Stimulation of banana *in vitro* shoot growth by yellow-cellophane-film shading

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Stimulation of banana *in vitro* shoot growth by yellow-cellophane-film shading.

Abstract — Introduction. Many shoots or bud-like structures are lost in the final stage of plant micropropagation because of being too short for handling. Yellow and red lights induce *in vitro* plant growth of some species, and blue light reduces it instead. Yellow cellophane film passes through from the yellow to red light spectrum but cuts off the blue one. Growth of banana *in vitro* shoots was thus compared with or without yellow-cellophane-film shading. **Materials and methods**. Field-grown banana normal and dwarf variant plants of Nanicão cv. (*Musa* sp., AAA group, Cavendish subgroup) derived from micropropagation were re-established *in vitro*. They were then transferred to MS medium without growth regulator in test tubes covered by yellow cellophane film under cool white fluorescent lamps at a light intensity of 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Shoot growth was measured as height was evaluated after (20 and 30) days of culture. **Results and discussion**. The yellow-cellophane-film shading stimulated the growth of banana *in vitro* shoots. When cultured under the yellow-cellophane-film shading for 20 days, the normal true-to-type plants showed an increase of about 25% in shoot height compared with the plants cultured without cellophane film. Similar growth response was also observed on the *in vitro* shoots of the dwarf plants but the increment was only about 12%. This shading technique can aid shoot elongation and consequently reduction of plantlet loss at the final stage of plant micropropagation. The differing sensibility of the normal true-to-type plants and dwarf variants to the yellow-cellophane-film shading may be used for reduction of the dwarf variant population in banana micropropagation. **Conclusion**. Yellow-cellophane-film shading stimulates growth of banana *in vitro* shoots.

Brazil / *Musa* / plant propagation / micropropagation / light requirements / radiosensitivity

Stimulation de la croissance *in vitro* de plantules de bananier sous film de cellophane jaune.

Résumé — Introduction. De nombreuses plantules ou autres structures analogues à des bourgeons sont perdues lors de la dernière phase de micropropagation des plants, car elles sont trop courtes pour être manipulées. Les lumières jaune et rouge induisent la croissance *in vitro* de certaines espèces, alors que la lumière bleue la réduit. Le film de cellophane jaune permet le passage d'un spectre de lumière du jaune au rouge mais arrête le bleu. La croissance *in vitro* des plantules de banane a alors été comparée avec ou sans film de cellophane jaune. **Matériel et méthodes**. Des plants normaux de bananiers et des variants nains du cv. Nanicão (*Musa* sp., groupe AAA, sous-groupe Cavendish), issus de micropropagation et cultivés en champ, ont été remis en culture *in vitro*. Les vitroplants ont été alors transférés, sur milieu MS sans régulateur de croissance, dans des tubes à essai obturés par un film de cellophane jaune, sous des lampes fluorescentes à lumière froide blanche présentant une intensité de 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$. La croissance des plantules mesurée par leur hauteur a été évaluée après (20 et 30) jours de culture. **Résultats et discussion**. L'ombrage effectué à l'aide du film de cellophane jaune a stimulé la croissance *in vitro* des plantules de bananiers. Après culture sous ce film jaune pendant 20 jours, les plantules issues des plants normaux ont présenté un gain de croissance d'environ 25 % par rapport à celles des vitroplants développés sans film de cellophane. Une réponse semblable a été observée pour la croissance des plantules *in vitro* issues des bananiers nains, mais le gain n'a été que de 12 % environ. Cette technique d'ombrage pourrait faciliter l'élongation des pousses et, par conséquent, réduire la perte de plantules lors de la phase finale de micropropagation des plants. La sensibilité différente observée vis-à-vis de l'ombrage du film de cellophane jaune entre les plantules issues de plants normaux et celles de plants nains pourrait être utilisée pour réduire la population de variants nains lors de la micropropagation du bananier. **Conclusion**. L'ombrage effectué avec un film cellophane jaune stimule la croissance *in vitro* des plants de bananiers.

Brésil / *Musa* / multiplication des plantes / micropropagation / besoin en lumière / radiosensibilité

1. Introduction

Shoot tip cultures are commonly used in banana micropropagation of a wide range of *Musa* genotypes [1–3]. Micropropagated banana plantlets grow faster, facilitate more synchronized harvesting and have higher yields than conventional propagules [4–6]. Although banana micropropagation is already well established, continuous improvement is needed for commercial plantlet production to increase efficiency and reduce production cost. One of the points to be improved upon is a reduction of *in vitro* plantlet loss in the subculturing procedure from multiplication to the rooting stage. Short shoots or bud–like structures at this stage are difficult to handle and need much more time for elongation and rooting [1, 2]. On the other hand, we are frequently faced with the problem of undesirable somaclonal variations [7]. About 80% of the somaclonal variations are dwarf-type variants [8], which are almost invariably linked to low productivity. Thus, many studies have been realized to detect dwarf variants at early stages of development, using gibberellic acid [9, 10], isozyme profile polymorphism [11] and molecular markers [12, 13]. The first technique is easily applicable to routine micropropagation, although it detects only gibberellin-sensitive variants. On the other hand, the second and third techniques may detect different kinds of variants, but they are not as easy to use for routine work and are costly, particularly in developing countries.

It is known that light quality influences plant growth behavior. Red light increases *in vitro* culturing plantlet developments in some species [14–16]. Yellow light also has a favorable influence on shoot elongation and leaf formation [17, 18]. On the contrary, blue light reduces *in vitro* plantlet growth [19, 20]. Yellow colored cellophane film transmits 550–720 nm wavelength lights that correspond from yellow to the red light spectrum. It is a good material for studying the light quality effect because of the selectivity of the light spectrum and its easiness to acquire. The present work aims to verify stimulation of *in vitro* shoot growth measured as height by yellow-cellophane-film shading in banana micropropagation.

2. Materials and methods

2.1. Origin of plant material

Banana shoot tips (*Musa* sp., AAA group, Cavendish subgroup, Nanicão cultivar) were cultivated *in vitro* for 2 years, and more than 500 plants of a 2-year-old banana plantation were established. The banana plantation had populations of normal true-to-type plants (18%), dwarf variants (67%) and intermediate characteristics (15%). Five of the true-to-type plants and five of the dwarf variant plants were randomly selected. Each parent plant was considered a separate line during subsequent *in vitro* culture.

2.2. *In vitro* establishment and culture

Two suckers from each parent plant were extracted and re-established *in vitro*. The explants were cultivated on MS medium [21] supplemented with 30 $g \cdot L^{-1}$ sucrose, 2 $g \cdot L^{-1}$ Phytagel and 3 $mg \cdot L^{-1}$ BA (6-benzylaminopurine). The cultures were maintained under a 16-h light / 8-h dark cycle at a light intensity of 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Photosynthetic Photon Flux Density- PPFD) at (28 ± 2) °C and subcultured at monthly intervals using culture medium of the same composition.

2.3. Yellow-cellophane-film treatment

After 1 month for establishment followed by 4 months of multiplication (four subcultures), when more than 50 explants of the 20-mm or longer shoots from each type of plant were obtained, the shoot explants were individualized, cut to 5 mm long and transplanted to the same MS medium but without BA (growth-regulator-free medium) in test tubes. Fifty test tubes, 25 from dwarf variant plants and 25 from normal plants, were covered by yellow cellophane film, and another 50 were not covered. Light intensities were 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the test tubes without cellophane film and 38 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the test tubes under cellophane film. After (20 and 30) days of

culture, two parameters of shoot height, which were the lengths from the base of the pseudostem to the leaf tip and from the base of the pseudostem to the emerging point of the final leaf, were measured. On the other hand, aiming to verify only the effect of the reduced light intensity on the shoot growth, another 40 true-to-type shoot explants were cultured on the growth-regulator-free medium under light intensities of 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 38 $\mu mol \cdot m^{-2} \cdot s^{-1}$ without cellophane film. The light intensities were controlled by adjusting distances between the light bulbs and the culture test tubes. The growth evaluation method was the same as used for the above experiment.

The experimental design was completely randomized. Data were submitted to statistical analyses using *t*-statistics and Duncan's multiple range test ($p < 0.05$).

Table I.
Mean height growth of banana shoots evaluated by length from the base of the pseudostem to the leaf tip after (20 and 30) days of *in vitro* culture with or without yellow cellophane cover.

Type of plant	Treatment	Mean ± standard error (mm)	
		20 days	30 days
Normal	Yellow cellophane (Yc)	54.3 a ± 2.05	71.9 a ± 2.49
	Control (Ct) (without cellophane)	43.5 b ± 1.81	64.6 b ± 2.55
	Ratio in % [(Yc / Ct) × 100]	125	111
Dwarf	Yellow cellophane (Yc)	42.5 b ± 1.56	59.1 bc ± 1.56
	Control (Ct) (without cellophane)	37.8 c ± 1.56	54.5 c ± 1.68
	Ratio in % [(Yc / Ct) × 100]	112	108

a, b, c: 5% significance level ($p < 0.05$).

3. Results and discussion

Although the heights of both dwarf variants and normal true-to-type plants were significantly increased by the yellow-cellophane-film shading ($p < 0.05$), the growth of the normal plants was much more increased over the dwarf variants. When the normal plants were cultured under the yellow-cellophane-film shading for 20 days and their heights measured from the base of the pseudostem to the leaf tip, their shoot height was 125% (about 25% higher) of the plants cultured under a normal illumination system, that is, without cellophane film (*table I*). On the other hand, it was 112% (about 12% higher) in the dwarf plants. Similar results were obtained when the heights from the base of the pseudostem to the emerging point of the final leaf were measured, counting 128% and 114%, respectively (*table II*). The differences in the height with and without cellophane film were reduced after 30 days of culture, but the normal true-to-type plants under cellophane film shading were still significantly higher than those not shaded (*tables I, II*).

Table II.
Mean height growth of banana shoots evaluated by length from the base of the pseudostem to the point of emergence of the final leaf after (20 and 30) days of culture with or without yellow cellophane cover.

Type of plant	Treatment	Mean ± standard error (mm)	
		20 days	30 days
Normal	Yellow cellophane (Yc)	23.5 a ± 1.05	32.5 a ± 1.10
	Control (Ct) (without cellophane)	18.3 b ± 0.99	29.5 b ± 1.01
	Ratio in % [(Yc / Ct) × 100]	128	110
Dwarf	Yellow cellophane (Yc)	19.1 b ± 0.69	26.7 c ± 0.87
	Control (Ct) (without cellophane)	16.7 c ± 0.79	24.4 c ± 0.63
	Ratio in % [(Yc / Ct) × 100]	114	109

a, b, c: 5% significance level ($p < 0.05$).

This shoot height growth, however, might be caused by the reduced light intensity through shading but not necessarily yellow- or red-wavelength light. To answer this question, the shoots of the normal true-to-type plants were cultured under light intensities of 46 $\mu mol \cdot m^{-2} \cdot s^{-1}$ and 38 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The shoot growths of the two different light intensities were not significantly different (*table III*). The standard errors of the data of

Table III.
Mean height of normal true-to-type banana shoots after (20 and 30) days of *in vitro* culture according to two intensities of white light.

Height measured	Light intensity ($\mu mol \cdot m^{-2} \cdot s^{-1}$)	Mean ± standard error[1] (mm)	
		20 days	30 days
To leaf tip	46	38.9 ± 3.4	53.1 ± 3.4
	38	40.2 ± 4.5	52.8 ± 4.8
	Significance (*p*)	0.8266	0.9688
To point of emergence	46	19.9 ± 1,2	24.7 ± 1.5
	38	22.0 ± 2.1	26.9 ± 2.1
	Significance (*p*)	0.3779	0.3827

[1] Each value represents the mean of 20 replications.

this experiment were relatively high, so we still cannot eliminate the possibility of the difference becoming significant if a higher number of shoot heights was evaluated. However, considering that each mean data of this experiment was collected as the average number of 20 shoots, the effect of the reduced light intensity on the shoot elongation, if any, must be small. It means that the growth differences observed by the yellow-cellophane-film shading should be mainly caused by yellow- and red-wavelength light but not by the reduced light intensity. Similar results have been reported on micropropagations of other species. Savithri Bhat *et al.* [19] observed that red and yellow lights stimulated shoot production of menthol mint. Glowacka [17] also observed shoot and internode elongation under red and yellow lights in tomato micropropagation. Chang *et al.* [16], however, observed that, besides red light, blue light stimulated calla lily (*Zantedeschia albomaculata*) shoot elongation. Furthermore, for this species, red light increased shoot weight, but not blue light. The red + blue light suppressed the elongation. In menthol mint and lettuce, the shoot number was reduced by blue light [19, 20]. It seems blue light acts negatively on shoot proliferation.

The yellow-cellophane-film shading stimulates the shoot elongation of the normal true-to-type plants more than of the dwarf plants. The differing sensibility of the *in vitro* plants allows the use of this treatment to reduce dwarf variant populations in banana micropropagation, by simply subculturing more elongated shoots. We still do not know why the dwarf plants showed less sensibility to yellow and/or red light. We also do not know what the mechanism of dwarfism of the banana variants is. However, we already know that dwarf somaclonal variants are less sensitive to GA_3 (active gibberellin) than the original banana cultivar [9], and red light increases the transcription of the gene for $GA3_{ox,}$ which converts an inactive gibberellin (GA_{20}) into an active one (GA_1) [22]. Similar mechanisms might be occurring in the banana normal true-to-type and dwarf variant plant growths under the yellow-cellophane-film shading, but we need more detailed studies to confirm it.

The yellow-cellophane-film-shading system may also be suggested for use at the final stage of micropropagation, when elongated explants are normally desirable because their handling, rooting and plantlet formation are facilitated.

4. Conclusion

The yellow- and red-wavelength lights provided by the yellow-cellophane-film shading stimulated the growth of banana *in vitro* shoots. The stimulation is greater in normal true-to-type plants than in dwarf somaclonal variants.

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Estímulo del crecimiento *in vitro* de plántulas de plátano bajo película de celofán amarillo.

Resumen — Introducción. Una gran cantidad de plántulas u otras estructuras análogas a las yemas se pierden durante la última fase de micropropagación de los plantones, debido a que son demasiado cortas para ser manipuladas. La luz de color amarillo y rojo inducen el crecimiento *in vitro* de ciertas especies, mientras que la luz azul lo reduce. La película de celofán amarillo permite el pasaje de un espectro de luz desde el amarillo hasta el rojo, pero el color azul no lo permite. Acto seguido, se comparó el crecimiento *in vitro* de plántulas de plátano con y sin película de celofán amarillo. **Material y métodos**. Se volvieron a poner bajo cultivo *in vitro* plantones normales de plátanos así como variantes enanas del Nanicão (*Musa* sp., grupo AAA, sub-grupo Cavendish), procedente de la micropropagación y cultivados en campo. Seguidamente se transfirieron los vitroplantones en un medio MS sin regulador de crecimiento, en tubos de ensayo obturados por una película de celofán amarillo, bajo lámparas fluorescentes de luz fría blanca y con una intensidad de 46 $mmol \cdot m^{-2} \cdot s^{-1}$. Se evaluó el crecimiento de las plántulas medido por la altura tras (20 y 30) días de cultivo. **Resultados y discusión**. La sombra efectuada gracias a la ayuda de la película de celofán amarillo estimuló el crecimiento *in vitro* de las plántulas de plátanos. Tras el cultivo bajo película amarilla durante 20 días, las plántulas procedentes de plantones normales presentaron un aumento de crecimiento de alrededor del 25 % en comparación con aquellas procedentes de vitroplantones desarrolladas sin película de celofán. Se observó una respuesta parecida para el crecimiento de plántulas *in vitro* procedentes de plátanos enanos; sin embargo el aumento aquí sólo fue aproximadamente del 12 %. Esta técnica de sombra podría facilitar la elongación de los brotes y, consecuentemente, reducir la pérdida de plántulas durante la fase final de micropropagación de plantones. La sensibilidad diferente observada con respecto a la sombra de la película de celofán amarilla entre las plántulas procedentes de plantones normales y entre aquellas procedentes de plantones enanos, podría emplearse con el fin de reducir la población de variantes enanas durante la micropropagación del plátano. **Conclusión**. La sombra efectuada gracias a una película de celofán amarillo estimula el crecimiento de vitroplantones de plátanos.

Brasil / *Musa* / propagación de plantas / micropropagación / necesidades de luz / radiosensibilidad