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EFFECT OF REPEATED SUBCULTURING ON SOMACLONAL VARIATION IN BANANA (*MUSA* AAB GROUP) CV. NENDRAN

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Abstract: Continuous subculturing of regenerated shoot initials of banana cv. Nendran was carried out at two-week interval to assess the variation induced due to repeated subculturing. It was found that the number of shoots produced per culture varied in different subcultures. Chromosome counts made at the root tips of the plaatlets from ten subcultures indicated that all the plants were triploids (2n = 33). However, the plantlets from the different subcultures varied significantly with respect to the rate of growth in height and leaf area.

Key words: Banana, Nendran, somaclonal variation, triploid.

INTRODUCTION

In a crop like banana where natural variability is limited because of continuous vegetative propagation, *in vitro* methods can be used with advantage to induce variability. It is reported that variability can be obtained through repeated subculturing (Pierik, 1987). Also it is noted that continuous subculturing modifies the 'physiological' state of the plant in such a way that it favours revitalisation of innate dormant vegetative buds (Litz and Conover, 1978; Franclet, 1979; David, 1982). Evidences from cytological and molecular studies show that plant genome is relatively unstable and subject to various changes in developing tissues. Any genetic variation obtained in Nendran variety through *in vitro* culture may be useful, as selection could be made for desirable characters such as a shorter stature of the plant, higher yield, better colour of the bunches and resistance to diseases and pests.

MATERIALS AND METHODS

Cultures derived from shoot tip explants of Nendran on semi-solid medium containing BA 5.0 mg l^{-1} and NAA mg 1^{-1} were used to assess the effect of continuous subculturing on the multiplication rate of shoots. Observations were recorded, on three replications per treatment, on the number of shoots produced / culture and the percentage increase in number of shoots over the initial culture. Somatic chromosome counts were made on the root squashes of plantlets from different subcultures, following the method by Darlington and La Cour,(1976).

In order to study the morphological variation among the plantlets derived from different subcultures, they were maintained separately in different plastic pots. Sterile sand was used as the medium. **Observations** like survival percentage, height of the plant, number of leaves, length and breadth of the leaves and girth **of** the plantlets at 15 days interval were recorded and the rate of growth was calculated (Pause and Sukhatme, 1985).

RESULTS AND DISCUSSION

During the culture period, there was an increase or decrease in the multiplication rate of axillary shoots as given in the Table 1. It was found that when subculturing was done at two-week interval, the number of shoots produced per culture varied in the subcultures.

The shoots produced per explant per culture vessel increased at a mean rate of 5.9. Increase in multiple shoot production continued from the 1st subculture until the 6th subculture. There was a decline in the shoot production at the 7th subculture, followed by a sudden increase in shoot production at the 8th subculture. After the 8th subculture, there was a reduction in shoot production at the 9th and 10th subcultures. It is suggested that continuous subculturing modifies the

Table 1. Influence of subculturing on *in vitro* multiplication and *ex vitro* growth rate of shoot tip explants from the initial shoot proliferating cultures *Musa* (AAB) Nendran (MS*** basal medium and sand potting medium)

No. of culture	*In vitro*		*Ex vitro* Mean growth rate, cm		Leaf area, cm^2
	*Shoot / culture	Increase in* number of culture over the initial culture, %	" Height	Girth	
1	2.2	0	0.38	0.35	13.07
2	2.4	16.88	0.88	0.30	14.22
3	3.4	36.00	0.50	0.22	16.02
4	3.2	14.28	0.10	0.53	19.20
5	6.4	52.38	0.37	0.21	16.50
6	6.6	53.49	1.03	0.13	11.37
7	2.8	3.70	0.36	0.42	4.07
8	7.4	57.44	0.67	0.23	8.72
9	3.2	23.00	0.84	0.29	11.67
10	3.4	35.99	0.46	0.15	9.90
CD (0.05)	NS	-	0.32	NS	-
SEm t	8.3	-	0.15	0.23	-

* Mean of four replication
** MS medium containing lull concentration of both inorganic salts and organic growth factors

physiological state of the plant in such a way that it favour revitalisation of innate dormant vegetative buds (Litz and Conover, 1978; Davi, 1982). The results obtained in the present investigations were also in favour of the report.

Differences in the growth rate of plantlets from the different subcultures at 15 days interval are presented in Table 1.Plantlets from the subcultures differed significantly in the rate of growth with respect to plant height and leaf area. Maximum growth rate with respect to height (1.03 cm) was recorded by the 6th subculture. Maximum growth rate with respect to leaf area (19.20 cm^2) was recorded by the 4th subculture. But the effect of subculturing on the growth rate with respect to girth of the plants was not significant. Minimum growth rate with respect to height (0.10 cm) was recorded by the 4th subculture and with respect to leaf area (4.07 m^2), by the 7th subculture.

Growth parameters, recorded sixty days after transplanting, are presented in Table 2. Maximum plant height (14.42 cm) and girth (2.87 cm) was recorded at the 10th subculture. Maximum length (8.50 cm) of the leaf was observed at the fifth subculture, maximum width of the leaf (2.73 cm) at the 4th subculture and maximum leaf production by the 9th subculture.

Somatic choromosome counts made on the root tip squashes of the plantlets from the ten subcultures revealed that there was no variation in chromosome number due to subculturing. All the plantlets were triploids (2n =33).

Table 2. Effect of subcultures on growth parameters of *Musa* (AAB) Nendran sixty days after transplanting (Planting medium : sand)

Culture	Plant height, cm	Girth of the plant, cm	Length of the* leaf, cm	Width of the* leaf, cm	Number of leaves
1	7.52	2.10	6.30	2.48	3.00
2	9.12	2.13	7.25	2.35	2.75
3	10.57	2.00	7.37	2.60	3.00
4	7.45	2.20	8.30	2.73	3.00
5	9.18	2.17	8.50	2.70	2.75
6	13.07	1.85	7.45	1.85	3.00
7	11.32	1.87	4.30	1.18	3.00
8	10.25	1.95	6.13	1.86	3.00
9	13.95	2.47	6.40	2.18	3.25
10	14.42	2.87	6.66	1.78	3.00
Mean	10.69	2.16	6.87	2.17	3.57

Values taken as mean of four observations; Culture period: 8 weeks;
* Observations were taken from the 3rd leaf

Induction of somaclonal variation by utilization *of in vitro* techniques should substantially contribute to the broadening of the spectrum of genetic variation among *Musa* clones with obligate vegetative reproduction. Integration of methods of early screening for disease resistance (Stover, 1986; Epp, 1986) with subsequent rapid propagation of desirable plants leads to an alternative breeding scheme, which should be applied for banana improvement.

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REFERENCES

David, A. 1982. *In vitro* propagation of gymnosperms, Bonga, J. M. and Durzan, D. J. (Eds.). *Tissue Culture in Forestry.* Martinus Nijhoff, Dr. W. Junk Publishers, London, p. 72-101

Darlington, C. D., and La Cour, LF. 1976. *The Handling of Chromosomes,* 6th ed. George Allen ami Unwin Ltd., London, p. 36-39

Epp, M. D. 1986. Somaclonal variation in banana - A case study with fusarium wilt. *Banana and Plantain Breeding Strategies.* Parsley, G. J. and De Langhe, E. (Eds.), Canberra, ACIAR Proceedings No. 21, 140-150

Franclet, A. 1979. *Reje unnissement des arbres adultes en vue de leur propagation vegetative. Micropopagation des Abres Forestiers, Annales* AFOCEL No. 12, 6/79 AFOCEL, Nangis, France, p. 3-18

Litz, R. E. and Conover, R. A. 1978. *In vitro* propagation of papaya. *Hort. Sci.* 13 : 241-242

Panse, V. G. and Sukhatme, P. V. 1985. *Statistical Methodsfor Agricultural Workers.* 4th ed. 1CAR, New Delhi, p. 131-143

Pierik, R. L. M. 1987. *In vitro Culture of Higher Plants.* Martinus Nijhoff Pub., Dordrecht, p. 231-232

Stover, R. H. 1986. Measuring response of *Musa* cultivars to sigatoka pathogens and proposed screening procedures. *Hanana and Plantain Breeding Strategies.* Persley, G, J. and De Langhe, E. (Eds.), Canberra, ACIAR Proceedings No. 21, 114-118