro* de cana-de-açúcar exposta a diferentes fontes de luz. *Pesquisa Agropecuária Brasileira, 48*(9), 1303-1307.

Monteiro, L. A., & Sentelhas, P. C. (2014). Potential and actual sugarcane yields in Southern Brazil as a function of climate conditions and crop management. *Sugar Tech, 16*(3), 264-276.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3), 473-497.

Ramel, F., Mialoundama, A. S., & Havaux, M. (2013). Nonenzymic carotenoid oxidation and photooxidative stress signalling in plants. *Journal of Experimental Botany, 64*(3), 799-805.

Rocha, P. S. G., Oliveira, R. P., & Scivittaro, W. B. (2013). Sugarcane micropropagation using light emitting diodes and adjustment in growth-medium sucrose concentration. *Ciência Rural, 43*(7), 1168-1173.

Silva, M. M. A., Oliveira, A. L. B., Oliveira Filho, R. A., Gouveia Neto, A. S., Camara, T. R., & Willadino, L. (2014). Effect of blue/red LED light combination on growth and morphogenesis of *Saccharum officinarum* plantlets *in vitro*. *SPIE, 8947*, 1-7.

Singh, R. K., Kumar, P., Tiwari, N. N., Rastogi, J., & Singh, S. P. (2013). Current status of sugarcane transgenic: an overview. *Advancements in Genetic Engineering, 2*(112), 1-7.

Snyman, S. J., Meyer, G. M., Koch, A. C., Banasiak, M., & Watt, M. P. (2011). Applications of *in vitro* culture systems for commercial sugarcane production and improvement. *In Vitro Cellular &Developmental Biology – Plant, 47*(2), 234-249.

Vieira, L. N., Fraga, H. P. F., Anjos, K. G., Puttkammer, C. C., Scherer, R. F., Silva, D. A., & Guerra, M. P. (2015). Light-emitting diodes (LED) increase the stomata formation and chlorophyll content in *Musa acuminata* (AAA) 'Nanicão Corupá' *in vitro* plantlets. *Theoretical and Experimental Plant Physiology, 27*(2), 91-98.

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