DOI: 10.13140/RG.2.1.2456.5366
http://dx.doi.org/10.13140/RG.2.1.2456.5366
Cultivos Tropicales, 2016, vol. 37, no. 1, pp. 57-64 January-March

ISSN print: 0258-5936
ISSN online: 1819-4087

Ministry of Higher Education. Cuba
National Institute of Agricultural Sciences
http://ediciones.inca.edu.cu

EFFECT OF INOCULATION DENSITY OF SOMATIC EMBRYOS FOR OBTAINING PLANTAIN 'FHIA-21' (AAAB) CULTIVARS

Efecto de la densidad de inoculación de embriones somáticos en la obtención de plántulas de plátano cv. 'FHIA-21' (AAAB)

Leyanis García-Águila[1]✉, Rafael Gómez-Kosky[1], Yelenys Alvarado Capó[1], Zoe Sarría Hernández[1], Nilca Albany[2], Jorge Vílchez[2], Maritza Reyes Vega[1], Blanca Pérez Pérez[1] and Alexis Rodríguez Concepción[1]

ABSTRACT. Plant regeneration through somatic embryogenesis in plantain and banana may become a commercial propagation technology, due to the high multiplication coefficient of somatic embryos. However, it is important to get quality embryos with a synchronous morphological development to achieve higher rates of germination and plant conversion. Therefore, the aim of this study was to determine the effect of inoculation density of somatic embryos for obtaining plantain 'FHIA-21' (AAAB) cultivars. Then, four inoculation treatments were studied: 0,2; 0,4; 0,6 and 0,8 grams of fresh mass (gMF) of embryos on liquid culture medium of maturation. The morphological and histological characteristics of somatic embryos were evaluated after 30 days and transferred to a semisolid germination culture medium. Results showed a better synchronization in the morphological development of somatic embryos when cultured with 0,6 Gmf, which was evident by their length uniformity, the apical and root meristem formation, as well as the accumulation of reserve substances. Such embryos reached a higher percentage of germination and whole plant formation, with statistical differences compared to other treatments. These plants were characterized by a longer pseudostem, more than two open leaves and greater root number. This study is essential for the mass propagation of plantain cv. 'FHIA-21' by somatic embryogenesis.

Key words: somatic embryogenesis, histology, maturation, morphology, synchrony

RESUMEN. La regeneración de plantas por embriogénesis somática en plátanos y bananos puede convertirse en una tecnología para la propagación a escala comercial, debido al elevado coeficiente de multiplicación de los embriones somáticos. Sin embargo, para lograr tasas superiores de germinación y conversión de plantas, es importante obtener embriones de calidad con un desarrollo morfológico sincrónico. Por tanto, el objetivo del trabajo fue determinar el efecto de la densidad de inoculación de embriones somáticos en la obtención de plántulas de plátano cv. 'FHIA-21' (AAAB). Para ello, se estudiaron cuatro tratamientos correspondientes a la inoculación de 0,2; 0,4; 0,6 y 0,8 gramos de masa fresca (gMF) de embriones en medio de cultivo líquido de maduración. A los 30 días, se evaluaron las características morfológicas e histológicas de los embriones y se transfirieron a un medio de cultivo semisólido de germinación. Los resultados mostraron una mejor sincronización en el desarrollo morfológico de los embriones somáticos cuando se cultivaron con 0,6 gMF, aspecto que se evidenció por la uniformidad de su longitud, formación de los meristemos caulinar y radical, así como la acumulación de sustancias de reserva. Estos embriones alcanzaron un mayor porcentaje de germinación y formación de plantas completas, con diferencias estadísticas respecto al resto de los tratamientos. Las plantas se caracterizaron por una mayor longitud del pseudotallo, más de dos hojas abiertas y mayor número de raíces. El presente estudio es fundamental para la propagación masiva de plátano cv. 'FHIA-21' por embriogénesis somática.

Palabras clave: embriogénesis somática, histología, maduración, morfología, sincronía

[1] Instituto de Biotecnología de las Plantas (IBP), Universidad Central "Marta Abreu" de Las Villas, carretera a Camajuaní km 5.5, Santa Clara, Villa Clara, CP 54 830, Cuba.

[2] Facultad de Agronomía, Universidad del Zulia, AP 15205, Maracaibo, Estado de Zulia (4005ZU), República Bolivariana de Venezuela.

✉ leyanis@ibp.co.cu

INTRODUCTION

Somatic embryogenesis is a process by which cells differentiate into somatic embryos and fully retain somatic genotype donor plant. Methods for performing this type of morphogenesis have been described in a large number of plant species, each with its own characteristics (1).

In banana and plantain, plant regeneration by somatic embryogenesis has been used as a tool in breeding programs for genetic transformation (2), as well as to increase germination of hybrid progeny used in conventional breeding strategies (3). However, the greatest interest is in its practical application for the propagation of plants on a commercial scale (4) due to high coefficient of multiplication of somatic embryos. Although to achieve higher rates of germination and plant conversion, it is important to obtain quality embryos with a synchronous morphological development.

Asynchrony that characterizes the embryogenic cultures is considered the main disadvantage of the method for use in the mass propagation of plants. Though this phenomenon, it is a more efficient propagation system than conventional micropropagation through meristemaric apex culture (5). The high costs of production during banana micropropagation generally limited its commercial use (6), due to phenolic oxidation, slow growth and low proliferation of explants (7).

In the process of somatic embryogenesis, maturation phase it is crucial because favors the development of the embryo and its conversion plant. Experimental evidence suggests that culture conditions can modulate the development of embryogenic cultures. Different authors have referred to the role of inoculation density and its relation to the differentiation of somatic embryos, regardless of the culture medium composition (6, 8).

In *Musa* spp., studies related to the effect of inoculation density not describe their influence on the morphological development of the somatic embryos and their relationship to reduce asynchrony and germination efficiency. This is because the embryos pass quickly through the different stages of ontogeny, without major morphological changes, which requires a detailed characterization of morphology and histology (9).

Based on the above, this study aimed to determine the effect of the inoculation density of somatic embryos in obtaining seedlings of plantain cv. 'FHIA-21' (AAAB).

MATERIALS AND METHODS

Vegetal material

Somatic embryos were obtained in liquid culture medium, from banana embryogenic cell suspensions from cv. 'FHIA-21' (AAAB), following the procedure described by other authors (10). A culture medium composed of 100 % of the inorganic salts Schenk and Hildebrandt was used (11), of which Murashige and Skoog vitamins (12) were added to 100 %, 0,5 mg L^{-1} biotin, 100 mg L^{-1} malt extract, 100 mg L^{-1} of L-glutamine, 230 mg L^{-1} of L-proline, 10 mg L^{-1} of lactose, 0,05 mg L^{-1} of zeatin, 100 mg L^{-1} of myo-inositol, 0,2 mg L^{-1} of naphthaleneacetic acid (NAA), 0,2 mg L^{-1} isopentylaminopurine (2ip), 0,1 mg L^{-1} of Kinetin and 45 g L^{-1} of saccharose. The pH was adjusted to 5,3 before sterilization. Embryos were in globular stage and were characterized by a length of between 0,20 to 0,50 mm.

Effect of inoculation density in the somatic embryos maturation

Four inoculation densities were studied during the maturation of somatic embryos. To this, they were added 0,2; 0,4; 0,6 and 0,8 grams of embryos fresh mass (gMF) in Erlenmeyer flasks of 250 mL capacity that containing 30 mL of liquid medium. The culture medium was composed of 100 % of salts and MS vitamins (12), 1,0 mg L^{-1} of biotin, 0,5 mg L^{-1} of 6-benzylaminopurine (6-BAP), 1,0 mg L^{-1} of indole-3-acetic acid (IAA) and 30 g L^{-1} sucrose.

The Erlenmeyer flasks were placed on orbital shaker (INFORS HT) at 90 rpm of rotation speed, in complete darkness and 27±2.0 °C. Five repetitions for each treatment were established with a completely randomized design. At 30 days of culture, morphological and histological characteristics of somatic embryos in the four inoculation densities were evaluated. Subsequently, they were transferred to semisolid medium germination proposed by other investigators (13), which was composed of salts and MS vitamins, 0,5 mg L^{-1} of 6-BAP, 2,0 mg L^{-1} of IAA, 100 mg L^{-1} of myo-inositol, 0,01 mg L^{-1} of Biobrás-6 (brassinosteroid analogue from the Faculty of Chemistry at the University of Havana) and 30 g L^{-1} sucrose. In both culture media the pH was adjusted to 5,8±0,01.

Morphology and histology of somatic embryos

A morphological description of somatic embryos was performed in order to identify its main features, during the maturation phase. Observations were made

under a stereoscopic microscope (OLYMPUS) (10x) and measuring its length (mm) through a micrometric scale coupled to ocular. To facilitate observation and measurement of the embryos they were removed from each treatment, 1,0 mL of liquid culture medium containing the embryos. The samples were placed in beakers (50 mL capacity) with 30 mL of a solution of Gelrite (Sigma) (2,5 g L^{-1}) and deionized water. Subsequently, solutions beakers were poured into Petri dishes of 70 mm diameter and embryos were immobilized after the solution solidified. The measurement values were grouped into three ranges of lengths, from 1,0 to 3,0; from 3,1 to 5,0 and from 5,1 to 7,0 mm, to determine its frequency of occurrence in the different treatments and thereby identify inoculation density provides greater cultivation synchrony.

Histological analysis was performed in order to identify anatomical and biochemical structures, confirming the development of somatic embryos and their preparation for germination. For this, two samples of each treatment were removed and somatic embryos were placed in a fixative solution containing 37 % formaldehyde (v/v) acetic acid 100 % (v/v) and 70 % ethanol (v/v), in a proportion 5:5:90 for 24 hours. Next, these were dehydrated in an ascending gradient of ethanol and embedded in paraffin. Serial sections of 10 µm thick with a rotation microtome (Zeiss, Germany) were performed, tey were fixed on glass slides, were hydrated and safranin staining was performed at 0,5 %. Histological sections of the samples were examined under an optical microscope (Axioskop) (40x) and images were captured with a (OLYMPUS DP70) digital camera that was connected to the microscope.

Germination of somatic embryos

Mature somatic embryos, for each inoculation density, were placed in plastic culture flasks (total capacity 500 mL) over 50 mL of semisolid medium germination. The bottles were placed in a growth chamber artificial light (fluorescent tubes of white light), with a photoperiod of 16 hours of light at a density photosynthetic photon flux (FFF) of 62-68 µmol m^{-2} s^{-1}) and 27±2,0 °C. Eight replicates were established for each inoculation density with somatic embryos 20 each in a completely randomized design.

During the germination phase of somatic embryos, observations at 10, 20 and 30 days of culture, with the aid of a stereomicroscope (OLYMPUS) (10x) were performed; further 30 days the number of germinated embryos and quantitated values were expressed in percent germination. At the same time, the morphological characteristics of plants, the number of open leaves and number of roots were evaluated as pseudostem length (cm) from the base to the insertion of the first leaf. Subsequently, the plants were transferred and maintained for 30 days in semisolid culture medium elongation prior to their transfer to *ex vitro* culture conditions at home.

Statistical analysis

For statistical analysis the SPSS software package (Statistical Package for Social Sciences) version 20 on Windows (14) was used. The experimental data were tested cases of normal distribution and homogenety of variance. The comparison of the average values was performed using the Kruskal Wallis, with a significance level of 0,05 %.

Para el análisis estadístico se utilizó el paquete computacional SPSS (Statistical Package for the Social Sciences) versión 20 sobre Windows (14). A los datos experimentales se les comprobaron los supuestos de distribución normal y homogeneidad de varianza. La comparación de los valores medio se efectuó mediante la prueba de Kruskall Wallis, con un nivel de significación de 0,05 %.

RESULTS AND DISCUSSION

Effect of inoculation density in the somatic embryos maturation

The results showed the inoculation density influence in its morphology, histology and germination of banana somatic embryos from cv . 'FHIA-21' (AAAB).

At 30 days of culture maturing, differences in morphology of the embryos were observed. The major difference occurred in length, with variations in the frequency of the ranges established for this study (1,0 to 3,0; 3,1 to 5,0 and from 5,1 to 7,0 mm). In this regard, it was noted that somatic embryos cultured with lower densities (0,2 and 0,4 gMF) showed greater heterogeneity in length, represented in the three ranges of lengths (Figure 1). They also showed partial germination root primordium presence (Figure 2A). In the histological examination of these treatments, an irregular epidermis by forming rounded structures at its periphery and defining cauline and root meristems in the central region of the embryo was observed. No reserve structures were observed in the scutellum region (Figure 2B, C).

Despite the asynchronous nature of embryogenic cultures, the inoculation density of 0,6 gMF provided greater synchrony because greater uniformity achieved in the length of their somatic embryos in relation to the rest of the treatments. The highest percentage of these embryos (87,2 %) was located in one of the ranges set with a length of 3,1 to 5,0 mm (Figure 1).

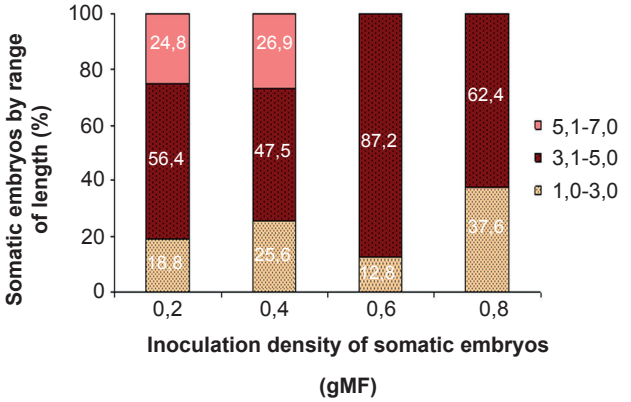
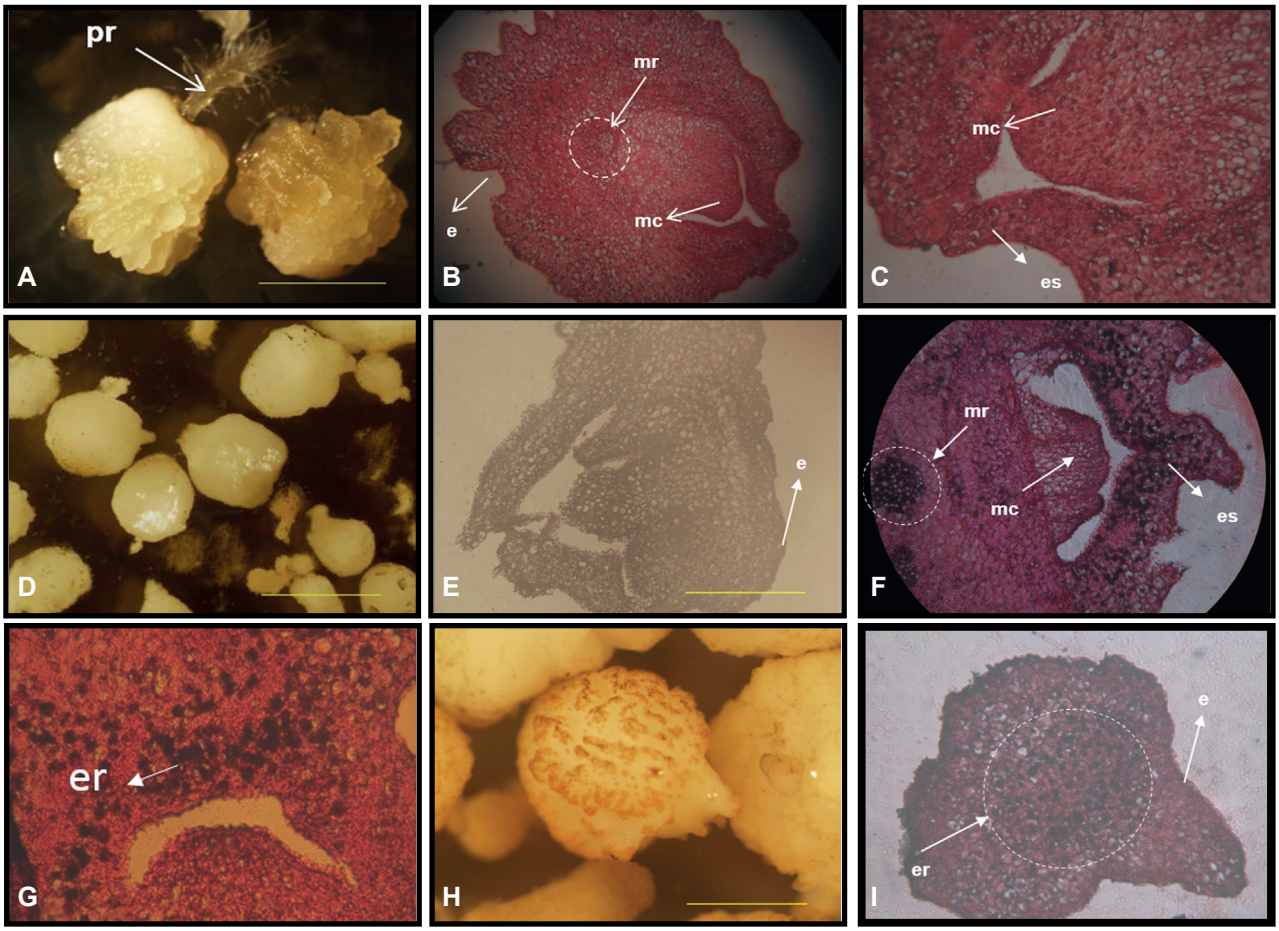


Figure 1. Effect of inoculation density in the frequency of occurrence length range of plantain somatic embryos cv. 'FHIA-21' (AAAB), after 30 days of culture in maturing phase

These embryos were characterized by present circular form with a pale yellow color (Figure 2 D). Histological sections showed a regular epidermis of these embryos, besides the presence of cauline and root meristems. It was also observed in the region scutellum accumulation reservation structures (Figure 2E, F, G).

Moreover, embryos cultured with 0,8 gMF of inoculation density showed greater frequency of smaller embryos (37,6 %), between 1,0 and 3,0 mm long, but 62,4 % of embryos started off half-length range between 3,1 and 5,0 mm (Figure 1). In this treatment damage on the epidermis of the embryos were observed, possibly because the friction between them due to the high density of inoculation (Figure 2H). In addition, darkening of the culture medium was observed, which may be related to the exudation of phenolic compounds and subsequent oxidation.



A,B,C: embryos cultures with 0,2 gMF showing partial germination of root primordium (pr) (bar=4,0mm) (250x) and histological sections where an irregular epidermis (e) is observed, the cauline (mc) and root (mr) meristems and scutellum region (es) without the presence of reserve structures (400x)
D,E,F,G: embryos cultured with 0,6 gMF (250x) (bar=4,0 mm) and histological sections showing the cauline (mc) and root (mr) meristems and scutellum region (es) with the presence of reserve structures (400x)
H,I: embryos cultures with 0,8 gMF with mechanical damage to the epidermis (250x) (bar=2,5 mm) and histological section showing definition of the epidermis (e) (400x) and presence of reserve structures (sr) in the central region of the embryo

Figure 2. Somatic embryos of banana cv. 'FHIA-21' (AAAB) in liquid culture ripening medium

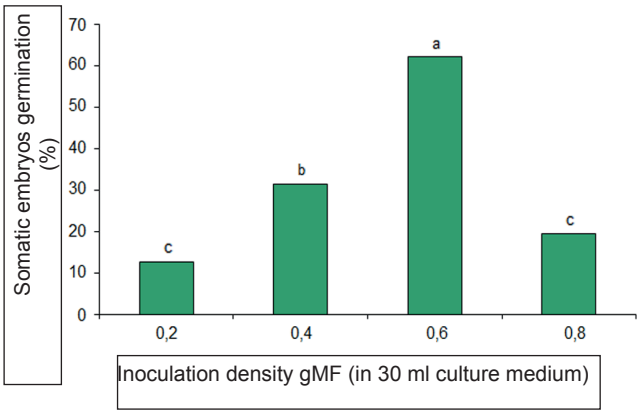
Histological sections of somatic embryos cultured with 0,8 gMF showed defining regular epidermis and the presence of reservation structures in the center embryo. However, the cauline and root meristems (Figure 2 I) were observed.

Germination of somatic embryos

During the somatic embryos germination, differences in morphological appearance, related inoculation density used during the maturation phase were observed. At 10 days, the embryos cultured with 0,6 gMF showed the cauline apex emission with small shoots green. These elongated with increasing days of culture until the formation of complete plants, which were observed at 30 days of culture (Figure 3 A, B, C). The highest percentage of germination corresponded to these embryos (62,0 %), with significant differences with the other treatments (Figure 4).

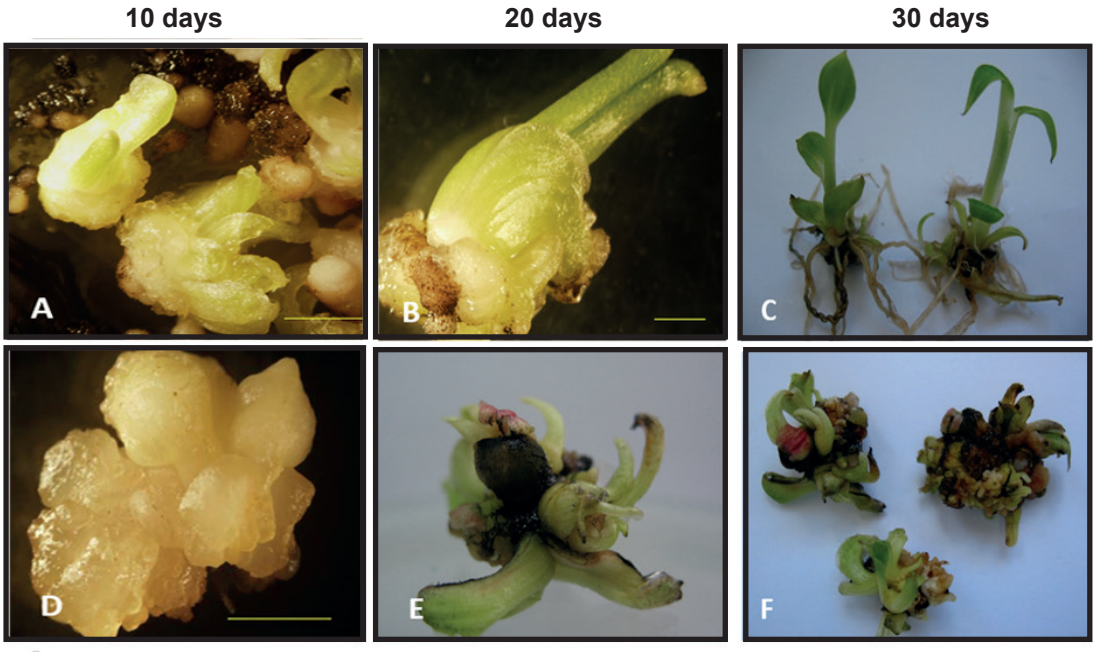
Germination of somatic embryos cultured with 0,8 gMF showed morphological development, similar to embryos cultured with 0,6 gMF; but its low germination percentage (19,3 %) could be given by the phenolization and death of the embryos on semisolid culture medium germination. This response should be related to the mechanical damage that presented in the epidermis and little ontogenetic development achieved at the end of the maturation phase.

Meanwhile, somatic embryos cultured with 0,2 and 0,4 gMF presented during germination morphological differences compared to other treatments. At 10 days of culture, an increase in size, with irregular epidermal growth, resulting in white rounded structures (Figure 3D) was observed. Subsequently, after 20 days these irregularities differ in small green sprouts and 30 days plants were observed growing in a rosette form (Figure 3 E and F).



Bars with different letters differ significantly by Kruskal Wallis test, p<0,05

Figure 4. Effect of inoculation density used in the ripening stage on germination (%) of somatic embryos plantain cv. 'FHIA-21' (AAAB) at 30 days of culture in semisolid medium



Somatic embryos cultured with 0,6 gMF (A, B, C) and 0,2 gMF (D, E, F) during the maturation phase

Figure 3. Germination of somatic embryos of plantain cv. 'FHIA-21' (AAAB), at 10, 20 and 30 days of culture in germination semisolid medium

When analyzing the morphological characteristics of the plants, was observed that the one that came from culture with 0.6 gMF had better definition and pseudostem length, more than two open leaves and more roots, with significant differences compared to plants from the other treatments (Table and Figure 3 C). However, somatic embryos originating from plants grown with 0,2 and 0,4 gMF showed disorders in morphology, growing in a rosette form by no definition of pseudostem and the presence of multiple buds of adventitious appearance (Figure 3F).

In the genus Musa, studies related to the density of inoculation have estimated the culmination of the somatic embryos development through germination. For example, somatic embryos of banana cv. 'FHIA-18' (AAAB) showed better germination when they were cultured with 0,8 gMF (13).

Moreover, in the banana cultivar 'Navolean' (ABB) embryo maturation occurred at a density of 0,5 to 46,8 % gMF germination (15). In this regard, the mature embryos of banana cv. 'Da Jiao' (ABB) showed 2,0 to 3,0 mm in diameter after three months of cultivation with a germination rate of 40,0 % (2). As noted there are differences in relation to the inoculation density and the germination percentage of somatic embryos, aspects that have been linked to the influence of genotype. In this study, 62,0 % of germinated embryos were obtained when embryos were cultured to 0,6 gMF, suggesting the desirability of spreading the cv. banana 'FHIA-21' by somatic embryogenesis.

The successful application of modern biotechnology depends on efficient and reproducible plant regeneration protocols from somatic embryos and embryogenic cell suspensions (16). Many remain limiting factors in somatic embryogenesis of banana as the length, unpredictability and a high degree of dependence on genotype (17). For this reason, it is very important to conduct studies that allow us to establish the conditions of good crops to standardize protocols depending on the cultivar.

The effect of inoculation density and the genotype influence are among the factors that determine the formation of somatic embryos of banana cv. 'FHIA-21' from embryogenic cell suspensions (10). However, the experimental results of this study also showed the importance of inoculation density in the morphological development of these embryos during maturation phases, germination and formation of complete plants. However, several researchers believe that for the proper selection of the inoculation density, determine its effect on reducing asynchrony; critical aspect for the advancement of somatic embryogenesis on a commercial scale (10). This can be simplified protocols and substantially reduce production costs (18). In this sense, other authors suggest that the protocols should be broad enough to capture the full range of possible genetic differences in the development of each genotype (19).

The effect of inoculation density is related to the availability of mineral nutrients in the culture medium. Although in a previous study found no relationship between the factor inoculation density and mineral nutrient requirements during the somatic embryos maturation of banana cv. 'FHIA-21' (20).

According to existing information, during the maturation phase somatic embryos undergo morphological and biochemical changes due to cell expansion and accumulation of reserve substances (1). In this study the cell expansion was observed through the increase of somatic embryos length, which occurred in all the treatments studied. Although only embryos that were cultured in 0,6 gMF of inoculation density showed reservation structures and defining cauline and root meristems. These features are considered anatomical evidence of preparing somatic embryos to germinate. The results of this study are the first report where the morphological and histological characteristics of somatic embryos of banana cv. 'FHIA-21' (AAAB) relate to the synchronization and subsequent crop germination.

Morphological characteristics of plants obtained from somatic embryos of plantain cv. 'FHIA-21' (AAAB) at different inoculation densities in maturation phase

Inoculation density (gMF)	Pseudostem length (cm)	Number of leaves	Number of roots
0,2	0,29 c	1,00 c	2,21b
0,4	0,65 b	1,72 b	0,40 c
0,6	1,07 a	2,46 a	3,68 a
0,8	0,64 b	1,23 bc	2,11 b

Means with different letters in a column differ significantly according to the Kruskal-Wallis test for p≤0,05

CONCLUSIONS

The results showed the inoculation density influence in morphological development of somatic embryos plantain cv. 'FHIA-21' (AAAB). Embryos were cultured at 0,6 gMF presented greater uniformity of its length, forming the caulinae and radical meristem, and the accumulation of reserve substances. These embryos reached higher germination and formation percentage of complete plants. This study is essential for the *in vitro* propagation of banana cv. 'FHIA-21' on a large scale by somatic embryogenesis.

ACKNOWLEDGEMENT

The authors thank to Dr.C. Novisel Veitía (IBP, Cuba), for her collaboration in the statistical analysis of the data. This research was supported by the Ministry of Science, Technology and Environment of Cuba, under the project "Implementation of somatic embryogenesis as an alternative way for mass propagation cultivar of plantain FHIA-21 '(*Musa* AAAB) ".

BIBLIOGRAPHY

1. von Arnold, S. ''Somatic embryogenesis'' [en línea]. En: eds. George E. F., Hall M. A., y Klerk G. J. D., *Plant Propagation by Tissue Culture*, edit. Springer Netherlands, Dordrecht, 2007, pp. 335-354, ISBN 978-1-4020-5004-6, [Consultado: 8 de diciembre de 2015], Disponible en: <http://link.springer.com/10.1007/978-1-4020-5005-3>.
2. Dai, X. M.; Xiao, W.; Huang, X.; Zhao, J. T.; Chen, Y. F. y Huang, X. L. ''Plant regeneration from embryogenic cell suspensions and protoplasts of dessert banana cv. 'Da Jiao' (*Musa paradisiacal* ABB Linn.) via somatic embryogenesis''. *In Vitro Cellular & Developmental Biology - Plant*, vol. 46, no. 5, 5 de octubre de 2010, pp. 403-410, ISSN 1054-5476, 1475-2689, DOI 10.1007/s11627-010-9314-7.
3. Uma, S.; Lakshmi, S.; Saraswathi, M. S.; Akbar, A. y Mustaffa, M. M. ''Plant regeneration through somatic embryogenesis from immature and mature zygotic embryos of *Musa acuminata* ssp. *burmannica*''. *In Vitro Cellular & Developmental Biology - Plant*, vol. 48, no. 5, 13 de septiembre de 2012, pp. 539-545, ISSN 1054-5476, 1475-2689, DOI 10.1007/s11627-012-9462-z.
4. Meenakshi, S.; Shinde, B. N. y Suprasanna, P. "Somatic embryogenesis from immature male flowers and molecular analysis of regenerated plants in banana 'Lal Kela'(AAA)". *Journal of Fruit and Ornamental Plant Research*, vol. 19, no. 2, 2011, pp. 15–30, ISSN 1231-0948.
5. Deo, P. C.; Tyagi, A. P.; Taylor, M.; Harding, R. y Becker, D. ''Factors affecting somatic embryogenesis and transformation in modern plant breeding''. *The South Pacific Journal of Natural and Applied Sciences*, vol. 28, no. 1, 1 de enero de 2010, pp. 27-40, ISSN 1013-9877, 1726-0787, DOI 10.1071/SP10002.
6. Monteiro, T. R.; Luis, Z. G.; Freitas, E. de O.; Matsumoto, K. y Scherwinski, P. J. E. ''Cell differentiation and plant regulators on banana''. *Revista Brasileira de Fruticultura*, vol. 33, no. SPE1, octubre de 2011, pp. 213-221, ISSN 0100-2945, DOI 10.1590/S0100-29452011000500025.
7. Korneva, S.; Flores, J.; Santos, E.; Piña, F. y Mendoza, J. ''Plant regeneration of plantain 'Barraganete' from somatic embryos using a temporary immersion system''. *Biotecnología Aplicada*, vol. 30, no. 4, 2013, pp. 267–270, ISSN 0864-4551, 1027-2852.
8. Ducos, J. P.; Lambot, C. y Pétiard, V. ''Bioreactors for coffee mass propagation by somatic embryogenesis''. *International Journal of Plant Developmental Biology*, vol. 1, no. 1, 2007, pp. 1–12, ISSN 1749-4753.
9. Jalil, M.; Chee, W. W.; Othman, R. Y. y Khalid, N. ''Morphohistological examination on somatic embryogenesis of *Musa acuminata* cv. Mas (AA)''. *Scientia Horticulturae*, vol. 117, no. 4, 18 de agosto de 2008, pp. 335-340, ISSN 0304-4238, DOI 10.1016/j.scienta.2008.05.018.
10. García, Á. L.; Gómez, K. R.; Alvarado, C. Y. y Reyes, Z. S. y M. ''Effect of inoculum density on formation and morphology of plantain somatic embryos (*Musa* spp. AAAB, cv. Hybrid 'FHIA-21')''. *Revista Colombiana de Biotecnología*, vol. 12, no. 2, diciembre de 2010, pp. 240-247, ISSN 0123-3475.
11. Schenk, R. U. y Hildebrandt, A. C. "Medium and techniques for induction and growth of monocotyledoneous and dicotyledoneous plant cell cultures". *Canadian Journal of Botany*, vol. 50, no. 1, 1 de enero de 1972, pp. 199-204, ISSN 0008-4026, DOI 10.1139/b72-026.
12. Murashige, T. y Skoog, F. "A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures". *Physiologia Plantarum*, vol. 15, no. 3, 1 de julio de 1962, pp. 473-497, ISSN 1399-3054, DOI 10.1111/j.1399-3054.1962.tb08052.x.
13. Kosky, R.; Gilliard, T.; Barranco, L. y Reyes, M. ''Embriogénesis somática en medios líquidos. Maduración y aumento de la germinación en el cultivar híbrido «FHIA-18» (AAAB)". *InfoMusa*, vol. 9, no. 1, 2000, pp. 12-16, ISSN 1023-0076.
14. *IBM SPSS Statistics* [en línea]. Versión 20, [Windows], edit. IBM Corporation, U.S, 2011, Disponible en: <http://www.ibm.com>.
15. Cabrera, M.; López, J.; Kosky, R.; Montano, N.; Reyes, M.; Reinaldo, D.; Ventura, J. C.; Medero, V.; Santos, A.; García, M.; Basail, M. y Espinosa, E. ''Multiplicación, histo-diferenciación y regeneración de suspensiones celulares embriogénicas en plátanos vianda «Navolean» (AAB)". *Biotecnología Vegetal*, vol. 2, no. 2, 2002, pp. 115-117, ISSN 2074-8647.
16. Ramírez, V. M. y de García, E. ''Características marcadoras en suspensiones celulares embriogénicas de banano cien bta-03 (AAAA) y su parental williams (AAA)''. *Bioagro*, vol. 24, no. 2, agosto de 2012, pp. 73-82, ISSN 1316-3361.
17. Remakanthan, A.; Menon, T. G. y Soniya, E. V. "Somatic embryogenesis in banana (*Musa acuminata* AAA cv. Grand Naine): effect of explant and culture conditions". *In Vitro Cellular & Developmental Biology - Plant*, vol. 50, no. 1, 13 de agosto de 2013, pp. 127-136, ISSN 1054-5476, 1475-2689, DOI 10.1007/s11627-013-9546-4.

18. Gupta, P. K. y Timmis, R. ‘‘Mass propagation of conifer trees in liquid cultures — progress towards commercialization” [en línea]. En: eds. Hvoslef E. A. K. y Preil W., *Liquid Culture Systems for in vitro Plant Propagation*, edit. Springer Netherlands, 2005, pp. 389-402, ISBN 978-1-4020-3199-1, [Consultado: 10 de diciembre de 2015], Disponible en: <http://link.springer.com/chapter/10.1007/1-4020-3200-5_30>.

19. Youssef, M.; James, A.; Mayo, M. A.; Ku, C. J. R.; Grijalva, A. R. y Escobedo, G. R. M. ‘‘Influence of genotype and age of explant source on the capacity for somatic embryogenesis of two Cavendish banana cultivars (*Musa acuminata* Colla, AAA)’’. *African Journal of Biotechnology*, vol. 9, no. 15, 2010, pp. 2216-2223, ISSN 1684-5315, DOI 10.4314/ajb.v9i15.

20. García, Á. L.; Alvarado, C. Y.; Kosky, R. G.; Sarría, Z.; Chong, P. B.; Reyes, M.; Pérez, B.; Concepción, A. y Mollineda, Á. ‘‘Análisis del contenido de nutrientes minerales durante la formación y maduración de embriones somáticos deFHIA-21 (*Musa* AAAB)’’. *Biotecnología Vegetal*, vol. 12, no. 1, 2012, pp. 33-40, ISSN 1609-1841, 2074-8647.

Received: October 7th, 2014
Accepted: April 16th, 2015