

CALLUS INDUCTION AND PLANT REGENERATION OF *ANTHURIUM ANDRAEANUM* LIND.

W. M. ABEYARATNE and A. YOGARAJAH

Royal Botanic Gardens, Peradeniya

ABSTRACT

In vitro propagation of *Anthurium andraeanum* Lind. (red and orange) through callus culture was studied using immature seeds and modified Murashige and Skoog medium. In the absence of light callus induction and growth occurred at low concentrations (0.1 to 1.0 mg/l) of 2,4—dichlorophenoxyacetic acid (2,4-D) while higher concentrations (4.0 to 5.0 mg/l) seem to be detrimental to callus growth. The media containing coconut milk (10% v/v) and/or 2,4-D (0.5–1.0mg/l) were favourable for the propagation of callus. Shoot formation in callus grown on such media was observed after exposure of the cultures to light (3000 lux, 16 h) for a period of 2 weeks. Root induction of these shoots was achieved with the media containing indole -3- butyric acid (IBA). The plantlets were successfully established in soil under high humid conditions.

KEY WORDS: *Anthurium andraeanum*, Callus culture, Plant regeneration

INTRODUCTION

The genus *Anthurium* has a tropical American origin and is now distributed in many tropical countries. *A. andraeanum* Lind. commonly referred to as anthurium or painters palette, is a well known floricultural crop. The wide adaptability for floral decorations and the long shelf-life of *A. andraeanum* flowers have contributed to its success as a cut-flower. During the past few decades there has been a sharp rise in the consumption of cut-flower in European countries. In Sri Lanka export oriented cut-flower production began in an organized manner in the early 1970s. This plant thrives in the wet zone from sea level up to about 1370 m elevation (Hagen and Ekanayake, 1977). Among the range of colours found in anthurium, the highest demand is for red and orange flowers. Bicolours also have a good demand since they are widely used in floral decorations. Medium sized heart-shaped flowers with a reclining spadix are ideal for the export market.

However, flowers with a large spathe could be sold at a considerable price if they could be transported without damage. Lack of planting material with desirable qualities is the greatest obstacle in the anthurium industry in Sri Lanka. In the late 1960's the Royal Botanic Gardens started an anthurium breeding programme using a heterogenous stock of plants and produced considerably good quality planting material. However propagation of such material using conventional methods was difficult.

Plant production through the tissue culture technique is an avenue to mass propagation of selected varieties. *A. andraeanum* can be propagated through callus culture using juvenile leaf tissues (spathe, petiole) as explants (Pierik. *et al.*, 1974; Pierik. 1975; 1976). The main objective of this study was to investigate the feasibility of *in vitro* propagation of *A. andraeanum* using immature seeds.

MATERIALS AND METHODS

Immature berries of *A. andraeanum* Lind. (red and orange varieties) were collected from the selected stock of mother plants in the Royal Botanic Gardens, Peradeniya. The berries were rinsed in tap water several times, squeezed and the immature seeds were isolated. These immature seeds were surface-sterilized by dipping in 70% (v/v) ethanol (20 seconds) followed by 10% (v/v) commercial clorox solution containing one drop of tween-20 (15 minutes). They were finally rinsed (three times) with sterilized distilled water.

Murashige and Skoog (1962) salts (half strength) with pyridoxin HCl (1.0mg/l), thiamin HCl (0.2mg/l), nicotinic acid (1.0mg/l) meso-inositol (100mg/l), glycine (2.0mg/l) and sucrose (20,000mg/l) were used as the basal medium for all experiments. This medium was modified with 2,4 — D (0.1, 1.0, 2.0, 4.0 and 5.0mg/l) and used for initiation of callus from the sterilized immature seeds. Basal medium and basal medium with 10% (v/v) coconut milk (CM) and/or 2,4 — D (0.5, 1.0, 2.0 and 5.0 mg/l) were tested for maintenance of callus. These cultures were incubated at $26 \pm 1^{\circ}\text{C}$ in the dark. The calli surviving in the media (maintenance media) were exposed to light

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(fluorescent; 3000 lux, 16h) for shoot induction. Shoots (2 cm in length) were isolated and cultured in basal medium with or without addition of IBA (0.5, 1.0, 2.0 and 5.0mg/l) for root initiation. Each culture medium was solidified with agar (Difco bacto, 0.75% w/v) after adjustment of pH (5.6) with a solution of 0.1N HCl or NaOH and was dispensed (10 ml aliquots) into test tubes. Media were sterilized by autoclaving for 20 minutes at 120°C with 1.06 kg/cm² steam pressure.

Plantlets formed were established in a sandy soil under high humidity conditions.

RESULTS

The growth response of anthurium (red and orange variety) seeds, cultured in callus induction media is summarized in Table 1. Callus could be established from immature seeds within 8—12 weeks of culture. The callus was nodular and yellow in colour and it originated from the embryo of the seed.

The seeds placed on media containing higher levels (4.0—5.0mg/l) of 2,4—D degenerated. Callus, established from the orange variety grew well when subcultured in the medium containing CM with or without 2,4—D (0.5 or 1.0 mg/l). The growth of callus of the red variety was prominent in the media containing CM and / or 0.5mg/l 2,4—D (Table 2). Shoot regeneration was observed when callus cultures were exposed to light for 2 weeks. In the presence of light, growth of the shoots was rapid compared to that of callus. Shoots produced roots when they were cultured in media tested for rooting. The highest number of roots was observed with the shoots of the orange variety in the hormone—free basal medium. However, maximum root growth was obtained with the basal medium containing 2 mg/l IBA. In the case of the red variety, the highest number of roots was observed in basal medium containing 1mg/l IBA, while maximum root growth was recorded with the medium supplemented with 2mg/l IBA.

DISCUSSION

Callus establishment in a number of monocotyledonous tissues *in vitro* has been achieved using the auxin 2,4—D in the culture medium (Fridborg, 1971). Callus induction in *Anthurium* species has also been achieved with the use of auxin (2,4—D) and cytokinin, either BAP (6—benzylamino purine) or PBA (6—(benzylamino)—9—(2—tetrahydropyranyl)—9H—purine) in the medium (Geier, 1982; Pierik, 1976). Pierik *et al.* (1974) reported that most of the embryos produced callus on medium enriched with 1mg/l cytokinin.

In the present study, callus induction from immature embryos required very low concentrations of 2,4—D while callus growth was achieved with the use of CM or both CM and low concentrations of 2,4—D in the medium. Incorporation of CM (15% v/v) and auxin into the culture medium enhanced callus growth in pineapple (Mathews and Rangan, 1981). To induce shoot formation in tissue cultures of *Acacia koa*, the callus was transferred to auxin—free Murashige and Skoog (1962) medium with 10% (v/v) CM (Skolmen and Mapes, 1976). Similar results were obtained in the present study. It is known that CM contains chemical compounds which have similar action to that of cytokinins. Therefore such compounds might have favoured the growth and regeneration of anthurium callus.

It is not known if all the seeds used in the study have the same genetic make up. If the embryos are genetically different from each other a considerable variation in characters within the regenerated plant population can be expected. This is very important in the genetic improvement of anthurium as plants with good characters can be screened from such plant populations. Although *in vitro* propagation of anthurium using seed—derived callus was achieved the variation for characters in the regenerated plant population could not be studied.

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Table 1. Effect of 2,4-D concentration on callus induction from immature seed of *A. andraeanum* (varieties: red and orange)

Medium	2,4-D (mg/l)	Callus induction	
		Red variety	Orange variety
$\frac{1}{2}$ M S	0.1	**	***
$\frac{1}{2}$ M S	1.0	***	***
$\frac{1}{2}$ M S	2.0	**	**
$\frac{1}{2}$ M S	4.0	*	—
$\frac{1}{2}$ M S	5.0	—	—

* Poor; ** Good; *** Excellent; - Explant degenerated

Table 2. Growth of *A. andraeanum* (varieties: red and orange) callus upon subculture to different media

Medium	Callus growth	
	Red variety	Orange variety
$\frac{1}{2}$ M S	*	*
$\frac{1}{2}$ MS + CM (10%)	***	***
$\frac{1}{2}$ MS + CM (10%) + 2,4-D (0.5 mg/l)	**	***
$\frac{1}{2}$ MS + CM (10%) + 2,4-D (1 mg/l)	*	***
$\frac{1}{2}$ MS + CM (10%) + 2,4-D (2 mg/l)	*	**
$\frac{1}{2}$ MS + CM (10%) + 2,4-D (5 mg/l)	*	**

* Poor; ** Good; *** Excellent