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MICROPROPAGATION AND FIELD PERFORMANCE OF VIRUS-FREE WHITE COCOYAM (*XANTHOSOMA SAGITTIFOLIUM* L. Schott) IN COSTA RICA

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ABSTRACT

Apical meristem (dome with one or two leaf primordia) explants of a local virus infected white cocoyam were culture on modified Murashige and Skoog (MS) medium containing 0.1 mg L⁻¹ 6-Benzylaminopurine (BA). After 120 days, complete plants were developed and above 90% of them were Dasheen Mosaic Virus (DMV) free according to ELISA test. To induce axillary shoot formation, corm section of *in vitro* plants were culture on modified MS medium supplemented with 3 mg L⁻¹ 6-BAP. An average of 4 shoots were formed every 42 days. Shoots produced *in vitro* were rooted on MS without growth regulators with 100% success and after 42 days, complete plants were obtained. About 98% of the rooted *in vitro* plants survived transfer to the greenhouse and were successfully transplanted outdoors. Field evaluation of two generations of *in vitro* virus-free white cocoyams was done. The results indicated that virus-free white cocoyams had twice the yield and better cormel quality than virus-infected plants. The establishment of a certification 'seed' program for white cocoyam is discussed.

INTRODUCTION

Edible aroids, cocoyam (*Xanthosoma* spp), taro (*Colocasia esculenta* (L) Schott) and edible yams (*Dioscorea* spp), represent an important source of food for the developing world. They are an important staple food in Africa, the Caribbean, Asia and many Pacific islands (O'Hair and Asokan, 1986; Volin and Zettler, 1976). In spite of their fundamental role in the diets of many people in these areas, little research has been done on them and the policy makers and researchers have only recently become interested in them (Gómez, 1994 personal communication).

After its nomination as non-traditional crop, the cocoyam production area and exports were expanded. According with the Costa Rican government data, the total production area was increased from 359 to 1023 ha in nine years (1983 to 1992). Consequently, exports increased from 540 to 5,661 metric tons in the same period. The estimated value increased from US \$ 214,121 to 3,567,000 (Rodríguez, 1993). The main export markets have been The United States (Latin American and Oriental communities), Puerto Rico, and other countries, such as Canada, Holland and England.

Cocoyam like most cultivated aroids, is propagated exclusively by vegetative means, using cormels and corm sections. Therefore, it is very sensitive to virus and other pathogens, which are transmitted with the propagation material.

Due to continued asexual propagation of edible aroids, growers are experiencing serious disease losses. Dasheen Mosaic Virus (DMV) is the most important virus disease. DMV has been found around the world associated with all edible aroids (Zettler and Hartman, 1986). It has been detected in Florida (Hartman 1974,) Egypt (Abo El-Nil and Zettler, 1976), Venezuela (Debrot and Ordosgoitti, 1974) and Costa Rica (Monge and Arias, 1984).

DMV belongs to the potyvirus group, its size is around 750 nm, is aphid-transmitted in a stylet-borne manner and it induces cytoplasmatic inclusions (Zettler *et al.*, 1970; Abo El-Nil, Zettler and Hiebert, 1977). DMV symptoms on leaves include "feathering," mosaic and distortion. Their expression depends on the genotype and the season in which it is grown. Normally they are

intermittently expressed, which make the virus difficult to detect (Zettler and Hartman, 1986; Hartman, 1974).

In Costa Rica, the DMV occurs in at least 80% of all commercial plantations (Ramirez, 1983). Preliminary studies on yield and quality of white and purple cocoyam, showed that plants with symptoms yielded 25% and 41% less, respectively, than non-symptom plants (Monge and Arias, 1984).

Root-rot, known as 'mal seco' in Puerto Rico and Costa Rica and cocoyam or tannia leaf-burning disease in the English-speaking Caribbean, is the most serious disease on *Xanthosoma* production in Africa, the Caribbean and now in Costa Rica. Potentially contaminated soil on the propagules and systemically infected planting material carried from one planting region to another are the two major ways the disease spreads. The cause of this root rot has been extremely difficult to identify because it is a soil borne disease, and speculation has prevailed in the absence of research. Nzietchueng (1983) and Rodriguez-Marcano and Rodriguez-Garcia (1986) found that *Rhizoctonia solani* and *Phytophthora* spp. which are commonly found in soils, seem to be causal agents of root-rot disease. However, cocoyam root-rot in Cameroon, possibly the same disease, is caused by *P. myriotilum* (Nzietchueng, 1985). In Costa Rica, bacteria of the genera *Erwinia* and *Pseudomonas* has been associated with the disease (Vargas, 1989; Bosque-Vega, 1991).

In Puerto Rico, Dominican Republic and Costa Rica as well as Cameroon, cocoyam production has been steadily declining as a consequence of this disease and has reduced yields up to 90% in some cocoyam plantations. A strategy to control mal seco is urgently needed. Clean 'seed' is an important part of this strategy.

Tissue culture techniques have been shown to be an effective alternative for producing large amounts of pathogen-free cocoyam planting material (Hartman, 1974; Salazar *et al.*, 1985; Gómez *et al.*, 1989 and Zettler *et al.*, 1991). However, the unit cost per tissue culture plant is high for direct use as planting material and the rapid reinfection limits the use of the new technology.

A more cost effective approach to produce *Xanthosoma*, whereby the benefits of micropropagation can be realized, is through the establishment of mother blocks, where pathogen-free stocks can be grown in areas with little inoculum pressure and ultimately released as certified stock for planting. This study was designed to show the results of a research program on producing cocoyam pathogen-free planting stock through tissue culture technique and field multiplication on mother blocks.

MATERIAL AND METHODS

Plant material and surface sterilization

Shoot-tips were excised from plants of (*Xanthosoma sagittifolium* L Schott) collected in the Atlantic area of Costa Rica. Shoots were trimmed to approximately 2 cm³ rinsed in flowing tap water, submerged 15 min in 1.25% sodium hypochlorite and rinsed three times in sterile distilled water in an aseptic room.

In vitro culture

With the aid of a dissecting microscope, each shoot was then trimmed until only the apical dome and one or two primordial leaves remained. Finally, each shoot was transferred to 18 x 150 mm test tubes containing 10 ml of establishment medium composed of Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) plus 0.1 mg. L⁻¹ 6-benzylaminopurine (BA), 3% sucrose and 0.18% gelrite. For axillary bud development, explants were cultured in a 125 ml baby food jar containing 25 ml multiplication medium composed of MS plus 3 mg. BA, 3% sucrose and 0.18% gelrite and for plantlet growth, explants were cultured in a 200 ml baby food jar containing 25 ml of a MS medium without growth regulators plus 4% sucrose and 0.18% gelrite. The pH was adjusted to 5.7 before autoclaving for 15 min at 121 °C. Explants were cultured at 23 ± 2 °C under 12 hr photo-

period. The light source was cool white fluorescent tubes (Phillips 60W) providing 50 $\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$.

Greenhouse culture

Plantlets were removed from the baby food jars and washed thoroughly with tap water to remove the gelled medium from the roots. They were submerged 30 seconds in a solution containing 5 ml Kilol L DF-100 and 1 ml Phosphorus per liter and transplanted to 72 compartment plastic trays, filled with sterilized substrate composed of soil and coconut fibre (1:1).

Plantlets were grown in the greenhouse under a plastic tunnel with intermittent mist (> 80% RH). Three days after transplanting and every weeks thereafter, the plantlets were fertilized (foliar applications) using 20:20:20 (N:P₂O₅:K₂O). Fertilizer mixed with water at the rate of 5 g L⁻¹. After three weeks, plantlets were moved from the plastic tunnel to a bench. The plants were ready for moving to the field four weeks after they were removed from the baby food jars.

Field establishment

At the field location the plastic trays with the plants were placed in shelter covered with plantain or palm leaves ('rancho'). After one week, they were removed from the plastic trays and transplanted to a polyethylene bags filled with 1 Kg. of soil and 10 g of 10:30:10 (N:P₂O₅:K₂O) granular fertilizer. The plants were ready for transplanting to the field three weeks after the shipment arrived.

Field experiment

One experiment was performed on a traditional production area (Los Diamante research station, Guápiles) from Nov. 1987 to Sept. 1988. Four treatments; 1. *in vitro* plants, 2. second generation planting material, cormels from *in vitro* plants and 3. third generation planting material, cormels from second generation of *in vitro* plants and 4. planting material (cormels) from local commercial plantations, were arranged in a randomized complete block design with four replications on 44 plants per plot.

Additional experiments were established on non-traditional production areas. One was set up on Bagaces, Guanacaste from Nov. 1989 to Nov. 1990 with 1000 *in vitro* plants. Another experiment was performed at Naranjito research sub-station, Quepos from Sept. 1990 to Nov. 1991. Two harvest treatments, 12 and 14 months, were arranged in a randomized complete block design with two replications on 250 *in vitro* plants per plot.

The plant spacing for the Guápiles experiment was 1 x 0.5 m, about 20,000 plants ha⁻¹. The distance for the Bagaces and Naranjito experiments was 1.5 x 0.4 m, about 16,666 plants ha⁻¹.

Guápiles site

Los Diamantes research station is located in the Atlantic zone, known as Limón. The mean monthly rainfall was 355 mm, the average daily temperature was 26 °C and 86% relative humidity. The soil texture was loam with good fertility.

Bagaces site

The Bagaces experiment was carried out in a private farm in the north-wester zone, known as Guanacaste. The mean monthly rainfall was 166.6 mm, the average monthly minimum and maximum temperature were 18.5 and 32.1 °C and 73.4% relative humidity. The soil texture was loam with good fertility.

Naranjito site

The Naranjito research sub-station is located in the South Pacific zone, known as Quepos. The mean monthly rainfall was 382.8 mm, the average daily temperature was 28 °C and 80% relative humidity. The soil texture was loam with low fertility.

Field practice

The Guápiles experiment was fertilized with 600 Kg. ha⁻¹ of a 10:30:10 (N:P₂O₅:K₂O) granular fertilizer. The fertilizer was applied at the rate of about 200 Kg. ha⁻¹ at planting, 1 and 2 months after planting. The Bagaces experiment was fertilized with 332 Kg. ha⁻¹ of 10:30:10 granular fertilizer, which was applied at the rate of about 166 Kg. ha⁻¹ at 1 and 3 months after planting. At the Naranjito experiment, 471.6 Kg. ha⁻¹ of 10:30:10 was employed one month after planting and after the third month, 471.6 Kg. ha⁻¹ of 20:3:20 was added.

Hand and chemical weed control was applied to all the experiments.

Data collection

The plants were measured for cormel production or yield, which was divided on exportation, local market and animal feed according to González classification (González, 1987) and corm diameter, weight and length. In addition, plants also were measured for DMV and 'mal seco' infection. Visual evaluation and ELISA test were carried out to evaluate DMV infection.

RESULTS AND DISCUSSION

In vitro responses

The attempt to culture pathogen-free plants of white cocoyam was successful. Within 16 weeks, shoot-tips developed into a plantlet with roots. Then each plantlet was tested for DMV infection using the ELISA test and 90% of them were virus-free.

For axillary bud development, virus free plantlets were trimmed at 1.5 cm from the corm-like structure. Then four explants were placed into a baby food jar containing 25 ml of multiplication medium. After six weeks, an average of four well developed axillary buds per explant was obtained. Then, each axillary bud was transferred to basal medium lacking growth regulators for rooting and growth. After six weeks, a well developed plantlet was produced. Then, plantlets can be recultured for axillary bud development or transferred to a greenhouse.

Field experiments

Preliminary experiments on cocoyam production areas showed that 100% of virus-free plants could be reinfected after a year. Therefore, 45% yield reduction was observed between reinfected plants and clean stock (data not shown).

The severity of DMV and 'mal seco' was reported in Table 1 for three generations of pathogen-free planting stock and a commercial 'seed'. The results showed that DMV severity did not increase in plants, which came from clean planting material (*in vitro*); however, it increased in plants which came from a commercial plantation (Table 1). The 'mal seco' severity increased during the evaluation time, in which plants from the commercial plantation were more affected (Table 1). The yield of all the treatments were affected by the DMV and 'mal seco' disease (Table 2). However, plants from clean stock showed higher total yield and marketable cormels than those from the commercial plantation.

The low yield obtained by first generation (*in vitro* plants) and from commercial stock

could be explained by 'mal seco' infection. Experiments with infected soil showed a rapid root destruction on *in vitro* plants, which lead to their death (data not shown). Planting material from the commercial plantation was already infected with 'mal seco.' Therefore, a rapid infection occurred affecting the plant development and yield.

The results of this experiment has shown the necessity of using pathogen-free planting material in order to get better yield. However, clean planting stock can not be produced on traditional producer zones due to DMV and 'mal seco' reinfection. Therefore, the establishment of mother blocks, where pathogen-free plants can be grown in isolated areas, were recommended.

Two isolated regions were selected to establish the first mother blocks for cocoyam pathogen-free production. The first area was located in Guanacaste, a dry area. During the experiment, no visual DMV and 'mal seco' symptoms were observed. In addition to that, ELISA tests were carried out on approximately 5% of the plants. All tested plants showed negative results.

The total yield was 0.88 Kg. plant⁻¹, about 14.6 ton ha⁻¹. The marketable yield (exportation cormels) was 7.69%. In addition to yield parameter, the average corm head length, diameter and weight per plant was 13.89 cm, 7.99 cm and 0.84 Kg. respectively.

The low yield of this experiment could be due to several factors, such as the cormels sprouting. Most of the cormels sprouted and average of 11 sprouts per plant was reported. Another factor was the leaf destruction due to strong wind, which may have affected the plants photosynthetic rate. The last factor was water stress. During the experiment, a drought was reported. Therefore, the plants were irrigated three times a week for 2 hrs a day with a sprinkler system. However, it may not have been enough to fulfill the plant water requirements. Water stress has been reported as one of the main causes of low cocoyam yield and its quality decreases in areas with low rainfall rates (O'Hair and Asokan, 1980; Silva and Irizarry, 1980; Onwueme, 1978).

The other non-traditional producer area evaluated was at Quepos, the Naranjito site. During the 14 months evaluation period, no DMV or 'mal seco' symptoms were observed and a sample of 10% of the total plants was selected for ELISA test. All of them were virus-free plants. In addition to ELISA test, 50 corm heads were taken for pathogen isolation tests, but none of them were reported as a 'mal seco' infected plant.

The harvest time (12 and 14 months) affected the total yield, cormel quality and corm head measurements (Table 3 and 4). Plants harvested at 12 and 14 months showed a total yield of 2.12 and 2.62 Kg. plant⁻¹, about 35.3 and 43.7 ton. ha⁻¹ respectively. This means an increase of 8.1% on total yield, about 8.4 ton. ha⁻¹, on plants harvested at 14 months. These plants showed a 7.1% increase on exported type cormels and a 3.81% decrease on waste or animal feed cormels. In addition to yield parameter, the corm head length and weight also increased on those plants 19.1 and 7.4% respectively, but a decrease of 8.1% of corm head diameter was reported in respect to plants harvested at 12 months (Table 4).

In conclusion, research has demonstrated conclusively the potential benefits of micropropagation for cocoyam pathogen-free production. However, the direct production of cocoyam planting stock through micropropagation is too expensive and therefore, small farmers can not get this type of material. Consequently, the establishment of isolated mother blocks for pathogen-free cocoyam production is an alternative to reduce the cost and to transfer the planting stock to small farmers.

A Costa Rica certification cocoyam pathogen-free program can be established, in accordance with the results presented in this paper. However, the application of this program will depend on a governmental decision.

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Table 1. DMV and 'mal seco' severity evaluation of three generations of pathogen-clean and a commercial planting material. Guapiles, 1988.

Treatments	Time after planting (months)					
	3		6		9	
	DMV	MS ¹	DMV	MS	DMV	MS
1 st Genera.	2.4	2.0	2.1	3.0	2.1	4.2
2 nd Genera.	2.6	2.0	2.2	3.0	2.2	4.0
3 rd Genera.	2.4	1.0	2.3	2.3	2.2	3.0
Commercial 'seed'	2.9	3.5	3.1	4.5	4.0	4.8

¹Mal Seco

Escape 1 - 5

1. No visual symptoms
2. Feathering on one leaf for DMV
Yellow leaves for 'mal seco'.
5. Feathering on more than two leaves or leaf deformed for DMV
Plant with just one yellow leaf alive for 'mal seco'

Table 2. Yield, by categories and total yield (ton ha⁻¹) of three generations of pathogen-clean and a commercial planting material. Guapiles, 1988.

Treatments	Yield (ton ha ⁻¹) ¹			
	Exportation Market	Local Market	Reject	Total Yield
1 st Generat.	0.31	2.4	2.41	5.12
2 nd Generat.	2.28	4.29	3.67	10.24
3 rd Generat.	6.83	6.40	2.65	15.88
Commercial 'seed'	0.00	0.59	0.96	1.55

¹2000 plants ha⁻¹

Table 3. Yield, by categories, total yield (ton ha⁻¹) of *in vitro* white cocoyam harvested at 12 and 14 months after planting. Quepos, 1991.

	Corm yield (Kg plant ⁻¹) ± SD	
	12 months	14 months
Exportac. Market	0.74 ± 0.27	1.10 ± 0.45
Local Market	1.09 ± 0.39	1.27 ± 0.53
Reject	0.29 ± 0.22	0.25 ± 0.22
Total Yield	2.12 ± 0.66	2.62 ± 0.96
Ton ha ⁻¹	35.3	43.7

¹16666 plants

Table 4. Corm head length, diameter and weight on *in vitro* white cocoyam harvested at 12 and 14 months after planting. Quepos, 1991.

Treatments	Corm head ± SD		
	Length (cm)	Diameter (cm)	Weight (Kg)
12 months	21.77 ± 3.61	8.89 ± 1.89	1.50 ± 0.41
14 months	26.92 ± 3.85	8.17 ± 1.68	1.62 ± 0.59

¹16666 plants