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In vitro regeneration of *Caladium bicolor*

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Abstract

The present experiment was conducted to determine the ideal concentration of different plant growth regulators (BA, Kin, IBA, IAA, and NAA) for *in vitro* regeneration of *Caladium bicolor* using shoot tip explants. The work was designed in CRD with three replications. Shoot tip explants gave rise to multiple shoots when cultured on MS medium supplemented with different concentration of BA with IBA. The highest (90%) response of shoot multiplication was obtained in MS medium containing 0.25-1.0mg/L BA + 2.0-2.5mg/L IBA. The regenerated shoots were then rooted on MS medium with different concentrations NAA, IAA and IBA. The maximum frequency of rooting and highest number of roots was produced on medium containing 2.0mg/L IAA. In accordance with average growth characteristics, it was revealed that the combined effect of BA and IBA appeared to be better to individual performance. The plantlets, thus developed were hardened and successfully established in soil. The plants raised through tissue culture exhibited normal growth. Reliable protocols for micropropagation of *Caladium bicolor* were established, which could be used for large scale production of disease free, high-yielding, and premium quality planting material.

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Introduction

Caladium bicolor is a member of Araceae family (arum family) and commonly known as angel wings, heart of Jesus and fancy-leaved caladium (Ali *et al*., 2007; Syedi *et al*., 2016). It is an important ornamental plant valued for its long-lasting colorful foliage, and is commonly grown in containers and in the landscape (Syedi *et al*., 2016; Deng, 2018; Zhang *et al*, 2019). They are 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. Ornamental value of *Caladiums* depends to a great extent on leaf characteristics, including shape, color, color pattern, and venation pattern (Deng and Harbaugh, 2005).

Generally, Caladium is 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, requires hand pollinations, very high mortality and very difficult to keep plant true to type and pathogen free. Moreover, plants grown from seeds are very expensive. It has also been reported that seed propagation results in variability (Ali *et al*., 2007; Deng *et al*., 2007). Concerns have been raised about possible loss of genetic diversity due to a drastic decline in the number of cultivars in the last century. Moreover, this method is very difficult to keep plant true to type and pathogen free (Siddiqui *et al*., 1993; Deng *et al*., 2007). Consequently, seed propagation is not used in commercial production of caladium plants.

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. The success of the micropropagation method depends on several factors like genotype, media, PGRs and type of explants (Pati *et al*., 2005; Nhut *et al*., 2010). Some investigations were done on micropropagation of Caladium spp. using leaf, apical meristem, inflorescences and other explants and a high number of treatments, plant growth regulators (PGRs), and dosages (Mujib *et al*., 2000; Chu and Yazawa, 2001; Ahmad *et al*., 2004; Ali *et al*., 2007; Thepsithar *et al*., 2010).

Therefore, the present investigation was carried out to identify the best hormonal combination in *Caladium bicolor* regeneration as well as rapid and easy *in vitro* propagation of *Caladium bicolor*.

Materials and methods

In the present study, *Caladium bicolor* plants were used as planting materials and were collected from different nurseries around Sher-e-Bangla Nager, Dhaka-1207. The plants were then grown in pots, and healthy, disease free shoot tips of 2 to 3cm in length and 2cm in width were used as explants for *in vitro* regeneration. Explants were sanitized by washing them in running tap water and then rinsing them again after adding a few drops of Tween-20 for 15 minutes. After that, they were sterilized for one minute with 70% ethanol after being washed several times with distilled water. Following 2 minutes of surface sterilization in a 0.2% mercuric chloride solution, they were rinsed four times with double-distilled water inside the laminar air flow chamber. Finally, 0.5–1.0cm sized explants (Fig. 1.) were cultured on MS media supplemented with specific concentrations of growth regulators (BA, IAA, IBA, KIN and NAA) singly or in combination, containing 30g/L sucrose, 5g of MS media and 0.8% agar. The pH of the media was adjusted to 5.8 with 0.1 NaOH or HCl before autoclaving at 1.06 kg/cm2 and 121°C for 20 minutes. Afterwards, the cultures were incubated at 25 ± 2°C for 16 hours of photoperiod. Three different sets of experiments were conducted

following the Completely Randomized Design (CRD) with three replications. The nodal segments from the proliferated shoots were sub-cultured again for further multiple shoot induction. Regenerated multiple shoots were cut and individual shoots were placed in MS media containing different concentrations of IBA, IAA and NAA for root induction. Ultimately, regenerated plantlets were transplanted to pots (10×15cm) containing soil and cowdung in 1:1 ratio and soil mixture were treated with a solution of 1% IBA.

Data for multiple shoot induction and rooting frequency were collected after 3, 5, and 8 weeks. Only data which showed some advantageous effect were included in the tables and 30 explants were used per treatment and repeated three times. The collected data on different parameters were analyzed using the MSTAT-C computer program. The analysis of variance was performed, and the mean was compared by the Least Significant Difference (LSD) test for interpretation of the results.

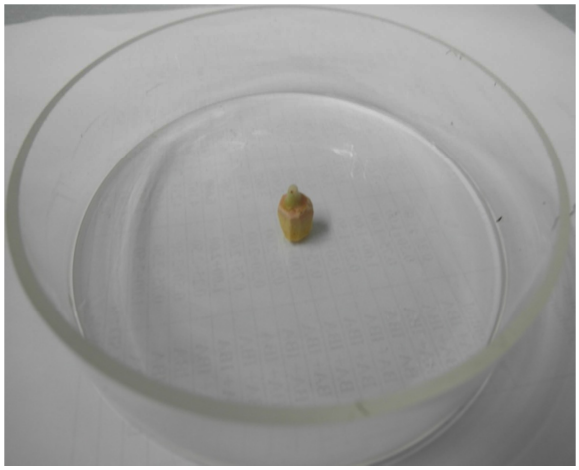

Fig. 1. *Caladium bicolor* explant (0.5–1.0cm sized shoot tip) prepared for placement in MS media.

Results and discussion

The *in vitro* regeneration of *Caladium bicolor* was studied in three separate sets of experiments. In the first experiment, the ability of *Caladium bicolor* to induce shoots from a single hormonal concentration was studied. In the second experiment, different hormone doses were used to examine the shoot induction potentiality and shoot morphology. The third experiment investigates the root morphology and capability of root development with different hormonal concentration.

Effect of BA and KIN on shoot induction potentiality in Caladium bicolor

Healthy shoot tips were used as explants for shoot multiplication and proliferation on MS medium supplemented with 4 different concentrations (0.25, 0.50, 0.75 and 1.0mg/L) of BA and KIN. *In vitro* culture of shoot tip resulted highest (16.67%) shoot induction at 1.0mg/L BA (Table 1) followed by 13.33% at 1.0mg/L KIN, which is the highest for different concentration of KIN. In both cases of BA, decreasing the concentrations had resulted both increased and decreased root induction. Whereas, decreasing the concentrations of KIN, resulted the reduced root induction. At control 3.33% shoot induction observed, which is lower than tested all concentration of both hormone. Again, maximum numbers of shoots (5) were initiated when MS media was supplemented with 1.0mg/L BA and minimum (1) was at control. According to Mujib *et al.* (2008), adding BA alone or in conjunction with NAA significantly improved the growth of *C. bicolor*'s shoots, somatic embryos, and following growth stages.

Table 1. Effect of BA and KIN on shoot induction potentiality of *Caladium bicolor*.

Name of phytohormone	Phytohormone concentration (mg/L)	Shoot induction potentiality		
		Explants cultured (Number)	Initiated shoot (Number)	Explants showing shoot induction (%)
Control	0	30	1	3.33
BA	0.25	30	2	6.66
BA	0.50	30	3	10.0
BA	0.75	30	2	6.67
BA	1.00	30	5	16.67
KIN	0.25	30	2	6.67
KIN	0.50	30	2	6.67
KIN	0.75	30	3	10.0
KIN	1.00	30	4	13.33

Ahmed *et al.* (2004) noticed a comparable high shoot induction response in BA modified MS medium. Chan *et al.* (2001) found that MS medium containing 2mg/L BAP provided the best shoot multiplication response.

The application of 2,4-D along with BA and BAP is the most popular combination among all other PGRs combinations in relation to the combination of auxins and CKs for callus induction (Kaviani, 2015). To boost shoot proliferation, some species may need to combine a high amount of CKs with a low concentration of auxin (Van Staden *et al.*, 2008; Kaviani *et al.*, 2015).

Combined effect of BA and IBA on shoot

Shoot tip explants were inoculated on MS medium fortified with combination of different concentration of BA (0.25-1.0mg/L) with IBA (1.0 and 2.5mg/L) (Table 2). Within three weeks of culture multiple shoots emerged directly from the explants. The highest number of shoots (19.67) were observes at 1.00mg/L BA + 2.00mg/L IBA hormonal combination. Again, 0.5mg/L BA + 2.0mg/L IBA hormone combination showed 90% explants induction but shoot number were maximum (19.67) when BA concentration was increased at 1.0mg/L and combined with 2.0mg/L IBA. Average length of shoot was highest (11.43cm) when the lowest BA concentration (0.25mg/L) combined with highest IBA concentration (2.5mg/L).

In the present study under the moderate combination of BA and IBA (0.5mg/L BA + 2.0mg/L IBA) was found to be the ideal combination for high frequency multiple shoot induction (Fig. 2.). Eight weeks after shoot induction, the leaves were counted; the highest number of leaves (30.67) was found at 1.00mg/L BA + 2.00mg/L IBA, and the lowest number (5.43) was in the control.

Fig. 2. Maximum multiple shoot induction at 0.5mg/L BA + 2.0mg/L IBA supplemented MS media

Among the evaluated PGRs, BA is the one utilized for shot induction the most frequently (Kaviani, 2015). Ahmad *et al.* (2004) found that MS medium containing a combination of BAP and NAA had the best shoot induction response. Again, Ali *et al.* (2007) found that the combination of 1.0mg/L BAP with 0.5mg/L NAA gave 100% shoot formation. They also reported that the shoot induction response was greatly influenced by MS media with various concentrations and BAP with NAA or Kin combinations. On the other hand, Al-Taleb *et al.* (2011) obtained their best results on MS medium containing IBA. The developing shoots were elongated by subculturing on the same combinations of growth regulators. Later on elongated shoots were excised and used for root induction.

Table 2. Effects of different concentrations of BA with IBA on shoot induction from shoot tip explants of *Caladium bicolor*.

BA+IBA	% Explant showing shoot induction	No. of shoots/ explant (8WAI)	Average length of shoot (cm) (8 WAI)
0.25 +1.00	40.00	8.913	3.900
0.50+1.00	50.00	9.680	4.067
0.75+1.00	56.67	10.13	3.867
1.00+1.00	60.00	8.490	4.100
0.25+1.50	66.67	8.080	4.233
0.50+1.50	70.00	9.633	4.533
0.75+1.50	76.66	9.440	4.767
1.00+1.50	80.00	8.967	5.100
0.25+2.00	80.00	9.960	6.100
0.50+2.00	90.00	9.080	6.600
0.75+2.00	83.33	10.35	7.100
1.00+2.00	83.33	19.67	8.700
0.25+2.50	76.66	10.26	11.43
0.50+2.50	73.33	8.550	7.333
0.75+2.50	70.00	9.760	7.133
1.00+2.50	70.00	8.370	6.467

Effect of different concentrations of NAA, IAA and IBA on root

Well-developed multiple shoots were shifted for rooting. For *in vitro* rooting four different concentrations (1.0, 1.5, 2.0 and 2.5mg/L) of NAA, IAA and IBA were used. Among different investigated concentrations of NAA, IAA and IBA, 2.0mg/L IBA proved to be the most suitable for root induction with 29.87 roots per explant (Table 3; Fig. 3A.); whereas, the average roots length was highest in slightly lower concentration (1.5mg/L).

The longest root was also found at 1.5mg/L IAA concentration (Fig. 3B.). It was observed that among different concentrations of NAA at 1.00mg/L, maximum percentage (70%) of explants showed root induction as well as maximum number of roots (23.53 at 8WAI) were observed. On the other hand, maximum average length (7.53cm) of root was observed when NAA was used at 2.50mg/L concentration.

For root induction and growth, NAA is required (Jain and Ochatt, 2010). NAA is a potent auxin and just small quantities are required for root development (Pierik, 1987). In case of IAA, maximum (90.00%) root induction and Maximum average roots (29.87) at 8 WAI were found at 2.0mg/L IAA, whereas, maximum average root length (10.20cm) was recorded at 1.50mg/L IAA. Moreover, when MS media was supplemented with IBA, maximum percentage of explants (83.33%) showed root induction and Maximum (22.87) roots were observed at 2.50mg/L concentration, while maximum average length of root was found when IBA was used at 2.0mg/L concentration.

Table 3. Effects of different concentrations of NAA, IAA and IBA on *in vitro* rooting of shoots of *Caladium bicolor* after 8 weeks of culture.

Growth hormone conc. (mg/L)	% of micro cutting rooted	No. of roots (8WAI)	Average root length (cm at 8WAI)
NAA			
1.00	70.00	23.53	3.800
1.50	66.67	17.37	5.400
2.00	60.00	14.47	6.200
2.50	50.00	11.23	7.533
IAA			
1.00	66.67	21.33	4.53
1.50	76.66	24.77	10.20
2.00	90.00	29.87	5.23
2.50	70.00	12.23	3.26
IBA			
1.00	50.00	12.80	3.433
1.50	66.67	15.67	5.233
2.00	70.00	19.37	6.533
2.50	83.33	22.87	4.367

In every case, control treatment (only MS media) showed the lowest performance. Mujib *et al.* (2000) found that the best rooting occurred in MS medium with 2.0mg/L IBA. On the contrary, Ahmed *et al.* (2004) reported that Caladium was most successfully propagated *in vitro* by NAA. According to Webb *et al.* (1983), the combination of GA3 and IAA increased shoot elongation. Dhital *et al.* (2010) observed that 1.0 mg/L NAA gave rise to a greater number of roots (9.5) than 1.0 mg L^{-1} IAA (4.0). Several investigations on IBA-containing media have shown that longest roots were produced on IAA (Al-Taleb *et al.*, 2011). The use of NAA reduced the length of potato roots from 6cm on control to 4cm on 1.5mg/L NAA containing MS medium, as shown by Sanavy and Moeini (2003).

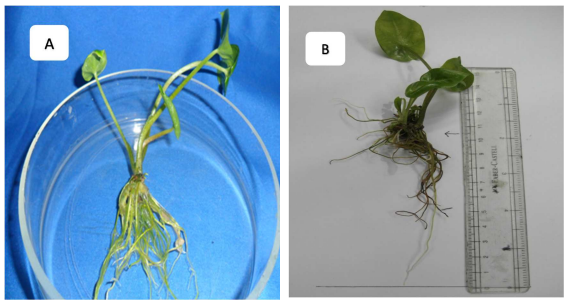

Fig. 3. *In vitro* regeneration of *Caladium bicolor*: A. Maximum average number of roots at 2.0mg/L IBA; B. Longest root in 1.5mg/L IAA at 8 WAI.

Ex vitro acclimatization and establishment of plantlets on soil

Rooted plantlets were taken out from culture tubes and washed thoroughly with tap water to remove the culture medium from the roots. Then, the washed plantlets were sprayed with fungicide and planted to normal and sterilized soil in small pots in growth chamber (Fig. 4.). Ghehsareh *et al.* (2020) reported that the substrate's root environment must be free of plant diseases and provide enough water, air, and nutrients for plant growth for acclimatization.

After 7 days the hardened plantlets were transferred to net house. Then 15 days later the hardened plantlets were planted in open field. Survival rate was 100% in growth chamber, 87% in net house and 92% in open field condition (Table 4). Numerous authors have documented effective plant acclimatization using peat moss and aglaonema; in addition to promoting plant growth, peat moss also enhanced leaf pigment synthesis (Barakat and Gaber, 2018).

Table 4. Survival rate of *in vitro* regenerated plantlets of *Caladium bicolor*.

Acclimatization	No. of plants transplanted	No. of plants survived	Percentage of survival rate
In growth chamber	30	30	100
Shade house with less humidity and indirect sunlight	30	26	87
In open atmosphere	26	24	92

Fig. 4. Regenerated plantlets of *Caladium* in shade house after 20 days of transplantation.

Conclusion

Caladiums are lovely landscape and container plants with a significant international market. Caladium *in vitro* propagation makes it possible to meet this export demand since it generates a significant amount of planting materials of the highest quality. Maximum number of shoot (5) initiated when MS media was supplemented with 1.0mg/L BA and minimum (1) was at control. Maximum percentage (90.00%) of explants showed shoot induction when 0.50mg/L BA was combined with 2.00mg/L IBA and minimum percentage (3.33%) was recorded at control and minimum (40.47) days required when 1.0mg/L BA was combined with 2.00mg/L IBA and maximum was recorded (85.50 days) in control (0.00mg/L). From the findings of the study it is very clear that the combination of 1.0mg/L BA+2.0mg/L IBA showed good performance in terms of shoot formation and 2.0mg/L IAA showed good performance in root formation. Reliable protocols for micropropagation of Caladium bicolor were developed and could be used for large-scale production.

Recommendation(S)

The following suggestions could be taken into consideration in light of the current experiment:

- To shorten the shoot induction time, further studies are needed to evaluate various hormones and hormone combinations at suitable doses.
- Research should include more cytokinin and auxin types besides BA, KIN, NAA, IAA, and IBA.
- In addition to shoot tip culture, petiole, leaf, and callus culture could be practiced.

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Conflict of interests

The authors declared that they have no conflict of interest related to this research.

Abbreviation

Benzyl Adenine= BA; Indole Butyric Acid= IBA; Indole-3-Acetic Acid= IAA, Kinetin= KIN
Murashige and Skoog medium= MS medium
Naphthalene acetic acid= NAA; Plant Growth Regulators= PGRs

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