In vitro propagation of plantain (*Musa acuminata* x *M. balbisiana* AAB) and banana (*M. acuminata* AAA) in Puerto Rico[1,2]

Lii J. Liu, Evelyn Rosa, Enid Lizardi, Angel Arocho, Norma Díaz, and José A. Rodríguez

Some 7,000 plantlets were produced in vitro from six plantain cultivars (Enano Común, Congo Enano, Maricongo, Congo, Clone 12 and Clone 7), and from three banana cultivars (Grand Nain, Valerie and Ziv) by supplementing Murashige and Skoog (MS) basal medium with 0.5 mg/L kinetin and 0.5 mg/L 6-benzylamino purine. Calli were induced by supplementing the MS basal medium with 0.05 mg/L 2,4-D, 0.1 mg/L NAA, 0.5 mg/L BA, 15% coconut water and 20 mg/L ascorbic acid (filter sterilized). Data obtained from a replicated field trial at the Corozal Substation with the above-mentioned plantain cultivars indicated that the bunch weight from plantain cultivar Congo Enano, regenerated from axillary meristem with in vitro culture, was significantly greater than that from plantains derived from conventional suckers in the plant crop (P = 0.1). Similarly, the individual fruit weight from Congo Enano derived from meristem culture was significantly greater than that from plantains derived from the conventional planting materials (P = 0.5).

RESUMEN

Propagación in vitro del platanero y el bananero en Puerto Rico

Alrededor de 7,000 plántulas de plátano de 6 cultivares (Enano común, Congo enano, Maricongo, Congo, Clon 12 y Clon 7) se obtuvieron por cultivo de tejidos, suplementando el medio basal de Murashige & Skoog (MS) con cinetina (KIN) 0.5 mg./l. y benzilaminopurina (BA) 0.5 mg./l. Se obtuvo la inducción de callo suplementando el medio basal de MS con ácido 2,4-diclorofenoxiacético (2,4-D) 0.05 mg./l., ácido naftalenoacético (NAA) 0.1 mg./l. benzilaminopurina (BA) 0.5 mg./l., agua de coco 15% (v/v) ácido ascórbico (filtrado por esterilización) 20 mg./l.

Los datos obtenidos del experimento de campo realizado en la Subestación de Corozal, con las variedades de plátano arriba mencionadas, indicaron que el peso del racimo de la cultivar Congo enano propagado por cultivo de tejidos fue significativamente (P = 0.1) mayor que el del racimo propagado por el método corriente. Similarmente, el peso de las frutas

[1]Manuscript submitted to Editorial Board 26 May 1988.

[2]This study was part of an investigation supported by USDA, Cooperative Agreement No. 83-CRSR-2-2157 (TAD Research Grant).

[3]Plant Pathologist, Research Assistants and Agronomist, respectively, Agricultural Experiment Station, University of Puerto Rico, Mayagüez Campus, Río Piedras, PR 00928.

[4]Trade names in this publication are used only to provide specific information. Mention of a trade name does not constitute a warranty of equipment of materials by the Agricultural Experiment Station of the University of Puerto Rico, nor is this mention a statement of preference over other equipment or materials.

individuales del Congo enano provenientes de plátanos propagados por tejido meristemático fue significativamente mayor (P = 0.5) que el de los que provenían del método corriente.

INTRODUCTION

Plantains (*Musa acuminata* x *M. balbisiana*, AAB and ABB groups) and banana (*M. acuminata* AAA) are major food staples in Puerto Rico, Central America, the Caribbean Basin and elsewhere in the tropics. The value of plantain production alone in this region has surpassed $600 million annually (8). In Central America and the Dominican Republic, plantains are grown by small farmers using low-level technology. In Puerto Rico, plantains are also grown by small farmers of the mountain region because they adapt well to uplands, marginal soil, and minimum tillage. In 1986, the farm value of the crop reached $35 million, representing 5.6% of the gross agricultural income of the island (1). The recent expansion of a plantain processing plant in Puerto Rico has increased expectations for new employment in rural areas, and increased the industry capacity for plantain export.

Plantains and bananas are normally propagated vegetatively with suckers. Low ratoon productivity of plantains is commonplace in Puerto Rico. Nematodes (*Radopholus similis*) and corm weevil (*Cosmopolites sordidus*) are thought to be causes of the ratoon deterioration (14,15,17). The need for producing pathogen-free foundation stocks has recently stimulated interest in the production of plantain and banana via meristem culture. Several investigators (2,3,4,5,9,10,11,12,16) have reported rapid clonal multiplication techniques for plantains and bananas. Herein described are in vitro propagation procedures utilizing axillary meristem tips, and statistical evaluations of bunch weight, individual fruit weight, and height of plants obtained from plantains regenerated from tissue culture, together with those derived from conventional planting material. Field observations on growth of semi-commercial field plantings of banana plants derived from in vitro axillary meristem culture were also performed.

MATERIALS AND METHODS

Shoot tips of six plantain cultivars (Enano Común, Congo Enano, Maricongo, Congo, Clone 12 and Clone 7) and three banana cultivars (Grand Nain, Valerie and Ziv) were isolated by removing the sheathing leaf bases which make up the pseudo-stem, i.e., the vegetative growth above ground. The isolated shoot tips were surface-sterilized for 15 minutes in 10% Clorox and rinsed three times with sterile distilled water. The sterile shoot tips were then transferred to the modified culture media of Murashige and Skoog (13), and of Gamborg B_5 (6), for callus induction and shoot proliferation. In addition, the following combinations of hormones and cytokinins were used: (a) MS basal medium (13) + 0.5 mg/L

2,4-D + 0.5 mg/L NAA + 0.5 mg/L BA + 0.5 mg/L folic acid (PMC_2); (b) MS basal medium + 0.5 mg/L 2,4-D + 1 mg/L NAA + 0.5 mg/L BA + 0.5 mg/L folic acid (PMC_3); (c) MS basal medium + 0.1 mg/L 2,4-D + 0.1 mg/L NAA + 0.5 mg/L BA + 400 mg/L casein hydrolysate + 15% coconut water (PMC_4); and (d) MS basal medium + 0.05 mg/L 2,4-D + 0.1 mg/L NAA + 0.5 mg/L BA + 20 mg/L ascorbic acid (filter sterilized) + 15% coconut water (PMC_5).

For the field study on productivity by meristem-cultured plants, two sources of planting material were used: plants regenerated from axillary meristem by in vitro culture, and conventional suckers. Twenty-four plantlets from meristem culture were secured for each of the six plantain cultivars. Similarly, 24 suckers from each cultivar were planted. These plantlets and suckers were established in a field experiment with a randomized block design with four replications. Each plot received six plantlets or suckers planted separately. Plot size was approximately 25′ × 20′. The experiment for the plant crop was established 17 May 1983 and harvested 1 June 1984, 13 months after planting. The ratoon crop with 16.5 months growth was harvested 14 October 1985.

Nematicide (Furadan$^{(cm)}$[1] was applied at the rate of 28 g/plant every four months, both in the plant and ratoon crops. Fertilizer, in a 10-5-30 formulation, was applied five times (140-450 g/plant) in the plant crop and four times (280-450 g/plant) in the first ratoon. Fungicide (Chlorothalonil) (Bravo-500) was administered as an aqueous spray solution at the rate of 1.8 kg/ha every four months in both the plant and ratoon crops.

Some 3,000 plantlets obtained from in vitro axillary meristem culture of bananas (Grand Nain and Valerie) were planted in the fields at the Corozal Substation. Because banana suckers for direct comparison were lacking, the plantlets were established on a nonexperimental scale for field observations.

RESULTS

Some 7,000 plantlets were produced in vitro from the six plantain and the three banana cultivars (Fig. 1). Calli were induced by supplementing the MS basal medium (13) with 0.05 mg/L 2,4-D, 01 mg/L NAA, 0.5 mg/L BA, 15% coconut water and 20 mg/L ascorbic acid filter sterilized (Fig. 2).

Plant crop data from the field trial indicate that bunches from meristem-cultured plantains (cv. Enano Común) were significantly heavier than those from the non-meristem cultured (table 1.) Similarly, the individual fruit weight from Enano Común was significantly greater than that of individual fruit from the non-meristem culture ($P = 0.5$). The in vitro cultured plantains appeared to grow faster than those from suckers

FIG. 1.—Growth of plantains (clone 12) derived from meristem culture. A-in Murashige and Skoog's basal medium supplemented with 0.5 mg/L BA and 0.5 mg/L Kinetin. B and C, in hardening stage in the greenhouse. D-in a replicated field trial at the Corozal Substation.

FIG. 2.—Plantain calli induced in the Murashige and Skoog basal medium supplemented with 0.05 mg/L 2,4-D, 0.1 mg/L NAA and 0.5 mg/L BA.

(table 2). Other plantain cultivars produced plantlets with normal bunch- and fruit-yield potentials.

Reductions occurred for bunch weight in the first ratoon crop. These reductions ranged from 34.1% (cultivar Congo Enano) to 61.6% (cultivar Congo). The Congo were derived from meristem culture (table 3). Similarly, the reduction in bunch weight in the first ratoon from that in the plant crop ranged from 7.6% for cultivar Congo Enano to 49.3% for cultivar Congo derived from conventional suckers.

Field growth observations of the semi-commercial plantings of banana plantlets indicated that only about 4% of the plants were off-type. The remainder produced vigorous growth with uniform fruit settings.

DISCUSSION

The data indicate that plantain and banana cultivars can be propagated in vitro by supplementing the Murashige and Skoog basal medium with low concentration of cytokinin and hormones, in accordance with Banergee and Langhe (2), and with Ma and Shii (12,13). In addition, the

TABLE 1.—*Bunch weight, fruit weight and fruit number of plantains derived by meristem culture and by conventional vegetative propagation*

Cultivar	Weight of bunch (kg)		Percent increase	Weight of fruit (kg)		Percent increase	Number of fruit		Percent increase
	Meristem	Non-meristem[1]		Meristem	Non-meristem		Meristem	Non-meristem	
Enano Común	15.5	13.4	15.8	0.31	0.30	1.4	48.6	43.2	1.25
Congo Enano	19.7	11.3	72.9**[3]	0.20	0.12	70.3*[4]	98.5	96.5	0.25
Maricongo	18.0	—[2]	—	0.21	—	—	85.2	—	—
Congo	22.7	20.1	11.8	0.26	0.25	1.7	89.1	79.7	1.17
Clone 12 (Maricongo)	16.8	—	—	0.30	—	—	54.2	—	—
Clone 7 (Maricongo)	17.4	13.6	26.6	0.35	0.29	2.0	50.2	48.0	0.45

[1]Propagated from suckers using the traditional procedures.

[2]Plants were harvested for seed purposes. Complete data not available.

[3]Difference significant at the 1% probability level.

[4]Difference significant at the 5% probability level.

TABLE 2.—*Height of plantain plants derived by meristem culture and from suckers with traditional procedures*

Cultivar	Height of plants (cm), from -	
	Meristem	Suckers
Enano Común	275.4[1]	239.9
Congo Enano	256.1	227.1
Maricongo	345.16	333.5
Congo	346.4	327.7
Clone 12	349.1	342.5
Clone 7	356.7	334.2

[1]Average of 24 plants. Data were recorded June 1, 1984, 13 months after planting.

culture medium developed in this study is capable of producing a large number of plantlets from axillary meristem using in vitro culture in a short period of time (three to four months) without contamination and oxidation problems.

Cronauer and Krikorian (5) observed in 1983 somatic embryos from cultured tissues of triploid plantains. In our work, we induced and regenerated calli in plantains by supplementing the MS basal medium (13) with low concentrations of 2,4-D and NAA.

Hwang et al. (7) demonstrated that banana plantlets derived from meristem culture could be used successfully for commercial cultivation in Taiwan. Similarly, field observations on growth of meristem-cultured bananas showed vigorous growth and uniform fruit settings. For one plantain cultivar, Congo Enano, plantlets yielded significantly greater bunch weight than conventional planting materials in the plant crop. Although the increase was not significant in the first-ratoon crop, production from plantlets in general remained better or equal to those derived from vegetative suckers. It is possible that other cultivars were less

TABLE 3.—*Bunch-weight reduction in the plantain first-ratoon crop, AES-UPR Corozal Substation*

Cultivar	Weight of bunch (kg)					
	Meristem-cultured			Non-meristem culture		
	Plant crop	1st ratoon	Percent reduction	Plant crop	1st ratoon	Percent reduction
Enano Común	15.5	9.54	37.6	13.4	11.2	38.6
Congo Enano	19.7	12.73	34.1	11.3	10.3	7.6
Maricongo	18.0	9.58	45.3	—	8.0	—
Congo	22.7	8.64	61.6	20.1	10.0	49.3
Clone 12 (Maricongo)	16.8	8.64	48.1	—	8.04	—
Clone 7 (Maricongo)	17.4	10.75	37.1	13.6	7.56	44.0

affected by nematodes, viruses and diseases. Therefore, their increases in bunch weight in response to the meristem culture were not significant.

"Plantain decline" is local terminology for severe loss of productivity in ratoon crops planted with suckers. It is not known with planting materials derived from axillary meristem using in vitro culture. This study confirmed evidence that plantain plants derived from in vitro axillary meristem culture are not exempt from ratoon deterioration.

LITERATURE CITED

1. Anon., 1987. Situación de la empresa de plátanos y guineos. Datos publicados. Esta. Exp. Agric., Univ. P. R.
2. Banergee, N. and E. de Langhe, 1985. A tissue culture technique for rapid clonal propagation and storage under minimal growth conditions of *Musa* (banana and plantain). *Plant Cell Rep.* 4: 351-54.
3. Bower, J. P. and C. Fraser, 1982. Shoot tip culture of William's bananas. *Subtropica* 3: 13-6.
4. Cronauer, S. and A. D. Krikorian, 1985. Aseptic multiplication of banana from excised floral apices. *Hort Science* 20 (4): 770-1.
5. ____ and ____, 1983. Somatic embryos from cultured tissues of triploid plantains (Musa 'ABB'). *Plant Cell Rep.* 2: 289-91.
6. Gamborg, O. L., R. A. Miller and K. Ojima, 1968. Plant cell cultures. 1. Nutrient requirements of suspension cultures of soybean root cells. *Exp. Cell Res.* 50: 151-8.
7. Hwang, S. C., C. L. Chen, J. C., and H. L. Lin, 1984. Cultivation of banana using plantlets from meristem culture. *Hort Science* 19 (2): 231-3.
8. Jaramillo, C. R., 1986. Banana and plantain production in Latin America and the Caribbean. Banana and Plantain Breeding Strategies, Aust. Cent. Int. Agric. Res. *Proc.* 21: 39-49.
9. Liu, L. J., P. P. Gupta, E. Rosa-Márquez, E. Lizardi, A. Arocho, and N. Díaz, 1986. Protoplast isolation, culture and yield of plantain and banana derived from meristem-tip culture. Proc. 1986 Tropical-Subtropical Agriculture Research, January 16-17, 1986, Gainesville, Florida, pp. 47-51.
10. Ma, S. S. and C. T. Shii, 1974. Growing banana plantlets from adventious buds (In Chinese) Chung-Kuo Yuan 1, Hseoh Hui. China Hort. 20: 6-12.
11. ____ and ____, 1972. In vitro formation of adventitious buds in banana shoot apex following decapitation (In Chinese). *J. Chinese Soc. Hort. Sci.* 18: 135-42.
12. Mohan, H. Y. and F. C. Steward, 1964. The induction of growth in explanted tissue of the banana fruit. *Can. J. Bot.* 42: 1559-79.
13. Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol. Plant.* 15: 473-97.
14. Román, J., X. Rivas, J. Rodríguez, and D. Oramas, 1976. Chemical control of nematodes in plantains (*Musa acuminata* x *M. balbisiana* AAB). *J. Agric. Univ. P. R.* 60 (1): 36-44.
15. ____, ____, D. Oramas, and J. Rodríguez, 1977. Further experiments on the chemical control of nematodes in plaintains (*Musa acuminata* x *M. balbisiana* AAB). *J. Agric. Univ. P. R.* 61 (2): 192-9.
16. Vessey, J. C. and J. A. Rivera, 1981. Meristem culture of bananas. Turrialba 31: 162-3.
17. Wilson, G. F., 1986. Status of bananas and plantains in West Africa. Banana and Plantain Breeding Strategies, Aust. Cent. Int. Agric. Res. *Proc.* 21: 29-35.