Plant Tissue Cult. & Biotech. **32**(1): 13-20, 2022 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v32i1.60468

PTC&B

Micropropagation of *Alocasia amazonica* through Indirect Shoot Organogenesis

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Key words: Micropropagation, *Alocasia amazonica*, Indirect shoot organogenesis.

Abstract

The present experiment was conducted to establish an efficient *in vitro* regeneration protocol for *Alocasia amazonica* through indirect organogenesis from young leaf segment. For callus induction, leaf explants were inoculated on MS medium containing different concentrations of 2,4-D. Highest callus induction frequency (90%) was recorded on MS medium supplemented with 4.0 mg/l 2,4-D. Remarkable results on indirect shoot organogenesis and multiplication (86.67%) with maximum shoot number per unit callus (11.64 ± 0.37) and shoot length (10.13 ± 0.24 cm) were observed when the leaf derived callus were transferred on MS medium fortified with 1.5 mg/l BAP and 0.5 mg/l NAA. Additionally, the incorporation of 10% coconut water with the medium showed satisfactory shoot growth and development. The best response towards root induction (85%) was achieved on ½ MS medium fortified with 3.0 mg/l IBA, 2.0 mg/l IAA and 2.0 mg/l NAA with an average 22.20 ± 0.87 roots per unit shoot. Well rooted plantlets were successfully acclimatized to the soil where the survival rate was 90%.

Introduction

Alocasia is one of the largest and morphologically most diverse genera in the family Araceae comprises about 100 species (Boyce 2008). *A. amazonica* is one of the most widely used ornamental species in the genus *Alocasia*. It is very popular as garden plants and can grow quite successfully indoors as long as a humid atmosphere is maintained. It makes an excellent foliage plant with shiny dark green leaves with a contrasting white or pale green veins and white scalloped margins, with a shape similar to an elephant's ear. The back of the leaves are as interesting as the front, they are a deep shade of purple (Jo et al. 2008). So, due to their foliar charm, patterns of leaf variegation and texture, as well as tolerance to limited sunlight *A. amazonica* is a very popular ornamental plant among plant collectors and landscape gardeners (Jo et al. 2008).

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Planting material of ornamental plants is in great demand for commercial production. The better quality planting material is a basic need of growers for boosting productivity (Kadu 2013). Alocasia species are conventionally propagated through seeds and corms, which provide an extremely slow rate of multiplication. The inefficiency of the vegetative propagation method is one of the problems in commercial production (Jo et al. 2008).

For this, it is necessary to develop an efficient and economically viable *in vitro* propagation method to meet the commercial demand for *Alocasia* species as well as to produce disease-free plants throughout the year (Sanatombi and Sharma 2008). Although the commercial demand for *Alocasia* species is high, few reports exist on their *in vitro* propagation (Bhatt et al. 2013, Thao et al. 2003) and associated protocols (Abdel-Baset et al. 2020; Jo et al. 2008, 2009, Adelberg and Toler 2004). Therefore, in the present research work an attempt was been made for establishing an efficient protocol for the rapid propagation of *A. amazonica* through indirect shoot organogenesis.

Materials and Methods

In the present experiment young, tender and disease-free leaves were used as explants collected from a small potted *Alocasia amazonica* plant purchased from local market. For surface sterilization, explants were washed under running tap water for 20 min, followed by washing in sterilized distilled water with several drops of a liquid detergent for 10-15 min and then by 3 times washing with autoclaved distilled water. Further sterilization of the explants was done under laminar airflow with 0.2% $HgCl_2$ solutions for 4 min and then washed thrice with sterilized distilled water. Cut ends of the explants were removed by cutting with a sterilized scalpel to avoid toxic effect of $HgCl_2$ on exposed explants tissues.

Leaf disc of convenient size (1 cm^2) were then inoculated singly in the culture bottles containing MS basal medium supplemented with different concentrations of 2,4-D (1.0-5.0 mg/l) for callus induction. The calli were transferred to MS medium containing different concentrations and combinations of cytokinins (BAP, TDZ and Kn) and auxin (NAA) for shoot induction and proliferation. For rapid shoot multiplication, the effects of different concentrations of Coconut water (0-20%) on the growth and development of *in vitro* raised shoots of *Alocasia amazonica* were also examined. The cultures were exposed to 16 hours light period with constant temperature at 24 ± 2°C. The light intensity of the growth chamber was 3000 lux. Overall, an aseptic environment was maintained throughout the whole process.

The *in vitro* raised shoots obtained from the multiplication media when grew about 5-7 cm in length, they were excised aseptically from the culture vessels and implanted separately on freshly prepared rooting media containing different concentrations and combinations of auxins (IBA, IAA and NAA). For root induction, the newly transferred cultures were kept in dim light for 3 days and then they were kept under full light. After

25-30 days of culture, the number of roots per shoot cluster and rooting percentage were recorded. The plantlets with well-developed root systems were then acclimatized. The plantlets with sufficiently developed roots were taken out from the culture vessels, and the roots were washed under running tap water. Immediately after washing the plantlets were transferred to small polybags containing the soil mixture garden soil, sand and compost with (1:1:1) ratio. The plants along with pots were covered with transparent polythene bags to prevent sudden desiccation. The inner side of the polythene bags was sprayed with water at every 8 hours to maintain high humidity around the plantlets. The polythene bags were gradually perforated to expose the plantlets to the outer normal environment and subsequently removed after 14 days. By this the plantlets became established in the soil.

Results and Discussion

Young disease-free leaf-tissues of *Alocasia amazonica* were used as potential sources of explants for callus induction. Similarly, Akin-Idowu et al. (2009) demonstrated that the leaf tissues have high rates of cell division and declared as the most preferable explants for tissue culture technique. MS gelled medium supplemented with different concentrations of 2,4-D (1.0 - 5.0 mg/l) was used to induce callus from the sterilized leaf-tissues. The callus initiation started at 2.0 mg/l 2,4-D and highest efficiency (90%) was noted at 4.0 mg/l of 2,4-D after 24 days of inoculation (Table 1). In this concentration the nature of leaf derived calli were whitish-friable (Table 1). The findings are in accordance with the results of Ling et al. (2009) in *Mirabilis jalapa*; Bajaj and Pierik (1974) in *Freesia* spp. and Chang and Yang (1995) in *Taxus mairei.*

Table 1. Effect of different concentrations of 2, 4-D in gelled MS medium on callus induction from leaf explants of *Alocasia amazonica*.

Concentrations of 2,4-D (mg/l)	Callus formation time (days)	Callus induction frequency (%)	Nature of callus	
			Color	Texture
1.0	-	-	-	-
1.5	-	-	-	-
2.0	38	26.67	Whitish	Granular
2.5	35	46.67	Whitish	Granular
3.0	30	68.00	Whitish	Compact
3.5	32	76.00	Greenish	Friable
4.0	24	90.00	Whitish	Friable
4.5	27	72.50	Pinkish	Compact
5.0	28	65.00	Brownish	Compact

- = Indicate no response.

According to the previous study on different species of *Alocasia*, shoot induction from callus tissues were influenced by the concentrations of cytokinins. In this study, the friable callus was transferred to shoot induction and multiplication media containing different concentrations and combinations of BAP, TDZ, Kn and NAA. The callus become greenish and appeared highly competent for shoot induction and multiplication particularly on the MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA where the number of shoots per unit callus and shoot lengths were 11.64 ± 0.37 and 10.13 ± 0.24 cm, respectively (Table 2). The results were supported by the earlier reports on micropropagation of different species of *Alocasia* (Thao et al. 2003, Jo et al. 2009, Chan and Chong 2010) and *Aglaonema* (Yeh et al. 2007).

Table 2. Effect of different concentrations and combinations of plant growth regulators on shoot morphogenesis and shoot multiplication.

Plant growth regulators (mg/l)	% of responsive explant	No. of shoots per explant (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
BAP + Kn			
3.0 + 1.0	46.67	2.37 ± 0.76	2.05 ± 0.57
3.0 + 2.0	53.33	2.56 ± 0.43	2.37 ± 0.82
4.0 + 1.0	66.67	2.56 ± 0.43	3.57 ± 0.52
4.0 + 2.0	60.00	4.27 ± 0.07	3.34 ± 0.03
5.0 + 1.0	56.67	3.82 ± 0.66	3.27 ± 0.39
5.0 + 2.0	46.67	3.47 ± 0.71	3.20 ± 0.09
BAP + TDZ			
3.0 + 0.5	40.00	4.45 ± 0.08	4.78 ± 0.51
3.0 + 1.0	50.00	4.89 ± 0.80	5.32 ± 0.72
3.5 + 0.5	56.67	5.26 ± 1.07	5.68 ± 1.21
3.5 + 1.0	70.00	5.86 ± 0.33	5.91 ± 0.43
4.0 + 0.5	63.33	5.35 ± 0.79	5.54 ± 0.87
4.0 + 1.0	60.00	4.93 ± 0.67	5.37 ± 0.58
BAP + NAA			
1.0 + 0.5	60.00	8.09 ± 0.61	6.86 ± 0.07
1.0 + 1.0	70.00	9.74 ± 0.38	7.95 ± 0.92
1.5 + 0.5	86.67	11.64 ± 0.37	10.13 ± 0.24
1.5 + 1.0	76.67	10.53 ± 0.11	9.18 ± 0.42
2.0 + 0.5	70.00	10.04 ± 0.70	8.73 ± 0.57
2.0 + 1.0	73.33	9.12 ± 0.63	8.15 ± 1.03
2.5 + 0.5	60.00	7.94 ± 0.81	7.34 ± 0.96
2.5 + 1.0	66.67	8.27 ± 1.02	7.41 ± 0.79
BAP + TDZ + NAA			
0.5 + 0.5 + 0.2	75.00	9.50 ± 1.31	8.46 ± 0.84
0.5 + 0.5 + 0.5	70.00	8.21 ± 0.39	6.78 ± 0.22
0.5 + 1.0 + 0.2	70.00	7.67 ± 0.07	6.31 ± 0.29
0.5 +1.0 + 0.5	60.00	7.13 ± 0.78	6.07 ± 0.55
1.0 + 0.5 + 0.2	65.00	6.84 ± 1.07	5.91 ± 0.87
1.0 + 0.5 + 0.5	60.00	6.94 ± 0.32	5.76 ± 0.09
1.0 + 1.0 + 0.2	50.00	6.88 ± 0.71	5.80 ± 0.43
1.0 + 1.0 + 0.5	55.00	6.45 ± 0.09	5.32 ± 1.06

SE* = Standard error of mean; Values are the mean of five replications.

A significant increase in the shoot multiplication rate with more healthy cultures were observed when 10% coconut water was added with 1.5 mg/l BAP and 0.5 mg/l NAA, showing a maximum rate of multiplication with an average 15.5 ± 1.08 shoots per unit callus which were 11.48 ± 0.94 cm long (Figs 1 and 2). The results obtained from this study support the earlier reports that the addition of coconut water as organic growth supplement in the media could improve plant's growth in tissue culture (Kalpona et al. 2000, Aktar et al. 2008).

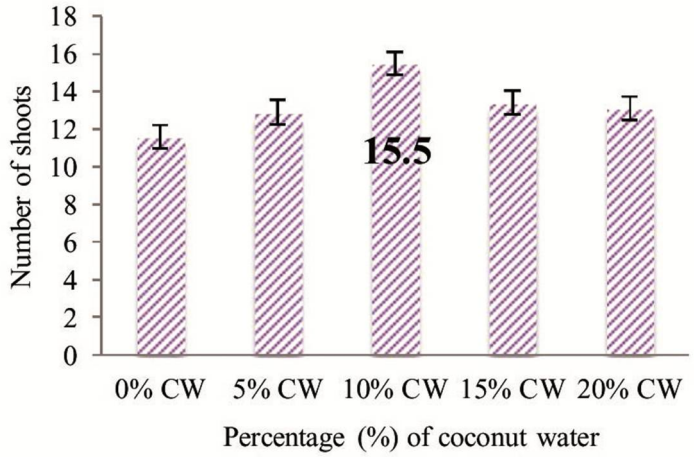

Fig. 1. Effects of coconut water (0-20%) along with constant 1.5 mg/l BAP + 0.5 mg/l NAA on number of regenerated shoots per unit callus

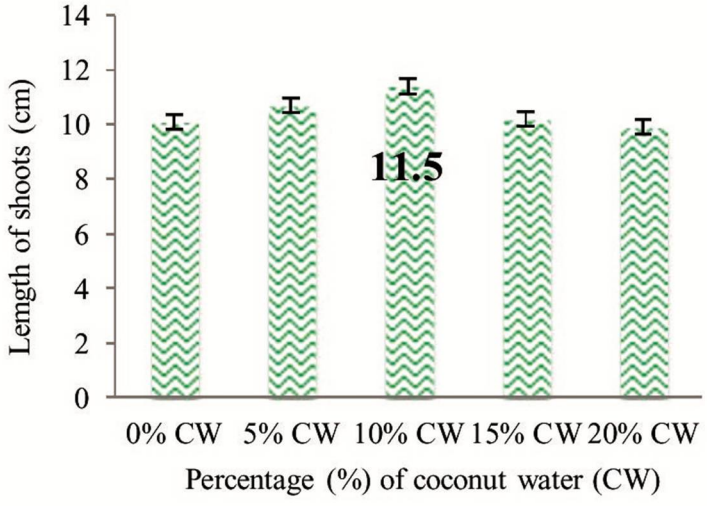

Fig. 2. Effects of coconut water (0-20%) along with constant 1.5 mg/l BAP + 0.5 mg/l NAA on length of shoots per unit callus.

In the current case, root induction occurred when regenerated shoots were sub-cultured on half-strength of gelled MS medium supplemented with different concentrations and combinations of auxins (IAA, IBA and NAA). A significant rooting (85%) was obtained within 30 days in *A. amazonica* when 3.0 mg/l IBA was added in conjunction with 2.0 mg/l IAA and 2.0 mg/l NAA where the number of roots per unit shoot was 22.20 ± 0.87 and root length was 12.11 ± 1.40 cm (Table 3). However, Thao et al. (2003) achieved 100% rooting efficiency within 4 weeks of sub-culture on half-strength MS gelled medium supplemented with BAP and NAA in case *A. micholitziana*. In the present study, IBA seemed to be having more efficient for rooting in regenerated shoots of *A. amazonica* than IAA and NAA, which was justified in accordance with the findings of Jahan et al. (2009) in case of *Anthurium andraeanum* and Faisal et al. (2007) in case of *Tylophora indica*.

Table 3. Effect of different concentrations and combinations of auxins in half-strength of gelled MS medium on rooting of *in vitro* raised shoots.

Concentrations of auxins (mg/l)	Percentage (%) of shoots forming roots	No. of roots per unit shoot (Mean ± SE*)	Root length (cm) (Mean ± SE*)
IBA + IAA			
1.0 + 1.0	20.00	3.62 ± 1.48	2.95 ± 0.94
1.0 + 2.0	25.00	6.04 ± 0.90	5.24 ± 1.08
2.0 + 1.0	30.00	9.71 ± 0.83	7.68 ± 1.40
2.0 + 2.0	50.00	12.41 ± 0.20	8.77 ± 0.75
3.0 + 1.0	40.00	13.67 ± 0.21	8.86 ± 0.35
3.0 + 2.0	55.00	14.49 ± 0.61	10.30 ± 0.53
IBA + IAA + NAA			
2.0 + 1.0 + 1.0	45.00	11.56 ± 0.83	8.04 ± 0.08
2.0 + 1.0 + 2.0	60.00	12.90 ± 0.36	8.63 ± 1.06
2.0 + 2.0 + 1.0	65.00	16.70 ± 1.21	9.85 ± 0.32
2.0 + 2.0 + 2.0	75.00	19.05 ± 0.82	10.08 ± 0.76
3.0 + 1.0 + 1.0	50.00	14.03 ± 0.93	9.20 ± 1.08
3.0 + 1.0 + 2.0	65.00	15.52 ± 0.09	10.67 ± 0.28
3.0 + 2.0 + 1.0	70.00	18.33 ± 0.40	10.84 ± 0.48
3.0 + 2.0 + 2.0	85.00	22.20 ± 0.87	12.11 ± 1.40

SE* = Standard error of mean; Values are the mean of five replications.

The rooted plantlets were acclimatized successfully with 90% survival rate to the soil condition. But, Thao et al. (2003) and Jo et al. (2008) observed 100% survival rate in the same soil mixture in case of *A. micholitziana* and *A. amazonica*, respectively. Bhatt et al. (2013) showed a survival rate of more than 90% in five different species of *Alocasia* in a mixture containing organic soil and sand in a ratio 1:1. The acclimatized plants were transferred to the larger pots containing garden soil and compost with 1:1 ratio for sufficient growth and finally transplanted to the field. Different stages of *in vitro* regeneration of *A. amazonica* through indirect organogenesis have been depicted in Fig. 3.

Fig. 3(a-i): *In vitro* regeneration of *Alocasia amazonica* through indirect organogenesis. (a) Callus induction from leaf explants (arrow pointed induced callus) in MS medium supplemented with 4.0 mg/l 2,4-D. (b) Whitish friable callus (45 days old). (c) Multiple shoot induction from the leaf-derived callus on MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA. (d) Induced shoots with leaf from callus. (e) Multiplication with elongated shoots in MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA + 10% CW. (f) Plantlets with well-developed multiple roots. (g) Complete plantlets. (h) Regenerated plants grown in a poly bags containing soil, sand and compost (1:1:1) in a shaded chamber. (i) Hardened plant in the larger pot.

The present study provides an efficient, well-established and cost effective micropropagation protocol for *Alocasia amazonica* from the young leaf-tissues of field grown plants. This indirect regeneration protocol can hence be used for commercial production of *A. amazonica* and also as a tool for germplasm conservation.

Acknowledgements

The authors gratefully acknowledge the Bangladesh University Grants Commissions (UGC) for providing financial support.

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(Manuscript received on 10 February, 2022; revised on 29 March, 2022)