TISSUE CULTURE OF *CURCULIGO LATIFOLIA* DRY. *ex* W.T. AIT. (HYPOXIDACEAE)

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ABSTRACT

This paper reports the success in clonal propagation of *Curculigo latifolia* at the Tissue Culture Laboratory, Singapore Botanic Gardens. Experiments using leaf blades, petioles, apical shoots, and rhizomes were carried out and the best results were obtained from rhizome cultures. The medium used contained the Murashige and Skoog inorganic salts (half strength), sucrose (30 g per litre), thiamine (0.4 mg per litre), coconut milk (150 ml per litre), kinetin (5 mg per litre), and IAA (2.5 mg per litre). Mass propagation of this species is in progress.

INTRODUCTION

Singapore, often called the Garden City, is famous for its parks, gardens and, most of all, the refreshing sight of the green canopy of big trees and luxuriant ornamental plants that grace the city especially along the roadsides. Rapid urban development has created many new environments, such as open spaces, under flyovers, or reclaimed land, where many plants find it difficult to grow and thrive. There has been considerable effort in trying to cultivate more species of native plants that can grow in these various new growth conditions. *Curculigo latifolia* is one species which has been found suitable for landscaping needs under flyovers which are shaded and sometimes rather dry.

Curculigo latifolia is an elegant native herb which grows under fully shaded forest conditions. It is a stemless herb with long leaves and short, thick rhizomes. The inflorescences, which are produced throughout the year, are compact, head-like, close to the ground, and have numerous flowers with bright yellow tepals (Fig. 1). Although this species offers good prospects for ornamental purposes in Singapore, the problem of obtaining large numbers of this species has yet to be solved. *Curculigo latifolia* is not abundant in Singapore's forests, thus collection of many living plants would have an undesirable effect on the floristics and ecology of their native habitats. In an effort to find alternative means to supply abundant material of this species for roadside planting, tissue culture techniques were investigated. This paper reports the success in mass propagation of *Curculigo latifolia* using tissue culture techniques at the Tissue Culture Laboratory, Singapore Botanic Gardens.

METHODS AND MATERIALS

Young shoots of *Curculigo latifolia* about 8–10 cm in height were used for tissue culture. The plants were thoroughly washed to remove dirt on the leaves and soil on the rhizomes. The outer leaves and roots were then removed, leaving the apical tissue and the rhizomes.

The apical shoots, rhizomes, and leaves were then sterilized by soaking sequentially in:

(1) 10% detergent for 10 minutes,
(2) 75% ethyl alcohol for 30 minutes,
(3) 15% chlorox for 30 minutes, and
(4) three changes of distilled water for five minutes each time.

The following three different plant parts were excised for tissue culture:

(1) Shoot apices – The sterilized apical tissue was further trimmed to a small cube of about 1 cubic mm in size containing the apical meristematic tissue of the shoot.

(2) Leaves – The whole leaf, blade and petiole, was cut into 5mm segments and placed proximal side down on agar medium.

(3) Rhizomes – The outer layer of the rhizomes was removed to reduce risk of contamination. The remaining part was then cut into sections and transferred onto an agar medium.

The agar medium used contained the inorganic salts of Murashige and Skoog (1962) at half strength with the addition of sucrose (30 g per litre), thiamine (0.4 mg per litre), coconut milk (150 ml per litre), and various hormones.

All cultures were incubated at a temperature of 20–28°C and exposed to Grolux light for 8 hours per day.

RESULTS

A total of 11 plants were used in the experiment. The number of cultures prepared using the various plant parts and their state of growth at the end of a 3 month period are listed in Table 1.

Table 1: Results of the tissue culture experiment of *Curculigo latifolia*

Explant	no. of cultures prepared	only callus formed	callus and shoots formed	callus, shoots, & roots formed	callus and roots formed	no. growth	conta–minated
Leaf blade	18	3	0	0	0	7	8
Petiole	20	3	6	0	0	8	3
Rhizome	20	3	1	7	2	3	4
Apical shoot	11	1	6	0	0	3	1

The most unsuccessful results were from those using leaves. Except for a few cases where callus formed (Fig. 2), the leaf cultures either showed no sign of growth or were contaminated. The difficulty is probably in the sterilization technique. To kill all the fungus spores stuck on the leaves, it was found necessary to use a sterilization duration which was probably too long in that the leaf tissue was also damaged.

The petiole and apical shoot cultures formed callus and shoots quite readily (Fig. 3), but were slow to form roots (root formation was observed 2–3 months after the end of the experiment).

The best results obtained were from rhizome cultures. Satisfactory growth of callus, shoots, and roots were observed in 7 out of the 20 cultures prepared (Fig. 4).

In order to encourage root formation, it was decided that hormones such as kinetin and IAA should be added to the agar medium used for incubation. A simple factorial experiment was conducted with three levels of kinetin (1, 2.5, 5 mg per litre) and five levels of IAA (0, 1, 2.5, 5, 10 mg per litre). Five replicates were prepared. In each test a single rootless shoot was introduced and incubated for three months. The results are listed in Table 2.

Table 2 shows that the best root formation and shoot multiplication were obtained at 5 mg per litre kinetin concentration. At 5 mg per litre kinetin concentration, although root formation improved with increasing concentration of IAA, shoot multiplication reached a clear peak at 2.5 mg per litre IAA concentration. Increasing the IAA concentration further appeared to strongly favour the formation of callus at the expense of shoot multiplication. Media discolouration also appear to become worse with increasing concentrations of kinetin or IAA.

Based on the above results, it was decided to use a medium containing the Murashige and Skoog (1962) basic salt (half strength), sucrose (30 g per litre), thiamine (0.4 mg per litre), coconut milk (150 ml per litre), kinetin (5 mg per litre), and IAA (2.5 mg per litre) for mass propagation work.

MASS PROPAGATION

Starting from the tissue taken from 11 plants, a stock of about 70 flasks of cultures were obtained after about 10 months. A production programme was then planned and put into operation. A culture flask normally contains 2–8 shoots at different stages of development. At 3 month intervals the shoots were taken out and separated into indivi-

Table 2 : Results of the factorial experiment on hormone-induced root formation of *Curculigo latifolia* shoots.

IAA (mg/L)	kinetin (mg/L)	medium discolouration*	callus formation*	root formation*	shoot multiplication (average no.)
0	1	none	+	none	1 (1)
1	1	none	+	none	1-2 (1.1)
2.5	1	none	+	none	1-3 (1.8)
5	1	+	+	none	1-2 (1.1)
10	1	++	+	+	2-3 (1.8)
0	2.5	+	+	+	1-2 (1.1)
1	2.5	+	++	++	1-2 (1.1)
2.5	2.5	++	++	++	0 (0)
5	2.5	++	++	++	2 (2)
10	2.5	++	++	++	2-5 (2.6)
0	5	++	+	++	1-2 (1.1)
1	5	++	+	++	3-5 (3.6)
2.5	5	++	++	+++	2-8 (5)
5	5	+++	+++	+++	1-5 (3)
10	5	+++	+++	+++	1-5 (3)

*+ = slight ++ = moderate +++ = high

dual plants. Those taller than 8 cm with strong roots were rinsed in distilled water and potted out. The smaller plants were transferred individually onto agar medium for multiplication.

The plants potted out were protected under a plastic cover for one week to help them adjust to nursery conditions. About 90% of the plants potted out have survived. After another 5-6 months these plantlets are normally ready to be planted in field. Fig. 5 shows some plants in the nursery.

About 90% of the plants produced so far have remained identical to the parent plants. The rest seem to produce more leaves and show some variation in leaf colour.

Based on the observed growth rate, it has been estimated that about 5,000 plants from tissue culture can be produced within the first year of mass propagation, sufficient to meet the current landscaping needs of the Parks and Recreation Department.

ACKNOWLEDGEMENT

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REFERENCE

Murashige, T. and F. Skoog 1962. A revised medium for rapid growth and bioassays with tobacco culture. Plant Physiol. 15, 473-497.

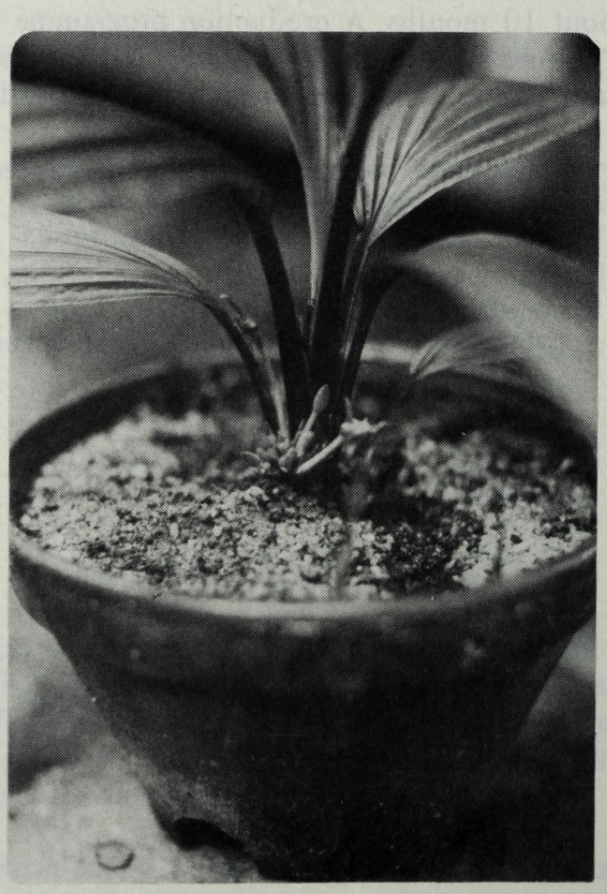

Fig 1. *Curculigo latifolia*, mature plant.

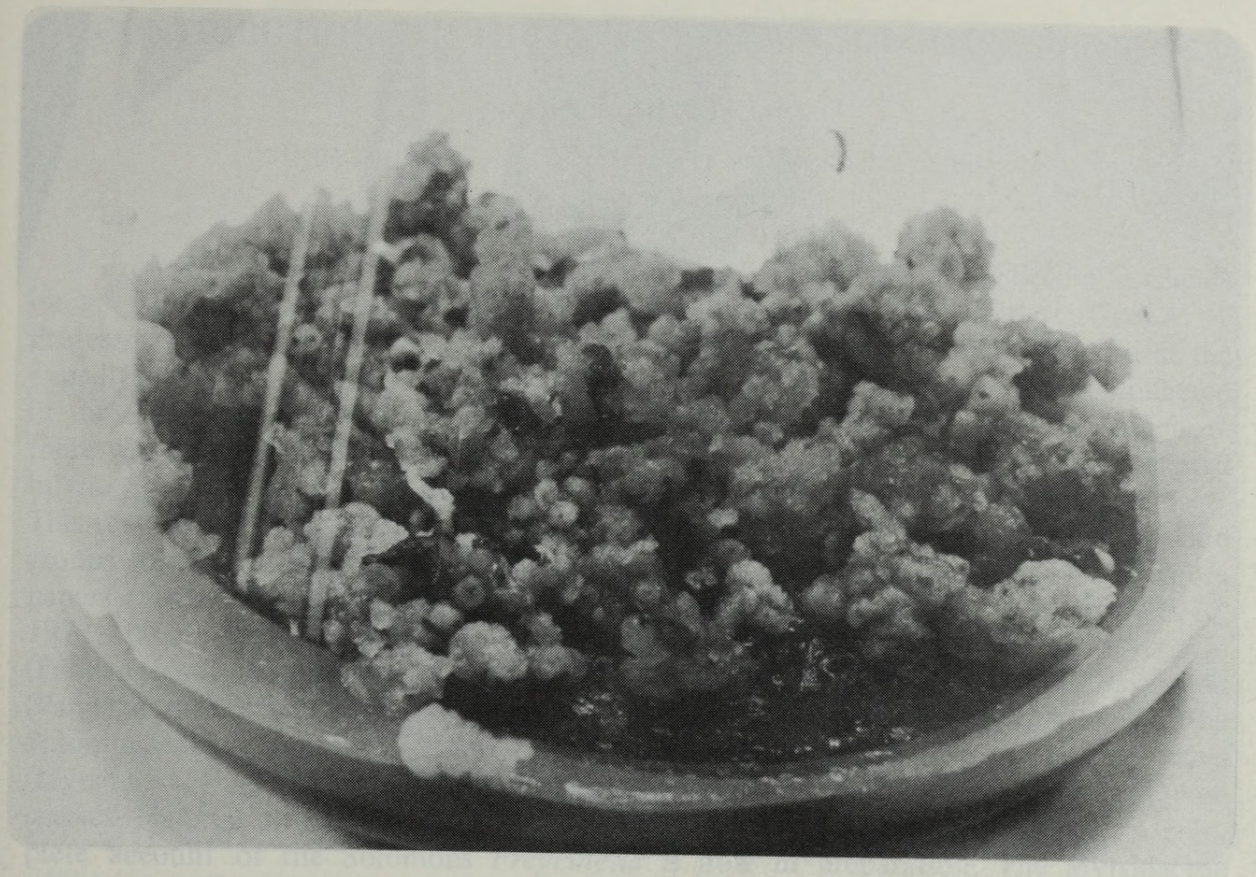

Fig. 2. Callus formed from leaf culture.

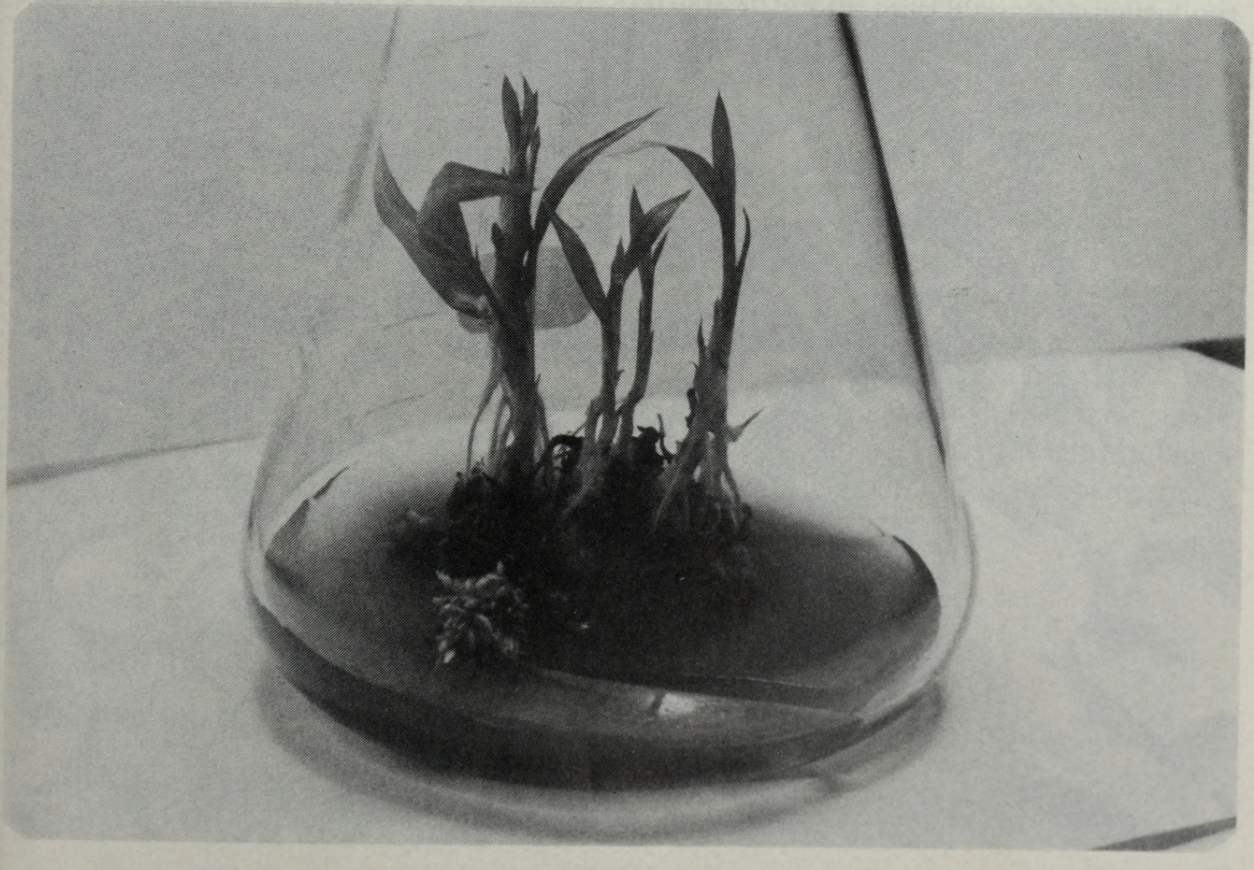

Fig. 3. Callus and shoots formed from apical shoot.

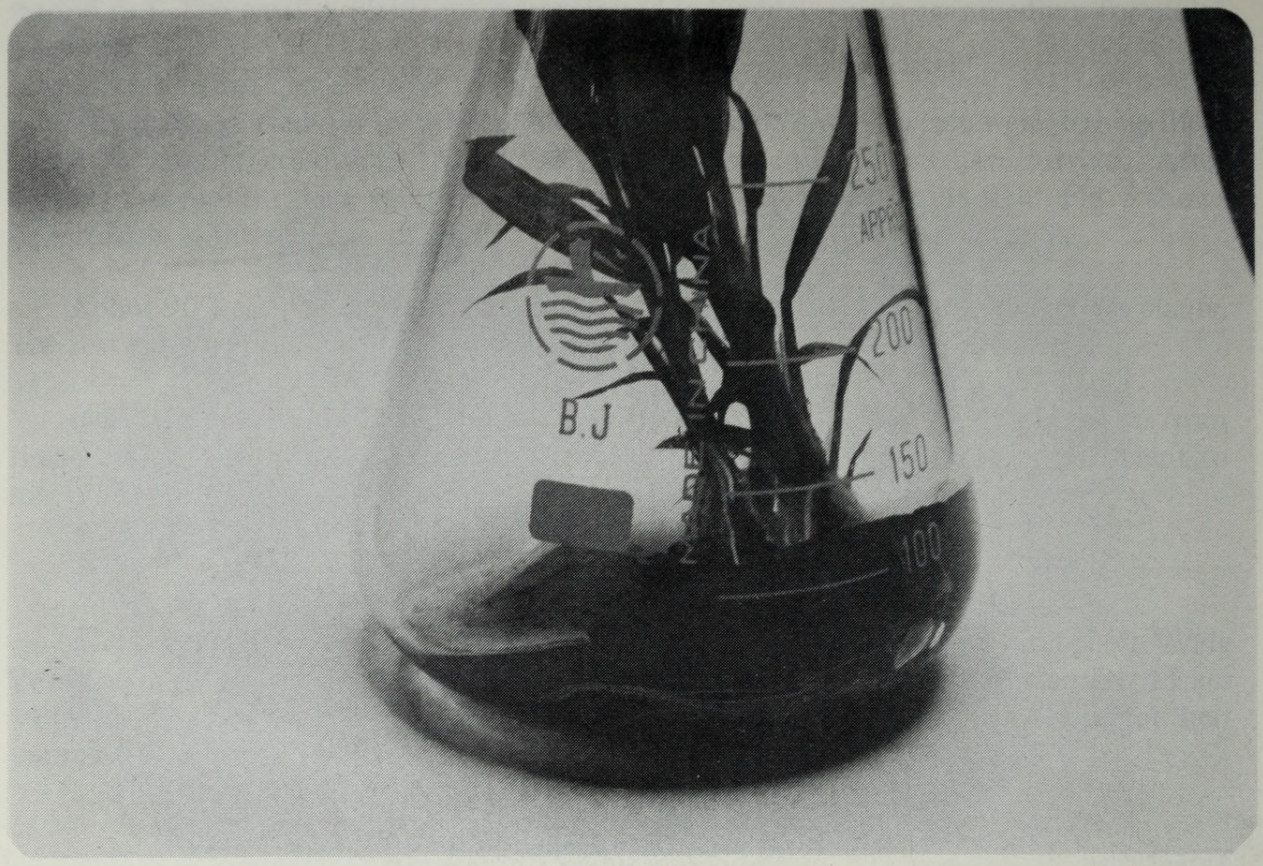

Fig 4. Rhizome cultures showing satisfactory growth of callus, shoots, and roots

Fig 5. *Curculigo latifolia* produced by tissue culture technique.