PAB
Pesquisa Agropecuária Brasileira

Plant Physiology/ Scientific Notes

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

In vitro rhizogenesis of blueberry microcuttings under different LED light spectra

Abstract - The objective of this work was to evaluate the effect of different LED light spectra on the in vitro rhizogenesis of microcuttings of blueberry 'Jewel', without the use of growth regulators. The plantlets were subjected to four LED light treatments, as follows: R, 100% red; R2B, 70% red and 30% blue; B, 100% blue; and CW, control white. After six weeks incubated in a culture room, R and R2B treatments show statistically significant effects on the in vitro rooting of blueberry.

Index terms: rooting, tissue culture, plantlets.

Rizogênese in vitro de microestacas de mirtilo sob diferentes espectros de luz de LED

Resumo – O objetivo deste trabalho foi avaliar o efeito de diferentes espectros de luz de LED sobre a rizogênese in vitro de microestacas de mirtilo 'Jewel', sem a utilização de reguladores de crescimento. As mudas foram submetidas a quatro tratamentos de luz de LED, conforme a seguir: R, 100% vermelho; R2B, 70% vermelho e 30% azul; B, 100% azul; e CW, controle – luz branca. Após seis semanas de incubação em sala de cultivo, os tratamentos R e R2B apresentam efeitos estatisticamente significativos sobre o enraizamento in vitro de mirtilo.

Termos de indexação: enraizamento, cultura de tecidos, plântulas.

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Received
October 26, 2023

Accepted
August 07, 2024

How to cite
FLORÊNCIO, M.T.; KREYCI, P.F.; JACOB, J.F. de O.; AMBROSANO, G.B.; DEMÉTRIO, C.A.; RODRIGUES, P.H.V. In vitro rhizogenesis of blueberry microcuttings under different LED light spectra. **Pesquisa Agropecuária Brasileira**, v.59, e03561, 2024. DOI: https://doi.org/10.1590/S1678-3921.pab2024.v59.03561.

Highbush blueberry (*Vaccinium corymbosum*) with low chill requirement was introduced in Brazil in 2011. Pioneering studies of southern highbush blueberry group were carried out under semi-protected cultivation conditions in the city of Piracicaba, São Paulo state, Brazil. These studies led to the development of the cultivars 'Jewel', 'Emerald', and 'Showchaser' (Cantuarias-Avilés et al., 2014). In subtropical regions, these cultivars vegetate continuously along the year. The propagation process of these cultivars uses cuttings and herbaceous microcuttings, which are sensitive to variations of temperature and humidity. Therefore, more complex processes are required for rootings, such as intermittent mist chambers and environments with controlled temperature, humidity, and shading (Cantuarias-Avilés et al., 2014).

The use of in vitro plants in new production areas is essential to guarantee the productivity and effective use of orchards (Schuch & Pinto Tomaz, 2019). Growing blueberry plants is a costly activity involving high investments. In vitro propagation facilities incur maintenance costs, which vary depending on the stage of plant development. The costs are associated with the time the explants remain in vitro and with

the time it takes to establish them in the greenhouse for consumers. In order to reduce time, laboratories eliminates the rooting phase, transferring the plantlets straight into the acclimatization phase (Lebedev et al., 2019).

During the acclimatization, high losses may occur, since the plantlets are not fully formed, which may not justify the early removal of plantlets bypassing the rooting phase. The lack of viable roots, presence of epicuticular wax on the leaves, and the reduced size of the plantlets can hinder the successful acclimatization phase of woody plants (Noé & Bonini, 1996; Pospíšilová et al., 2007).

Light is one of the most important factors for explant development under in vitro conditions. The possibility of using different light spectra during the in vitro process is crucial as it directly affects the development of explants. Studies on the effects of LED spectra on different crops – *Saccharum* sp., *Anthurium* spp., and *Cunninghamia lanceolata* – have been carried out to increase their production and to improve the quality of plantlets (Maluta et al., 2013; Martínez-Estrada et al., 2016; Xu et al., 2019).

Studies on the blueberry culture involving in vitro and ex-vitro propagation techniques have been conducted to evaluate the effects of different substrates, culture media, auxins, containers, and in vitro genetic variability (Isutsa et al., 1994; Litwińczuk et al., 2005; Debnath et al., 2012; Arencibia et al., 2013; Fan et al., 2017). However, few studies have researched the effect of environmental conditions such as temperature, light, and humidity on the in vitro rooting phase (Zhang et al., 2022; Correia et al., 2024).

The objective of the present work was to evaluate the effect of different LED light spectra on in vitro microcuttings rhizogenesis of blueberry 'Jewel', without the use of growth regulators.

Shoots of *V. corymbosum* 'Jewel' were used as the initial source of explants. They were subcultured twice every six weeks in full-strength woody plant medium (WPM) (Caisson, Smithfield, Utah, USA) (Lloyd & McCown, 1980), amended with 0.3 mg L^{-1} of zeatin (Sigma-Aldrich, St. Louis, MO, USA). The cultured shoots were excised into approximately 1.5 cm long microcuttings, containing two nodes each. The microcuttings were transferred to 250 mL flasks (10 cm × 8 cm) with a T60 cap (6 cm) containing 40 mL each of full-strength WPM (Caisson, Smithfield, Utah, USA) culture medium without growth regulators, plus 3% sucrose (w/v) (Synth, São Paulo, SP, Brazil), semi-solidified at 2% (w/v) Phytagel (Sigma-Aldrich, St Louis, Missouri, USA). pH was adjusted to 4.7, and the media was autoclaved at 121°C for 20 min at 105 kPa.

The microcutting cultures were incubated in a culture room for six weeks at 25°C, under photoperiod of 16/8 hours (light/dark). Culture flasks containing microcutting explants were distributed into four treatments of adjustable LED light settings (GreenPower TLED, Philips, Netherlands). The following combinations of light settings were applied: R, 100% red light (645–675 nm); B, 100% blue light (450–465 nm); R2B, 70% red and 30% blue light; and CW, control white (6000k, Philips), using a photosynthetic photon flux density (PPFD) set for all treatments at 50 µmol m^2 s^{-1} (LI-250A, LI-COR, Illinois, USA).

At the end of 45 days of light treatment, the plants were removed from the flasks and washed in running water for phytagel removal. Then, the plantlets were evaluated for plantlet height (H, mm), number of leaves (LN), leaf area (LA, cm^2), percentage of rooting (%), length of the largest root (RL, mm), and plantlet fresh mass (PFM, g). For the analysis of LA, a nondestructive method was used. Measurements were performed in the third leaf (length and width) from the apex of the plant, using a caliper. Based on the elliptical shape of the leaf, 37.5% of the area of the rectangle was discounted, using the equation $LA = 0.625 (L \times W)$ (Schmildt et al., 2014), where: LA is the leaf area; L is the length; and W is the weight.

The contents of chlorophyll a, b, and total were estimated using leaf samples. The amount of 200 mg fresh-weight tissue samples were subjected to the extraction with 80% acetone (Synth, São Paulo, Brazil) (Lichtenthaler & Buschmann, 2001). Absorbance was measured using a UV/VIS Helios Alpha spectrophotometer (Unicam Ltd, Cambridge, UK), at the following maximum wavelengths (A_{max}): chlorophyll a at 663.2 nm; and chlorophyll b at 646.8 nm.

The experiment consisted of three flasks containing ten explants per flask. The data of H, LN, LA, RL, PFM, chlorophyll a, chlorophyll b, and chlorophyll total were subjected to exploratory and descriptive analyses. In order to carry out the analysis of variance, the assumptions of normality, homoscedasticity, and

independence of residuals were verified. Data was subjected to the Shapiro-Wilk's test and graphical analysis for normality checking. Graphical analyes were used to check the homoscedasticity. Durbin-Watson's test was used to check independence of residuals. LN, LA, RL, PFM, chlorophyll b, and chlorophyll total did not met the assumptions. LN had outliers removed and subjected to a quadratic transformation using the Box-Cox's method. LA had four outliers removed. PFM was subjected to transformation using the square rooting of the data. Chlorophyll b and chlorophyll total data were transformed using $(\sqrt{x})^{-1}$. Finally, no transformation was possible to adjust LN and RL data, and nonparametric tests were used. The Kruskal-Wallis' test was chosen to detect the differences in the medians of LN and RL, and the Dunn's test was applied as a post-hoc test to find differences between specific treatments pairs. The data of H, LA, PFM, chorophyll a, chlorophyll b, and chlorophyll total were analyzed using the one-way analysis. The Tukey's test was used for multiple comparisons. All analyses were performed using R software v.4.4.1 (R Core Team, 2024), considering the significance at 5% probability.

Light spectra had significant effects on the in vitro rhizogenesis of southern highbush blueberry 'Jewel'. Shoot formation was not observed during the rooting phase using different light spectra. In R and R2B samples, there were significant differences for plantlet height, number of leaves, leaf area, rooting percentage, and root length, in comparison with those of treatments B and CW, following a gradient of most to least performing samples: R>R2B>CW>B (Figure 1). Light treatments R and R2B did not differ from each other for LA and RL. However, both treatments showed higher values of B and CW, which also did not differ from each other. R light presented higher LN values than the others did.

Treatments R and R2B, compared with B and WC showed significant differences for the estimations of plantlet fresh mass under different spectral treatments. Changes were observed in the order of most to least performing samples: R2B>R>B>CW (Figure 1). However, statistically, R2B did not differ from R; R did not differ from B; and B did not differ from CW. Light treatments had noticeable effects on H, LN, RL, and on R% (Table 1). An increase of R% was observed in R and R2B samples at 63.33% and 76.66%, respectively. For B and CW, R% was 30.00% and 26.66%, respectively.

Significant differences of leaf chlorophyll content were observed between different light spectra. The R treatment showed significantly higher chlorophyll total levels than the CW light. There were no significant differences between the other pairs of treatments (Figure 1). However, no visible color change was observed in the plantlets, even in the monochromatic treatments of R and B (Table 1).

Studies for the effect of LED light spectra on in vitro cultures of woody plants are scarce; however, blueberry culture studies under LED light spectra have been performed using highbush and rabbiteye groups. In experiments with different light spectra and ventilation for the propagation of rabbiteye 'Titan', the combinations R, R8B2, and R5B5 were the most promising both during the in vitro development and ex-vitro phases (Hung et al., 2016a). The same combinations of spectra were used for the in vitro propagation of highbush 'Huron' in MW culture medium (50% MS + 50% WPM), without PGR and with zeatin (Hung et al., 2016b). Even though there was no shoot formation in the treatment without PGR,

Figure 1. Plantlets of blueberry southern highbush 'Jewel', after 45 days of in vitro cultivation under different light spectrum treatments, as follows: R, 100% red; RB (R2B), 70% red and 30% blue; B, 100% blue; and CW, control treatment, white. Bar: 7.5 mm. Photo by João Geraldo Brancaleon.

Table 1. Means, standard deviations, and coefficient of variation (CV) of plantlet height (H), leaf area (LA), plantlet fresh mass (PFM), chlorophyll a (Cl a), chlorophyll b (Cl b), and chlorophyll total (Cl T); medians (minimum, maximum) values of leaf number (LN) and root length (RL), and means of rooting percentage (R). All measurements were from plantlets of southern highbush 'Jewel' (*Vaccinium corymbosum*), after 45 days of in vitro cultivation under different light spectrum treatments.

Light spectrum[(1)]	H[(2)] (mm)	LA[(2)] (cm^2)	PFM[(2)] (g)	Cl a[(2)]	Cl b[(2)]	Cl T[(2)]	LN[(3)]	RL[(3)] (mm)	R (%)
R	50.7 ± 14.2a	0.23 ± 0.07a	0.045 ± 0.021ab	1.41 ± 0.17a	27.5 ± 4.8b	28.9 ± 4.7b	10 (6; 12)a	17.6 (0; 28.9)a	63.33
R2B	41.6 ± 14.3b	0.26 ± 0.08a	0.052 ± 0.020a	1.31 ± 0.83a	31.1 ± 2.8b	32.4 ± 3.6ab	9 (4; 11)b	9.8 (0; 29.7)a	76.66
B	20.7 ± 9.3d	0.13 ± 0.05b	0.035 ± 0.022bc	1.27 ± 0.16a	30.4 ± 4.0b	31.7 ± 4.2ab	7 (3; 11)c	0 (0; 19.3)b	30.00
CW	29.3 ± 10.3c	0.14 ± 0.07b	0.024 ± 0.013c	1.85 ± 0.91a	37.0 ± 1.6a	38.8 ± 2.5a	8 (3; 9)bc	0 (0; 24.6)b	26.66
CV (%)	34.4	35.5	26.0	42.9	6.3	6.4	-	-	

[(1)]Light treatments: R, 100% red; R2B, 70% red and 30% blue; B, treatment 100% blue; and CW, treatment control white. [(2)]Different letters, in the same column, indicate significant differences by Tukey's test, at 5% probability. [(3)]Different letters, in the same column, indicate significant differences, by Dunn' test, at 5% probability).

the combinations R, R8B2, and R5B5 were statistically superior to the fluorescent control and B. These results support the present work, as the combination of R and R2B was the most effective for southern highbush 'Jewel'. The study involving highbush 'Huron' did not obtain microcuttings rooted in R, R8B2, or R5B5, in a complete MW culture medium without PGR (Hung et al., 2016b). In contrast, in the present study, when using complete WPM medium, southern highbush 'Jewel' showed root formation even in the absence of PGR. The southern highbush 'Jewel' showed higher R%, RL, and H, in the R and R2B spectra, than the B and CW treatments.

In vitro rooting induced exclusively by R and R2B spectra has not been described for the southern highbush 'Jewel', and it did not occur in the highbush group 'Huron'. In vitro adventitious root development involves a complex biological process regulated endogenously and by environmental factors (Hartmann et al., 2002).

Therefore, genetic factors (southern highbush vs. highbush) and the culture medium used may have contributed to rooting in the cultivar. While evaluating the results of the monochromatic spectra, some similarities were observed between the groups. Under the R spectrum, southern highbush 'Jewel' showed a statistically significant difference for H, LN, LA, and PFM, which was superior to the B spectrum. The same trend has been observed for the highbush 'Huron', which showed similar results for plant height, leaf area, and fresh mass to the B spectrum. Despite the similarities described above, only southern highbush 'Jewel' was able to form roots in vitro, without the use of PGR, induced exclusively by the R and R2B spectra.

Plants detect ambient light conditions to increase their fitness and modulate developmental processes using photoreceptors, such as phytochromes (red/far red spectrum), cryptochromes, and phototropins (blue spectrum) (Smith, 2000; Christie & Briggs, 2001). Therefore, although many plant responses are dependent on the light quality regulation, their effect on in vitro rooting remains unknown and genotype-dependent. Thus, the induction of roots during the 45 days in vitro in R and R2B treatments, without the use of PGR, is a new information and a significant result for southern highbush 'Jewel'. The monochromatic red spectrum and the combination spectra of R2B induce in vitro rooting of microshoots in southern highbush 'Jewel'.

Acknowledgments

To Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Finance Code 001), for granting a scholarship to the first author.

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