Micropropagation of Two *Caladium* Species

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ABSTRACT

Great differences occur in cell division and regenerative capacity between plants even within a single species. Therefore difference in callus induction and plant regeneration abilities of two *Caladium* species – *Caladium bicolor* and *Caladium humboldtii* was studied by culturing them on different combinations of growth regulators. *Caladium humboldtii* was found to be the more responsive genotype for callus induction while *Caladium bicolor* was the more responsive genotype for plant regeneration. Roots and shoots were more readily generated on corm explants in combinations of Kinetin and Naphtalene Acetic Acid (NAA) than in media containing different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) and 1 mg/L Kinetin. Callus was generated on tubers of both species on media supplemented with 0.8 mg/L 2,4-D and 1 mg/L Kinetin.

KEY WORDS

Callus induction, Plant regeneration, *Caladium* sp., genotype, 2,4-D.

INTRODUCTION

Caladium is a genus of herbaceous monocotyledonous, understory plants which occur in humid to wet primary forests. It is made up of 17 species (Croat, 1994) which are mainly tuberous. *Caladium humboldtii* Schott differs from *Caladium bicolor* (Aiton) Vent by its small size, freely suckering habit and lack of inflorescences (Croat & Lambert, 1986).

Caladiums are used successfully as pot-ted and landscape plants throughout much of the United States and much more than before in other parts of the world including Nigeria. Horticulturists are increasingly employing propagation by tissue culture because of the fact that rapid introduction of new valuable plants to the market is very important in the horticulture industry, and because the cultures can be kept free of disease which aids production. This is done especially with foliage plants where a greater number of identical plants can be produced and grown to maturity within a given time than can be achieved by conventional propagation methods.

A successful application of a tissue culture system depends on a judicious choice of variables such as explant type (Hol & Petersen, 1996) and growth regulators, among others (Brown, 1990). Since great differences occur in cell division and regenerative capacity between plants even within a single species, different species are likely to respond differently to growth regulators. Xie & Hong (2000) reported that different species have a different requirement for Thidiazuron (TDZ). Large genotype and medium effects have been observed for callus production (Remotti & Loffler, 1995). Explant source of callus and the growth regulator inducing the callus have also been shown to exhibit a significant influence on organogenesis (Martin, 2002). This paper reports the comparative study of the callus induction and plant regeneration vigor of two species of *Caladium* - *Caladium bicolor* (fancy leaved) and *Caladium humboldtii* (lance-leaved). The morphogenic responses of different explants of the two species on

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edium supplemented with different rowth regulators are also reported.

ATERIALS AND METHODS

Tubers, root, leaf, and petiole explants were collected from screen house grown *Caladium* plants. Soil particles were removed from the tubers by washing under running tap water. This also helped in reducing microbial population. Tubers without malformations or presence of necrotic spots were then selected after dehusking. Explants from other sources were also rinsed. The explants were then surface-sterilized for 10 minutes with 0.7% (w v) sodium hypochlorite solution in which two drops of Tween 20 was added as a surfactant. They were thereafter rinsed three times in sterile distilled water. The explants were trimmed to suitable sizes, cultured on full strength MS medium and incubated at 25°C ± 2°C. All operations were performed under the laminar flow chamber.

MEDIA AND CULTURE CONDITIONS

Murashige & Skoog's (1962) medium supplemented with 3% (w v) sucrose was used in all experiments. The media was solidified with 0.8% (w v) agar. pH adjusted to 5.7 ± 0.1. The medium was dispensed into 50 ml culture flasks which were sealed with non-absorbent cotton wool, and then with aluminum foil before autoclaving at 121°C and 15 lb/in^2 pressure for 15 minutes. Cultures were maintained at 25°C ± 2°C in the dark.

Various concentrations of kinetin (0–2 mg L) in combination with NAA (1–10 mg L) and three concentrations of BA (0.5–5 mg L) in combination with NAA (1–5 mg L) were tested for their effect on direct regeneration and callus initiation from tuber and root explants. Three different concentrations of 2,4-D were combined with 1.0 mg L kinetin to test their effect on direct regeneration and callus initiation of tuber, leaf and petiole explants. This was also used to study the effect of different explants on regeneration and callus induction. The cultures were monitored weekly for the effect of each treatment on the induction of callus or organogenesis.

Organogenesis was evaluated as the number of shoots produced per explant and the time required for generation of shoot (Rita & Floh 1995). The explants were also evaluated visually and scored for the presence and type of callus (Remotti & Loffler 1995; Mencuccini & Rugini, 1993). Responses were expressed as percentages (%) ± Standard Error.

RESULTS

Comparison of Explant Type On Callus Induction and Direct Organogenesis

Only the petiole of *C. humboldtii* generated callus (Fig. 1A). More callus (both friable and watery) was induced from tuber explants than from petiole or leaf explants (Table 3). Of all callus produced on media supplemented with 2,4-D, 58.3% was generated on tubers, 25% on leaves and 16.7% on petioles. In all media, conditions and species tested, the root and petioles did not regenerate any shoots. Tuber explants were found to generate roots and shoots more readily than leaf explants (Tuber – 62.5%, leaves – 37.5%) on media supplemented with 2,4-D. Leaf explants of *C. bicolor* generated plantlets on media supplemented with combination of 0.4 mg L 2,4-D plus 1 mg L kinetin and 0.8 mg L 2,4-D plus 1 mg L kinetin (Fig 1B).

Comparison of Species Type On Callus Induction and Direct Organogenesis

Only 0.8 mg L 2,4-D in combination with 1 mg L kinetin induced callus in tuber and leaf explants of *C. bicolor* and callus which was white and compact was scanty. With 67% of callus induced generated on *C. humboldtii*, callus appears to be more readily induced in *C. humboldtii* than *C. bicolor* on which only 33% of the calli was induced (Table 3 and 2). Callus in *C. bicolor* was white/creamy and compact while different types of callus developed on *C. humboldtii* explants varying from creamy coloured to yellow or greenish, big

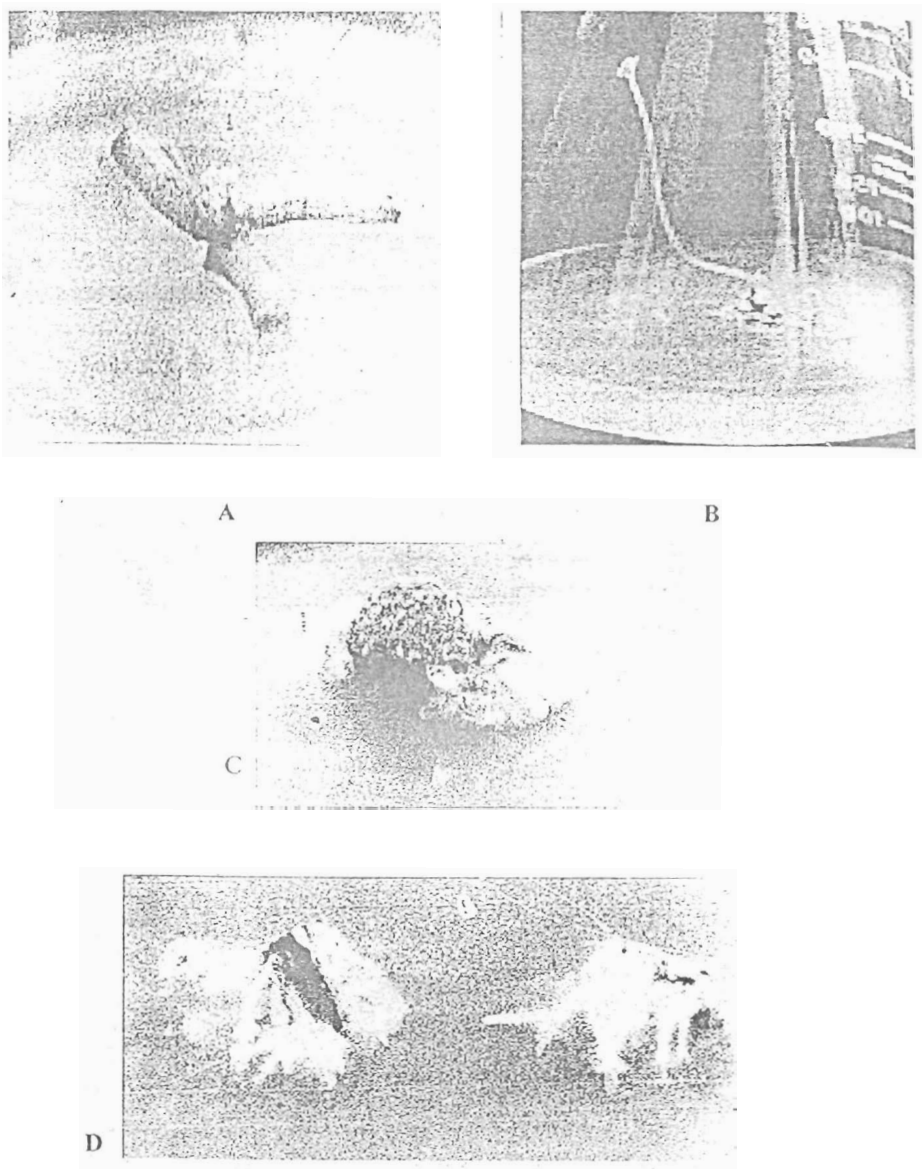


Fig. 1. Plant regeneration and callus induction in *C. bicolor* and *C. humboldtii*. A. Callus on petiole explant of *C. humboldtii* in 0.4 mg L 2,4-D. B. plantlet from leaf explant of *C. bicolor* in 0.8 mg L 2,4-D. C. *C. humboldtii* tuber in K1N5 - roots. D. *C. bicolor* tuber in K2N10 -generated roots.

and small callus lobes to compact and wet looking to watery.

Of the responses that yielded roots and shoots, 75% were generated on *C. bicolor* with only 25% in *C. humboldtii*. Only the leaves of *C. bicolor* generated roots and shoots compared with the leaves of *C. humboldtii*.

Table 1 Effect of BA and NAA on callus induction and plant regeneration

	C. humboldtii						*C. bicolor*					
	5.0 mg BA & 1.0 mg l NAA		1.0 mg L BA & 1.0 mg L NAA		5.0 mg BA & 5.0 mg L NAA		5.0 mg L BA & 1.0 mg L NAA		1.0 mg L BA & 1.0 mg L NAA		5.0 mg BA & 5.0 mg NAA	
Explant	Tuber	Root	Tuber	Root	Tuber	Root	Tuber	Root	Tuber	Root	Tuber	Root
Time of response	—	—	—	—	—	—	—	—	Callus	—	—	—
% Response ± standard error	—	—	—	—	—	—	—	—	33 ± 11.24	—	—	—
No of Shoots ± Standard Error	—	—	—	—	—	—	—	—	—	—	—	—
Time of Initiation in wks	—	—	—	—	—	—	—	—	3	—	—	—
Size of Callus (diameter)	—	—	—	—	—	—	—	—	<1 c	—	—	—

Comparison of Growth Regulator effects On Callus Induction and Direct Organogenesis

It appears that the concentrations of kinetin, NAA and BA used were not suitable for the induction of callus. The only exceptions being the combinations of 1 mg L NAA and 1 mg L BA which induced callus on the tuber of *C. bicolor* and 1 mg L kinetin in combination with 3 mg L NAA which induced callus on the tuber of *C. humboldtii*. All the concentrations of 2,4-D used in combination with 1 mg L kinetin induced callus in tuber explants of *C. humboldtii* (Table 3). The combination of 0.8 mg L 2,4-D and 1 mg L kinetin showed more incidence of callus generation than the use of 0.4 mg L or 1.6 mg L 2,4-D. Callus was generated in tubers of both species.

Callus from tuber explants of *C. humboldtii* induced on 0.8 mg L 2,4-D was maintained for more than a year. The callus generated on 1.6 mg L 2,4-D was slow growing and did not survive.

Combinations of BA and NAA used did not yield direct organogenesis in the two species of *Caladium*. Among the concentrations of 2,4-D used, the combination of 0.4 mg L 2,4-D and 1 mg L kinetin favoured direct regeneration of plantlet from tuber explants of both species (Table 3). Among the combinations of kinetin and BA used, 1 mg L kinetin and 5 mg L NAA was the only combination that elicited response from both species with the generation of plantlets (Table 2). Roots and shoots were more readily generated on tuber explants in combinations of kinetin and NAA (61.5%) than in media containing different concentrations of 2,4-D and 1 mg L kinetin (38.5%). In most cases roots were first generated before shoots (Fig 1C).

DISCUSSION

In this study, tuber and petiole explants of *C. humboldtii* formed more callus than tuber and petiole explants of *C. bicolor*, while leaf explants of both species did not show any difference in callus induction. Tuber and leaf explants of *C. bicolor*

Table 2. Effect of kinetin and NAA on callus initiation and plant regeneration

	C.humboldtii					*C. bicolor*			
	Explant	Type of response	% Response ± standard error	No of shoots ± standard error	Time of initiation in wks (Average)	Type of response	% Response ± standard error	No of shoots ± standard error	Time of initiation In wks (Average)
K_0N_0	TUBER	—	—	—	—	Roots	33 ± 0.24	0 ± 0	4
	ROOT	—	—	—	—	—	—	—	—
K_0N_3	TUBER	—	—	—	—	—	—	—	—
	ROOT	—	—	—	—	—	—	—	—
$K_{0.25}N_1$	TUBER	Roots/Shoot	33% ± 0.33	1 ± 0.33	16	—	—	—	—
	ROOT	—	—	—	—	—	—	—	—
$K_{0.5}N_3$	TUBER	—	—	—	—	Roots/Shoot	33 ± 0.24	2 ± 0.25	5/12
	ROOT	—	—	—	—	—	—	—	—
K_1N_3	TUBER	Callus	33% ± 0.47	—	15	—	—	—	—
	ROOT	—	—	—	—	—	—	—	—
K_1N_5	TUBER	Roots/Shoot	67% ± 0.67	1 ± 0.33	8	Root/Shoot	67 ± 0.33	2.5 ± 0.69	11.5/26
	ROOT	—	—	—	—	—	—	—	—
K_2N_{10}	TUBER	—	—	—	—	Roots	33 ± 0.24	0 ± 0	4
	ROOT	—	—	—	—	—	—	—	—

Table 3. Effect of different concentrations of 2,4-D in combination with 1 mg/l kinetin on callus initiation and plant regeneration of different explants of *C. humboldtii* and *C. bicolor*

1 mg/ l kinetin + 2,4-D >	*C. humboldtii*									*C. bicolor*								
	0.4 mg L			0.8 mg L			1.6 mg L			0.4 mg L			0.8 mg L			1.6 mg L		
Explant	Tuber	Petiole	Leaf	Tuber	Petiole	Leaf	Tuber	Petiole	Leaf	Tuber	Petiole	Leaf	Tuber	Petiole	Leaf	Tuber	Petiole	Leaf
Type of Response	Roots	Callus	—	Callus	—	Callus	Callus	—	—	Roots/ Shoot	—	Roots/ Shoot	Roots/Callus	—	Callus/ Roots&Shoot	Roots	—	—
% Response ± Standard Error	33 ± 0.3	33 ± 0.3		67 ± 0.67	0 ± 0	33 ± 0.3	33 ± 0.3	—	—	33 ± 0.3	—	67 ± 0.67	50 ± 0.5/ 17 ± 0.03	—	17 ± 0.7/ 50 ± 0.47	33 ± 0.3	—	—
No of Shoots ± Standard Error	16 ± 5.3	—	—	—	—	—	—	—	—	1 ± 0.17	—	5 ± 2.36	—	—	2 ± 0.33	—	—	—
Time of Initiation (in wks)	12	4	—	7	—	3	8	—	—	9	—	10.5	8.5/9	—	9/13	6	—	—
Size of Callus (diameter)	—	<1 cm	—	>1 cm	—	<1 cm	<1 cm	—	—	—	—	—	>1 cm	—	<1 cm	—	—	—

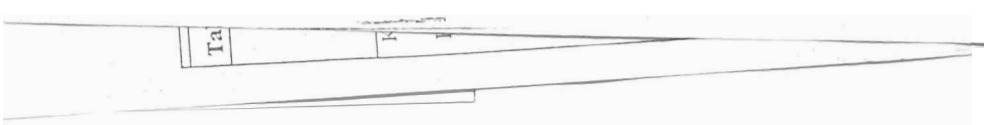
however, regenerated more plantlets than explants of *C. humboldtii* indicating that *C. humboldtii* species are more efficient in callus production than regeneration of plantlets and *C. bicolor* appears to be more favourably disposed to direct organogenesis than the induction of callus. Of all the responses that involved direct organogenesis, *C. bicolor* had the highest percentage response compared with *C. humboldtii*. On the other hand, induction of callus occurred more frequently in *C. humboldtii* as compared to *C. bicolor*. Similarly, Sharma & Rajam (1995), reported that genotype, explant and genotype-explant interaction have highly significant effects on both organogenesis and somatic embryogenesis in *Solanum melongena* L. with genotype exerting maximum effect on both processes. Differences in ability for *in vitro* shoot regeneration and elongation have also been reported to be dependent on variety and explant type (Dabauza & Pena 2001).

The concentrations of kinetin and NAA that induced responses from *C. humboldtii* tubers were different from those that induced responses from *C. bicolor* tubers except for 1 mg L kinetin and 5 mg L NAA which induced responses in both species. Different concentrations of 2,4-D used also elicited different responses in the two species. This suggests that there is a correlation between the concentration of growth regulator and genotype. Magioli & Mansur (2005), also reported that the type and concentration of a given growth regulator in association to specific genotypes can cause significant differences in morphogenetic responses in eggplant. According to Xie & Hong (2001), different responses of different species to plant growth regulators may be due to variations in their genetic background.

Of all the explants used, tuber explants responded better to all growth regulators applied. This finding is in agreement with that of Satish *et al.* (2003), who reported that, bulbils of *Pinellia ternata* showed better response to tissue culture as compared to leaf blades and petiole explants. Genotype appears to have an effect on the order of response. In *Medicago littoralis*, an annual legume species, leaves were reported to show a better morphogenic response compared with hypocotyls, roots and cotyledons in that order. These findings were also in agreement with those reported for *M. sativa* (Zafar *et al.*, 1995).

Li *et al.* (2005) induced callus formation in *C. bicolor* on medium supplemented with 0.5 mg L 6-BA and 0.1 mg L 2,4-D and induced shoot generation in MS medium with 2 mg L 6-BA and 0.2 mg L NAA. This corresponds to results obtained in this study where only 9.8% of callus produced was produced on medium supplemented with NAA while 90.2% was produced on medium supplemented with 2,4-D. For plant regeneration, 61.5% regeneration occurred on NAA supplemented media and 38.5% on media supplemented with 2,4-D indicating that 2,4-D is more efficient in callus formation than shoot regeneration, and NAA more efficient in direct organogenesis than callus formation for this species. A lower concentration of 2,4-D (0.4 mg L) was also observed to induce generation of plantlets in *C. humboldtii* while at higher concentrations, callus was obtained although with percentage generation of callus formation decreasing with increasing concentration of 2,4-D. Similarly, in an experiment using African violet plants, lower concentrations (<2.5 um) of TDZ induced shoot organogenesis (Mithila *et al.*, 2003), whereas at higher doses (5–10 um) somatic embryos were formed.

In conclusion, callus of both species can be induced and maintained on MS medium supplemented with 0.8 mg L 2,4-D plus 1 mg L kinetin although *C. humboldtii* appears to be the most responsive genotype for callus induction as compared to *C. bicolor* which has more vigor for direct organogenesis. Tuber explants are the best explants compared to leaf and petioles. This result gives an insight into the correlation between the concentration of growth regulator, explant type and genotype as it affects these species.



ACKNOWLEDGMENTS

Patricia Frank was very helpful in supplying literature during this study. I should also like to thank Dr S.O Oke and Mr B.E Ayisire for helping to go through the manuscript at the initial stage, and the editor and reviewers for valuable comments on the drafts of the manuscript.

LITERATURE CITED

Brown, J. T. 1990. The initiation and maintenance of callus culture, pp. 57–63 *In* J. W. Pollard & J. M. Walker (eds.), *Methods in Molecular Biology 6, Plant Cell and Tissue Culture*. Humana Press, Clifton, New Jersey.

Croat, T. B. 1994. Taxonomic Status of Neotropical Araceae. *Aroideana* 17: 33–60.

——— & N. Lambert. 1986. The Araceae of Venezuela. *Aroideana* 9(1–4):59–62.

Dabauza, M. & L. Pena. 2001. High efficiency organogenesis in sweet Pepper (*Capsicum annum* L.) Tissues from different Seedling Explants. *Plant Growth Regulation* 33(3): 221–229.

Deng, Z. & B. K. Harbaugh. 2005. Inheritance of Leaf Shape and Main Vein Color Caladium. Document ENH1006, Environmental Horticulture Department, Florida Cooperative Extension Service, I. F. A. S., University of Florida. http://edis.ifas.ufl.edu.

Holme, I. & K. K. Petersen. 1996. Callus Induction and Plant Regeneration from Different Explant types of *Miscanthus* x *ogiformis* Honda 'Giganteus.' *Plant Cell Tissue and Organ Culture* 45:43–52.

Larkin, P. J. & W. R. Scowcroft. 1981. Somaclonal variation - a novel source of variability from cell cultures for plant improvement. *Theor. Appl. Genet.* 60:197–214.

Li, S. J., X. M. Deng, H. Z. Mao & Y. Hong. 2005. Enhanced anthocyanin synthesis in foliage plant *Caladium bicolor*. *Plant Cell Reports* 23(10–11):716–720.

Magioli, C. & E. Mansur. 2005. Eggplant (*Solanum melongena* L.): tissue culture, genetic transformation and use as an alternative model plant. *Acta Botanica Brasilica* 19(1):139–148.

Martin, K. P. 2002. Rapid propagation of *Holostemma ada-kodien* Scult., a rare medicinal plant, through axillary bud multiplication and indirect organogenesis. *Plant Cell Reports* 21:112–117.

Mencuccini, M. & E. Rugini. 1993. *In vitro* shoot regeneration from olive cultivar tissues. *Plant Cell, Tissue and Organ Culture* 32:283–288.

Mithila, J., J. Hall, J. M. R. Victor & P. Saxena. 2003. Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). *Plant Cell Reports* 21(5):408–414.

Murashige, T. & F. Skoog. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. *Physiol. Plt.* 15:473.

Remotti, P. C. & H. J. M. Loffler. 1995. Callus Induction and Plant regeneration from *Gladiolus*. *Plant Cell, Tissue and Organ Culture* 42:171–178.

Rita, I. & E. I. S. Floh. 1995. Tissue culture and micropropagation of *Cuphea ericoides* a potential source of medium chain fatty acids. *Plant Cell, Tissue and Organ Culture* 40:187–189.

Satish, M. N., A. P. Sagare, C. Lee, C. Kao & T. Tsay. 2003. Studies on tissue culture of Chinese medicinal plant resources in Taiwan and their sustainable utilization. *Bot. Bull. Acad. Sin* 44:79–98. http://ejournal.sinica.edu.tw/bbas/content/2003/2/bot442.01.pdf.

Sharma, P. & M. V. Rajam. 1995. Genotype, explant and position effects on organogenesis and somatic embryogenesis in eggplant (*Solanum melongena* L.). *J. Exp. Bot.* 46:135–141.

Xie, D. & Y. Hong. 2001. *In vitro* regeneration of *Acacia mangium* via