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THE INFLUENCE OF AUXIN AND CYTOKININ COMBINATIONS PLANT REGENERATION OF COCOYAM (Xanthosoma sagittifolium)

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ABSTRACT

The standard Murashige and Skoog (1962) culture medium was proven effective for initiation of meristem in cultures of cocoyam (tannia; Xanthosoma sagittifolium cv. Rabess) but was found not to be so useful for plantlet regeneration. However, when cultures at 6-8 weeks old were transferred to an amended medium /with the exclusion of benzylaminopurine (BAP)/ calli formation was high along with organogenesis, resulting in extensive shoot and root development. Plantlet regeneration from meristems was achieved within 16-20 weeks. There was no significant variation in plant morphology of plantlets regenerated using this two-step production system.

INTRODUCTION

Cocoyam /tannia; Xanthosoma sagittifolium (L.) Schott/ is important to Dominica as a foreign-exchange earner and holds fourth place after banana, plantain, and dasheen (taro) as an export crop (Central Statistics Unit, 1987).

Tannia, like dasheen (taro) is a member of the family Araceae. It is plagued by destructive diseases such as dasheen mosaic virus (DMV) and pythium root rot (Pythium myriotylum). Pythium root rot, more commonly known as tannia rapid yellowing disease (TRYD) is still the most desctructive disease. It has the potential for causing catastrophic losses to tannia production in Dominica and elsewhere in the Windward Islands group, including St. Lucia, St. Vincent, and Grenada. An importnat element of the technological package (tech-pack) for control of pythium root rot disease is the planting of clean, healthy and vigorous plant material (CARDI, 1986). Because the crop is propagated vegetatively, the disease is easily spread by plant propagules.

The advancement of plant tissue and cell culture techniques over the past two decades has enabled plant propagators to achieve not only recovery of pathogne-free clones but also rapid clonal multiplication.

There are several reports (Hartman, 1974; Liu et al., 1988; McDonald et al., 1990; Strauss and Ariditti, 1980; Zettler and Hartman, 1987) that have demonstrated major successes in micropropagation of several species of Xanthosoma. These successes were largely due to the refinement of culture media over the years. The achievements also depended on the understanding of the influence of growth hormones (in particular auxins) and cytokinins, in plant regeneration from meristems.

The aim of this study was to devise a system for rapid clonal multiplication of X. sagittifolium that is not only efficient but also economical.

MATERIALS AND METHODS

Apical meristems were obtained from corms of 9-month-old cocoyam (tannia) mother plants cv. Rabess) known to be disease-free and from a farm with a history of high yields. Shoot tips were trimmed to $\leq 1.5cm^3$ and rinsed in flowing tap water for about 10 minutes. The meristem apices were then surface-sterilized in 0.52 per cent sodium hypochlorite (containing 1 drop of Tween^R 20 per 100ml) for 10 minutes, trimmed further and then submerged in 0.26 per cent sodium hypochlorite (containing Tween^R 20; 1 drop per 100ml) for 5 minutes. Three washings with sterile distilled water preceded the removal under a dissecting microscope in a laminar flow chamber of the outer layers of the sterilized shoot tips. Each shoot was then trimmed until the apical dome and two leaf primordia, 0.2-0.4mm long, remained. The meristematic dome was then transferred aseptically and cultured in solid Murashige and Skoog (MS) nutrient agar (Murashige and Skoog, 1962).

The MS basal culture medium was used for the initiation of meristems. Six to eight weeks after initiation, cultures with undifferentiated calli, as well as those with green calli and shoot differentiation, were transferred aseptically to magenta GA 7 vessels each containing 50ml of the test media. There were six replicates for each medium composition.

Twelve concentrations and combinations of cytokinin (BAP) and auxin (NAA) were tested in media compositions for callus differentiation, shoot and root development, and consequent plantlet regeneration, including the MS basal medium which was used as the standard medium for comparisons.

0.5mg per l BAP / 0.5mg per l NAA
0.4mg per l BAP / 0.5mg per l NAA
0.3mg per l BAP / 0.5mg per l NAA
0.2mg per l BAP / 0.5mg per l NAA
0.1mg per l BAP / 0.5mg per l NAA
0.0mg per l BAP / 0.5mg per l NAA
0.5mg per l BAP / 0.4mg per l NAA
0.5mg per l BAP / 0.3mg per l NAA
0.5mg per l BAP / 0.2mg per l NAA
0.5mg per l BAP / 0.1mg per l NAA
0.5mg per l BAP / 0.0mg per l NAA
0.0mg per l BAP / 0.1mg per l NAA

All media were adjusted to pH 5.8.

All plantlets which had differentiated in cultures were transplanted to 7.5cm plastic cups containing a sterile mixture of sand:soil:perlite (1:1:1 ratio). All plantlets were weaned in a plastic cloche for 2-3 weeks before being transferred to a field nursery. Counts were made of the number of plantlets developed for each medium composition and then this was expressed as a percentage of six replicates.

RESULTS AND DISCUSSION

The standard MS basal culture medium does not seem to be the most suitable for rapid multiplication of cocoyam (Xanthosoma sagittifolium cv. Rabess) from meristems, giving only slight induction of green callusing (Table 1). Callus formation was less than 25 per cent and

Table 1. Morphogenetic responses from green calli and shoots of X. sagittifolium after 6-8 weeks of culturing on MS medium amended with various combinations of growth hormones.

Plant hormones (mg/l)		Nature of response[ab]			
BAP	NAA	G[c]	S[c]	R[c]	PR[c]
0.5	0.1 (standard)	+	+	+	+
0.5	0.5	++	+	-	-
0.4	0.5	++	+	+	+
0.3	0.5	++	+	-	-
0.2	0.5	++	+	-	-
0.1	0.5	++	+	-	-
0.0	0.5	++	+	+	-
0.5	0.4	+++	++	+	+
0.5	0.3	+++	++	+	+
0.5	0.2	+++	++	+	+
0.5	0.1	+++	++	++	++
0.0	0.1	++	+++	+++	+++

a. Average of six replicates per treatment.
b. No. of + signs and - signs indicate cultures showing varied amount of the respective forms of differentiation as a percentage:

- = 0
+ = 0 - 25%
++ = 26 - 50%
+++ = > 50%

c. G = Green callus; S = Shoot differentiation; R = Root differentiation; PR = Plantlet regeneration.

subsequent organogenesis was even lower, resulting in only 2 per cent organ differentiation and poor plantlet regeneration (Table 1). Similar results were reported by McDonald and Royer (1989) when a system was being developed for in vitro propagation of cocoyam in Dominica. Several workers have for some time now demonstrated that the MS standard medium requires amendment for satisfactory regeneration of tannia plantlets from meristems (Gupta, 1985; Liu et al., 1988; McDonald et al., 1990). All the investigators studied the effect of types and concentrations of plant hormones in culture media for plant regeneration. Gupta (1985) established parameters for

initiating and maintaining callus cultures and subsequent regeneration of plantlets from apical meristems in two species of Xanthosoma.

High concentration of the auxin, naphthaleneacetic acid (NAA) seems to cause extensive callusing (Table 1), as was reported by Gupta (1985). However, high NAA and low concentrations of BAP seemed not to be the most satisfactory combination for plantlet regeneration from indirect organogenesis (Table 1). A high percentage of plantlet regeneration was likewise not derived from high concentrations of both NAA and BAP.

Organogenesis from differentiated calli in amended medium with no BAP and a NAA concentration of 0.1mg per l was repeatedly high. The induction of green callus was also high and continually produced leaf clumps after repeated sub-cultures. Shoot and root differentiation occurred from the leaf clumps within 8 weeks, and subsequently plantlet regeneration. Highest plantlet regeneration (> 50 per cent) was obtained and completed within 16-20 weeks (Table 1).

The 2-step system (i.e. 1. initiation in standard MS medium and 2. transfer after 6-8 weeks to amended medium, for plant regeneration of X. sagittifolium cv. Rabess proved to be efficient and there was no indication of any significant variation of plant morphology in over 150 regenerated plantlets. There were also no visible symptoms of dasheen mosaic virus infection.

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