



Effects of different light spectra on the slow-grown in vitro storage and quality of banana plantlets cv. Prata Catarina (AAB)

Paulo Hercílio Viegas Rodrigues¹ · Emerson Luis Oliveira² · Christian Aparecido Demetrio¹ · Guilherme Bovi Ambrosano¹ · Sonia Maria Stefano Piedade³

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Abstract

Continues subculturing of in vitro plant subcultures is essential for commercial micropropagation and tissue culture research. In unusual situations, the subcultures can be delayed, and the slow-growth in vitro storage technic could be applied to reduce the loss of plant material. The present study aimed to evaluate the slow-growth in vitro storage of banana plantlets ('Prata Catarina'; group AAB) under different light spectra. Shoot cultures in MS medium without plant growth regulators were maintained under blue (B), red (R), red plus blue (R2B), and white (CW) light spectra ($25\text{ °C} \pm 2\text{ °C}$; $50\text{ }\mu\text{mol m}^{-2}\text{ s}^{-1}$) for up to 140 days. The plantlets maintained under the R, CW, and R2B spectra suffered severe root damage and browning after 140 days of in vitro slow-growth storage (SGS). All plantlets maintained under the B spectrum survived after 140 days of in vitro SGS and showed little browning.

Key message

The blue spectrum reduces root browning and showed greater tolerance of the in vitro environment for 140 days of banana plantlets (group AAB).

Keywords Tissue culture · Acclimatization · Somaclonal variation · Plantlet · LED

Abbreviations

MS	Murashige and Skoog medium
BA	6-Benzylaminopurine
CML	Commercial micropropagation laboratory
SGS	Slow grown storage
LS	Light spectra

In commercial micropropagation laboratories (CML), operational difficulties may arise during commercial micropropagation processes when the in vitro-produced plantlets that are ready to be transplanted cannot be moved to the greenhouse because they are full of unsold plants or because the customer is not ready to receive the plants. Such operational difficulties can result in financial losses to both CMLs and customers (Watt et al. 2000). Recently, many CMLs and tissue culture research centers (TCRC) were temporarily closed due to the COVID-19 pandemic, which reduced the workforce, thereby limiting the subculture frequency during the lockdown. In such unusual situations, CMLs and TCRCs can employ in vitro slow-growth storage (SGS).

During in vitro SGS, to increase the period between subcultures, the development of plants is retarded by suppressing their metabolism, albeit without altering the genetic identity and quality of micropropagated plantlets (Kamińska et al. 2016). Culture conditions (temperature, photoperiod, and light intensity) and culture medium components (organic and inorganic nutrients, osmotic, and plant growth regulators) can be adjusted to suppress plant metabolism (Oliveira

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✉ Paulo Hercílio Viegas Rodrigues
phrviegas@usp.br

- ¹ Laboratory of Tissue Culture Ornamental Plants, Crop Science Department, Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, Piracicaba, São Paulo CEP: 13400-900, Brazil
- ² Herminio Ometto Foundation, University of Araras, Av. Dr. Maximiliano Baruto, 500, Jd. Universitário, Araras, São Paulo CEP: 13607-339, Brazil
- ³ Statistics Analysis Laboratory, Luiz de Queiroz College of Agriculture, University of São Paulo, Av. Pádua Dias, 11, Piracicaba, São Paulo CEP: 13400-900, Brazil

et al. 2000; Gopal and Chauhan 2010; Kaur et al. 2012; De Carvalho et al. 2014; Thakur et al. 2015).

In the banana germplasm collection at the International Transit Centre (ITC) in Belgium, over 1600 banana accessions sourced from 38 countries have been maintained under in vitro SGS. At ITC, in vitro SGS is achieved under a combination of low temperature (16 °C) and limited light ($25 \mu\text{mol m}^{-2} \text{s}^{-1}$) in a growth room (Van den houwe et al. 1995; Panis et al. 2020). Therefore, this method is appropriate to decrease the frequency of subculture by retarding the growth of plantlets, ranging between 3 and 22 months, depending on the genomic group and in vitro browning reaction (Panis et al. 2020). At banana CMLs the subculture frequency is shorter (around 3–4 weeks). The plants maintained between 12 and 16 weeks in vitro does not continue subculturing cause severe explant damages. In this case, additional time is required for the staff to work to cut the roots and the frequency of contamination increases (Pers. Comm).

To date, few studies have explored different light spectra for in vitro SGS. The use of different light spectra for in vitro SGS of *Heliconia champneiana* ‘Splash’ plantlets was evaluated and promising outcomes were achieved using the blue spectrum for up to 12 weeks (Rodrigues et al. 2018). To the best of our knowledge, however, there are no reports on the use of this technique for banana tissue culture.

To this end, the present study proposes to evaluate the effect for banana in vitro SGS under different light spectra without compromising plantlet quality.

Fifty shoot tips (ST) were obtained from uniform sword suckers of ‘Prata Catarina’ (SCS451; group AAB) with 3–4-month age. For the asepsis process, the STs were immersed in 70% alcohol for 5 min to break the surface tension. In a solution of 2.5% sodium hypochlorite and autoclaved distilled water (add three drops of Tween 20®) the STs were immersed for 20 min. Followed by them were washed three times in autoclaved distilled water. After this procedure, the excess tissue was removed from each ST before introduction in test tubes (one ST per tube) containing a semi-solid culture medium MS salts and vitamins (Murashige and Skoog 1962) plus 30 g L^{-1} sucrose, 1.0 mg L^{-1} 6-benzylaminopurine (BA; Sigma-Aldrich), and 2.0 g L^{-1} Phytigel® (Sigma-Aldrich); the pH of the medium was adjusted to 5.8 before autoclaving for 20 min at $121 \text{ }^\circ\text{C}$. The STs were incubated in a growth room for 30 days at $25 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$, photoperiod of 14 h; was supplied by LEDs (6000 k, Philips®) at a photosynthetic photon flux density (PPFD) of $50 \mu\text{mol m}^{-2} \text{s}^{-1}$ (LI-250A, LI-COR). Forty healthy STs were induced to multiply under the same conditions as described above, but in jars containing 40 mL of a semi-solid culture medium MS salts and vitamins, plus 30 g L^{-1} sucrose, 3.5 mg L^{-1} BA,

2.0 g L^{-1} Phytigel®, and subculture was performed four times.

Next, 400 in vitro-produced shoots were selected, cut to obtain uniform explants ($1.5 \text{ cm} \times 0.5 \text{ cm}$), inoculated into test tubes ($15.0 \text{ cm} \times 2.5 \text{ cm}$) containing 7.0 mL of the semi-solid MS medium as described above, no plant growth regulators added. The tubes were incubated with the above described conditions and under four different light treatments (GreenPower TLED®; Philips®): R = 100% red light (645–675 nm); B = 100% blue light (450–465 nm); R2B = 70% red + 30% blue light; and CW = white control (6000 k, Philips®).

Each light treatment included 100 explants, divided into five-time intervals with 20 explants per interval: I1 (28 days), I2 (56 days), I3 (84 days), I4 (112 days), and I5 (140 days) without subculture. Every 28 days, 20 plantlets to 100 were selected at random from each treatment to be evaluated for survival, height (from the base of the pseudostem to the cigar leaf tip, cm), fresh weight (g), pseudostem diameter immediately above the original cut (cm), number of leaves, number of primary roots, the longest root length (cm), and chlorophyll content (expressed as the SPAD index) of the third top leaf, measured with a CFL1030- chlorophyll meter (Falker®).

Concluded the time period (I1 to I5), in vitro plantlets were transferred to 24-cell plastic trays (163 cm^3) containing a commercial horticulture substrate- BASE® (50% pine bark, 30% fibrous peat, and 20% vermiculite) and acclimatized in a greenhouse with fogging for 28 days, at $28 \text{ }^\circ\text{C} \pm 2 \text{ }^\circ\text{C}$, 80–90% humidity (Full Gauge® Controller—MT-530 Super) and 50% shading (Aluminet®). In this period, the plantlets were evaluated for monitored survival, plantlet height (cm), pseudostem diameter (cm), and leaf number.

During in vitro SGS, and acclimatization period, the experimental design was completely randomized, with plots representing the light treatments and subplots representing the time intervals. All data were subjected to analysis of variance (*F* test) in the split-plot arrangement with 20 replications, and the mean values among treatments and across time intervals were compared with Tukey’s test at 5% significance.

There was a significant difference in plant height between different light spectra ($p = 0.0359$) (Fig. 1a). The cigar leaf length of explants increased steadily up to I4 but decreased during I5 (Fig. 2c). The height of explants grown under different light spectra was in the order $B < R2B < CW < R$ (from the shortest to tallest) across time intervals (Fig. 1a).

Light spectrum affected the number of leaves ($p = 0.0005$) and the thickness of the pseudostem ($p = 0.0285$) significantly. These characteristics steadily increased until I5 (Fig. 1b and c). No symptoms of leaf senescence were observed under any light spectrum at I5 (Fig. 2a). There

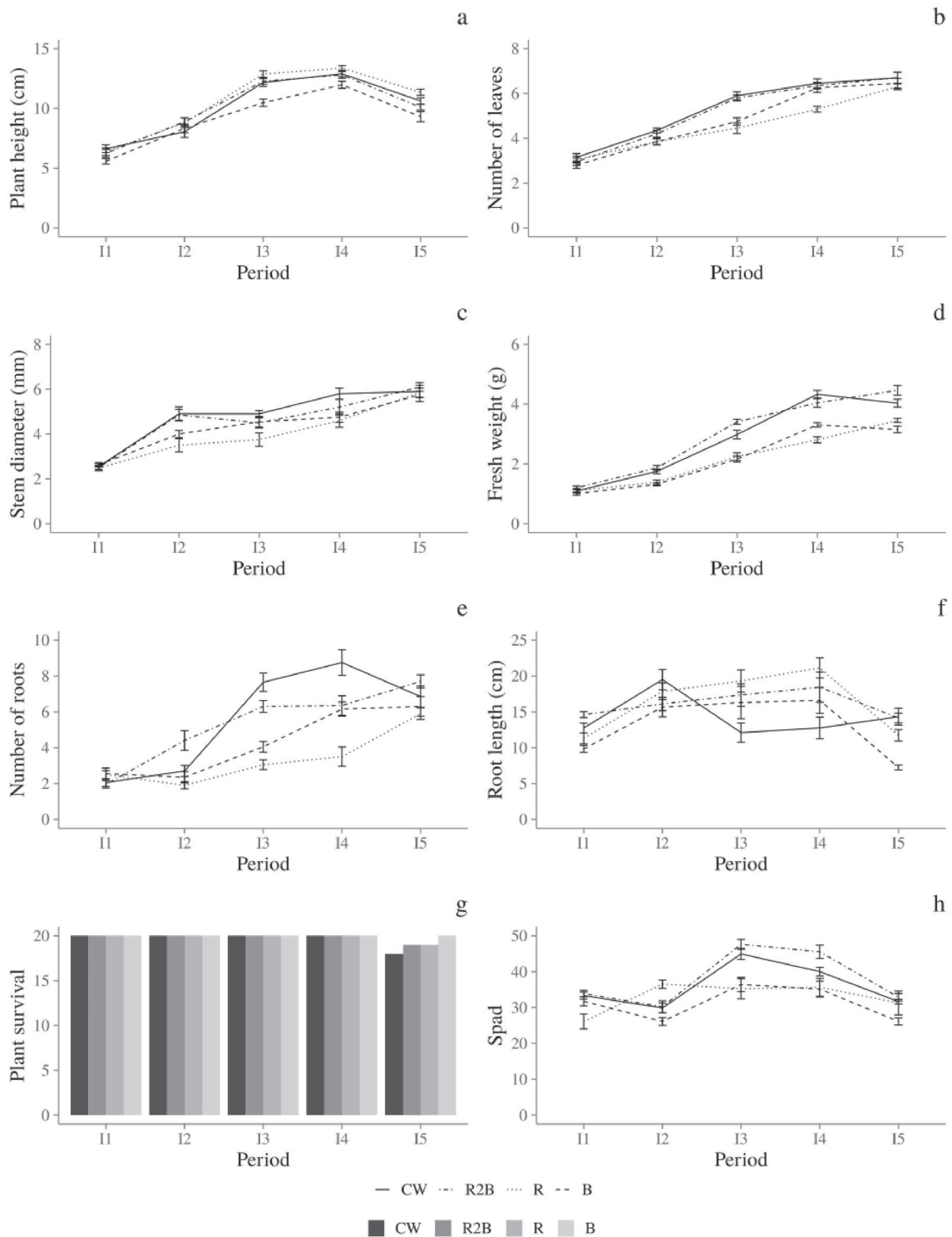


Fig. 1 Plantlets in SGS in different LS. Evaluation of banana shoots from cv. Prata Catarina in SGS for five periods of time in different LS. **a** Plant height-cm, **b** number of leaves, **c** stem diameter-mm, **d** Fresh weight-g, **e** number of roots, **f** root length-cm, **g** plant survival

and **h** SPAD. CW control white, R2B 70% Red + 30% Blue, R 100% Red and B 100% Blue. I1 (28 days), I2 (56 days), I3 (84 days), I4 (112 days), and I5 (140 days). (Color figure online)

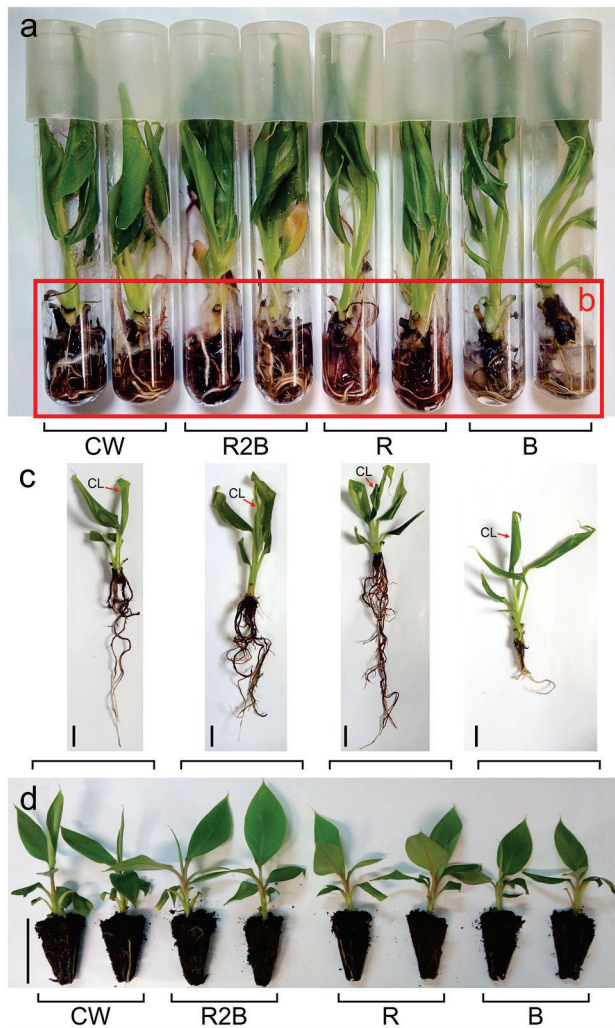


Fig. 2 **a** Plantlets at different LS in SGS after 140 days (I5). **b** Roots of in vitro plants after 140 days in SGS. **c** I5 Plantlets before acclimatization. **d** Acclimatized plantlets after 4 weeks. *CL* cigar leaf, *CW* control white, *R2B* 70% Red+30% Blue, *R* 100% Red and *B* 100% Blue. Barr (c) = 1.5 cm, Barr (d) = 6.0 cm. (Color figure online)

was a significant difference in fresh weight for the different light spectra ($p < 0.0001$). During the experimental period, fresh weight was the lowest under the B spectrum, and the value fluctuated under the R spectrum during I4. In contrast, fresh weight was the highest under the CW and R2B spectra from I2 to I5 (Fig. 1d).

There was a significant difference in root number between different light spectra ($p < 0.0001$). During I2, there was a significant increase in growth under the R2B spectrum compared with that under the remaining spectra (Fig. 1d). The number of primary roots increased from I2 to I4 under all evaluated spectra, except R2B.

There were significant differences in evaluated root number under the CW and other spectra during I3 and I4. During I5, primary root number under the CW spectrum and growth

under the R2B and R spectra were significantly reduced, but the values were stable under the B spectrum. Of note, under the CW spectrum, primary root number increased from I2 to I4 but decreased during I5.

There was a significant difference in the length of the longest root (Fig. 1e) between the light spectra ($p < 0.0001$). This length increased from I1 to I4 under all spectra, except CW. Under the CW spectrum, there was a significant reduction in this length from I2 to I3 and a further significant reduction from I3 to I4, and the values were lower than those under all the remaining spectra. During I5, there were significant reductions in the length of the longest root under the R, B, and R2B spectra, but the values were stable under the CW spectrum. The roots suffered the effect of in vitro senescence over time, in the order of $CW > R2B > R > B$. Under the B spectrum, characteristic young root color was observed even after 140 days of in vitro SGS (Fig. 2a and red rectangle in b). Although we did not quantify the consumption of the culture medium, a larger volume of the culture medium, which was colorless/translucent even during I5, was remaining under the B spectrum.

All plants survived SGS until I4 (Fig. 1g). The explants were grown under the CW, R2B, and R spectra died soon after removal from the test tubes. Excessive browning resulted in the death of some explants. At the intersection of the pseudostem and root, tissue breakage occurred during explant wash, subsequently leading to death, under the R (1 explant), R2B (1 explant), and CW (2 explants) spectra. No explant loss occurred under the B spectrum.

There was a significant difference in SPAD index between light spectra ($p < 0.0001$). The trends were similar under the CW, R2B, and B spectra, with a lower index recorded under B; the opposite trends were observed during I1 and I2, but they remained stable from I3 to I5 (Fig. 1h).

Following SGS, all transplanted plantlets survived the acclimatization period. During SGS, light spectrum affected plantlet height ($p = 0.0181$), leaf number ($p = 0.0053$), and pseudostem diameter ($p < 0.0001$) significantly. Plant height and leaf number increased from I1 to I4. During I5 (Fig. 2d), plants grown under the R and B spectra showed reduced development. The fewest leaves (Fig. 3a), the shortest plants (Fig. 3b), and the smallest pseudostem diameter (Fig. 3c) were observed under the R and B spectra (Fig. 3).

The present study demonstrated that different light spectra affected all evaluated parameters during in vitro SGS. Regarding plantlet height, there was a reduction from I4 to I5 under all spectra, which was caused by the shortening of the cigar leaf. In a previous study, the effects of the B and R spectra and their different combinations during in vitro cultivation *Musa paradisiac* ‘Nam Dinh’ were assessed using a sugar-free technique with CO_2 injection (Nhut et al. 2002). In that study, the plant height was in the order of $B < PGF$ (fluorescent lamp) $< R2B$ (70% red + 30% blue) $< R$ (from

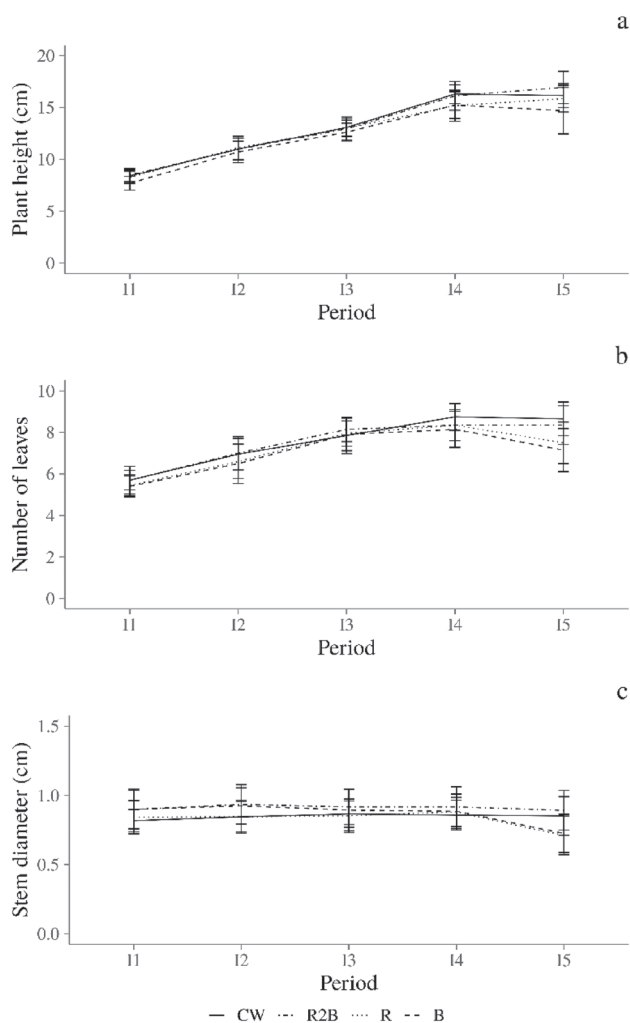


Fig. 3 Evaluated banana plantlets cv. Prata Catarina in SGS for five periods after 4 weeks of acclimatization. **a** Plant height-cm, **b** number of leaves, **c** stem diameter-cm. I1 (28 days), I2 (56 days), I3 (84 days), I4 (112 days), and I5 (140 days)

the shortest to tallest). These results were similar to those for ‘Prata Catarina’ under 140 days of SGS in the present study ($B < CW < R2B < R$) as well as for *H. champneiana* ‘Splash’ under 84 days of in vitro culture under different light spectra (Rodrigues et al. 2018).

Similar trends were observed for fresh weight, which was lower under the B spectrum, for ‘Prata Catarina’, ‘Nam Dinh’, and *H. champneiana* ‘Splash’. Thus, different species exhibited similar behaviors during SGS under different light spectra. The plantlets were shorter and showed a lower fresh weight under the B spectrum. Leaf number and explant diameter were not affected by light spectrum during SGS in the present study.

In the plantlets, no yellowing or leaf necrosis, a typical symptom of in vitro stress caused by the gas-saturated in vitro environment and/or nutrient shortage in the culture

medium, was observed (Rodrigues et al. 2018; Pimenta et al. 2013). In a restricted environment without regular subculture, gas accumulation due to explant growth can be detrimental to plant development in vitro. Ethylene is one of the gases that significantly affect different physiological processes. Numerous studies have demonstrated the involvement of ethylene in callus proliferation, somatic embryogenesis, and rhizogenesis (Buddendorf-Joosten and Woltering 1996; Biddington 1992; Marota et al. 2001). In *H. champneiana* ‘Splash’, leaf senescence was observed under the CW, R2B, and R spectra after 84 days of SGS. The leaves showed discoloration and necrosis, which are typical symptoms of long-term in vitro growth (Rodrigues et al. 2018). In the present study, even under the conditions of restricted gaseous exchange, the ‘Prata Catarina’ explants were not affected by SGS under different light spectra.

Under in vitro conditions from I2 to I4, root growth was suppressed under the CW spectrum but compensated for by the formation of more primary roots. A similar result was observed, albeit to a lesser extent, under the R2B and R spectra. During the explant wash for data collection, browning of the tissues at the root tips was observed, which prevented further growth from I3 onward. Meanwhile, this favored an increase in the number of primary roots under these treatments. During I5, the degree of browning in the roots was higher, which results in the death of some explants under the CW, R2B, and R spectra. Under the B spectrum, root development was stable, and no symptoms of senescence were observed until I5. The browning of plant tissues is caused by the oxidation of tannins and polyphenols and the formation of highly reactive and toxic quinones. This process can start due to successive subcultures, cuts that generate injuries, or senescence of plant tissues in vitro (Ahmad et al. 2013). Marked root senescence during I5 under the CW, R, and R2B spectra, with the loss of some plantlets, limits the use of these light spectra for 140 days in vitro.

Plantlets grown under the B spectrum showed greater tolerance of the in vitro environment for 140 days, indicating that a longer period in SGS under this is feasible. The culture medium under the B spectrum remained translucent without the darkening effect observed in the other treatments. Darkening of the culture medium occurs as a result of the oxidation of polyphenol exudates from the roots. The absence of this effect under the B spectrum suggests that blue light, directly or indirectly, inhibits the oxidation of tannins and polyphenols from root exudates in vitro.

The effect of light quality on the cellular redox balance in vitro has been poorly studied and is restricted to only a few species (Dutta Gupta and Agarwal 2017). A previous study evaluated the antioxidant capacity in *Rehmannia glutinosa* grown in vitro under different light conditions; the highest total antioxidant capacity of the leaf and root extracts was observed in the presence of blue light (Manivannan et al.

2015). At ITC, some banana accessions did not achieve satisfactory development during SGS, and there may be large differences in subculture conditions such as transfer interval, ranging between 3 and 22 months (Panis et al. 2020). The browning effect during in vitro banana culture varies among different genomic groups and even within the same subgroup (Van den houwe et al. 1995). The prevention of root browning during SGS under blue light is an important result, which may contribute to controlling browning during banana tissue culture.

The lowest SPAD index was observed from I2 to I5 under the B spectrum, indicating lower photosynthetic activity and, consequently, lower metabolic activity during SGS. These results are similar to those reported previously for ‘Nam Dinh’, in which blue light completely inhibited plantlet growth (Nhut et al. 2002).

The acclimatized plants showed no morphological variation. In the present study, plants under the B spectrum showed reduced growth during I5 of the acclimatization period. In a previous study, no differences in the height, fresh weight, or SPAD index of acclimatized ‘Nam Dinh’ plants propagated in vitro in PGF under 80% red + 20% blue light were observed (Nhut et al. 2002). The results of the present study are unprecedented and demonstrate that prolonged in vitro environmental conditions affect plant development during the acclimatization period.

Factors related to in vitro culture techniques, such as stress caused by the accumulation of gases and extreme humidity in the flasks, growth regulator concentrations stimulating bud formation or growth, number of subcultures, and the total duration for which the explant remains in vitro, can induce epigenetic alterations or true mutations (Krikorian et al. 1993; Karp 1994; Santos and Rodrigues 2004; Bairu et al. 2006). After acclimatization, the plantlets during I5 under the B spectrum showed normal development, indicating a temporary physiological effect. In the present study, no morphological variations were observed among the evaluated light treatments.

In conclusion, the use of the B spectrum for banana (AAB group) cultivation in a growth chamber or room may be used either exclusively or in combination with other techniques aimed at reducing the growth of the explant without compromising the material quality. This approach may provide more time to resolve operational problems or maintain an active germplasm bank in a CML.

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Declarations

Conflict of interest The authors have not disclosed any competing interests.

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