VII^th Symposium of the International Society for Tropical Root Crops,
Gosier (Guadeloupe), 1-6 July 1985, Ed. INRA, Paris, 1988.

VIRUS-FREE PLANTS OBTAINED BY THERMOTHERAPY AND MERISTEM CULTURE OF WHITE (Xanthosoma saggitifolium (L.) Schott.) AND PURPLE (X. violaceum Schott.) COCOYAMS

(Obtention de plants débarrassés de virus par culture de méristème et prolifération de pousses axillaires chez le Malanga blanc et violet)

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SUMMARY

Apparently virus-free plants of white and purple cocoyams were obtained by a combination of thermotherapy treatment at 38 1 C for 5 to 6 weeks followed by meristem culture on a modified Murashige and Skoog (MS) liquid medium supplemented with N -benzyladenine (BA). After 5 weeks of culture, emerging buds were dissected out, cut in half longitudinally through their apices, and each piece was transferred to fresh semi-solid (0.7 per cent Difco Bacto agar) MS medium containing elevated concentrations of BA which promoted the development of axillary buds. Excision of individual axillary buds and transfer to hormone-free semi-solid (0.7 per cent agar) MS medium resulted in rapid and extensive root formation. Plantlet survival after transfer to methyl bromide-treated soil approached 100 per cent. Initial establishment of plants in the field was accomplished following procedures normally used for vegetative propagation of these crops.

RESUME

Des plants de Malanga blanc et violet (Xanthosoma sagittifolium (L.) Schott et X. violaceum Schott) apparemment indemnes de virose sont obtenus par culture de méristèmes sur un milieu liquide modifié de Murashige et Skoog (MS) supplémenté avec 0,1 mg/l de N benzyladenine (BA). Après 4 semaines de culture les pousses qui émergent sont coupées longitudinalement à travers leur apex, en 2 moitiés et chaque partie est transférée sur un milieu MS, à moitié solide, contenant 5 mg/litre de BA, qui provoque le développement des bourgeons axillaires.

L'excision et le transfert individuel des bourgeons axillaires sur le milieu MS de base conduisent à une initiation et à un développement rapide des racines.

Figure 1 : Complete plant of Xanthosoma saggitifolium (L.) Schott.

Figure 2: Plant dwarf of X. saggitifolium (L.) Schott infected with Dasheen Mosaic Virus (DMV), with 3 months after planting in field conditions.

INTRODUCTION

Cocoyam or Tiquisque (Fig. 1), as it is locally referred to in Costa Rica, is an important staple of subsistence farmers in tropical areas. Together with "malanga" (*Colocasia esculenta* var. esculenta) and "nampi" (*C. esculenta* var. antiquorum), tiquisque is recognized as having considerable potential for feeding cattle and for fuel production (14).

Plants are generally grown for their edible corms or cormels and in some cultivars the young leaves and petioles are eaten in salads (12). Cocoyam is propagated vegetatively by planting the main stem cutting which consists of the apical portion of the corm and the lower part of the petioles. More frequently used are pieces of the corm and the optimal size is about 250 g (4). These propagation methods are ineffective for maintaining pathogen-free stock plants and result in disease dissemination (8). In addition, maintaining field collections of these crops is expensive, and the threat of attack by pathogens, principally viruses, results in the loss of desirable genotypes (9). For example, Dasheen Mosaic Virus (DMV) (Fig. 2), detected in the aroids collection of CATIE (13) has been responsible for the loss of about 50 per cent of the *Xanthosoma* spp (15).

Virus-free plants of these and other crops can be obtained, either through seed propagation (6, 17, 18), or through shoot tip culture (1, 2, 5, 6). Virus-free plants of *X. brasiliensis* have been obtained by in vitro propagation (16). Also, many plants of *X. caracu* have been recovered from cultured shoot tips (3). However, the literature contains little information on the application of meristem culture and micropropagation techniques for virus elimination from and propagation of white and purple cocoyams.

This research was initiated to test the effectiveness of thermotherapy treatment and meristem culture as virus eradication and to develop a culture media, which would support the growth of true meristems while stimulating. It is our intention to utilize these techniques for production and dissemination of certified planting material which would facilitate accurate field evaluation of these materials and regional and international exchange of this germplasm.

MATERIAL AND METHODS

Thermotherapy Treatment

White and purple cocoyam tuber pieces of about 250 g were taken from the head of the main tuber. These were washed, soaked for 15 minutes in a solution of commercial fungicide (1 per cent w/v Benomyl), planted in 85 x 120 mm pots containing sterile soil, and incubated in a thermotherapy

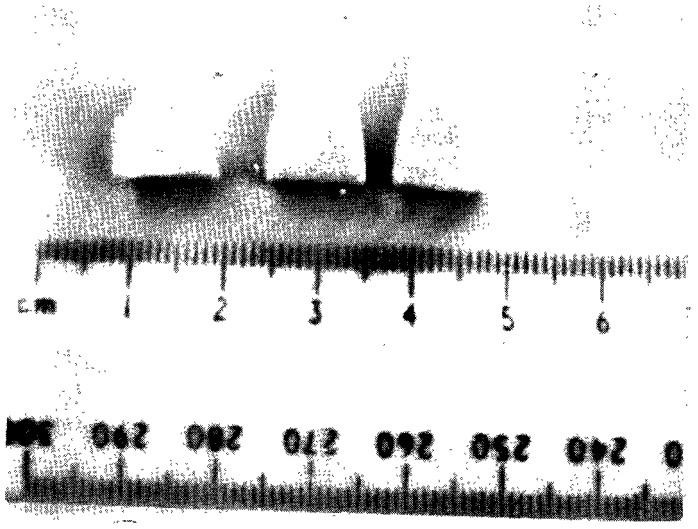

Figure 3: Explants of Cocoyam before surface sterilization.

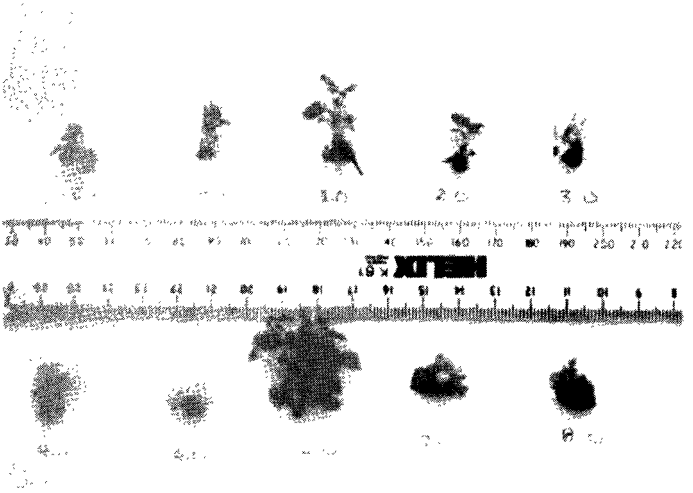

Figure 4: Effect of BA concentration (mg/liter) on the stimulation of axillary buds in Cocoyam.

chamber. Thermotherapy treatment consisted of continuous exposure to a temperature of 39 1°C constant for 6 to 8 weeks under 3.5 Klux illumination provided by white fluorescent lamps on a 16 hour photoperiod. Plants were irrigated daily and maintained under 60 to 80 per cent relative humidity. When the first petioles reached 20.0 cm in height, the shoot tips and subtending tissues were excised by cutting through the petioles above the shoot tips and through the starchy tissues below (Fig. 3). These were surface sterilized by immersion in a solution of 10 per cent (v/v) commercial laundry bleach containing 2 drops/100 ml of the surfactant Tween 80 with constant agitation for 10 minutes and rinsed 3x in sterile distilled water.

In vitro culture

With the aid of a dissecting microscope additional petiole tissue and immature leaves were removed until the meristem was visible. The meristem 1 leaf primordia was excised. Meristem were individually transferred to 125 x 10 mm screw cap vials containing 10 ml of liquid basal medium. Basal medium consisted of Murashige and Skoog (MS) salts (11), and the following in mg/liter : thiamine. HCl, 0.1., pyridoxine. HCl, 0.5., nicotinic, 0.5., glycine, 2.0., myo-inositol, 100., sucrose 30,000 (Table 2) and supplemented with BA at : 0.0, 0.1, 0.3, 1.0, 3.0 or 3.0 mg/l in combination with NAA at : 0.0 ; 0.1 ; 0.3 ; or 3.0 mg/l in a factorial experimental design with 10 replications per treatment. For stimulation of axillary bud development actively growing shoots tips were cut longitudinally, and the pieces were cultured individually into baby food jars (60 x 100 mm) capped with B-caps containing 10 ml of semi-solid basal medium supplemented with BA at : 0.0 ; 0.1 ; 2.0 ; 3.0 ; 4.0 ; 5.0 ; 6.0 ; or 7.0 mg/l. All media pH were adjusted to 5.7 0.1 with KOH or HCl and autoclaved for 15 minutes at 121°C at 15 psi. Cultures were maintained in a growth room at 28 2°C and a 16 hours photoperiod under 3 Klux illumination. Experiments were replicated 3 times. Cultures were scored at 2 day intervals for survival and further development.

RESULTS AND DISCUSSION

Thermotherapy treatment

When the cormel pieces were exposed to the hot air, they sprouted and shoots developed quickly. Each cormel produced 3 to 4 shoots which provided 3 to 4 meristems for use as primary explant until the emergence of more buds ended.

"In vitro" meristem initiation

Meristems floating in liquid basal medium began to enlarge and became chorophyllous in all treatments within 2 days after culture initiation. In the following 15 to 17 days the emergence and unfurling of the first green leaves was evident. In the subsequent 3 to 4 weeks, 4 to 6 small green leaves emerged. Swelling at the basal regions of the shoot tip was noted in explants on treatments containing BA at 0.1 mg/l alone or in combination with NAA at 0.3 or 3.0 mg/l BA at 0.3 mg/l alone or in combination with NAA at 0.3 or 3.0 mg/l (Table 1). The other treatments were also effective but less so. The control, devoid of BA and NAA, developed more slowly. At the end of the 5 week culture period, and of the explants cultured on the treatments described previously, those cultured on basal medium containing 0.1 BA mg/liter were the most vigorous. BA 0.1 mg/liter was therefore used as the standard for "initiation medium" (Table 2).

Shoot tips which developed in the "initiation medium" were transferred to semi-solid (0.7 per cent agar) MS media supplemented with BA in order to test the effects of the different concentrations of BA on the stimulation of axillary buds development.

Stimulation of Axillary Development

Shoot tips from plantlets which developed on initiation medium were cut longitudinally through their apex into 2 to 4 pieces, or scored with a series of cuts across their apex and transferred media for stimulation of axillary development (Table 2). A BA concentration of 5.0., 6.0., or 8.0 mg/l was sufficient to stimulate axillary bud growth after 2 to 3 weeks (Fig.4). Splitting shoots tips was more effective than scoring apices for stimulation of lateral branching. Similar results were obtained with several genotypes of *Musa* (8). A combination of BA at 6.0 mg/liter in combination with splitting of the shoot apex promoted maximal axillary bud emergence. Individual cultures contained 30 or more shoots. Other treatments resulted in less vigorous shoot development (Fig. 4).

Reculture of individual excised axillary buds onto fresh semi-solid basal media supplemented with 6.0 mg/liter BA inhibited the emergence of many buds. After 5 days on media containing 6.0 mg/l BA globular structures arose at the base of the shoot tip piece that were greenish-brown in color on their exterior and light green on their interior. After 6 months numerous small buds were evident in these cultures, however, their further development was arrested or very slow. The utilization of these responses for limited growth conditions, could be useful for germ-

Table 1 : Effect on the growth factor concentration on the development of shoots from cultured apical meristem of Xanthosoma saggitifolium (L.) Schott and Xanthosoma violaceum Schott.

NAA (mg/liter) \ BA (mg/liter)	0.0	0.1	0.3	1.0	3.0	10.0
0.0	+	++++	++++	++	+	+
0.1	+	++	++	+++	++	++
0.3	+	++++	+++	++	++	+
1.0		++++	+++	++	++	
3.0	+	+++	+++	+	+	+

\- nil

\+ very low

++ low

+++ middle height

++++ high

+++++ very high

Table 2 : Media constituents (mg/liter for culture initiation axillary bud development and complete plant regeneration in 24 genotypes of cocoyam.

Media constituents	MEDIA TYPE		
	Culture initiation[1]	Axillary bud development[1]	Complete plant regeneration[2]
Inorganic salts	MS[3]	MS	MS
i-Inositol	100	100	100
Thiamine-HCL	0.1	0.1	0.1
Piridoxine-HCL	0.5	0.5	0.5
Nicotinic acid	0.5	0.5	0.5
Glycine	2.0	2.0	2.0
Sucrose	30,000	30,000	30,000
Agar	0	7,000	7,000
			0
BA	0.1	3.0	0

1/ 25 x 95 mm screw cap vials, 1 explant per culture.

2/ Baby food jars (60 x 100 mm) capped with B-caps (Magenta Corp., Chicago, U.S.A.), 4-6 shoots per vessel of culture.

3/ MS = Murashige and Skoog, 1962.

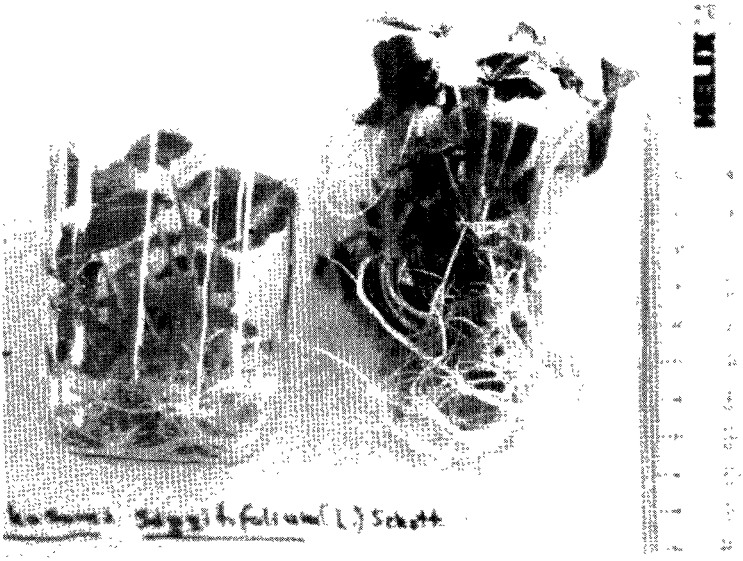

Figure 5: Complete plants of Cocoyam ready to be transferred to Bromide treated soil.

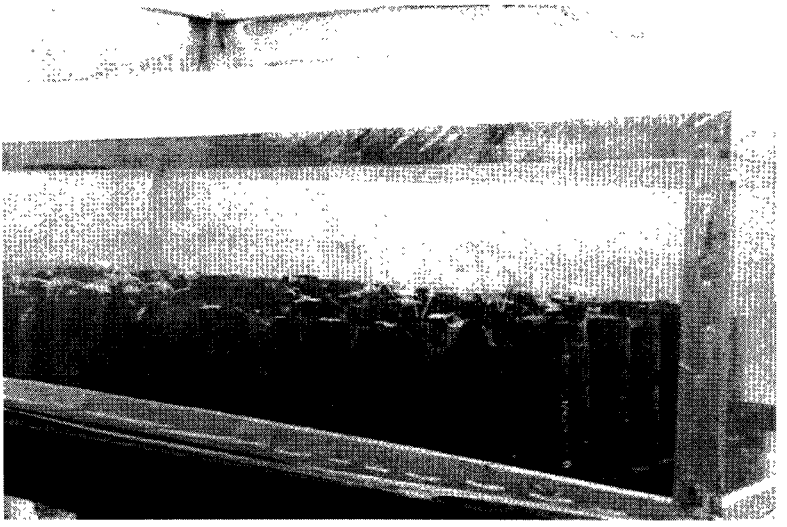

Figure 6: Plastic chamber in which the plantlets of Cocoyam are transferred and immediately planted in soil from the culture tubes.

plasm storage of this crop. Transfer of shoot tips from basal medium containing 6.0 mg/liter BA to basal medium containing 3.0 mg/l BA resulted in the rapid emergence of many buds. As many as 60 developing shoots were counted in a single culture on this media (Table 2). After 6 to 8 weeks, shoot tips from these were divided and recultured onto a fresh medium devoid of growth regulators (Table 2). This further promoted the development of complete and vigorous plants (Fig. 5).

Establishment of plantlets in soil

Well develop axillary buds of five of the 24 genotypes of *Xanthosoma* spp. (Table 3) propagated in vitro as described here rapidly developed a vigorous and extensive root system when transferred to basal medium lacking growth regulators (Fig. 5). These plantlets were subsequently removed from culture, transferred directly to methyl-bromide treated soil and placed in a plastic chamber in a screenhouse (Fig. 6). The temperature inside the chamber was approximately 35 and 30 C day and night respectively and the relative humidity was in excess of 80 per cent. Under these conditions survival exceeded 99 per cent. Survival of plants maintained in the screenhouse in the absence of plastic chamber was about 80 per cent. We suggest that the lower relative humidity in the absence of an enclosed chamber was responsible for the higher mortality. A period of 3 to 4 days inside the plastic chamber was sufficient for acclimatization.

Tissue culture propagated plants grow rapidly following transfer to soil (Fig. 7). In a 3 week period total leaf area increased about 4 times (Fig. 7a), and plant width 3 times (Fig. 7b). Plant height did not increase appreciably until the end of the 4th week (Fig. 7c). New leaf emergence was initiated after 3 weeks (Fig. 7d).

For further acclimatization, plants were placed outside under 30 to 40 per cent shade. Four weeks later they were transferred to full sunlight (Fig. 8). Subsequent growth was rapid.

Following this procedure plants are large enough for field establishment about 6 weeks after removal from culture. The time required, from the initiation of the thermotherapy treatment until the establisment of plants in the field, is approximately 24 weeks (Fig. 9).

Dasheen Mosaic Virus (DMV)

DMV (Fig. 10) was not detected in tissue culture propagated plantlets following serological tests. Eight months after removal from culture the appearance of these plants

Table 3 : Xanthosoma spp. cultivars from which meristem culture have been successfully initiated and whole plants recovered.

Introduction N°	Country of Origin	Date of Entry at CATIE
6115	Costa Rica	12 - 1976
6562	Honduras	7 - 1977
6568	Honduras	3 - 1977
7314	panamá	7 - 1977
7361	Panamá	7 - 1977
7368	Panamá	7 - 1977
7369	Panamá	7 - 1977
7372	Panamá	7 - 1977
8806	Costa Rica	7 - 1978
9492	Puerto Rico	1 - 1979
9505	Puerto Rico	1 - 1979
9509	Puerto Rico	1 - 1979
9523	Puerto Rico	1 - 1979
9856	Costa Rica	9 - 1979
9859	Costa Rica	9 - 1979
10017	Costa Rica	9 - 1979
10767	Panamá	1 - 1980
10876	Honduras	3 - 1980
11752	Costa Rica	6 - 1980
11972	Nicaragua	12 - 1980
12181	Honduras	12 - 1980
12469	Panamá	9 - 1981
12531	Costa Rica	2 - 1981
14330	Panamá	3 - 1983

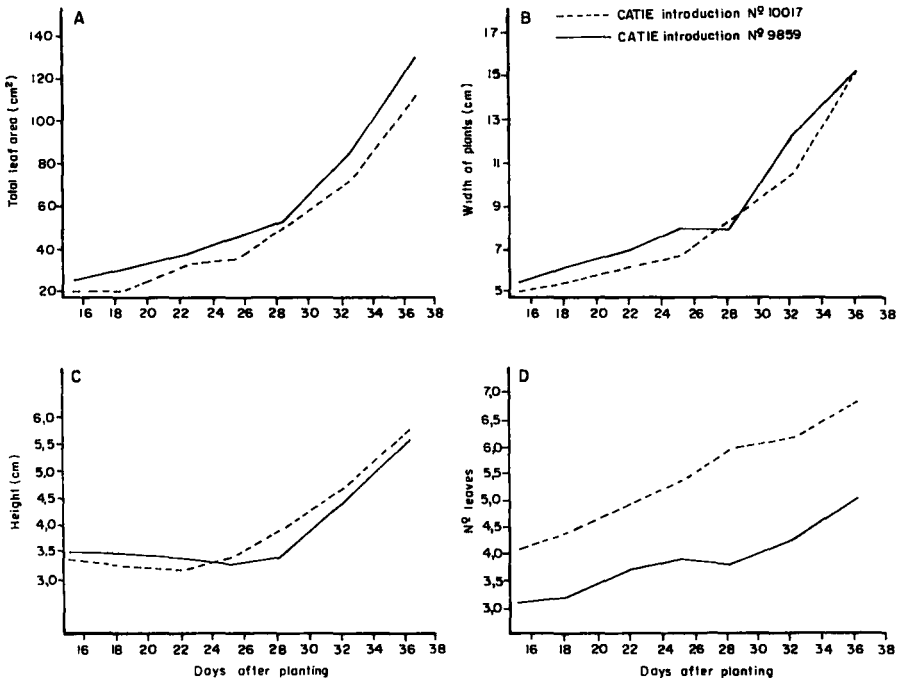

Figure 7: Growth of the CATIE Introduction #10017 after 36 days under greenhouse conditions: a) Foliar area, b) Width of plants, c) Height of plants and d) Number of leaves.

Figure 8: Plants of Cocoyam in conditions of full sunlight.

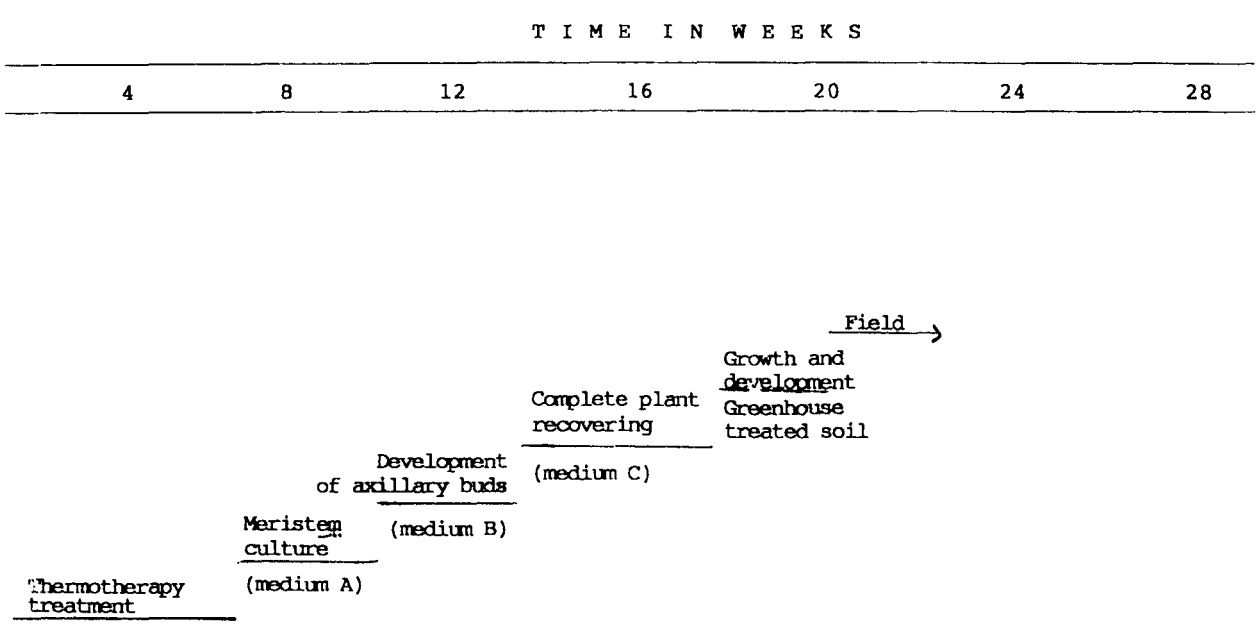

Figure 9: Time scale (approximate) for culture initiation in vitro micropropagation, complete plant recovering, growth and development and field plantation of 26 genotypes of cocoyam.

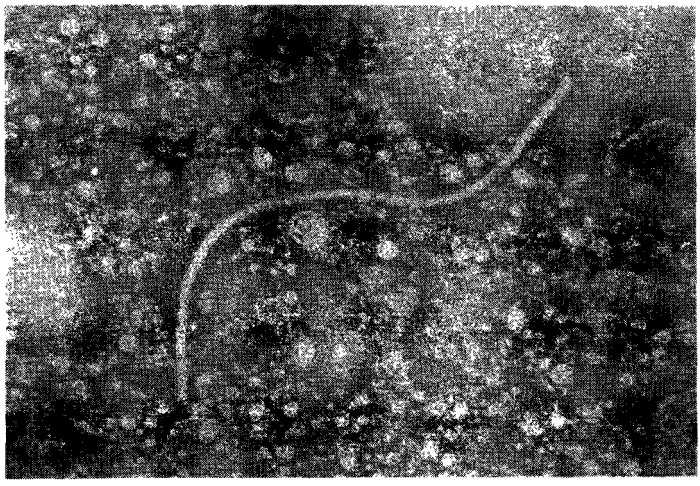

Figure 10 Electron micrograph of Dasheen Mosaic Virus. 30.000X.

Figure 11 Purple Cocoyam coming from meristem culture (left) and the same genotype with virus symtoms (right).

continues to suggest freedom from DMV. In addition, they are considerably more vigorous than plants of the same age and genotype propagated by conventional means (Fig. 11).

At present, the growth of these plants is being evaluated in several locations around Turrialba, Costa Rica. Attemps are being made to determine if reinfection by DMV can be avoided or delayed by planting at altitudes which would continue to allow for crop production.

ACKNOWLEDGEMENTS

This research work was financed by the German Agency for Technical Cooperation (GTZ). Grateful acknowlegements are made to HUMBERTUS HEINZE, formed Head of the Plant Genetic Resources Unit at CATIE, for his enthusiasm and support during this research project, to WERNER RODRIGUEZ for his continued interest and help in this work, to Dr. RAMON LASTRA for his assistance in virological techniques, and to Dr. ALEXANDER GRAF ZU STOLBERG for his assistance with the soil-establishment of plantlets. Special thanks go to E. SALAZAR and M.A. SALAZAR, two farmers from Guayabo of Turrialba, Costa Rica who provided us with the vegetative material for this study.

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