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An *In Vitro* Study on Micropropagation of C*aladium bicolor*

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ABSTRACT

Rapid propagation of *Caladium bicolor* was achieved *in vitro*. Excellent results for shoot induction from apical meristem were obtained when it was cultured on MS medium containing 1 mg L^{-1} BAP within 8 days of inoculation. For shoot multiplication maximum number of shoots was obtained when 0.25 mg L^{-1} NAA was added to shoot induction medium i.e., MS medium supplemented with 1 mg L^{-1} BAP + 0.25 mg L^{-1} NAA. Cent percent rooting was achieved by transferring an individual microshoot to modified MS medium containing combination of 2 mg L^{-1} IBA + 1 mg L^{-1} NAA. Plantlets were successfully transferred to greenhouse in sterilized sand and nourished with Hoagland's solution. After acclimatization these plants were shifted to natural conditions in pots containing growth mixture (sand + clay + peat at 1:1:1 ratio), where 100% survival was observed. We concluded that technique of micropropagation can successfully be used for large scale production of premium quality planting material and almost 2500 plants of Caladium could be generated from single apical meristem in a 12 weeks period.

Key Words: *Caladium*; Micropropagation; Multiple shoots; Acclimatization

INTRODUCTION

Ornamental plants like caladium (*Caladiums bicolor Vent.*) are important floriculture industry of South America where 95% of the world's caladium grows. From here they are shipped nationwide and across making an important source of income. Caladiums are excellent landscape and pot plants (Deng & Harbaugh, 2006) grown for their colorful leaves that have a combination of green and white, green and red, white with red blotches or green veins and some have lavender spots. The size of the heart-shaped leaves may vary from 6 inches to 2 feet in length. The plant needs bright light but no direct sunlight. The plants are very cold sensitive and prefer temperatures above 25ºC and ample of moisture. Michael and Ayebaemi (2006) reported that biomass of *C. bicolor* is also an alternative adsorbent for metal ion contaminants in aqueous system. Saviour (2005) reported an Increased elastic modulus and tensile strength of low density polyethylene by incorporating Caladium bicolor (ornamental cocoyam) and wild Dioscorea dumetorum (sweet yam) starches into low density polyethylene using a standard hot-melt compounding technique.

Caladium is generally propagated from tubers for commercial purpose but tuber propagation has limitations as tubers produce healthier plants for one season only and second year foliage is usually not as good as the first year. Therefore, more satisfactory results may be obtained by starting with new tubers each year. Commercial propagation can also be achieved through seeds but the seed propagation is difficult, being seeds very small, very high mortality and plants grown from seeds are very expensive. It has also been reported that seed propagation results in variability (Gill *et al.*, 1994). Concerns have been raised about possible loss of genetic diversity due to a drastic decline in the number of cultivars in the last century (Deng *et al.*, 2007). Moreover, this method is very difficult to keep plant true to type and pathogen free (Siddiqui *et al.*, 1993). Recently, many caladium companies and nurseries have started using tissue culture technology known as micropropagation for large scale production of true to type and disease free caladium.

In vitro techniques are powerful tools for plant breeders in improving the performance of agriculture, horticulture and floriculture plant species. Interest in tissue culture propagation of *Caladium bicolor* has evolved due to its ornamental importance throughout the world. Propagation through meristem has been identified as mechanism of rapid regeneration in Caladium. The plants produced through this technology provide much export potential as they are shipped internationally with limited quarantine restrictions and it has the prospective for developing new cultivars of Caladium species (Hyndman, 1987). Micropropagation of *C. bicolor* was for the first time reported by Hartman (1974) and later on a new Caladium cultivar was discovered from *C. humboldtii* named as "Marcel" by Lecouffe (1981) through somaclonal variation (Larkin *et al.*, 1981). Present investigation was undertaken to achieve large-scale multiplication and faster development of caladium through tissue culture technique using meristem as explants.

MATERIALS AND METHODS

In the foregoing study meristems of pot grown

caladium was taken from the green house of AgriBiotech nursery Farms. The explants were surface sterilized by cleaning initially with detergent and later on treated with 0.5% NaOCl for 15 min and subsequently washed three times with autoclaved double distilled water. The sterilized explants were then inoculated by proper dissecting and sizing the meristem (0.5 - 1.0 cm) on MS (Murashige & Skoog, 1962) medium supplemented with different concentrations of BAP either alone or in combination with kinetin (Kin).

For multiplication of induced shoots, hormonal concentration was decreased and shoot multiplication was observed after 24 days of shoot induction. For *in vitro* rooting MS medium containing different concentrations of NAA and IBA was used either alone or in combination with each other. Sucrose (3%) was used in all the media. The pH of the medium was adjusted to 5.74 with 0.1 N solution of NaOH or HCl. MS medium was used both in solid and liquid forms. For solidification 0.7% agar was used. In case of liquid medium autoclaved cotton was used to support the plant tissues. The medium was autoclaved at 121ºC and 15 Ibs $Inch^{-2}$ for 15 min. Cultures were maintained under fluorescent light having 2500 lux light intensity. The incubation temperature was 26°C ± 1°C with 16 h light and 8 h dark period. Sub-culturing was done after five week.

RESULTS

Shoot formation. Shoot formation was noticed after 8 days of inoculation (Plate 1). For shoot formation MS medium was supplemented either with BAP alone or in combination with Kin. or NAA. It was observed that shoot formation was obtained in almost all the media supplemented with BAP. However, best shoot formation was obtained in MS medium containing 1 mg L^{-1} BAP, within 8 days of inoculation. By increase or decrease in the concentration of BAP the rate of shoot formation was decreased (Table Ia).

Combination of BAP with NAA also showed good results for shoot formation from apical meristem. Among these combinations 1.0 mg L^{-1} BAP with 0.5 mg L^{-1} NAA showed 99.6% shooting, although, time taken was longer as compared to medium containing 1.0 mg L^{-1} BAP. As far as combination of BAP with Kin was concerned no good results were obtained as compared to BAP alone or combination of BAP and NAA (Table Ib).

Multiple shoot formation. For *in vitro* shoot multiplication it was observed that MS medium containing 1.0 mg L^{-1} BAP with 0.25 mg L^{-1} NAA gave best results. Total 32 shoots per culture vial were formed at this concentration after 28 days of incubation. By increasing the concentration of BAP or NAA shoot multiplication response was decreased (Table II; Plate 2 & 3). When BAP was used alone no good results for shoot multiplication were obtained. Maximum 24.6 shoots were formed after 28 days of incubation when 1.0 mg L^{-1} BAP was used. By increase or decrease in the concentration of BAP rate of shoot multiplication as well as number of shoots per culture were decreased. Similarly, combination of BAP with Kin also gave less number of shoots as compared to the combination of BAP and NAA.

Rooting. Well developed multiple shoots (5 cm long), were shifted for rooting. For *in vitro* rooting two different auxins NAA and IBA were used either alone or in combination. It was observed that among different concentration of NAA, 100% rooting was obtained at 1 mg L^{-1} of NAA within 12 to 13 days of incubation. By increase or decrease in the

Plate 1. Apical meristem inoculated for shoot formation

Plate. 2. Induction of *in vitro* shoot multiplication

Plate 3. Multiple shoot formation and proliferation

Table Ia. Effect of Different Concentration of BAP on Shoot Formation From Meristem

Sr. No	Media	Concentration (mg L^{-1})	No. of explants cultured	Days for shoot induction	No. of test tubes showing shoot induction	Rate of shoot induction	Shoot length
MS1		0.5	10	12.4 ± 0.738^{ab}	6.33 ± 0.272^{c}	63.3	0.8 ± 0.028^{c}
MS2		1.0	10	8 ± 0.774^{c}	10^{a}	100	1.2 ± 0.097^{b}
MS3	MS + BAP	1.5	10	9.4 ± 0.845^{bc}	9.33 ± 0.272^{ab}	93.3	1.56 ± 0.060^{a}
MS4		2.0	10	12.6 ± 0.84^{ab}	8.67 ± 0.272^{b}	86.7	0.78 ± 0.043^{c}
MS5		2.5	10	$14 .2 \pm 0.77^{a}$	7.33 ± 0.544^{c}	73.3	0.52 ± 0.033^{d}

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test

Table Ib. Effect of Different Concentration of BAP with Kinetin and NAA on Shoot Formation From Meristem

Sr. No	Media	Concentration (mg L^{-1})	No. of explants cultured	Days for shoot induction	No. of test tubes showing shoot induction	Rate of shoot induction	Shoot length
S1		0.5+0.25	10	14.0 ± 0.489^{a}	7.67 ± 0.272^{b}	76.7	0.6 ± 0.028^{c}
S2		0.5+0.5	10	14.2 ± 0.438^{a}	9.13 ± 0.720^{ab}	91.3	0.74 ± 0.035^{b}
S3	MS+BAP+Kin	1.0+0.5	10	12 ± 0.4^{b}	9.96 ± 0.272^{a}	99.6	0.88 ± 0.052^{a}
S4		2.0+0.5	10	12.4 ± 0.456^{b}	9.0 ± 0.471^{ab}	90	0.76 ± 0.035^{ab}
S5		0.5+0.25	10	17.4 ± 1.04^{b}	6.67 ± 0.544^{a}	66.7	0.54 ± 0.045^{b}
S6		0.5+0.5	10	21 ± 0.748^{a}	4.33 ± 0.720^{b}	43.3	0.54 ± 0.021^{b}
S7	MS+BAP+NAA	1.0+0.5	10	13.6 ± 0.606^{c}	8.0 ± 0.471^{a}	80	0.86 ± 0.045^{a}
S8		2.0+0.5	10	$15.2 \pm 0.521b^{c}$	8.37 ± 0.544^{a}	83.7	0.76 ± 0.021^{a}

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test

Table II. Effect of Different Hormones on *in vitro* Shoot Multiplication

Sr. No	Media	Concentration (mg L^{-1})	No. of explants cultured	No of shoots per culture	Average shoot length
M1		0.5	10	8.6 ± 0.219^{c}	3.5 ± 0.2^{a}
M2		1.0	10	24.6 ± 1.18^{a}	2.6 ± 0.126^{b}
M3	MS+BAP	1.5	10	20.8 ± 0.593^{b}	2.5 ± 0.132^{b}
M4		2.0	10	14.4 ± 0.456^{d}	2.5 ± 0.164^{b}
M5		0.5+0.25	10	21.4 ± 0.829^{b}	2.78 ± 0.091^{ab}
M6		1.0+0.25	10	32 ± 0.632^{a}	2.6 ± 0.048^{b}
M7	MS+BAP+NAA	0.5+0.5	10	20.6 ± 0.829^{b}	3.0 ± 0.084^{a}
M8		1.0+0.5	10	23.8 ± 1.425^{b}	2.6 ± 0.074^{b}
M9		0.5+0.25	10	20.4 ± 0.920^{b}	2.6 ± 0.063^{a}
M10		1.0+0.25	10	25.6 ± 0.829^{a}	2.5 ± 0.04^{a}
M11	MS+BAP+Kin	0.5+0.5	10	21.2 ± 0.769^{b}	2.5 ± 0.063^{a}
M12		1.0+0.5	10	27 ± 1.16^{a}	2.1 ± 0.089^{b}

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test

concentration of NAA the rate of root induction was decreased. Use of IBA at 2 mg L^{-1}, gave a maximum of 90% rooting. IBA less than 2 mg L^{-1} did not produce good results for *in vitro* rooting. The best response in this regard was obtained when 2 mg L^{-1} IBA with 1 mg L^{-1} NAA was used. At this concentration 100% rooting was noted in 8 days of incubation for shoots (Table III).

Hardening. Well developed *in vitro* plants were shifted in glass house in three different media compositions containing sand, sand + peat and sand + peat + soil (1:1:1) at temperature of 25°C ± 1°C to 27°C ± 1°C for hardening. Excellent hardening was achieved in a mixture containing sand + peat + soil (1:1:1) at 95% humidity level (Plate 5 & 6).

DISCUSSION

Meristem culture allows rapid clonal multiplication and virus elimination for the study of shoot and root development *in vitro* (Li *et al.*, 1991; Altaf *et al.*, 1993). The results of present study revealed that, meristem explant (Plate I), cultured on MS medium containing different concentrations and combinations of BAP with NAA or Kin greatly influenced the shoot induction response. Best shoot formation was obtained when 1.0 mg L^{-1} BAP was used in MS medium (Table Ia). At this concentration 100% shoot formation was obtained with in 8 days of inoculation. By increase or decrease in the concentration of BAP the rate of shoot formation was decreased. Mujib *et al.* (1996) also reported the best shoot formation response in MS medium supplemented with BAP. However, Li (1987), Zhu *et al.* (1993), Chu and Yazawa (2001) and Ahmad *et al.* (2004) reported best shoot induction response in MS medium containing a combination of BAP with NAA. Although in present investigation the combination of 1.0 mg L^{-1} BAP with 0.5 mg L^{-1} NAA gave 100% shoot formation response but time taken for shoot induction was higher than 1.0 mg L^{-1} BAP alone.

In case of shoot multiplication BAP alone did not give satisfactory results. Maximum (24 shoots) were obtained in MS medium containing 1.0 mg L^{-1} of BAP. Change in the concentration of BAP, the number of multiple shoot was decreased. Chan *et al.* (2003) reported best shoot

Table III. Effect of Different Hormones on Rooting

Sr. No	Media	Concentration (mg L^{-1})	No. of shoots cultured	Days for root initiation	No of Plants showing roots	Frequency of root formation	Number of roots per plant
R1		0.5	10	18.4 ± 0.606^{a}	7.0 ± 0.282^{b}	70	1.2 ± 0.178^{b}
R2		1.0	10	12.6 ± 0.829^{c}	9.4 ± 0.456^{a}	94	2 ± 0.282^{a}
R3	MS+NAA	1.5	10	14.6 ± 0.456^{b}	9.4 ± 0.536^{a}	94	2.2 ± 0.178^{a}
R4		2.0	10	14.8 ± 0.334^{b}	9.2 ± 0.334^{a}	92	1.6 ± 0.219^{ab}
R5		0.5	10	17.2 ± 0.769^{a}	5.2 ± 0.438^{c}	52	1.2 ± 0.178^{b}
R6		1.0	10	14.4 ± 0.456^{b}	7.2 ± 0.593^{b}	72	1.6 ± 0.357^{ab}
R7	MS+IBA	1.5	10	14 ± 0.489^{b}	8 ± 0.4^{ab}	80	2.2 ± 0.334^{a}
R8		2.0	10	12.6 ± 0.726^{b}	9 ± 0.489^{a}	90	2.4 ± 0.219^{a}
R9		0.5 + 0.5	10	16 ± 0.565^{a}	8.2 ± 0.219^{b}	82	1 ± 0.219^{b}
R10		0.5 + 1.0	10	14 ± 0.632^{b}	9.4 ± 0.219^{a}	94	2.4 ± 0.357^{ab}
R11	MS+NAA+IBA	0.5 + 2.0	10	12 ± 0.489^{c}	9.6 ± 0.357^{a}	96	2.2 ± 0.178^{ab}
R12		1.0 + 2.0	10	8.4 ± 0.669^{d}	10 ± 0^{a}	100	3 ± 0.282^{a}

Means followed by different letters in the same column differ significantly at P = 0.05 according to Duncan's new multiple range test

Plate 4. Well developed multiple shoots

Plate 5. Well developed *in vitro* plant shifted for hardening in green house

multiplication response in MS medium containing 2 mg L^{-1} BAP. However, in present study when 0.25 mg L^{-1} NAA was added in 1 mg L^{-1} of BAP, excellent multiple shoots were formed (Plate 2). At this combination total 32 shoots were formed (Plate 3 & 4). By increase or decrease in the concentration of either BAP or NAA rate of shoot multiplication was decreased (Table II). Many workers have reported best shoot multiplication response of Caladium on MS medium containing specific combination of BAP with

Plate 6. Well developed hardened plant ready for shifting in to the natural climate

Plate 7. Tissue cultured plant of Caladium bicolor growing under Natural climatic conditions

NAA (Zhu *et al.,* 1993; Chu & Yazawa 2001; Ahamd *et al.,* 2002). As far as combination of BAP with Kin is concerned it failed to give good shoot multiplication response.

Well developed multiple shoot when attain the size of 3.0 cm were shifted for rooting on MS medium containing different concentration of either NAA or IBA. Best rooting response was obtained on MS medium containing 1.0 mg L^{-1} NAA. At this concentration all plants showed excellent

rooting within eight days of inoculation. Mujib *et al*. (2000) reported best rooting in MS medium containing 2.0 mg L^{-1} IBA, but in present study the results of IBA were no better than NAA alone (Table III).

After rooting well developed *in vitro* plants were shifted for hardening into the green house (Plate 5). Better hardening was obtained in a mixture of sand, peat and soil (1:1:1), while in pure autoclaved sand seedling mortality was noticed. This may be due to less water holding capacity of pure sand. During hardening plants were watered with Hoagland solution (Cooper *et al.*, 1983). After 3 week of hardening plants were shifted into pots containing soil + peat and were brought to natural climate after one week (Plate 6 & 7).

CONCLUSION

MS medium containing 1 mg L^{-1} BAP gave best results for shoot formation within 8 days of inoculation. Excellent *in vitro* shoot multiplication was obtained on MS medium containing 1 mg L^{-1} BAP + 0.25 mg L^{-1} NAA. For rooting of *in vitro* shoots combination of 2 mg L^{-1} IBA + 1 mg L^{-1} NAA showed cent percent response. Reliable protocols for micropropagation of *Caladium bicolor* were established and could be used for large scale production of disease free, high yielding and premium quality planting material. An average of 2500 plants could be produced from a single meristem within 12 week.

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