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Effect of BAP and NAA on Micropropagation of *Caladium bicolor* (Aiton) Vent., an Ornamental Plant

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Abstract

Leaf explants of kaladium dwubarwne *Caladium bicolor* (Aiton) Vent., were cultured *in vitro* on MS media supplemented with 25 different concentrations of BAP and NAA in order to determine the appropriate concentrations for micropropagation. All combinations induced callus formation on explants. Callus production on leaves explants grown on control medium was very low. The medium enriched with 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA was the most effective for callus formation. The highest number of shoots (6.43 per explant) and roots (5.56 per explant) were regenerated on media containing 1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA and 3 mg l^{-1} BAP + 0.5 mg l^{-1} NAA, respectively. The regenerated plantlets were grown in a greenhouse and acclimatized successfully.

Keywords: Araceae, *In vitro* propagation, Plant growth regulators, Tissue culture.

Abbreviations: BA: 6-benzyladenine, BAP: 6-benzylaminopurine, CK: cytokinin, 2,4-D: 2,4-dichlorophenoxyacetic acid, MS: Murashige and Skoog, NAA: α-naphthalene acetic acid, PGRs: plant growth regulators.

INTRODUCTION

Caladium is a genus of flowering plants in the family Araceae. There are over 1000 named cultivars of *Caladium* bicolor from the original South American plant. The genus *Caladium* includes seven species that are native to South America and Central America. Several species are grown as ornamental plants for their large, arrowhead-shaped leaves marked in varying patterns in white, pink, and red. Caladiums are excellent landscape and pot plants grown for their colorful leaves (Deng and Harbaugh, 2006). *Caladium* is generally propagated via tubers for commercial purpose but tuber propagation has some limitations (Ali *et al*., 2007). Commercial propagation may also be done by seeds but the seed propagation is difficult because the seeds are very small and have a very high mortality and the plants grown from the seeds are very expensive, very difficult to keep plant true to type and pathogen free and with high risk of variability (Siddiqui et al., 1993; Gill *et al*., 1994; Deng *et al*., 2007; Ali *et al.*, 2007).

In vitro propagation techniques allow for the production of physiologically uniform clonal plants and potentially rapid multiplication. Micropropagation has been extensively applied for the rapid production of many plant species and cultivars especially ornamental plants. In many micropropagation studies, a high number of treatments, plant growth regulators (PGRs), and dosages are examined in an effort to find the best way to obtain a proper propagation protocol. Micropropagation is a powerful tool for *in vitro* propagation of *Caladium*. 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 BA, BAP, KIN, NAA, 2,4-D and IBA as PGRs (Zhu *et al*., 1993; Mujib *et al.*, 2000; Chu and Yazawa, 2001; Ahmad *et al.,* 2004; Ali *et al.*, 2007; Thepsithar *et al*., 2010). The present study was carried out to achieve large-scale multiplication of kaladium dwubarwne (*Caladium bicolor* (Aiton) Vent.) through tissue culture technique using leaf as explants and different concentrations of BAP and NAA as PGRs.

MATERIALS AND METHODS

The plants of kaladium dwubarwne (*Caladium bicolor* (Aiton) Vent.) were prepared from a greenhouse in Amol, Iran. Leaves were dissected from mother plants and used as explants for tissue culture establishment. The explants were washed under running tap water and 1-2 drops of hand washing liquid for 30 min. followed by 0.1% Carbendazim fungicide for 30 min. Explants were disinfected by immersion in 8% sodium hypochlorite (NaClO) (v/v) with two drops of Tween-20 for 10 min., followed by three rinses in sterile distilled water. Finally, explants were kept in 70% ethanol for 1 min. followed by three rinses in sterile distilled water. Surface sterilized leaves were inoculated in MS (Murashige and Skoog, 1962) medium with 3% (w/v) sucrose. The media were solidified with 0.70% Agar-agar. The concentrations of 0, 1, 2, 3 and 4 mg l^{-1} of BAP, and 0, 0.5, 1, 2 and 3 mg l^{-1} of NAA were added to the media individually or/and in combination. The pH of the medium was adjusted to 5.7 before adding agar to it. The medium (30 ml) was dispensed into 100 ml glass bottles. The culture glass bottles containing the media were autoclaved at 121°C and 104 kPa for 20 min. Experiments were carried out in five replications and one explants were inoculated in each glass bottles and plugged firmly. The cultures were maintained at temperature of 22 ± 2ºC, 16-h photoperiod (irradiance of 50 µmol m^{-2} s^{-1}), provided by cool daylight fluorescent lamps. Traits including shoot length, shoot number, leaf number, leaf long, petiole length, root number, root length, and survival percentage were measured after 55 days. For hardening and acclimatization, the glass bottles with plantlets (fully expanded leaflets) were kept open for a week after removing the plugs in the culture room. Then, the plantlets were removed from the culture media and washed with distilled water; then, they were transferred to plastic cups containing perlite. Plantlets were kept in a greenhouse at 22 ± 2°C and 80% RH with periodic irrigation (once each for four days).

The experimental design was RCBD with five replicates and each replicate includes one

specimens. Data processing of the results was carried out by MS-Excel Software Package. Analysis of variance (ANOVA) was done using SPSS and MSTATC statistical software and the means were compared using the Least Significance Difference Test (LSD) at the 5% probability level.

RESULTS

Effect of BAP and NAA on shoot length

There was a significant difference between the effects of BA ($P<0.01$), NAA ($P<0.01$) and BA + NAA ($P<0.05$) on shoot length (Table 1). The highest mean shoot length (5.83 cm per explant) was obtained from the treatment of 3 mg l^{-1} BAP + 1 mg l^{-1} NAA in *Caladium bicolor* (Aiton) Vent. (Table 2). 2 mg l^{-1} BAP + 1 mg l^{-1} NAA as well as 1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA were suitable for enhancing the shoot length (5.36 and 5.16 cm per explants, respectively) (Table 2, Fig. 1). The

Table 1. Analysis of variance (ANOVA) for the effect of different concentrations of NAA and BAP, in combination with each other, on shoot length, shoot number, leaf long, leaf number, petiole length, root length, root number and viability of *Caladium bicolor* (Aiton) Vent.

S.O.V.	df	MS							
		Shoot length	Shoot number	Leaf length	Leaf number	Petiole length	Root length	Root number	Viability
BAP	4	6.915**	1.722**	0.720*	40.29**	11.37**	2.268**	0.904^{ns}	142.8*
NAA	4	7.205**	18.03**	1.640**	1.51^{ns}	2.28^{ns}	12.97**	11.82**	267.8**
BAP × NAA	16	1.62*	1.272**	0.438*	1.843*	2.34*	1.282*	1.207*	101.7*
Error	50	0.719	0.406	0.226	0.924	1.182	0.576	0.563	53.66
CV (%)		25.04	15.03	42.38	15.25	31.55	42.29	29.59	9.93

* and **: Significant at α = 5% and 1%, respectively, ns: not significant

Table 2. Mean comparison of the effect of different concentrations of BAP and NAA, in combination with each other, on shoot length, shoot number, leaf long, leaf number, petiole length, root length, root number and viability of *Caladium bicolor* (Aiton) Vent.

PGRs (mg l^{-1})	Shoot length (cm)	Shoot number	Leaf length (cm)	Leaf number	Petiole length (cm)	Root length (cm)	Root number	Viability (%)
BAP 0.00 + NAA 0.00	2.53^{d-h}	3.53^{fg}	0.50^{fg}	3.20^{h}	1.83^{h}	1.06^{efg}	2.23^{c-h}	66.66^{d-f}
BAP 0.00 + NAA 0.5	3.20^{c-g}	4.86^{bcd}	1.25^{a-f}	3.66^{gh}	2.50^{f-h}	1.70^{d-g}	2.50^{c-g}	70.00^{c-f}
BAP 0.00 + NAA 1.00	1.86^{gh}	4.43^{def}	1.25^{a-f}	4.16^{gh}	2.46^{gh}	1.06^{efg}	2.93^{b-e}	70.00^{c-f}
BAP 0.00 + NAA 2.00	1.70^{h}	3.46^{fg}	0.41^{g}	3.96^{gh}	2.16^{h}	1.33^{efg}	2.13^{d-h}	76.66^{a-d}
BAP 0.00 + NAA 3.00	2.23^{fgh}	3.60^{efg}	0.66^{efg}	4.03^{gh}	2.50^{f-h}	0.70^{g}	2.50^{c-g}	63.33^{ef}
BAP 1.00 + NAA 0.00	3.23^{c-g}	3.66^{efg}	0.66^{efg}	5.06^{fg}	2.86^{d-h}	0.83^{fg}	1.43f ^{gh}	60.00^{f}
BAP 1.00 + NAA 0.5	5.16^{ab}	6.43^{a}	1.50^{a-d}	5.20^{e-g}	2.83^{d-h}	2.76^{bcd}	3.43^{bc}	70.00^{c-f}
BAP 1.00 + NAA 1.00	3.50^{c-f}	5.53^{abc}	1.41^{a-e}	5.10^{fg}	2.56^{f-h}	2.93^{bcd}	2.40^{c-h}	80.00^{abc}
BAP 1.00 + NAA 2.00	3.83^{bcd}	4.63^{cde}	1.00^{c-g}	6.23^{c-f}	2.76^{e-h}	1.40^{efg}	1.56f ^{gh}	83.33^{ab}
BAP 1.00 + NAA 3.00	3.23^{c-g}	3.46^{fg}	1.33^{a-e}	5.80^{d-f}	3.03^{d-h}	1.06^{efg}	1.86^{e-h}	76.66^{a-d}
BAP 2.00 + NAA 0.00	2.63^{d-h}	3.60^{efg}	0.83^{d-g}	6.40^{c-f}	3.96^{c-g}	0.96^{efg}	1.33^{gh}	71.66^{b-f}
BAP 2.00 + NAA 0.5	4.50^{abc}	5.76^{ab}	2.00^{a}	7.63^{bc}	3.46^{c-h}	3.00^{bc}	4.00^{b}	80.00^{abc}
BAP 2.00 + NAA 1.00	5.36^{a}	5.60^{abc}	0.66^{efg}	6.43^{c-f}	3.16^{d-h}	1.93^{c-g}	3.23^{bcd}	76.66^{a-d}
BAP 2.00 + NAA 2.00	3.66^{b-e}	2.36^{h}	0.66^{efg}	7.33^{b-d}	3.20^{d-h}	1.76^{c-g}	2.83^{b-e}	76.66^{a-d}
BAP 2.00 + NAA 3.00	2.80^{d-h}	3.36^{gh}	0.75^{d-g}	7.66^{bc}	4.43^{b-e}	0.90^{efg}	2.30^{c-h}	73.33^{b-d}
BAP 3.00 + NAA 0.00	3.30^{c-f}	2.83^{gh}	0.66^{efg}	7.53^{bc}	4.03^{b-g}	1.07^{efg}	1.40^{gh}	70.00^{c-f}
BAP 3.00 + NAA 0.5	4.53^{abc}	6.13^{a}	1.75^{abc}	7.26^{b-d}	4.23^{b-f}	5.13^{a}	5.56^{a}	73.33^{b-d}
BAP 3.00 + NAA 1.00	5.83^{a}	3.53^{fg}	2.10^{a}	7.76^{bc}	2.83^{d-h}	2.10^{c-f}	2.30^{c-h}	83.33^{ab}
BAP 3.00 + NAA 2.00	3.63^{b-e}	3.46^{fg}	1.33^{a-e}	7.63^{bc}	4.56^{b-d}	0.86^{efg}	2.36^{c-h}	83.33^{ab}
BAP 3.00 + NAA 3.00	2.43^{e-h}	3.53^{fg}	0.83^{d-g}	6.80^{b-e}	5.76^{ab}	1.80^{c-g}	1.83^{e-h}	76.66^{a-d}
BAP 4.00 + NAA 0.00	3.56^{c-f}	3.43^{fg}	1.83^{ab}	7.13^{b-d}	5.16^{a-c}	0.93^{efg}	1.23^{h}	70.00^{c-f}
BAP 4.00 + NAA 0.5	3.63^{b-e}	5.70^{ab}	1.66^{abc}	9.93^{a}	6.36^{a}	3.93^{ab}	4.00^{b}	86.66^{a}
BAP 4.00 + NAA 1.00	3.90^{bcd}	6.30^{a}	1.08^{b-f}	8.30^{b}	3.40^{d-h}	2.13^{cde}	3.13^{bcd}	66.66^{d-f}
BAP 4.00 + NAA 2.00	1.90^{gh}	3.66^{efg}	1.25^{a-f}	6.66^{c-e}	3.46^{c-h}	1.66^{d-g}	2.63^{c-f}	71.66^{b-f}
BAP 4.00 + NAA 3.00	1.95^{gh}	3.20^{gh}	0.75^{d-g}	6.56^{c-f}	2.56^{f-h}	1.70^{d-g}	2.23^{c-h}	66.66^{d-f}

In each column, means with the similar letters are not significantly different at 5% level of probability using LSD test.

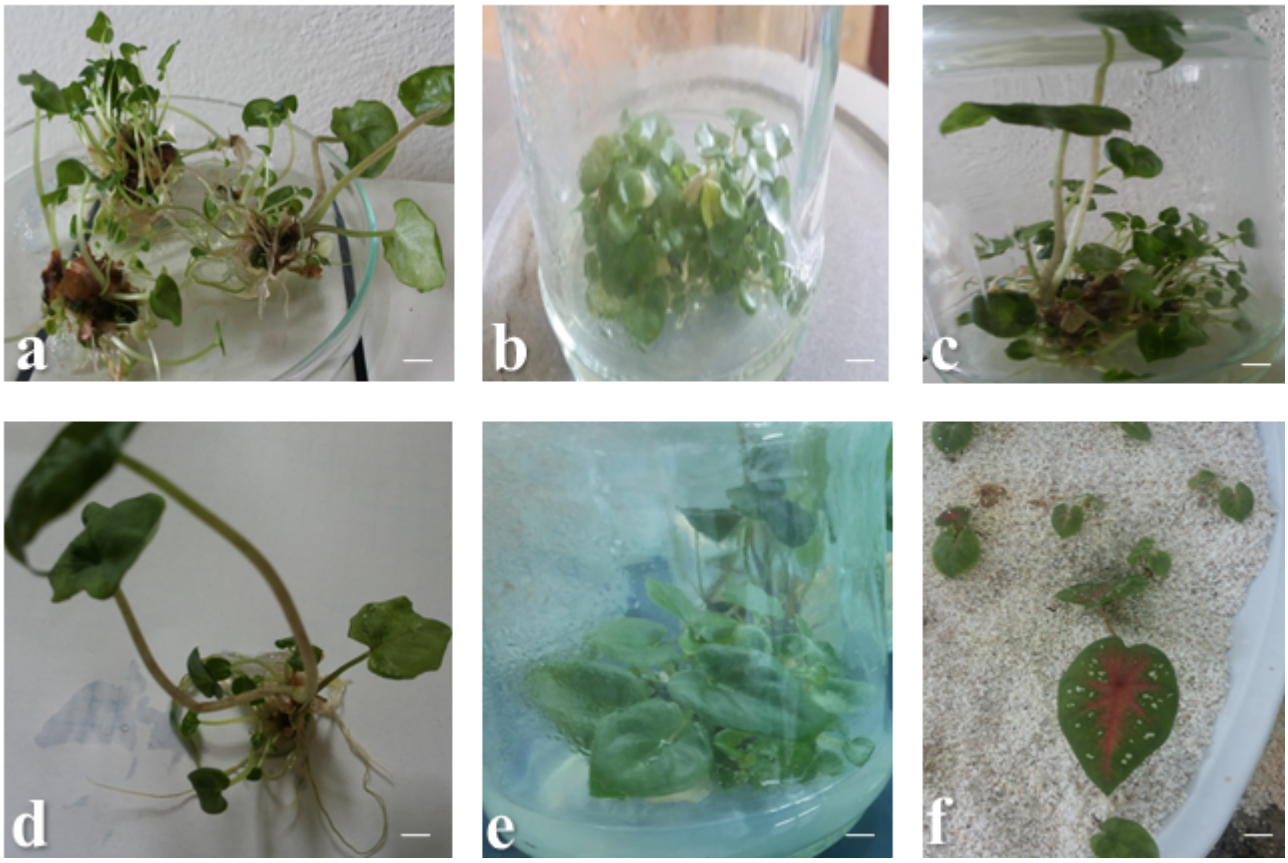

Fig. 1. Effect of different concentrations of BAP and NAA on shoot multiplication and root induction of *Caladium bicolor* (Aiton) Vent. (a) plantlets multiplied in MS medium (Bar = 1 cm); (b) shoot multiplication on medium enriched with 1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Bar = 1 cm); (c) petiole growth in medium containing 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Bar = 1 cm); (d) root induction and growth in shoots grown on medium supplemented with 3 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Bar = 1 cm); (e) expansion of leaf length in medium containing 3 mg l^{-1} BAP + 1 mg l^{-1} NAA (Bar = 1 cm); (f) plantlets hardening and acclimatization (Bar = 2 cm). Acclimatization was done in a greenhouse using pots filled with perlite.

lowest shoot length (1.70 and 1.86 cm per explants) was measured in plantlets treated with 2 and 1 mg l^{-1} NAA without BAP, respectively (Table 2). The highest concentrations of BAP and NAA in combination with each other were no good for the induction of shoot length (Table 2). Concerning the shoot length per plantlets, BAP at 4 mg l^{-1} induced the maximum length (3.56 cm per explant).

Effect of BAP and NAA on shoot number

Differences in shoot number in explants grown under combination of NAA and BAP were significant ($P<0.01$) (Table 1). Shoot number varied with NAA and BAP concentrations (Table 2). Minimum shoot number per explant (2.36 and 2.83) was recorded in the plantlets treated with 2 mg l^{-1} BAP + 2 mg l^{-1} NAA and 3 mg l^{-1} BAP without NAA, respectively (Table 2). The highest number of shoots (6.43/plantlet) was achieved on MS medium supplemented with 1 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Table 1). Concerning the shoot number per plantlets, BAP at 1 mg l^{-1} induced the maximum number (3.66). Among all concentrations of NAA, 0.5 mg l^{-1} induced the highest number of shoots per plantlets (4.86) (Table 2).

Effect of BAP and NAA on leaf length

Results presented in Table 2 show that the highest leaf length (2.10 cm) was calculated in plantlets treated with 3 mg l^{-1} BAP + 1 mg l^{-1} NAA. Plantlets treated with 2 mgl^{-1} BAP + 0.5 mg l-1 NAA containing 2 cm long was suitable treatment (Table 2). The lowest leaf length (0.41 and 0.5 cm) was obtained in plantlets treated with 2 mg l^{-1} NAA without BAP and control, respectively

(Table 2). Analysis of variance (ANOVA) showed significant differences between the effects of BAP ($P<0.05$), NAA ($P<0.01$) and BAP + NAA ($P<0.05$) on leaf length (Table 1).

Effect of BAP and NAA on leaf number

Leaf number varied with NAA and BAP concentrations (Table 2). Minimum leaf number (3.20/ plantlet) was recorded in the control plantlets (Table 2). The highest number of leaves (9.93/plantlet) was achieved on MS medium supplemented with 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Table 2). Concerning the leaf number per plantlets, BAP at 3 mg l^{-1} induced the maximum number (7.53). Among all concentrations of NAA, 1 mg l^{-1} induced the highest number of leaves per plantlets (4.16) (Table 2). Differences in leaf number in explants grown under BAP and BAP + NAA were significant ($P<0.01$ and $P<0.05$, respectively) (Table 1). NAA had no significant effect on leaf number.

Effect of BAP and NAA on petiole length

Differences in petiole length in explants grown under BAP and BAP + NAA were significant ($P<0.01$ and $P<0.05$, respectively) (Table 1). NAA had no significant effect on leaf number. The highest mean petiole length (6.36 cm) was measured with the treatment of 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Table 2). The lowest petiole length (1.83 cm) was measured in control plantlets (Table 2).

Effect of BAP and NAA on root length

PGRs (BAP and NAA) had a significant effect on increasing root length. Maximum root length (5.13 cm/plantlet) was observed on MS medium supplemented with 3 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Table 2). Minimum root length (0.7 mm) was recorded in the plantlets treated with 3 mg l^{-1} NAA without BAP (Table 2). Among all concentrations of BAP, 3 mg l^{-1} induced the longest root (1.07 mm/plantlets). Also, among all concentrations of NAA, 0.5 mg l^{-1} resulted in the longest root per plantlets (1.70 mm) (Table 2). All explants grown on media without NAA produced shorter roots than other treatments. Differences in root length in explants grown under various combinations of BAP, NAA and BAP + NAA were significant ($P<0.01$, $P<0.01$ and $P<0.05$, respectively) (Table 1).

Effect of BAP and NAA on root number

The data clearly show that root number was strongly affected by NAA ($P<0.01$) (Table 1). Maximum roots were induced on media with NAA (Table 2, Fig. 1). The highest number of roots (5.56/explant) was produced on the base of plantlets treated with 3 mg l^{-1} BAP + 0.5 mg l^{-1} NAA. The lowest number of roots (1.23 and 1.33/plantlets) was induced in media enriched with 4 and 2 mg l^{-1} BAP without NAA, respectively (Table 2).

Effect of BAP and NAA on survival percentage

Viability of plantlets varied with BAP ($P<0.05$), NAA ($P<0.01$) and BAP + NAA ($P<0.05$) (Table 1). The highest viability (86.66%) was seen in plantlets treated with 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA (Table 2). The lowest viability (60%) was recorded in plantlets treated with 1 mg l^{-1} BAP without NAA. Among all concentrations of BAP, the highest viability (71.66%) was induced in plantlets treated with 2 mg l^{-1} (Table 2). On the other hand, among all concentrations of NAA, the highest viability (76.66%) was induced in plantlets treated with 2 mg l^{-1} (Table 2).

DISCUSSION

Callus obtained from leaf is a suitable source for the establishment of many plantlets. However, the genetic variation is highly likely. Thus, the comparison of the treated and control plantlets is necessary. Current study showed that leaf explants grown on MS medium containing 4 mg l^{-1} BAP + 0.5 mg l^{-1} NAA produced the highest number of calluses. The production of calluses induced

by PGRs has been shown by many researchers (Jain and Ochatt, 2010). Among all types of auxins and cytokinins (CKs) used alone or in combination for callus induction, 2,4-D is at the top level (Kaviani, 2015). Callus formation is central to many investigative and applied tissue culture procedures (biotechnology) (Mineo, 1990). Callus is a good source for suspension culture, production of secondary metabolites, clonal propagation through somatic embryogenesis and organogenesis, and also, for the study of cell division, elongation and differentiation process and genetic transformation (Mineo, 1990). The auxin commonly used for callus induction is 2, 4-D, but NAA and IAA are also used (George *et al*., 2008). In relation to the combination of auxins and CKs for callus induction, the use of 2,4-D along with BA and BAP is the most widely-used combination among all other PGRs combinations (Kaviani, 2015). Thepsithar *et al*. (2010) studied the effect of PGRs (BA, NAA and 2,4-D) on micropropagation of *Caladium bicolor*. Explants first expanded the leaves fully. Their results revealed that the eleven out of the sixteen combinations promoted callus formation. The medium containing 17.76 μM BA with 2.69 μM NAA was the most effective for callus proliferation in *Caladium bicolor*.

In case of shoot multiplication, BAP alone did not give suitable results. So, most shoots were produced on media containing BAP and NAA. Many concentrations of BAP induced shoot multiplication. Chan *et al*. (2003) revealed that the best shoot multiplication response of *Caladium* was obtained in MS medium enriched with 2 mg l^{-1} BAP. Ali *et al.* (2007) demonstrated that when 0.25 mg l^{-1} NAA was added to 1 mg l^{-1} of BAP, excellent multiple shoots were formed for Caladium. At this combination, 32 shoots were formed. This finding is in complete agreement with ours. Many researchers have shown the maximum shoot multiplication response of *Caladium* on MS medium containing specific combination of BAP with NAA (e.g. Zhu *et al.*, 1993; Chu and Yazawa, 2001; Ahamd *et al.*, 2002; Ali *et al*., 2007). In a study on micropropagation of *Caladium bicolor*, Thepsithar *et al*. (2010) showed that the plantlets were regenerated on MS medium containing 11.1 μM BA. Some species may require a low concentration of auxin in combination with high levels of CKs to increase shoot proliferation (Van Staden *et al*., 2008; Kaviani *et al*., 2015). The success of the micropropagation method depends on several factors among which the most important one is PGRs (Gomes and Canhoto, 2003). CKs are usually used on the micropropagation media to stimulate shoot multiplication (Chawla, 2009; El-Agamy, 2009). Many researchers showed that BA induced multiple shoot formation and shoot length (e.g. Nhut, 2003; Fráguas *et al.,* 2004; Kaviani, 2015). BA is the most widely used among all PGRs for shoot induction (Kaviani, 2015). In spite of the more important role of CKs on shooting, the use of auxin NAA for shooting is more extensive than all other CKs except for BA (Kaviani, 2015).

Our findings revealed that the presence of NAA is necessary for root induction and growth. Similar findings were observed by some other researchers (Jain and Ochatt, 2010). In a study on micropropagation of *Caladium*, Ali *et al*. (2007) showed that the best rooting was achieved on medium supplemented with 1.0 mg l^{-1} NAA. At this concentration, all plants showed excellent rooting within eight days of inoculation. Mujib *et al.* (2000) reported the best rooting in MS medium containing 2.0 mg l^{-1} IBA. For any micropropagation protocol, successful rooting of microshoots is an important pre-requisite to facilitate their establishment in soil. Auxins enhance the germination, root induction and seedling growth of many species (Jain and Ochatt, 2010). Pierik (1987) indicated that NAA is a strong auxin and relatively low concentrations are needed for root formation. With high concentrations of NAA, root formation fails to occur and callus formation takes place. Rooting is an important process to the success of micropropagation. Without effective root system, plant acclimatization will be difficult and the rate of plant propagation may be severely affected (Gonçalves *et al.*, 1998). Auxin type and concentration significantly influenced rooting percentage and root length. The use of NAA along with BA is the most widespread among all other combinations of auxins and CKs across the world (Kaviani, 2015). There is a significant difference between the uses of NAA in combination with BA with other combinations of PGRs (Kaviani, 2015).

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