Tanier (*Xanthosoma* spp.) propagation in vitro[1, 2]

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ABSTRACT

The standard medium for in vitro propagation of taniers (*Xanthosoma sagittifolium* and *X. violaceum*) was improved by supplementing Murashige and Skoog's basic formula (MS) with 2 mg/L glycine, 5 mg/L indoleacetic acid (IAA), 2 mg/L kinetin and 10% coconut water (v/v). Gamborg's B_5 medium was found better than Abo-Zettler (AZ) and MS media for callus formation. Clorox 10% for 10 minutes, Clorox 5% for 5 minutes and Clorox 1% for 1 to 2 minutes consecutively was the best combination for surface sterilization. Bactericides, actidione 0.02% and sodium azide 1/40 were ineffective. Protoplasts of approximately 4 x 10^5 per ml were isolated and calcium oxalate crystals were eliminated with the modified Binding techniques. Data obtained from two replicated field trials at the Gurabo Agricultural Experiment Station indicated that the average weight of corms from apparently healthy tanier plants was significantly ($P < .01$) more than that of corms from plants with dasheen mosaic virus symptoms. Similarly, the average weight of corms produced by tanier plants free from the symptoms of "mal seco" disease was significantly ($P < .01$) more than that of corms obtained from plants affected by the disease. Variations in shape and size of the leaves as well as in dasheen mosaic virus symptoms among the plantlets are described.

RESUMEN

Propagación in vitro de la yautía (*Xanthosoma* spp.)

Los nutrimentos glicina (2 mg./l.), ácido indolacético (IAA, 5 mg./l.), cinetina (2 mg./l.) y agua de coco (10%, v/v) mejoraron grandemente la propagación de yautías (*Xanthosoma sagittifolium* y *X. violaceum*) *in vitro* al incorporarse al medio basal Murashige & Skoog (MS). El medio de Gamborg (B-5) fue mejor para desarrollar callos que el medio de Abo-Zettler (AZ) y el MS. El método más eficaz para esterilizar la superficie del tejido fue Clorox al 10% por 10 minutos, 5% por 5 minutos y 1% por 1 a 2 minutos. Los bactericidas actidiona 0.02% y azida sódica (1:40) para esterilizar no fueron eficaces. Se aislaron aproximadamente 4 x 10^5 protoplastos por ml. Se eliminaron los cristales de oxalato de calcio usando la técnica de Binding. El peso medio de los cormos de las plantas sanas fue mayor ($P < 0.01$) que el de las plantas con virus del mosaico de la malanga (dasheen mosaic virus—DMV). El peso medio de los cormos de las plantas libres de mal seco fue mayor que el de las plantas enfermas. Se describen

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variaciones en forma y tamaño de las hojas y la expresión de los síntomas del virus del mosaico de la malanga.

INTRODUCTION

The farm value of the tanier (*Xanthosoma* spp.) crop reached $5.3 million in 1986 (3). Tanier production has decreased recently because of high incidence of "mal seco" disease and possibly dasheen mosaic virus. The application of meristem culture methods to vegetatively propagated plants such as tanier has become increasingly important because pathogen-free stock plants can be generated, maintained and increased with relative ease. From 1974-1980, several investigators (1,11,26) initiated *in vitro* culture of *Colocasia* and *Xanthosoma* in Florida and California. In 1980, Licha et al. (14) initiated in Puerto Rico meristem culture and induced callus and shoot development of taniers. Variations in morphology and dasheen mosaic virus symptoms in tanier plantlets derived from callus culture were also reported (15,16,17). While working in this laboratory in 1985, Gupta described similar morphological variations and culture media for callus induction and plant regeneration of *Xanthosoma* (10). Difficulties are frequently encountered by growers who want to establish meristem culture of taniers in Puerto Rico because of the lack of comprehensive data on culture media, sources of materials used as well as sterilization techniques. This paper describes the methods developed in this laboratory for *in vitro* callus induction, shoot differentiation and protoplast isolation in taniers. The average weight of corms observed under field conditions from both apparently healthy tanier plants and those with dasheen mosaic virus or "mal seco" disease symptoms are also compared statistically.

MATERIALS AND METHODS

The corms of *Xanthosoma sagittifolium* cv. Blanca del País and *X. violaceum* cv. Morada (Vinola) used in this study were obtained from the Corozal Agricultural Experiment Station. Shoot tips were sterilized in 10% Clorox for 10 minutes, followed by 2 to 3 washings with sterile distilled water containing 0.1% ascorbic acid. Outer layers of the sterilized shoot tips were removed under a dissecting microscope in the laminar flow. The meristematic domes bearing 3 leaf primordia (0.2 to 0.4 mm long) were cut out and cultured on various culture media.

Studies were made in the attempt to determine the effect of culture media on callus formation and growth of tanier, disinfectants on surface sterilization, bactericides and fungicides on contamination, and plant growth regulators on callus induction and shoot differentiation.

For the culture media study, the following 6 substrates were evaluated: a) Murashige and Skoog's medium (21) with 20 g/L sucrose, 1 g/L myo-inositol and 1 p/m 2,4-dichlorophenoxyacetic acid (2,4-D); b) re-

FIG. 1.—Disease-free plantlet of tanier (*Xanthosoma* sp.) via meristem-tip culture (test tube stage).

vised tobacco medium with vitamins and increased sugar (5 g/L myo-inositol, 30 g/L sucrose) and 1 mg/L 2,4-D; c) Murashige and Skoog's medium plus increased sugar, no 2,4-D, but modified by adding (i) glycine, indoleacetic acid and 6-furfuryl amino purine (kinetin) and (ii) casein, naphthaleneacetic acid (NAA) and kinetin; d) Murashige and Skoog plus increased sugars (5 g/L myo-inositol, 20 g/L sucrose, 10 g/L glucose) and 1 mg/L 2,4-D; e) AZ (1) medium; and (f) Gamborg's B_5 medium (9).

For the study on surface sterilization, the following 4 methods were evaluated: 70% alcohol for 1 min, 10% Clorox for 1 min; 70% alcohol for 1 min, 2% Clorox for 15 min; 3% Lysol for 1 min, 70% alcohol for 1 min,

2% Clorox for 15 min; and 10% Clorox for 10 min, 5% Clorox for 5 min, 1% Clorox for 1 to 2 min, and by rinsing them afterwards 3 times with double-distilled sterile water.

For the study on protoplast isolation, plantlets previously incubated at 27° C, 380 footcandle light intensity and 16 h photoperiod were transferred to a dark chamber for 24 to 72 h, and subsequently used for isolating protoplasts. Approximately 2 to 5 g of leaf tissue were sterilized 3 min with 10% Clorox and Tween 80 (1 drop/25 ml) solution and rinsed 3 times with autoclaved distilled water. The sterilized leaf tissue was cut with a sterile scalpel and the resulting materials filtered through a 120 μ filter. After filtration, the fine pieces of leaf tissues were suspended overnight (15 to 17 h) in an enzyme solution consisting of 0.25% cellulase, 0.25% macerozyme, and 0.6 M mannitol. The suspension was adjusted to pH 5.8 with 0.1 *N* NaOH, diluted with seawater (730 millimol), poured into test tubes and centrifuged 3 times at 1,000 r/m. After centrifugation, the seawater in the suspension was replaced with a 0.6 M sucrose solution. As soon as the seawater was replaced, protoplasts rose to the surface of the sucrose solution forming a green band at the upper level of the test tubes. These floating protoplasts in the band were isolated with a fine pipette, cultured in paraffilm sealed petri dishes containing the liquid medium V-47 (4,5), and incubated at 22° C under 15 footcandle light.

The above-mentioned procedure was a modification of the Binding's method (4,5) for protoplast isolation. Ricardo Alfonso's method (2) for protoplast isolation was also tried. The Alfonso method consisted of a lower speed of centrifugation (700 r/m) than the modified Binding's method and a washing solution as described by Kao (13).

Two field experiments were established in 1981 at the Gurabo Agricultural Experiment Station to determine the effect of dasheen mosaic virus on corm weight and the effect of "mal seco" disease on corm weight.

For the study on the effect of dasheen mosaic virus on yield of corms, variety Blanca del País was selected. A total of 1,116 plantlets derived from meristem culture of taniers were used. Half were inoculated mechanically with the dasheen mosaic virus by the method of Monllor et al. (20) and the other half were not inoculated. The inoculated plantlets showing the dasheen mosaic virus symptoms were planted in the field for comparison with the non-inoculated plantlets (presumably mosaic-free). The weights of corms were recorded.

For the study on the effect of "mal seco" on yield of corms, an equal number of plantlets were planted in the area heavily infested by the disease. On the basis of symptoms produced, plantlets were divided into 2 groups: the apparently healthy and the "mal seco" affected plants. They were planted in 24 plots. Corm weights were recorded.

RESULTS

Effect of culture media on callus formation and plant regeneration

Of the 6 different culture media tested, Gamborg's B_5 medium (9) was found better than all the other media for callus induction. With this medium, calluses were induced 6 weeks after culturing. There were no significant differences in callus formation between varieties Blanca del País and Vinola. Callus formation seemed to be faster in the dark than in light. The effect of various hormones and amino acids on plant regeneration was also studied. The MS medium containing 2 mg/L glycine, 5 mg/L IAA and 2 mg/L kinetin plus 10% coconut water (v/v) appeared to be the best for shoot differentiation and proliferation (fig. 1).

Effect of disinfectants on surface sterilization

None of the 4 different combinations of disinfectants involving alcohol and Clorox was better than surface sterilization consisting of 3 changes in Clorox (10% for 10 min, 5% for 5 min and 1% for 2 min). The combination of alcohol with Clorox did not yield satisfactory results, nor Lysol with Clorox.

Effect of bactericides and fungicides on contamination

Actidione at 0.02% and sodium azide at 1/40 were added to the Murashige and Skoog (21) and AZ (1) media. Neither was effective for controlling surface contamination.

Protoplast isolation

Binding's method (4,5) as modified by us consists of a) 1,000 r/m centrifugation, b) 730 millimol seawater as washing solution and c) overnight (15-17 h) enzyme solution treatment for leaf materials. This modified method was found better for tanier protoplast isolation than other methods and combinations of methods. A large number of protoplasts (4×10^5/ml) were obtained, and at the same time calcium oxalate crystals were eliminated (fig. 2 and 3). The protoplasts were cultured in V-47 medium (4,5). Divisions beyond the single-protoplast stage were observed (fig. 4).

Yield trials

The two field experiments which had been established in 1981 at the Gurabo Substation were harvested in 1982. The corms from apparently healthy tanier plants derived from meristem cultures were significantly ($P < .01$) heavier than those of corms from plants with dasheen mosaic virus symptoms. Figure 5 shows that the average weight of corms from the plants free from the symptoms of dasheen mosaic virus ranged from 0.3 to 0.7 kg, whereas the average weight of corms from the plants affected by the virus ranged from 0.1 to 0.3 kg. The affected plants also

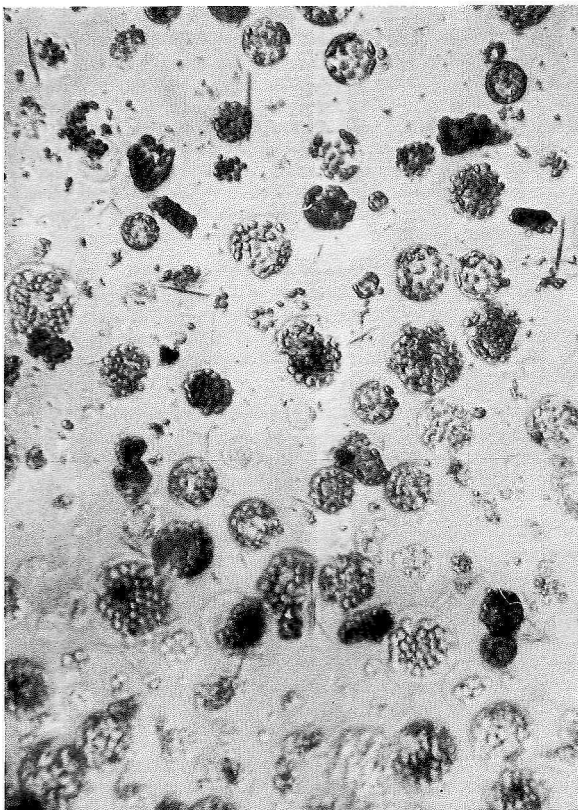

FIG. 2.—Protoplasts from the leaves of *Xanthosoma sagittifolium* (note the presence of calcium oxalate crystals).

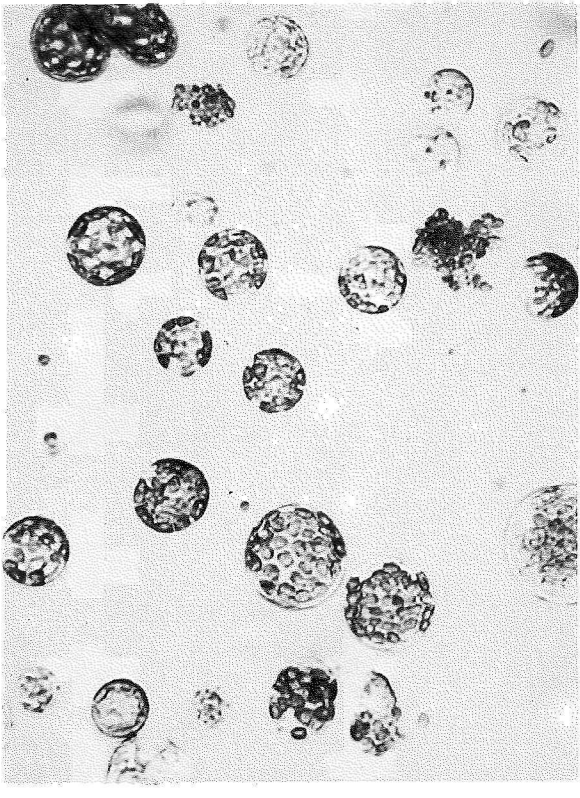

FIG. 3.—Protoplasts isolated from the leaves of *Xanthosoma sagittifolium* (note the absence of the calcium oxalate crystals).

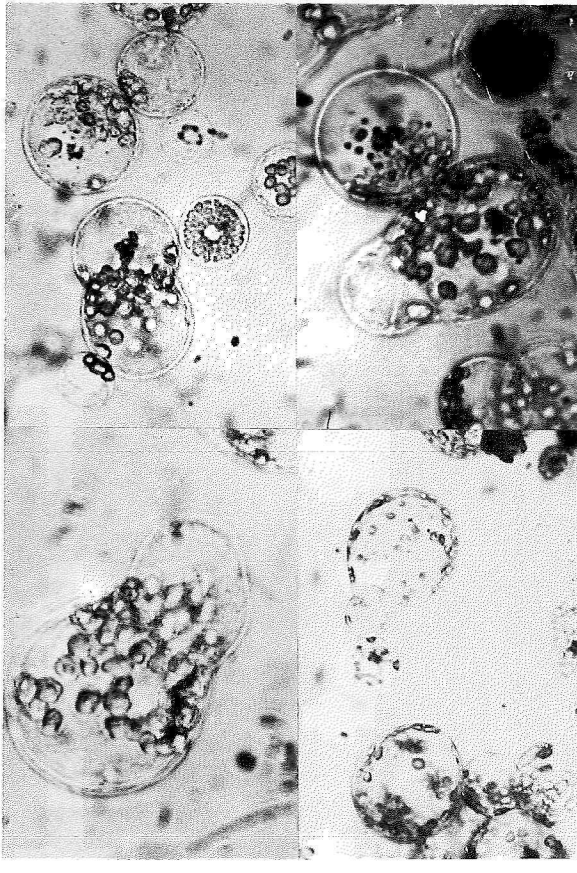

FIG. 4.—Protoplasts of tanier in the culture medium V-47 (note the elongation and division of the protoplasts).

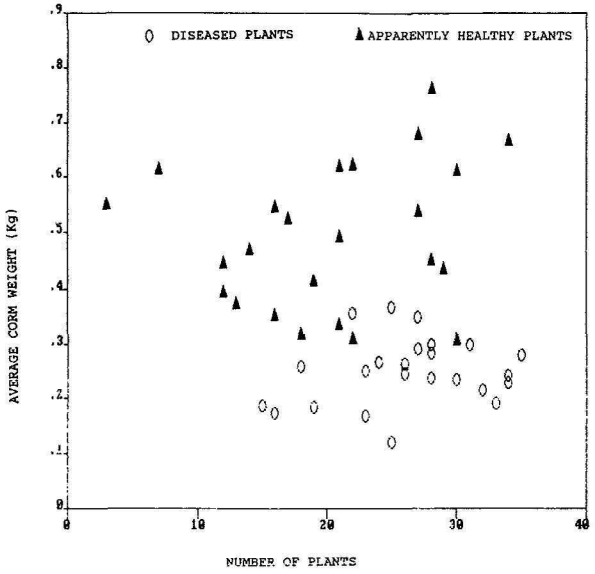

FIG. 5.—Average corm weight of apparently healthy (meristem culture) and dasheen mosaic virus-affected plants of *Xanthosoma sagittifolium*.

showed a general discoloration and depressed growth. In addition to the morphological variations (narrow elongated leaves, figure 6), we also observed variations in disease symptoms (mottling, vein chlorosis, feathery and malformation (fig. 7)).

Similarly, the corms from the apparently healthy taniers (free from the "mal seco") were significantly ($P < 1.01$) heavier than those of corms from plants affected by the "mal seco". The average weight of corms from the apparently healthy taniers ranged from 0.2 to 1.0 kg, whereas those of corms from "mal seco" affected taniers ranged from 0.2 to 0.5 kg (fig. 8).

DISCUSSION

Successful induction of calluses and shoots in taniers depends upon a number of factors, such as sources of materials and culture media as well

FIG. 6.—Variations in shape of leaves among plants of *Xanthosoma sagittifolium* derived from tissue culture.

as disinfection techniques. In general, high concentration of auxin and low concentration of cytokinins promote callus formation. On the contrary, high concentration of cytokinin and low concentration of hormones, especially 2,4-D, promotes shoot differentiation. The results obtained in this study indicated that Gamborg's B_5 medium (9) with high concentration of salts stimulates callus formation in taniers. It was found better than the AS medium (1) which was favored by other investigators (10).

The Murashige and Skoog medium (21), containing 20 mg/L glycine, 5 mg/L indoleacetic acid and 2.0 mg/L kinetin, appeared to be the best for shoot differentiation and proliferation.

Careful peeling of the leaves surrounding the meristem dome under aseptic conditions should keep the explants considerably free from contamination. In order to assure a maximum sterilization with minimum adverse effect on explant tissues or lateral buds, two or three changes of Clorox are needed. Clorox at 10% for 10 min, 5% for 5-30 min and 1% for 1 to 3 min with continuous agitation has been adopted as a routine procedure for tanier surface sterilization. Preconditioning the plants under aseptic conditions would also help to reduce some of the contamination and oxidation problems.

The size and condition of the explants should also be taken into consideration. The smaller the size of the explant, the shorter the time it needs for sterilization. Clorox and alcohol were found to be ideal disinfectants for surface sterilization of taniers. Bactericides, such as actidione and

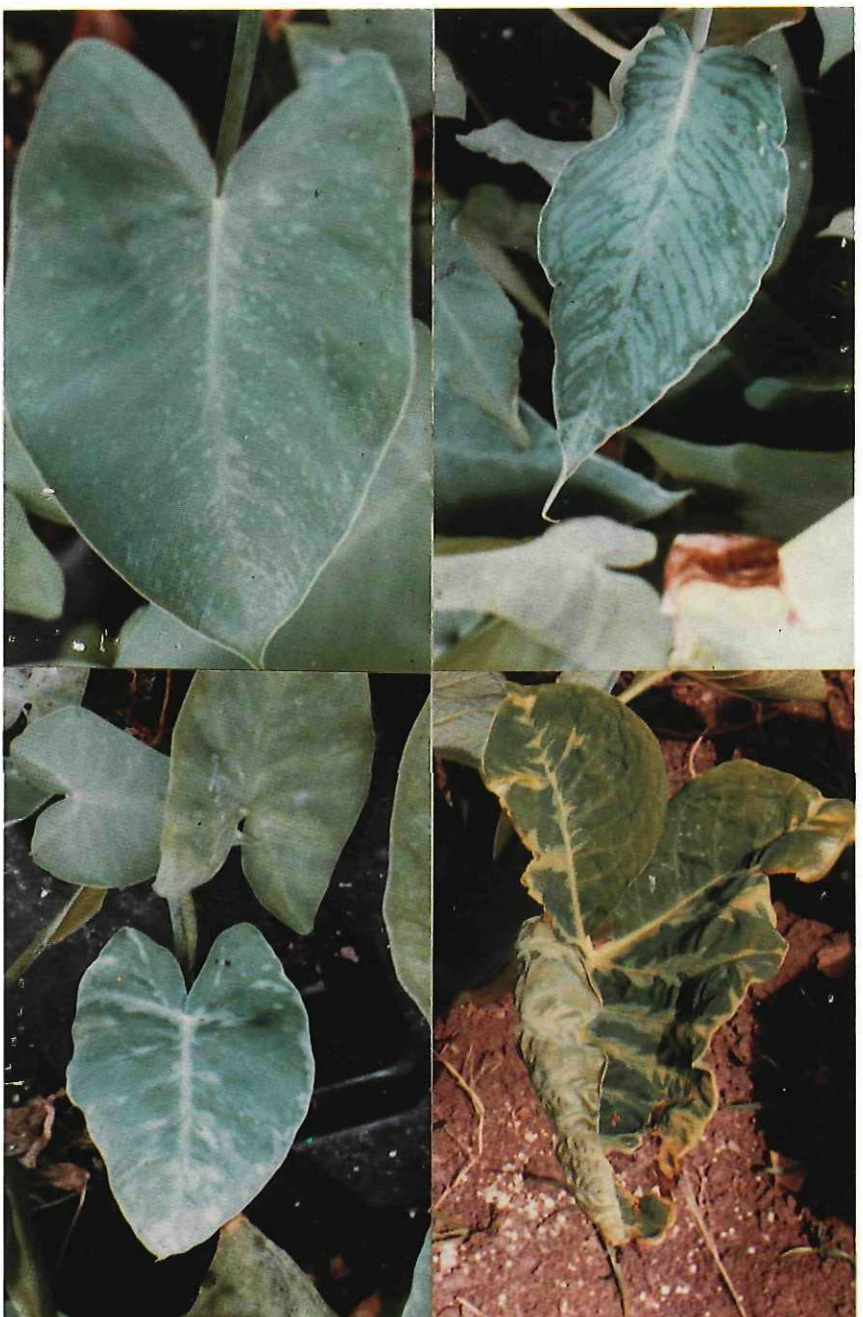

FIG. 7.—Variations in dasheen mosaic virus symptoms among plantlets of *Xanthosoma sagittifolium* derived from tissue culture. (Upper left: mottling and upper right: vein chlorosis. Lower left: feathery and lower right: malformation).

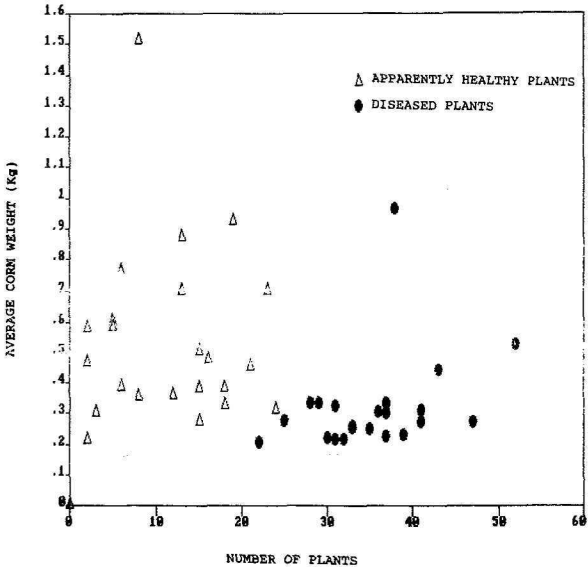

FIG. 8.—Average corm weight of apparently healthy (meristem culture) and "mal seco" diseased plants of *Xanthosoma sagittifolium*.

sodium azide, as well as fungicides such as mercuric chloride, were found to be inadequate for surface sterilization of taniers.

Protoplasts have been isolated from *Datura* (24), *Daucus* (8), *Nicotiana* (19,25,27), *Petunia* (22,27), rice (6,7), corn (21,22), and sugarcane (18). However, isolation of protoplasts from monocotyledonous plants such as tanier is a difficult task. Sugar and inorganic salts are known to influence cell regeneration and sustain division in plant protoplasts (12,18). With additional sugar and salts, Binding's V-47 medium (4,5) would probably sustain cell division better.

Yield increase obtained in this study should be of great interest to local growers because it provides evidence to indicate that quality of seeds may play an important role in commercial production of taniers in Puerto Rico. The variability in morphology as well as in disease

symptoms in the population of tissue culture taniers as observed in this study would also encourage plant breeders to use this technique for selecting clones resistant to "mal seco" and dasheen mosaic virus.

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