Micropropagation of *Musa* AAA cv. Poyo in the Ivory Coast

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An improved micropropagation method for the Poyo banana clone is described. Main apices of sucker buds and lateral buds are stimulated by a high concentration of benzyladenine in the absence of auxin. After transplantation, shoot proliferation is achieved with the same concentration of cytokinin. Shoot elongation and rooting are obtained simultaneously on a medium containing no phytohormone and 10 g l^{-1} sucrose. Rooting is improved when the bottoms of the culture tubes with the medium are set in darkness; rooting is then maximal at 97.9%.

Keywords: *Musa* cv. Poyo; Micropropagation; *In vitro* weanings

In the Ivory Coast, *Musa* AAA banana cv. Poyo, which is also called Robusta, Valery or Tall Mons Mari in other countries (Simmonds, 1982), is the most widely cultivated clone on about 10 000 ha with an annual average production of 100 000 t. As with other *Musa* cvs. Poyo can only be propagated by vegetative multiplication because of its parthenocarpy; successive generations of suckers guarantee the production of planting material, but multiplication rates are low, about five–six mother plant^{-1} culture cycle^{-1} (Lassoudière, 1979).

On the other hand, *in vitro* banana culture can provide a rapid and increased production of healthy material (Berg and Bustamante, 1974; Gupta, 1986) and improvement by clonal multiplication from selected varieties. Several authors have described micropropagation methods of different banana cultivars from buds (Vessey and Rivera, 1981; Bower and Fraser, 1982; Dore Swany *et al.*, 1982; Hwang *et al.*, 1984); other workers have shown some differences in behaviour between different banana clones and their needs in *in vitro* culture (Cronauer and Krikorian, 1984 a and b; Banerjee and De Langhe, 1985; Jarret *et al.*, 1985; Vuylsteke and De Langhe, 1985; Wong, 1986).

The aim of the present study was to apply and to simplify the existing *in vitro* culture methods to the Poyo banana cultivar to produce vigorous vitroplants under modest local technical conditions.

Materials and methods

Plant material

Young suckers of banana *Musa* AAA cv. Poyo, belonging to the Cavendish Robusta group, were used; these suckers, with 10 cm wide leaves, came from the first or second growth cycles of the banana plants. Lateral axillary and apical buds were removed from sucker corms with a hollow punch. Two or three outer bud leaves were removed; explants consisted of the apex, bud leaves and a 5 mm base fragment of the underlying corm.

About 50 explants were surface sterilized for 10 min in 100 ml of a 0.25% sodium hypochlorite solution with 0.1 ml Tween 80 added. After the explants were rinsed three times in sterile distilled water, any dead tissues were cut off. Before *in vitro* introduction, they were distributed into two or four groups according to their size.

In vitro culture

The basic medium was prepared according to Murashige and Skoog (1962). Salts were supplemented with 0.1 mg l^{-1} thiamine hydrochloride, 0.1 mg l^{-1} pyridoxine hydrochloride, 0.5 mg l^{-1} nicotinic acid, 100 mg l^{-1} *myo*-inositol, 2 mg l^{-1} glycine, 500 mg l^{-1} casein hydrolysate and 200 mg l^{-1} tyrosine.

To induce meristem tips, we added 50 mg l^{-1} ascorbic acid as an antioxidant to prevent media blackening, 20 g l^{-1} sucrose and 21.5 µM naphthalene acetic acid (NAA) in combination with four concentrations of benzyladenine (BA) 4.5 µM, 9 µM, 22.5 µM and 45 µM. This medium was solidified with 0.8% agar and the pH adjusted to 5.8 before autoclaving. For bud proliferation, the same medium was used. Initiation and proliferation cultures were set under continuous light (Gro Lux 20 ± 2 W m^{-2}) at 30°C.

Shoot elongation and rooting were best achieved by removing BA, and reducing sucrose concentration by half (10 g l^{-1}). This medium was solidified with 0.7% agar and pH adjusted to 5.8. To improve elongation, we studied the effect of 0.5% activated charcoal and the effect of dark conditions on rooting. On the top of the white medium, we laid 5 mm of charcoal (Figure 1) and placed the bottom of the culture tubes in wooden boxes, so that shoot leaves grew under light and root systems were formed in darkness. These cultures were incubated under a photoperiod of 12 h light (Gro Lux 20 ± 2 W m^{-2}) at 30°C and 12 h darkness at 27°C. When shoots were about 5 cm high, they were transferred to a glasshouse.

During each culture period, explants were never transplanted.

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Results

Sprouting and meristem tip initiation

First, we noticed that longitudinal cuts of buds induced a threefold increase in multiplication. Multiplication speed also depended on the type of sucker bud; those of lateral origin doubled in size within three weeks but apical buds reached three times their size; on the other hand, 95% of apical buds were green compared with only 78% of the lateral ones.

We tested eight different BA/NAA concentrations (Table 1) and noticed that NAA was not necessary and that a high concentration of BA to initiate caulogenesis was needed. Without NAA and with only 4.5 µM BA, main bud apices grew only after a long dormancy. With the double BA concentration, axillary tips were stimulated at the base of outer leaves only; then, with the 22.5 µM concentration, we observed a rapid sprouting of the buds and the appearance of more axillary tips; with the strongest concentration, adventitious meristems were initiated. When BA concentration was higher than 45 µM, explants underwent hypertrophy, became swollen and died.

Proliferation

The effects on proliferation of the four BA concentrations were compared. The two lower concentrations (4.5 µM and 9 µM) did not favor multiplication and only produced bud elongation; the highest one (45 µM) caused necrosis of the buds. Proliferation was best achieved with the 22.5 µM concentration, reaching a multiplication rate of 2.8 every three weeks.

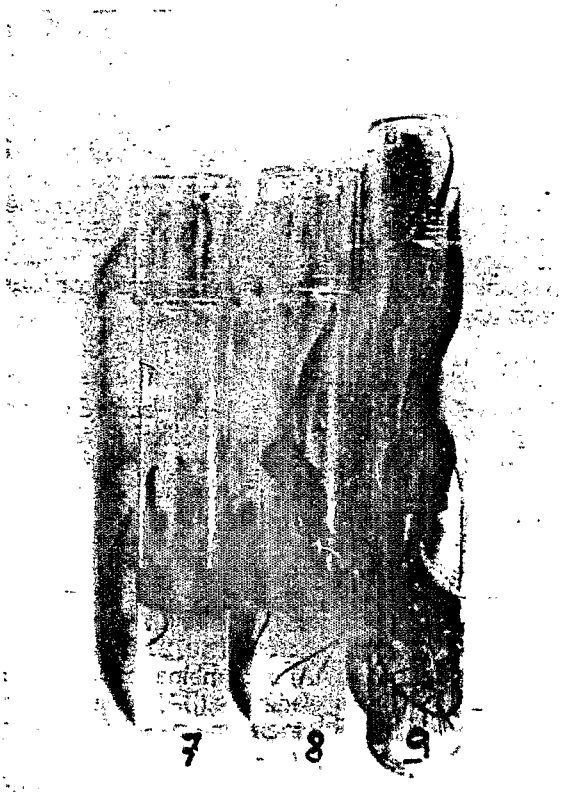

Figure 1 Elongation and rooting of banana cv. Poyo shoots

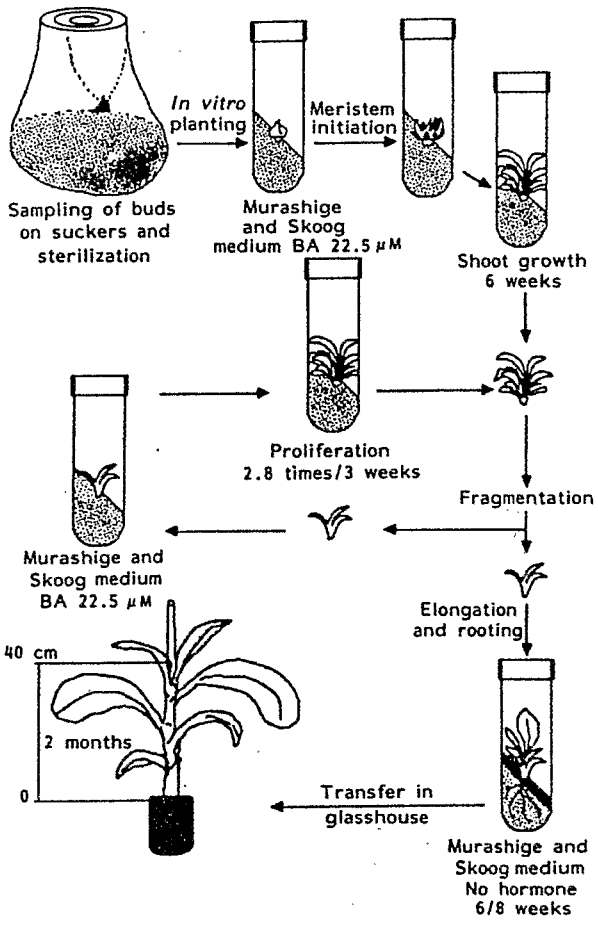

Figure 2 *In vitro* production organigram of banana cv. Poyo

Table 1 Effects of benzyladenine with naphthalene acetic acid on meristem initiation

Naphthalene acetic acid (µM)	Benzyladenine (µM)	Minimal period for caulogenesis (days)	Maximal multiplication rate
0	4.5	97	1.06
0	9	60	2.83
0	22.5	52	3.66
0	45	45	5.75
21.5	4.5	Callogenesis	no multiplication
21.5	9	Callogenesis	no multiplication
21.5	22.5	Callogenesis	no multiplication
21.5	45	56	1.1

Table 2 Effects of activated charcoal and darkness on shoot growth and rooting

Culture conditions	Necrosis (%)	Rooted shoots (%)	Primary roots			Secondary roots	
			No.	Length (cm)	With secondary roots (%)	No. per primary root	Distance between two roots (cm)
1. Elongating medium	18.7	64.6	7	5.92	42.86	5	3.55
2. Elongating medium + charcoal	35.4	58.3	8	6.07	37.5	11	1.49
3. Elongating medium in darkness	2.1	97.9	8	5.06	50	11	1.04
Comparison and classification of the means (Mann–Witney *U* test)			NS	NS	NS	S 2a 3a 1b	S 2a 3a 1b

NS = not significant; S = significant ($P < 0.05$)

Elongation and rooting

Buds cultivated during six months without any transplantation on a medium containing 22.5 µM BA and 20 g l^{-1} sucrose produced leafy shoots which rooted; assuming that depletion of medium nutrients could induce caulogenesis and then rhizogenesis, cytokinin was suppressed and sucrose concentration reduced by half (10 g l^{-1}). To improve elongation and rooting, we tested 0.5% activated charcoal in the medium (Table 2) and found that it increased the percentage of necrosed shoots, but living roots were better developed and that the primary root system did not change but the secondary one was significantly improved.

If rhizogenesis was induced in darkness without charcoal in the medium (Figure 1), primary and secondary root systems had the same conformation as with charcoal, but dark conditions increased the number of rooted shoots (Table 2).

Discussion and conclusions

Plant material

We observed that bud cuts caused a faster sprouting of the bud fragments; it would seem that concentrically arranged leaves, according to the phyllotaxy of the banana pseudostem (De Langhe, 1961) might inhibit growth of apical meristems. On the other hand, different budding and greening capacities between apical and lateral sucker buds could result from a physiological regulation which depends on dominance effects of apical buds on lateral ones.

Culture media

An auxin is usually used, either in combination with a cytokinin or alone, depending on the needed growing phase (caulogenesis or rhizogenesis). Our studies show that the use of an auxin was not essential and that organogenesis can be led towards caulogenesis only with a cytokinin, as shown by Cronauer and Krikorian (1984a, b) using also 22.5 µM benzyladenine with other banana clones. We found that the use of the strongest BA concentration (45 µM) initiated adventitious meristems but although this provided a greater and more rapid shoot tip production, genetic disturbances, which could induce physiological and morphological changes in such meristems after cell fusions, for example, undoubtedly cannot be avoided. Moreover, this high BA concentration cannot be used for long since it will be necrotic towards buds.

Shoots elongation and rooting were obtained without cytokinin; in fact, it seems that total phytohormone weaning has the same effects as a cytokinin/auxin ratio reduction by adding auxin in the medium. This weaning has advantages, first to harden shoots by hormone depletion and then to improve shoot growth and induce rooting.

Reduction of sucrose concentration also improved elongation so that impoverishment of the culture medium in carbohydrates can increase the whole foliar surface, improving photosynthesis in shoots.

Physical conditions for rooting

Exposure to light did not favour rooting. We have shown that putting cultures in darkness improved rooting, while activated charcoal reduced shoot life.

Conclusion

Following these results, the best conditions for *in vitro* production of banana plant cv. Poyo includes (Figure 2) (i) obtaining bud clusters from initial explants after six weeks with 22.5 µM benzyladenine, (ii) bud proliferation at an approximate multiplication rate of 2.8 every three weeks with the same benzyladenine concentration, (iii) growth of leafy-rooted shoots during six to eight weeks of culture, with leaves under photoperiodic light and roots in darkness, without any phytohormone and a half strength of sucrose in the medium.

In these micropropagation conditions, shoots were transferred to the glasshouse with an acclimatization rate reaching 100%. The best growth conditions for banana plants during the important period of their glasshouse culture before their field plantation need investigation. This experiment, described with the Poyo cultivar, shows that it is possible to produce banana *in vitro* plants, training shoots to get their own metabolism by nutrients or phytohormone weanings.

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