EFFICACY OF BANANA PLANTLET PRODUCTION BY MICROPROPAGATION[1]

BEATRIZ M. J. MENDES[2], FERNANDA J. MENDES[3], AUGUSTO TULMANN NETO[2]
CLARICE G. B. DEMETRIO[4] and OTTO RICARDO PUSKE[5]

ABSTRACT - The use of banana (*Musa* spp.) plant production by tissue culture is increasing throughout the world. However the occurrence of somaclonal variation has been one of the most important problems affecting the commercial value of the plants. A reason for the somaclonal variation can be the time of permanence of the culture *in vitro*, i.e., the number of subcultures. With the aim of evaluating the effectiveness of banana plant production from shoot tip *in vitro*, and the behavior of vegetative apices coming from different rhizomes of cv. Nanicão, nine shoot tips were cultured in MS medium supplemented with 4.5 mg/L BAP for six subcultures at four-week intervals. The evaluations were done at each subculture by counting the number of new shoots produced. The results show that shoot tips coming from different rhizomes behave differently under *in vitro* conditions, some being highly productive and others producing a much smaller number of plants under similar culture conditions. The average number of plants produced was 676 per shoot tip after six subcultures, with 1,850 plants produced by the most productive, against 143 plants produced by the lowest productive one. These data can be very useful for micropropagation companies in the screening of the most promissor shoot tips in the beginning of the culture and in the economic calculations of the individual plantlets prices.

Index terms: shoot tip culture, somaclonal variation.

EFICIÊNCIA DA PRODUÇÃO DE MUDAS DE BANANEIRA POR MICROPROPAGAÇÃO

RESUMO - A produção de mudas de bananeira (*Musa* spp.) através de cultura de tecidos está se tornando uma prática comum em diversas regiões do mundo. No entanto, a ocorrência de variação somaclonal tem sido um dos principais problemas na utilização deste tipo de material em grande escala. Uma das possíveis causas da ocorrência da variação somaclonal seria o tempo em que o material permanece na cultura *in vitro*, isto é, o número de subcultivos. Com o objetivo de se determinar qual o potencial de produção de mudas a partir do cultivo de ápices meristemáticos e o comportamento de diferentes clones *in vitro*, realizou-se a cultura de nove ápices meristemáticos, extraídos de rizomas, coletados no campo, da variedade Nanicão, em meio MS suplementado com 4,5 mg/L de BAP, por seis subcultivos, em intervalos de 4 semanas. As avaliações foram feitas em cada subcultura, pela contagem do número de mudas produzidas. Pôde-se verificar que os clones se comportam de maneira diferente no cultivo *in vitro*, existindo clones que apresentam alta taxa de produção de mudas e clones que produzem uma quantidade bastante baixa de mudas, embora todo o material tenha sido tratado de maneira homogênea. A produção média de mudas foi de 676 plantas por ápice meristemático em seis subcultivos, tendo-se obtido 1.850 mudas no clone mais produtivo e 143 mudas no clone menos produtivo. Estes dados podem ser úteis para as empresas, no momento de selecionarem os meristemas mais promissores no início da cultura, e nos cálculos econômicos do preço de cada muda individualmente.

Termos para indexação: cultura de ápices caulinares, variação somaclonal.

INTRODUCTION

The use of micropropagation for the clonal propagation of banana cultivars is increasing in different regions of the world, including, Israel (Israeli et al., 1991), France (Cote et al., 1990), Australia (Drew & Smith, 1990) and Cuba (Filipia, 1987). Recently, in Brazil, several private companies have entered

[1] Accepted for publication on August 9, 1996.

[2] Eng. Agr., Dr., Centro de Energia Nuclear na Agricultura (CENA), Caixa Postal 96, CEP 13416-970 Piracicaba, SP.

[3] Undergraduate student, ESALQ/USP, Piracicaba, SP. FAPESP assistantship.

[4] Eng. Agr., Dr., Dep. de Matemática e Estatística, ESALQ/USP, Caixa Postal 9, CEP 13414-970 Piracicaba, SP.

[5] Undergraduated student, ESALQ/USP, Piracicaba, SP. CNPq assistantship.

the business of large scale banana plant production using micropropagation.

Several authors report the occurrence of high levels of somaclonal variation in areas cultivated with micropropagated banana plants, thus affecting the commercial value and acceptance of these plants (Vuysteke et al., 1988; Smith & Drew, 1990; Israeli et al., 1991). The high level of variation is thought to be caused by one or more factors including the growth regulator concentration used in the culture medium, the subculture method, which may favor the development of adventitious or axillary buds (Hussey, 1986), the number of subcultures reflecting the period under *in vitro* conditions (Smith, 1988; Reuveni & Israeli, 1990), and the genetic background (Smith, 1988; Krikorian et al., 1993).

In order to minimize the occurrence of somaclonal variation in a large scale of banana micropropagation, a maximum of six subcultures has been suggested (Reuveni & Israeli, 1990). However, it is important to determine whether it is economically feasible to be continuously introducing field material to start new cultures.

The present work aims to determine the shoot tip capability of producing plants by micropropagation, considering the average number of plants that can be produced when the period under *in vitro* conditions is limited.

MATERIAL AND METHODS

Cultures were initiated from field material obtained from a commercial orchard in Sete Barras, in the south coast of São Paulo State, Brazil. Nine rhizomes of banana plants, cv. Nanicão, were collected and brought to the laboratory, where they were washed and had the external leaves removed until approximately 3 cm of the pseudostem diameter and 10 cm in height was left. These explants, composed of several leaf bases surrounding the shoot tip and a small rhizome segment, were then immersed in a 30% solution of commercial bleach for 20 minutes. Under aseptic conditions the material was then washed four times in sterile distilled water and dissected to obtain the explants, which were approximately 0.5 cm in diameter and 1.0 cm in height, containing the shoot tip surrounded by two or three leaf bases and a rhizome segment of approximately 0.3 cm.

The explants were individually placed in Magenta boxes containing MS medium (Murashige & Skoog, 1962) supplemented with 2.5 $mg.L^{-1}$ benzilaminopurine (BAP), and maintained at 27°C under a 16-hour photoperiod. After four weeks, the explants were subcultured to fresh MS medium supplemented with 4.5 $mg.L^{-1}$ BAP, when the oxidized tissue was removed. Five more subcultures followed every four weeks, when the number of shoots developed per explant was scored. At every subculture the explants were cut in several pieces, each containing at least one developing shoot. The developed shoots were rooted and the plantlets acclimatized under greenhouse conditions, and later planted in the field. All the subcultures were done by the same technician trying to avoid differences in treatment.

After six subcultures, the data were analyzed using descriptive statistics (Microsoft EXCELL 5.0). The mean, variance and variation coefficient were calculated for each subculture and a multiple box plot was drawn. Also considering the Poisson distribution for the counts a generalized linear model (McCullagh & Nelder, 1989) was fitted for the data with the linear predictor given by a regression line.

RESULTS AND DISCUSSION

Although there are many reports on banana micropropagation using the shoot tip as explant (Cronauer & Krikorian, 1984; Vuysteke & De Langue, 1985), data showing the number of subcultures and the number of plants actually obtained is practically inexistent. Recently, with the actual observations of somaclonal variation, the need for controlling the number of subcultures for a given material has been emphasized (Smith, 1988; Reuveni & Israeli, 1990).

Table 1 shows the number of shoots obtained for each clone in every subculture, the mean, the variance, and the variation coefficient for each subculture. The data show that in average one shoot tip cultivated *in vitro* under the conditions detailed earlier can produce a total of 676 plants after six subcultures. Table 2 shows the models adjusted for each clone and for the mean of all clones considering a Poisson model. Fig. 1A shows the adjusted model and the observed data. Krikorian (1990) estimates that in theory 4,136,000 banana plantlets could be produced from one single banana shoot tip in fourteen subcultures. Table 2 shows the estimated num-

TABLE 1. Number of shoots produced by banana clones (cv. Nanicão) after six subcultures.

Clones	Introduction	Subcultures					
		1st	2nd	3rd	4th	5th	6th
A	0	6	10	28	84	163	557
B	0	0	4	12	49	134	435
C	0	3	7	24	71	195	564
D	0	4	14	59	213	559	1850
E	0	3	5	15	34	60	143
F	0	4	7	31	67	199	572
G	0	6	9	17	38	87	243
H	0	4	9	33	94	269	876
I	0	2	9	47	109	324	845
Mean	0	3.6	8.2	29.6	84.3	221.1	676.1
Variance	0	3.5	8.7	237.0	2958.0	22890.9	251752.1
C.V. (%)		52.7	35.9	52.1	64.5	68.0	74.0

TABLE 2. Adjusted regression model using Poisson distribution for each clone and estimated numbers ($\hat{Y}_{14}$) of shoots after fourteen subcultures.

Clones	Model	$\hat{Y}_{14}$
A	$\hat{Y} = \exp[0.3183+0.9942.x]$	1 524 382.55
B	$\hat{Y} = \exp[-0.9095+1.165.x]$	4 879 239.86
C	$\hat{Y} = \exp[0.002915+1.055.x]$	2 604 926.45
D	$\hat{Y} = \exp[0.6329+1.148.x]$	17 982 327.00
E	$\hat{Y} = \exp[0.3182+0.7720.x]$	67 927.76
F	$\hat{Y} = \exp[0.1316+1.035.x]$	2 239 134.58
G	$\hat{Y} = \exp[0.2923+0.8590.x]$	223 753.44
H	$\hat{Y} = \exp[0.03837+1.121.x]$	6 799 570.46
I	$\hat{Y} = \exp[0.5977+1.026.x]$	3 146 181.33
Mean count	$\hat{Y} = \exp[0.1288+1.063.x]$	3 304 514.10

bers of shoots, with the adjusted models, if we had used fourteen subcultures. It can be seen that the mean number (3,304,514) is close to the theoretic prediction of Krikorian (1990). The author also emphasizes the fact that different explants show different behavior, some of them producing many buds, which develop readily into plants, and others forming a small number of buds, which usually develop into small plantlets. This same behavior was also observed in this current work, where clone D produced a total of 1,850 plantlets, while clones E and G produced 143 and 243 plantlets, respectively. This observation indicates that there must be either a genetic or a physiological factor affecting the production of new shoots *in vitro*, influencing either negatively or positively, considering that all the material was handled in a homogeneous way.

The statistical analysis clearly shows that the variability among clones increases at every subculture, being more evident after the fourth subculture when the variation coefficient increases from 64.5% to 68% and to 74% from the 4th to 5th and 6th subcultures, respectively. However, this increase in variability can be explained by the cumulative difference among clones, which is accentuated after the third and fourth subcultures. From Table 1 it can be observed that clone D, which was the most productive on the 6th subculture, had already shown this tendency in the 3rd subculture. The same fact could also be observed for clones B, E and G producing the smallest number of new shoots in the 3rd and 4th subculture. Analyzing the multiple box plot (Fig. 1B) the variability increase among clones can be visualized within every subculture, including the outliers, represented by values obtained from clone D.

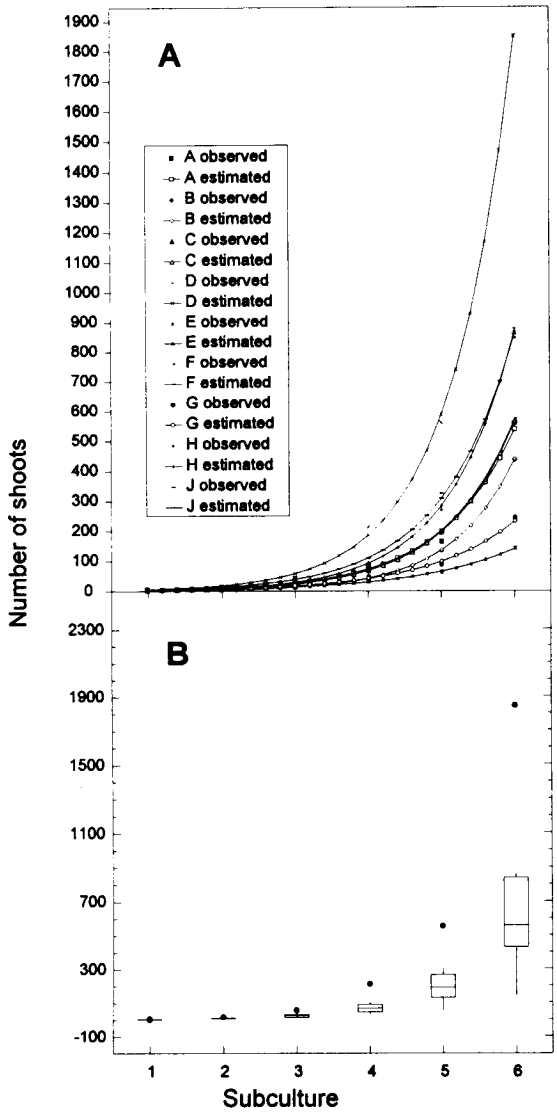

FIG. 1. Shoots produced by subculture (A) and Multiple Box-Plot for number of shoots (B).

CONCLUSIONS

1. The average number of plants produced from one shoot tip of cultivar Nanicão after six subcultures *in vitro* is approximately six hundred.

2. The banana explants show differences in their behavior in culture in terms of the number of plants produced.

3. As early as the third subculture it is already possible to identify the clones that will potentially be efficient plant producers.

REFERENCES

COTE, F.; ALVARD, D.; DOMERGUE, R.; NAVARRO-MASTACHE, L.; TEISSON, C. Micropropagation *in vitro* du bananier. **Fruits**, v.45, p.112-118, 1990.

CRONAUER, S.S.; KRIKORIAN, A.D. Multiplication of *Musa* from excised stem tips. **Annals of Botany**, v.53, p.321-328, 1984.

DREW, R.A.; SMITH, M.K. Field evaluation of tissue-cultured bananas in south-eastern Queensland. **Australian Journal of Experimental Agriculture**, v.30, p.569-574, 1990.

FILIPIA, R. Propagation intensiva de platano vianda y fruta. **Ciencia y Tecnica en la Agricultura. Série: Viandas Tropicales**, v.10, p.31- 43, 1987.

HUSSEY, G. Problems and prospects in the *in vitro* propagation of herbaceous plants. In: WITHERS, L.A.; ALDERSON, P.G. (Eds.) **Plant tissue culture and its agricultural applications**. London: Butterworths, 1986. p.69-84.

ISRAELI, Y.; REUVENI, O.; LAHAV, E. Qualitative aspects of somaclonal variations in banana propagated by in vitro techniques. **Scientia Horticulturae**, v.48, p.71-88, 1991.

KRIKORIAN, A.D. Baseline tissue and cell culture studies for use in banana. In: PLOETZ, R.C. (Ed.) **Fusarium wilt of banana**. St Paul: APS Press, 1990. p.127-133.

KRIKORIAN, A.D.; IRIZARRY, H.; CRONAUER-MITRA, S.; RIVERA, E. Clonal fidelity and variation in plantain (*Musa* AAB) regenerated from vegetative stem and floral axis tips *in vitro*. **Annals of Botany**, v.71, p.519-535, 1993.

McCULLAGH, P.; NELDER, J.A. **Generalized linear models**. 2.ed. Londres: Chapman & Hall, 1989. 511p.

MURASHIGE, T.; SKOOG, F.A. A revised medium for rapid growth and biossays with tobacco tissue cultures. **Physiologia Plantarum**, v.15, p.473-497, 1962.

REUVENI, O.; ISRAELI, Y. Measures to reduce somaclonal variation in *in vitro* propagated bananas. **Acta Horticulturae,** v.275, p.307-313, 1990.

SMITH, M.K. A review of factors influencing the genetic stability of micropropagated bananas. **Fruits,** v.43, p.219-223, 1988.

SMITH, M.K.; DREW, R.A. Growth and yield characteristics of dwarf off-types recovered from tissue-cultured bananas. **Australian Journal of Experimental Agriculture,** v.30, p.575-578, 1990.

VUYSTEKE, D.; De LANGUE, E. Feasibility of *in vitro* propagation of bananas and plantains. **Tropical Agriculture,** v.62, p.323-328, 1985.

VUYSTEKE, D.; SWENNEN, R.; WILSON, G.F.; LANGHE, E. de. Phenotypic variation among *in vitro* propagated plantain (*Musa* sp. Cultivar "AAB"). **Scientia Horticulture,** v.36, p.79-80, 1988.