Plant Tissue Cult. & Biotech. **34**(1): 35-47, 2024 (June) **ISSN 1817-3721, E-ISSN 1818-8745**
DOI: https://doi.org/10.3329/ptcb.v34i1.74343

PTC&B

In vitro Propagation of *Alocasia baginda* 'Silver Dragon' through Direct and Indirect Organogenesis

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Key words: *In vitro* propagation, *Alocasia baginda* 'Silver Dragon', Direct and indirect organogenesis.

Abstract

Direct and indirect *in vitro* organogenesis of *Alocasia baginda* 'Silver Dragon' was investigated using explants of shoot tips, leaf tissue, and petiole segments. For direct shoot induction from shoot tips, MS medium containing 1.5 mg/l BAP was shown to be the most efficient (80%), producing a maximum of 6.60 ± 0.93 shoots/explant with 3.66 ± 0.38 cm in length. Directly induced shoots were multiplied using a 2.5 mg/l BAP enriched medium, where the number and length of shoots/culture were 11.60 ± 1.12 and 9.20 ± 1.01 cm, respectively. Shoots gradually increased in number and length during the 1st three sub-culture cycles in the similar medium combination but declined after 3rd cycle. Following the 3rd sub-culture cycle, there were 14.80 ± 0.58 shoots per culture, with a shoot length of 9.90 ± 0.63 cm. The greatest frequency of callus induction was 97.67% for leaf tissue and 86.67% for petiole segments when they were cultured on the media containing 3.0 mg/l 2,4-D. Impressive results regarding indirect shoot organogenesis (90%) with maximum shoot number/unit callus (16.20 ± 1.62) and shoot length (11.06 ± 0.97 cm) were noted upon transferring the callus onto MS medium containing 3.5 mg/l BAP and 3% sucrose. Within 10-12 days, 100% rooting was observed in half-strength of gelled MS medium containing 2.0 mg/l IBA, with an average of 15.40 ± 1.17 roots/culture. After being acclimatized in a potting mixture containing garden soil, compost, coco peat, and moss in a 2:1:1:1 ratio, *A. baginda* 'Silver Dragon' exhibited a 100% survival rate. Established plantlets seem morphologically similar to mother plants and possess fully developed root and shoot systems, along with an adequate leaf number per plant.

Introduction

Ornamental plants are the silent architects of aesthetically pleasing landscapes in the field of horticulture. The most rapidly evolving ornamental plants are foliage plants for interior decoration or landscape (Mariani et al. 2011). These plants are distinguished by

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their appealing color, shape, texture and leaf variegation, and other visually attractive characteristics (Chen 2021). Thus, the propagation and enhancement of qualitative features and the introduction of novel variety are major commercial goals for the ornamental industry (Jain and Ochatt 2010). However, the commerce of ornamental foliage plants in Bangladesh is relatively new but growing in acceptance.

The Araceae (arum) family, whose members are often known as aroids, is one of the most popular foliage ornamental families due to its eye-catching look with large showy leaves and unique inflorescences. It is the third most diverse family of monocots after grasses and palms (Boyce and Croat 2011). *Alocasia*, is one of the largest genera in the Araceae family and a popular choice among collectors and landscapers worldwide, with 113 species spread over tropical and subtropical Asia, Southeast Asia, and parts of Australia (Sahagun 2005, Arbain et al. 2022). *Alocasia baginda* 'Silver Dragon' or African Gray Mask, is one of the most uncommon and exotic varieties of *Alocasia*, highly valued as a house plant for its exquisite foliage. This tropical plant from Borneo, Southeast Asia, has heart-shaped, light to silvery-green leaves with dark-green ribbed venation that resembles dragon scales (Kurniawan and Boyce 2011). Its rigid leaves contain purple-red venation on the underside and, fresher leaves are lighter green at first and become silver as they mature. However, as a 'Jewel Alocasia', well-established plants develop into gorgeous specimens with leaves that seldom grow to 2-3 feet (Johnstone 2022). In addition to being ornamental plants, silver dragon plants may be used as natural indoor air purifiers since they emit O_2 into the air and absorb excess CO_2 and other toxic gases, keeping the environment healthy and clean. They may also be used as humidifiers to provide atmospheric moisture, relieving dry skin complaints (Rankel 2023).

A. bagunda 'Silver Dragon' is becoming more popular among plant enthusiasts and collectors, although its conventional propagation methods are ineffective for commercial production. Conventional methods for propagating *Alocasia* species include seeds, corms, bulbs, and rhizome division which are time consuming and give an exceedingly sluggish rate of multiplication, restricting bulk production (Jo et al. 2008). Furthermore, seasonal specificity limits the availability of better-quality planting materials for residential and commercial gardens and landscaping (Chebet et al. 2003). In this situation, *in vitro* clonal propagation is ideal for this leafy ornamental plant to commercialize the planting material, as it has been done with other *Alocasia* species and other beautiful foliage Aroid species. So far we know, there is no report on the *in vitro* propagation of this particular *A. baginda* cultivar. Therefore, the current study established an efficient and reproducible *in vitro* regeneration protocol for *A. baginda* 'Silver Dragon' through direct and indirect shoot organogenesis.

Materials and Methods

This experiment employed young and disease-free leaf tissue, petiole, and shoot tip as explants collected from a locally purchased small potted *A. baginda* 'Silver Dragon' plant.

All kinds of explants were surface sterilized using the procedure followed by Raju et al. (2022). Disinfected shoot tips (1.5-2 cm) were inoculated individually on MS basal medium with BAP (0-4.0 mg/l) for direct shoot induction. Directly induced small shoots were aseptically removed from the culture vessels and cultured on freshly prepared medium with the same or different concentrations and combinations of BAP (0-3.5 mg/l) and NAA (0-0.5 mg/l) for shoot development and multiplication. This study also examined the consecutive sub-culture cycles that affected shoot multiplication.

Segments of petiole (2-3 cm) and leaf tissue (1-2 cm^2) of convenient size were inoculated singly in MS basal medium supplemented with 2,4-D (0-5.0 mg/l) for callus induction. Healthy calluses were excised into small pieces (1.5-2.0 cm^2) and transferred onto gelled MS medium with BAP (0-5.0 mg/l) alone or in combinations with Kn (0.5-1.0 mg/l) or NAA (0.5 mg/l) for shoot induction and immense shoot proliferation. The cultures were kept at 24 ± 2°C and subjected to 16 hrs of light and 8 hrs of darkness daily. The growth chamber was illuminated with 3000 lux light, and the air cooler's temperature was monitored. Overall, an aseptic environment was maintained throughout the whole process.

When directly and indirectly induced shoots reached 6-10 cm in length, they were aseptically excised and implanted separately on freshly prepared rooting medium containing different strengths of MS medium with various concentrations and combinations of IBA (1.0-2.0 mg/l) and/or NAA (0.5-2.0 mg/l). Newly transferred cultures were maintained in dim light for 3 days before being in light for rooting. Plantlets with sufficient roots were removed from the culture vessels and gently rinsed under tap water to remove the media from the roots. They were then transferred to small poly bags with different potting mixtures, such as Garden soil + Compost + Sand; Garden soil + Compost + Coco peat; Garden soil + Coco peat; and Garden soil + Compost + Coco peat + Moss, in 1:1:1, 1:1:1, 1:1:1, and 2:1:1:1 ratio, respectively. The plants and pots were covered with clear polythene bags to avoid desiccation, and the interior side was sprayed with water every 8 hrs to maintain high humidity. After 45- days, the plantlets were moved to bigger pots with the optimum potting mixture to grow. One-way ANOVA was performed using SPSS version 16.0 software, followed by a post hoc comparison of means using Duncan's Multiple Range Test (DMRT) with a 0.05 significant level.

Results and Discussion

The addition of cytokinin to the basal medium is essential to develop multiple shoots from shoot tips, as shoot tips did not respond well in the current experiment using hormone-free MS medium. Out of the different BAP concentrations used, gelled MS medium with 1.5 mg/l BAP produced fairly good results (80%) for direct shoot induction, producing the maximum number of shoots/explant (6.60 ± 0.93) with greatest shoot length (3.66 ± 0.38 cm) (Table 1; Fig. 1a and 1b). The present findings are consistent with Kepue et al. (2021) in *Colocasia esculenta*; Sayed (2021) in *Dracaena umbraculifera* and

Alawaadh et al. (2020) in *Philodendron* sp. However, BAP concentrations over 1.5 mg/l prevented shoot bud formation and elongation (Table 1). Unlike these, Bogale (2018) and Han et al. (2004) successfully accomplished direct shoot formation in *C. esculenta* and *Philodendron* sp. using 8.0 and 5.0 mg/l BAP, respectively.

Directly induced shoots were removed and grown on solidified MS medium with varying doses of BAP, either alone or with NAA to facilitate multiplication, as the shoots failed to multiply in zero MS medium. Solidified MS medium containing 2.5 mg/l BAP showed considerably satisfying results on shoot multiplication (92%), with a maximum of 11.60 ± 1.12 shoots per culture and 9.20 ± 1.01 cm shoot length (Table 2; Fig. 1c). Similarly, Duhoky and Al-Mizory (2010) observed that 2.5 mg/l BAP in MS medium enhanced shoot proliferation in *Codiaeum variegatum*, producing the most shoots and length. Nevertheless, Aslam et al. (2013) used a lower strength of BAP (1.75 mg/l) for *Dracaena sanderiana,* whereas Guerra et al. (2018) and Abdulhafiz et al. (2020) used a somewhat greater strength of BAP (3.0 mg/l) for shoot multiplication in the case of *Alocasia* 'verde picante' and *A. longiloba*, respectively.

The interaction effect of BAP with NAA in this experiment yielded less satisfying outcomes than BAP alone (Table 2). The results of the present investigation differed from those of Swaranjali and Abhishek (2023) for *Aglaonema commutatum*; El-Gedawey and Hussein (2022) for *Aglaonema* 'Lady Valentine'; Kalaivani et al. (2022) for *Dracaena sanderiana*; Raju et al. (2020) for *D. fragrans* cv. Victoria. They produced multiple shoots per culture bottle in MS medium with 2.0-5.0 mg/l BAP and 0.1-1.0 mg/l NAA.

Table 1. Impact of various strengths of BAP in solidified MS medium on direct shoot induction from shoot tips of *A. baginda*.

Concentrations of BAP (mg/l)	Responding explants (%)	Number of shoots/explant (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
0	10	$0.60^{d} \pm 0.40$	$0.88^{c} \pm 0.36$
0.5	25	$2.00^{bcd} \pm 0.55$	$2.34^{ab} \pm 0.20$
1.0	45	$3.20^{bc} \pm 0.73$	$3.02^{ab} \pm 0.56$
1.5	80	$6.60^{a} \pm 0.93$	$3.66^{a} \pm 0.38$
2.0	65	$4.00^{b} \pm 0.55$	$3.08^{ab} \pm 0.41$
2.5	55	$3.40^{bc} \pm 0.51$	$3.02^{ab} \pm 0.45$
3.0	55	$3.00^{bc} \pm 0.63$	$2.96^{ab} \pm 0.44$
3.5	30	$2.80^{bc} \pm 0.73$	$2.26^{b} \pm 0.30$
4.0	35	$1.40^{cd} \pm 0.40$	$2.12^{b} \pm 0.48$

At least 20 cultures were maintained in each concentration; data scored after 28-days of culture; values are mean ± SE of five separate treatments; according to DMRT at $P < 0.05$, values with the same letter in the same column are not statistically different.

Table 2. Impact of various strengths of BAP, either alone or in combinations with NAA on the proliferation of directly induced shoots of *A. baginda* in gelled MS medium.

Growth regulators (mg/l)		% of culture exhibited proliferation	Number of shoots/culture (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
BAP	NAA			
0	0	No new shoots; cultured shoot dies		
1.0	0	28	$2.60^{d} \pm 0.68$	$3.34^{e} \pm 0.21$
1.5	0	56	$4.20^{cd} \pm 0.73$	$4.76^{cde} \pm 0.43$
2.0	0	68	$6.00^{bc} \pm 1.22$	$5.92^{bc} \pm 0.72$
2.5	0	92	$11.60^{a} \pm 1.12$	$9.20^{a} \pm 1.01$
3.0	0	80	$7.20^{b} \pm 1.32$	$6.72^{b} \pm 0.74$
3.5	0	64	$5.40^{bcd} \pm 0.93$	$5.70^{bcd} \pm 0.65$
1.0	0.5	Cultured shoots become more green and callus induction from the shoot base		
1.5	0.5	44	$3.40^{cd} \pm 0.60$	$3.04^{e} \pm 0.62$
2.0	0.5	48	$3.80^{cd} \pm 0.92$	$3.56^{e} \pm 0.49$
2.5	0.5	64	$4.60^{bcd} \pm 1.03$	$3.84^{de} \pm 0.57$
3.0	0.5	60	$4.00^{cd} \pm 0.84$	$4.40^{cde} \pm 0.66$
3.5	0.5	52	$3.20^{cd} \pm 0.37$	$2.84^{e} \pm 0.45$

Data scored after 28-days of culture; for multiplication 25-shoot units (2-3) were inoculated in each combination; values are mean ± SE of five separate treatments; according to DMRT at $P < 0.05$, values with the same letter in the same column are not statistically different.

Fresh shoots from the multiplication stage were dissected into segments containing 2-3 shoots and grown on MS medium with the best concentration of BAP (2.5 mg/l) for periodic subculture to determine their influence on shoot multiplication. The number of shoots increased in the first 2-3 subcultures, which thereafter decreased (Table 3). In the 3rd subculture cycle, the highest number and length of regenerated shoots/culture were 14.80 ± 0.58 and 9.90 ± 0.63 cm, respectively (Fig. 1d). Compared to present experiment, Kakuei and Salehi (2015) in *Dracaena sanderiana* and Ramadevi et al. (2012) in *Boucerosia diffusa* reported a significant effect of sub-culturing on multiplication potential after the 3rd subculture. Meanwhile, Mariani et al. (2011) found the highest shoot multiplication rate in *Aglaonema* at the 5th subculture.

Callus induction requires appropriate growth hormones, since petiole segments and leaf tissue cultured on hormone-free gelled MS media did not respond. In this study, among the different strengths of 2,4-D (0-5.0 mg/l) employed, the MS medium with 3.0 mg/l of 2,4-D produced the maximum callus induction frequency, as evidenced by 96.67% for leaf tissue and 86.67% for petiole segments (Table 4; Fig. 1e and 1f). However, leaf tissue derived callus was more vigorous and developed faster (12-14 days) than

Table 3. Impact of consecutive subcultures on the proliferation of directly induced shoots of *A. baginda* in solidified MS medium containing 2.5 mg/l BAP.

Sub-culture	Number of regenerated shoots/culture (Mean ± SE*)	Shoot length (cm) (Mean ± SE*)
1st	11.60^{b} ± 1.12	9.20^{a} ± 1.01
2nd	13.20^{ab} ± 1.02	9.48^{a} ± 0.96
3rd	14.80^{a} ± 0.58	9.90^{a} ± 0.63
4th	13.00^{ab} ± 0.45	9.14^{a} ± 0.68
5th	11.20^{b} ± 1.20	8.28^{a} ± 0.61

Data scored after 28-days of culture; values are mean ± SE of five separate treatments; according to DMRT at $P < 0.05$, values with the same letter in the same column are not statistically different.

petiole segments derived callus (16-18 days). Furthermore, the nature of callus derived from various sources and combinations varied, as seen in Table 4. However, once 2,4-D concentration exceeds 3.0 mg/l, callus induction frequency decreases progressively. Oo et al. (2019) reported a somewhat different result in *Anthurium andraeanum*, showing that the highest degree of callus production was attained on MS medium containing just 0.6 mg/l 2,4-D for both types of explants (95.5% for petiole and 82.9% for leaf tissue). However, Haring et al. (2023) found that 2.0 mg/l 2,4-D increased callus induction from *Amorphophallus muelleri* petiole segments, whereas Raju et al. (2022) found that 4.0 mg/l was optimum for *Alocasia amazonica* leaf tissue. In this current experiment, petiole explants also showed a callogenic response followed by shoot induction at 2.0 mg/l 2,4-D (Table 4). Similarly, Chen et al. (2012) showed that petioles of *Philodendron* sp. can generate adventitious shoots at 0.5 mg/l 2,4-D.

The current study found that cytokinin strengths affected the induction of shoots from callus tissues, as no morphogenetic response was seen on hormone-free MS medium and the callus gradually turned brown. However, out of 9 BAP concentrations tested, gelled MS medium with 3.5 mg/l BAP provided the highest percentage of responding callus (90%) for indirect shoot induction (Fig. 1g, 1h and 1i). This BAP concentration resulted in an average of 16.20 ± 1.62 shoots per culture, with a maximum shoot length of 11.06 ± 0.97 cm (Table 5). Increasing or decreasing BAP concentrations after 3.5 mg/l decreased shoots/culture, length, and responsive callus. However, Heedchim et al. (2022) in *Caladium bicolor*, Bhavana et al. (2018) in *Anthurium andreanum*, and Thao et al. (2003) in *Alocasia micholitziana* found that BAP at 0.1-0.5 mg/l greatly increased the number of shoots from callus. Our investigation also demonstrated that single impact of BAP was significantly more beneficial than its combination with Kn or NAA (Table 5). In contrast, Raju et al. (2022; 2020) in *Alocasia amazonica* and *Dracaena fragrans*, Thokchom and Maitra (2017) in *Anthurium andreanum*, and Seydi et al. (2016) in *C. bicolor* revealed that BAP with NAA positively affected indirect shoot organogenesis.

Table 4. Impact of varying strengths of 2,4-D on callus induction from leaf tissues and petiole segments of *A. baginda*.

2,4-D (mg/l)	Type of explants	CIF (%)	Callogenic response (*)	Days required for callus initiation	Nature of callus	
					Color	Texture
0	Leaf	-	-	-	-	-
	Petiole	-	-	-	-	-
0.5	Leaf	10.00	+	28-30	Whitish	Friable
	Petiole	-	-	-	-	-
1.0	Leaf	46.67	++	23-25	Whitish	Friable
	Petiole	26.67	+	26-28	Whitish	Compact
2.0	Leaf	66.67	++	18-20	Whitish	Granular
	Petiole	53.33	Callus formation followed by shoot induction			
3.0	Leaf	96.67	++++	12-14	Greenish	Friable
	Petiole	86.67	+++	16-18	Whitish	Friable
4.0	Leaf	80.00	+++	20-22	Green	Granular
	Petiole	73.33	++	20-22	Whitish	Friable
5.0	Leaf	60.00	++	24-26	Greenish	Compact
	Petiole	63.33	++	24-26	Creamy	Compact

30 explants were inoculated in each concentration; Callogenic response (*): No response (-); Poor (+); Moderate (++); Good (+++); Excellent (++++); CIF (Callus Induction Frequency).

In this study, roots formed from the shoot base in most hormone combinations employed in multiplication, although not significantly. Similarly, Bhatt et al. (2013) observed well-developed roots in every *in vitro* plantlet throughout the multiplication stage in all five *Alocasia* species. However, the newly formed shoots were cultured on full and half strengths of gelled MS medium with varying combinations of IBA and NAA to promote adequate roots (Table 6). Using half-strength MS medium with 2.0 mg/l IBA yielded the best rooting percentage (100%) and number of roots/culture (15.40 ± 1.17) in the shortest period (10-12 days). At this concentration, excellent rooting with maximum length was also observed (Fig. 1j and 1k). These results are consistent with those of Kakuei and Salehi (2015), who, in the case of *Dracaena sanderiana* observed excellent rooting from the base of 81.25% shoots grown on half-strength MS medium containing 2.0 mg/l IBA. Furthermore, Klanrit et al. (2023) in *Philodendron erubescens*; Ali et al. (2022) in *Caladium bicolor* and Raju et al. (2020) in *D. fragrans* 'Victoria' also reported that the medium with just IBA (0.2-3.0 mg/l) resulted in the best root growth. Our experiment also revealed that combining IBA with NAA yields less satisfying results than utilizing them alone (Table 6). However, Swaranjali and Abhishek (2023) and Barakat and Gaber (2018) obtained rooted plantlets of *Aglaonema commutatum* on the medium containing both IBA and NAA.

Table 5. Impact of varying strengths of BAP, either alone or in combination with NAA or Kn on shoot morphogenesis from induced callus of *A. baginda* in solidified MS medium.

Plant growth regulators (mg/l)			Responsive callus (%)	Number of shoots produced/culture (Mean ± SE*)	Length of shoot (cm) (Mean ± SE*)
0			No new shoot buds; cultured callus turned brown in color		
BAP					
1.0			Callus continued to develop into more compact and rooty callus		
1.5			Few pale green colored shoot with rooty callus		
2.0			43.33	$4.80^{c} \pm 0.92$	$4.46^{c} \pm 0.26$
2.5			66.67	$6.60^{bc} \pm 1.33$	$6.74^{bc} \pm 0.45$
3.0			80.00	$10.40^{b} \pm 1.72$	$8.90^{ab} \pm 1.13$
3.5			90.00	$16.20^{a} \pm 1.62$	$11.06^{a} \pm 0.97$
4.0			76.67	$8.60^{bc} \pm 1.83$	$8.92^{ab} \pm 1.23$
4.5			63.33	$7.00^{bc} \pm 1.48$	$6.28^{bc} \pm 0.77$
5.0			53.33	$5.20^{c} \pm 0.97$	$5.00^{c} \pm 0.78$
BAP	NAA	Kn			
1.0	0.5	-	No new shoot buds; callus developed slowly and turned brown		
2.0	0.5	-	60	$6.00^{ab} \pm 0.84$	$5.10^{b} \pm 0.75$
3.0	0.5	-	75	$7.20^{a} \pm 1.02$	$6.88^{a} \pm 0.94$
4.0	0.5	-	50	$5.00^{abcd} \pm 0.32$	$4.80^{bc} \pm 0.52$
5.0	0.5	-	40	$3.60^{cde} \pm 0.75$	$3.76^{bc} \pm 0.35$
1.0	-	0.5	No new shoot buds; callus continued to develop		
1.0	-	1.0	30	$2.80^{de} \pm 0.49$	$3.16^{c} \pm 0.42$
2.0	-	0.5	65	$5.80^{abc} \pm 0.97$	$4.42^{bc} \pm 0.58$
2.0	-	1.0	50	$3.60^{cde} \pm 0.81$	$4.50^{bc} \pm 0.58$
3.0	-	0.5	50	$4.20^{bcde} \pm 0.73$	$3.82^{bc} \pm 0.69$
3.0	-	1.0	40	$3.20^{de} \pm 0.80$	$3.68^{bc} \pm 0.55$
4.0	-	0.5	35	$3.40^{de} \pm 0.40$	$3.56^{bc} \pm 0.36$
4.0	-	1.0	35	$2.80^{de} \pm 0.58$	$3.26^{bc} \pm 0.36$
5.0	-	0.5	25	$2.40^{e} \pm 0.51$	$3.02^{c} \pm 0.33$
5.0	-	1.0	More compact, green, and rooty callus with a few tiny shoots		

Data scored after 28-days of culture; at least 30 cultures were maintained in each concentration; values are mean ± SE of five separate treatments; according to DMRT at $P < 0.05$, values with the same letter in the same column are not statistically different.

Table 6. Impact of growth regulators and the strength of the MS medium on the *in vitro* rooting of *A. baginda* shoots derived from both direct and indirect regeneration.

Strength of MS medium	Concentrations of Auxins (mg/l)		Days required for root Initiation	Rooting (%)	Number of roots/culture (Mean ± SE*)	Root length
	IBA	NAA				
Full-MS	1.0	-	24-26	40	4.20cd ± 0.58	+
Full-MS	2.0	-	Development of callus at the base of the shoot			
Full-MS	-	1.0	24-26	32	4.40^{cd} ± 0.75	+
Full-MS	-	2.0	Development of callus at the base of the shoot			
½ MS	1.0	-	14-16	84	12.60^{a} ± 1.03	+++
½ MS	2.0	-	10-12	100	15.40^{a} ± 1.17	+++
½ MS	-	1.0	18-20	56	6.00^{cd} ± 0.71	++
½ MS	-	2.0	14-16	68	7.40^{bc} ± 0.87	++
Full-MS	1.0	0.5	28-30	24	3.80^{d} ± 0.58	+
Full-MS	1.0	1.0	28-30	16	3.40^{d} ± 0.60	+
Full-MS	2.0	0.5	Development of callus at the base of the shoot			
Full-MS	2.0	1.0	Development of callus at the base of the shoot			
½ MS	1.0	0.5	18-20	60	5.60^{cd} ± 1.29	++
½ MS	1.0	1.0	18-20	52	5.40^{cd} ± 1.50	++
½ MS	2.0	0.5	14-16	72	9.20^{b} ± 1.02	+++
½ MS	2.0	1.0	18-20	64	6.20^{bcd} ± 1.39	++

Culture duration 30 days; 25 individual shoots were inoculated in every combination for rooting; + = Small; ++ = Medium; +++ = Long; values are mean ± SE of five separate treatments; according to DMRT at $P < 0.05$, values with the same letter in the same column are not statistically different.

Well-rooted plantlets of *Alocasia baginda* were transplanted to various potting mixtures for acclimatization. According to the data in Table 7, the potting mixture combining garden soil, compost, coco peat and moss (2:1:1:1) had the best survival percentage (100%) (Fig. 1l). Using moss along with garden soil, compost, and coco peat enhanced moisture-holding capacity and root-zone aeration, which may have reduced plant mortality. Alawaadh et al. (2020) for *Philodendron* and Barakat and Gaber (2018) for *Aglaonema commutatum* observed similar survival rates when *in vitro* developed plants were transplanted to pots containing peat moss and perlite in a 1:1 ratio. Several more research on the acclimatization of other ornamental aroids (Klanrit et al. 2023 in *P. erubescens*; Ali et al. 2022 in *C. bicolor*; Abdulhafiz et al. 2020 in *Alocasia longiloba*) have revealed that peat moss surpassed all other forms of the potting substrate, possibly due

to its excellent water holding capacity. Following 45 days, established plants were moved to bigger pots exhibiting well-developed shoot and root systems and producing enough leaves (Fig. 1m).

Fig. 1(a-m): *In vitro* regeneration of *A. baginda* 'Silver Dragon' through direct and indirect organogenesis. (a-b) Multiple direct shoot inductions from shoot tip on MS + 1.5 mg/l BAP. (c-d) Proliferation of the directly induced shoots on MS medium with 2.5 mg/l BAP after 28 days of (c) 1st subculture, and (d) 3rd subculture. (e-f) Callus induction from (e) leaf tissue (greenish-friable), and (f) petiole segment (whitish-friable) on MS + 3.0 mg/l 2,4-D. (g-i) Multiple shoot induction from callus tissues following (g) 10-days, (h) 20-days, and (i) 28-days of inoculation on MS + 3.5 mg/l BAP. (j) Rooting of *in vitro* regenerated shoots on half-MS + 2.0 mg/l IBA. (k) Complete plantlets. (l) Acclimatization of regenerated plantlets in potting mixture combining garden soil, compost, coco peat, and moss (2:1:1:1). (m) A two-month-old hardened plant whose leaves have started to display their characteristic silvery-green color on its surface.

Table 7. Impact of different potting mixtures on the acclimatization of *A. baginda* 'Silver Dragon' plantlets grown *in vitro*.

Potting mixtures (PM)	Mixture ratio	Survival (%)
PM_1: Garden soil + Compost + Sand	1:1:1	80.00
PM_2: Garden soil + Compost + Coco peat	1:1:1	93.33
PM_3: Garden soil + Coco peat	1:1:1	73.33
PM_4: Garden soil + Compost + Coco peat + Moss	2:1:1:1	100

For acclimatization 30-rooted shoots were transplanted into each potting mixture; data scored after 45-days of culture on potting mixtures.

Alocasia baginda 'Silver Dragon' is a highly prized ornamental cultivar with distinctive leaf color and texture. Although plant lovers and collectors are becoming more and more interested in this plant, there are issues with the effectiveness and availability of planting materials with conventional propagation methods. This study, for the first time provides an effective micropropagation protocol for *A. baginda* 'Silver Dragon' employing both direct and indirect organogenesis. For direct shoot induction from shoot tips, MS medium with 1.5 mg/l BAP was best, while 2.5 mg/l BAP was optimal for the proliferation of directly induced shoots. MS medium with 3.0 mg/l 2,4-D was effective in inducing callus from leaf tissue and petiole explants, while 3.5 mg/l BAP was ideal for callus-to-multiple shoot development. ½ MS medium with 2.0 mg/l IBA improved root number and length. This protocol also offers a step toward applying this methodology to other significant leafy-ornamental plants.

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(Manuscript received on 02 April, 2024; revised on 01 June, 2024)