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CALLUS INITIATION AND PLANT REGENERATION OF *CALADIUM BICOLOR* (AITON) VENT. BY *IN VITRO* CULTURE

A.M.A. SAKPERE+ and A.C. ADEBONA
Department of Botany, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

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Abstract

A method for the direct plant regeneration of *Caladium bicolor* (Aiton) Vent. is described. Callus was induced from corm and leaf explants of *C. bicolor* on Murashige and Skoog (MS) medium supplemented with 0.8 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) in combination with 1 mg/l kinetin. The callus which was white and compact was scanty and shortlived. Rootlets and shootlets were generated on corm explants inoculated on medium supplemented with kinetin and NAA as well as various concentrations of 2,4-D. Corm and leaf explants had a 50 % response each to all the concentrations of 2,4-D used. More callus was induced from leaf explants than from petiole or corm explants.

Keywords: *Caladium bicolor*, 2,4-D, corm, plantlets, callus, kinetin

1. Introduction

Caladium bicolor (Aiton) Vent. is an ornamental plant of the Araceae family and is tropical American in origin. It is a succulent perennial monocotyledon of the order Arales. It is a tuber forming species with a cocoyam habit (Olorode, 1984). *Caladium bicolor* is characterized by its terrestrial habit, yellow corm, petiole 30-95 cm long, and its blades (18-46 cm long, 12-25 cm broad) with rounded, divergent posterior lobes.

Apart from being an ornamental plant, *Caladium bicolor* is used in healing poisonous bites and possibly neutralizing toxins. It has medicinal uses in tropical America, as well as being eaten as a vegetable and its' dried powdered leaves make a dressing for wounds (Bown, 2000). In Brazil the stem juice of *C. bicolor* is used as an enema to expel roundworms and destroys maggots when applied to the skin. Peasants use leaf decoctions to get rid of external cattle festers caused by worms (Balbach, 1980). In the West Indies, Hedrick (1919) observed that the corms are eaten roasted or boiled and the leaves are eaten, boiled as a vegetable.

In view of the multipurpose use of this species, developing an efficient protocol for the rapid propagation of *C. bicolor* is in order. Reddy *et al.* (2001) reported that *in vitro* propagation methods offer powerful tools for plant germplasm conservation and multiplication. The first and, to date, the most extensive practical application of tissue culture techniques to horticultural crops involves the multiplication of ornamental plant species (Torres, 1989). Plant tissue culture and micropropagation techniques allow a far greater number of plants to be produced in a given time than can be achieved by conventional propagation methods. These techniques can also play an important role in biotechnology since regeneration from cells or tissues cultured *in vitro* is a fundamental requirement for most applications of plant biotechnology.

This study describes the basic procedures for the generation of callus and plant regeneration of *C. bicolor*, as part of a study aimed at developing a procedure for efficient plant regeneration and multiplication of the species.

2. Materials and Methods

Corms of *Caladium bicolor* were collected from the Senior Staff Quarters of the Obafemi Awolowo University (OAU), Ile-Ife, and grown in the Screen House of the Department of Botany, OAU, Ile-Ife. Leaf, petiole, corm and root segments from these plants were washed under running tap water to remove dirt and reduce microbial population. Corms were dehusked and selected for a healthy appearance (i.e. without malformations or presence of necrotic spots). The explants were surface disinfested in 0.7 % (w/v) sodium hypochlorite solution with 2 drops of Tween 20 for 10 mins. and then thoroughly rinsed three times in sterile distilled water. Leaf explants (1 cm *x* 1 cm), petiole cylinders (about 1 cm long), small cubes of corm (about 1 cm *x* 1 cm *x* 1 cm) and root explants (about 1 cm long) were cultured aseptically on full strength MS medium and incubated at 25±2 °C in the dark. The culture medium

+ *corresponding author* (email: asakpere@oauife.edu.ng **or** asakpere@yahoo.com)

consisted of Murashige and Skoog's (1962) medium (MS medium) supplemented with 3 % (w/v) sucrose, 0.8 % (w/v) agar (Oxoid Agar No. 1, Code L11) and several combinations of auxins and cytokinins. The pH of the medium was adjusted to 5.7±0.1, dispensed into 50 ml. Erlenmeyer flasks which were stoppered with non-absorbent cotton wool wrapped with aluminum foil and then autoclaved at 121 °C and 15 lb/in^2 pressure for 15 minutes.

Four different concentrations of kinetin (0.25, 0.5, 1.0 and 2.0 mg/l) in combination with 1-naphthalene acetic acid (NAA) (1, 3, 5, and 10 mg/l) and two concentrations of 6-benzyladenine (BA) (1 mg/l and 5 mg/l) in combination with NAA (1 mg/l and 5 mg/l) were investigated for their effects on direct regeneration and callus formation from corm and root explants. Also, three different concentrations of 2,4-D (0.4, 0.8 and 1.6 mgs/l) were combined with 1.0 mg/l kinetin to investigate the callogenic and regenerative capacities of corm, leaf and petiole explants. MS medium lacking growth regulators served as control.

The explants were evaluated visually for the presence and type of callus (Remotti and Loffler, 1995) and the responses scored (Mencuccini and Rugini, 1993). Also, the number of shoots produced per explant and the time required for generation of shoot were observed.

3. Results

(a) Callus Studies

Callus induction was observed on corm explants of *C. bicolor* after 3 weeks on MS medium supplemented with 1.0 mg/l BA and 1.0 mg/l NAA (Table 1). The callus did not, however, survive for more than two weeks. On the medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (K0.5N3), roots were generated on one side of the corm explant and callus on the other side after 5 weeks in culture. However, by the 7th week, root growth had overshadowed the growth of callus and shoots were initiated on the explant. That was the only incidence of callus initiation using medium supplemented with kinetin and NAA. Callus was initiated on corm explant cultured on MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin. There was 17 % incidence of generation of roots from the explant and 17 % incidence of a combination of callus and roots (Fig 1B), however, growth of roots was more rapid and the callus was soon choked. The leaf of *C. bicolor* cultured on MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin showed various responses. There was 17 % incidence of generation of plantlets and 34 % initiation of callus (Fig 1A). Of the callus initiated, 50 % did not grow beyond the stage they were on transfer to a fresh medium of the same composition. The size of the callus remained constant but after 4 weeks, root, shoot and leaves developed from the explant. Root explants showed no response at all.

(b) Direct Regeneration

Regeneration of root was observed on corm explant on MS hormone-free medium. Rootlets and shootlets were generated on corm explants inoculated on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (Fig 1C) as well as 1.0 mg/l kinetin and 5.0 mg/l NAA though shootlets developed earlier on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA (Table 2). Root explants did not respond to any of the auxin and cytokinin combinations. The

Table 1: Effect of BA and NAA on Callus Induction and Plant Regeneration

	Control		5.0mg/l BA & 1.0mg/l NAA		1.0mg/l BA & 1.0mg/l NAA		5.0mg/l BA & 5.0mg/l NAA	
Explant	Corm	Root	Corm	Root	Corm	Root	Corm	Root
TOR	-	-	-	-	C	-	RL	- -
% Response ± SE	0 ± 0	0 ± 0	0 ± 0	0 ± 0	33 ± 0.24	0 ± 0	33 ± 0.24	0 ± 0
NOS ± SE	-	-	-	-	-	-	-	-
TOI in wks	-	-	-	-	3	-	-	-
SOC	-	-	-	-	< 1 cm	-	-	-

KEY

TOR - Type of Response
TOI - Time of Initiation In wks
SE – Standard Error
NOR - No of Roots
SOC - Size of Callus (diameter)
RL – Root-like structure
NOS - No of Shoots
C - Callus

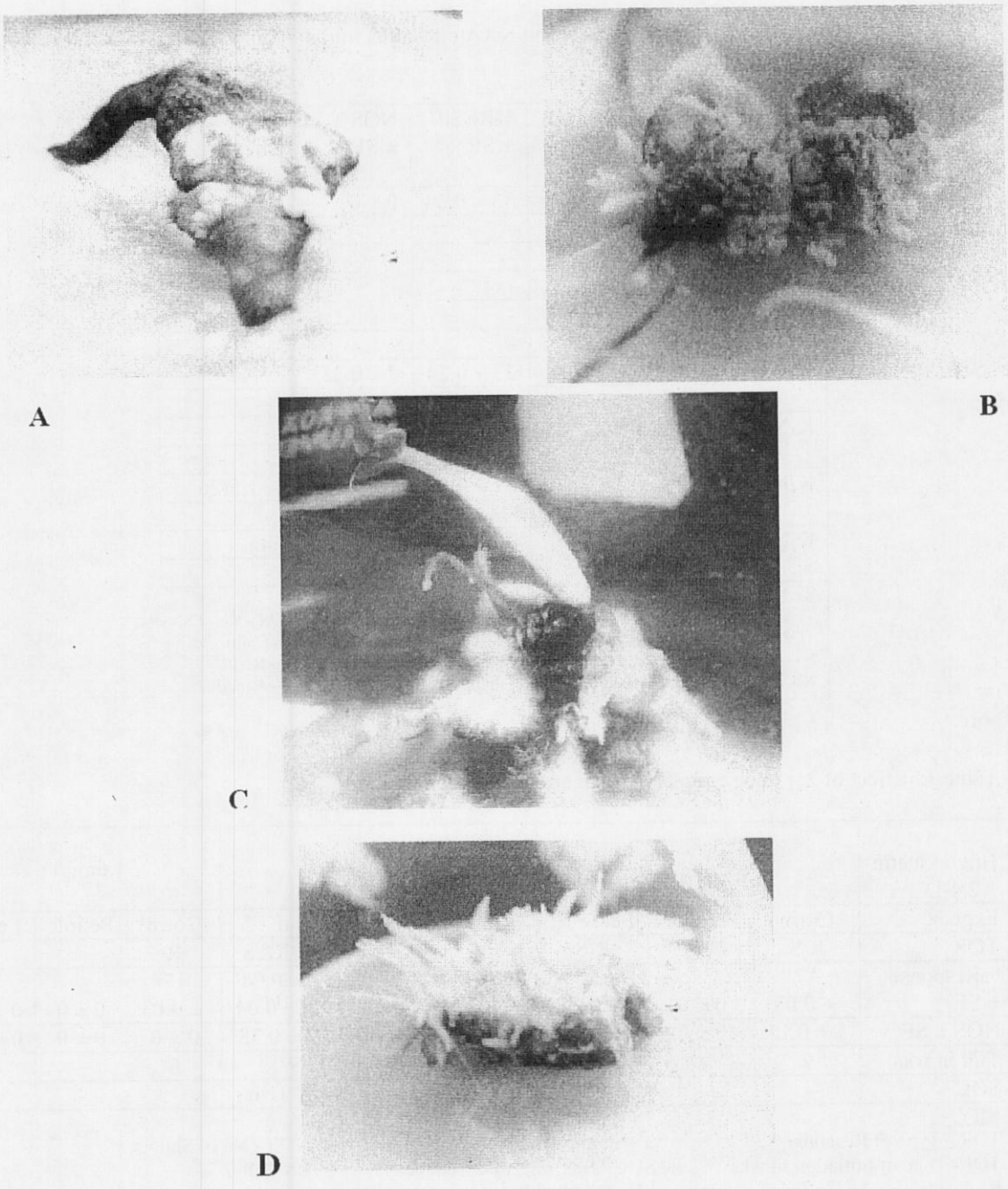

Fig. 1: (a) Callus on leaf explant of *C. bicolor* on 0.8mg/l 2,4-D, (b) Callus and root on tuber explant of *C. bicolor* on 0.8mg/l 2,4D, (c) *C. bicolor* tuber on K0.5N3 - roots and shoots developed, and (d) roots on tuber explant of *C. bicolor* on 1.6mg/l 2,4D.

leaf of *C. bicolor* cultured on medium supplemented with 0.4 mg/l 2,4-D in combination with 1 mg/l kinetin generated roots and shoots after an average of 19 weeks. Corm explants developed plantlets after 9 weeks on medium supplemented with 0.4 mg/l 2,4-D in combination with 1 mg/l kinetin much earlier than leaf explants. Roots and shoots were also generated on leaf explants cultured on medium supplemented with 0.8 mg/l 2,4-D in combination with 1 mg/l kinetin after 13 weeks. Thirty three percent rooting was observed on corm explants on the same concentration of supplements out of which 17 % was in combination with callus. On 1.6 mg/l 2,4-D in combination with 1mg/l kinetin, *C. bicolor* corm generated a lot of roots (Fig. 1D). No response was observed on petiole explants apart from swelling of the explants.

In general, more callus was induced from leaf explants than from petiole or corm explants (Table 3). Only 0.8 mg/l 2,4-D in combination with 1 mg/l kinetin induced callus in corm and leaf explants of *C. bicolor* and the callus which was white and compact was scanty.

Table 2: Effect of Kinetin and NAA on Callus Initiation and Plant Regeneration

	EXPLANT	TOR	%RESP. ± SE	NOS ± SE	TOI In wks (Average)
K_0N_0	CORM	R	33 ± 0.24	0 ± 0	4
	ROOT	-	-	-	-
K_0N_3	CORM	-	-	-	-
	ROOT	-	-	-	-
$K_{.25}N_1$	CORM	-	-	-	-
	ROOT	-	-	-	-
$K_{.5}N_3$	CORM	R/S	33 ± 0.24	2 ± 0.25	5/12
	ROOT	-	-	-	-
K_1N_3	CORM	-	-	-	-
	ROOT	-	-	-	-
K_1N_5	CORM	R/S	67 ± 0.33	2.5 ± 0.69	11.5/26
	ROOT	-	-	-	-
K_2N_{10}	CORM	R	33 ± 0.24	0 ± 0	4
	ROOT	-	-	-	-

KEY
TOR - Type of Response S - Shoot NOS - No of Shoots
TOI - Time of Initiation In wks SOC - Size of Callus (diameter) C - Callus
N – Naphthalene Acetic acid SE – Standard Error R – Roots
No. Subscript - concentration of growth regulator used in mg/l K – Kinetin

Table 3: Effect of 2,4-D on callus initiation and plant regeneration

1mg/l kinetin + 2,4-D >	0.4mg/l			0.8mg/l			1.6mg/l		
Explant	Corm	Petiole	Leaf	Corm	Petiole	Leaf	Corm	Petiole	Leaf
TOR	R/S	-	R/S	R/C	-	C/R&S	R	-	-
%Response ± SE	17 ± 0.03	0 ± 0	33 ± 0.06	33±0.06/ 17± 0.03	0 ± 0	25±0.04/ 25± 0.04	17 ± 0.03	0 ± 0	0 ± 0
NOS ± SE	1 ± 0.17	0 ± 0	4.5±1.07	0 ± 0	0 ± 0	2 ± 0.33	0 ± 0	0 ± 0	0 ± 0
TOI in wks	9	-	19	6	-	9/13	6	-	-
SOC	-	-	-	> 1cm	-	< 1cm	-	-	-

KEY
TOR - Type of Response S - Shoot NOS - No of Shoots
TOI - Time of Initiation In wks SOC - Size of Callus (diameter) C - Callus
R - Roots SE - Standard Error

4. Discussion

Among the wide range of auxins and cytokinins which have been used to induce callus, the auxins 2,4-D and NAA were always indicated as the best auxins for callus induction (Remotti and Loffler, 1995). Using 3 combinations of BA and NAA as supplements, callus was only induced on corm explant of *C. bicolor* cultured on MS medium supplemented with 1 mg/l BA and 1 mg/l NAA. This is in contrast to the work on *C. bicolor* by Ahmed *et al.* (2002) who found cultures of explants on 1 mg/l BA and 1 mg/l NAA yielded plantlets directly. Combinations of kinetin and NAA used did not induce callus except on medium supplemented with 0.5 mg/l kinetin and 3.0 mg/l NAA in which there was initiation of callus which was quickly overgrown by roots.

Among the combinations of 2,4-D used, MS medium supplemented with 0.8 mg/l 2,4-D in combination with 1.0 mg/l kinetin was the only one that induced callus (both on corm and leaf explants). There was induction of exclusively callus only on 17 % leaf explants. All other incidence of callus initiation was in association with root and shoot growth which soon overcrowded it. Satish *et al.* (2003) also reported

that in *Fritillaria hupehensis* (Lilaceae), protocormlike bodies (PLB) cultured on MS basal medium supplemented with either 0.8 mg/l 2,4-D or NAA in combination with 0.5 mg/l kinetin proliferated and produced bulblets and callus. The use of 1.6 mg/l 2,4-D in combination with 1 mg/l kinetin as supplement on the MS medium did not induce callus on the explant neither was shoot produced. Only roots were generated on the corm explants. This is in contrast to the statement of Carter *et al.* (1967) that a higher concentration of 2,4-D is required for callus induction in monocotyledons.

Combinations of BA and NAA used did not yield direct organogenesis but most of the combinations of kinetin and NAA used generated roots and shoots. *C. bicolor* appears to be more capable of direct organogenesis since callus induction formed only 27.27 % of the responses. Roots were developed even in the control which had no growth regulator. The incidence of root formation on auxin free medium may be due to the availability of high quantity of endogenous auxin in explant (Minocha, 1987). The induction of callus on MS hormone-free medium has also been reported on internode and leaf explants of *Holostemma ada-kodien* (Martin, 2002). In explants that generated roots and shoots, roots were usually generated first and shoots later developed on the same medium without need for transfer into any special medium for either root or shoot initiation. Combinations of growth regulators that formed only roots had a high concentration of auxin. This indicates that the high auxin probably suppressed the growth of shoots since there was a higher auxin to cytokinin ratio.

Corm and leaf explants had a 50 % response each to all the concentrations of 2,4-D used although corm explants responded across board while leaf explants responded to only two of the concentrations. This is in agreement with Satish *et al.* (2003) who worked on *Pinellia ternate* (Araceae) a perennial medicinal herb that grows wildly in Japan and China. They observed that maximum response from tissue culture occurred in bulbils followed by leaf blades, and petiole explants. The root explants used had 0 % response. This could be due to a number of factors since root explants of other monocots generated callus and plantlets in culture, for example barley (Malepszy and Gay, 2003) and *Cymbidium ensifolium* (Chang and Chang, 1998) which even regenerated plantlets from root induced calli. The fact that the root explant used was not the root tip could be a deciding factor. Ahmed *et al.*, (2002) regenerated plants from root tips of *C. bicolor*, though Malepszy and Gay (2003) generated callus sporadically from non meristematic root explants of Barley.

In conclusion, the present communication presents a procedure for the micropopagation of *C. bicolor* through direct organogenesis in the dark. In future work, an attempt will be made to develop an efficient procedure for callus induction of the species.

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