Current Botany 2024, 15: 25-28
doi: 10.25081/cb.2024.v15.8617
https://updatepublishing.com/journal/index.php/cb

Research Article

ISSN: 2220-4822

In vitro regeneration of *Arisaema leschenaultii* (Blume) using leaf explants

V. Vijayan, K. K. Vijayakumar*, S. Nigesh

Department of Botany, Kandaswami Kandar's College, Namakkal-638182, Tamil Nadu, India

Received: September 26, 2023
Revised: February 12, 2024
Accepted: February 22, 2024
Published: March 06, 2024

***Corresponding Author:**
K. K. Vijayakumar
E-mail: kkvijay4@yahoo.com

ABSTRACT

A standard *in vitro* regeneration protocol from leaf explants was developed for a potential medicinal plant *Arisaema leschenaultii*. Maximum callus induction (78%) was achieved on MS medium fortified with BAP (1.5 mg/L) and NAA (0.9 mg/L). The maximum percentage of shoot proliferation (75%) was attained on MS medium supplemented with 2.0 mg/L of BAP and 0.5 mg/L of GA_3. The highest root induction (70%) was achieved on MS medium prepared with the combination of NAA (1.5 mg/L) and IBA (0.6 mg/L). The well-rooted plantlets that successfully followed acclimatization in the green house survived. The present efficient and optimized protocol provides mass propagation as well as the possibility of germplasm conservation for this study species.

KEYWORDS: *Arisaema leschenaultii*, Leaf explant, MS medium, Growth regulators, Acclimatization

INTRODUCTION

The tissue culture technique is mostly used for clonal propagation of valuable indigenous medicinal plants, and *in vitro* conservation of germplasm is threatened with extinction (Boro *et al.*, 1998). The micropropagation method is applied specifically to species that are needed for clonal propagation (Gamborg & Phillips, 1995). The common name of the medicinal plant *Arisaema leschenaultii* Blume is cobra. In the Ayurveda system of medicine, it is traditionally used to cure colitis, eczema, fistulas, gonorrhea, hemorrhoids, purging, piles, roundworms, sinuses, syphilis, and urinary disorders (Mathew, 1999). The Kani tribes of the Western Ghats in Tirunelveli district have used tender leaves and corms with tamarind fruit paste as vegetables. The Singhalese people in Sri Lanka have used it as a substitute for A. *leschenaultia*, whose juice is good for curing ringworm infections. The leaf paste is used to cure skin disease, and corms are used for the treatment of piles. A. *leschenaultii* is a well-known endemic species to South India and Sri Lanka, distributed in the understories of evergreen forests at 1200-2000 m altitude, and it is also reported as a rare and threatened species in the silent valley regions of the Western Ghats. Hence, conventional micropropagation is an important source to meet the demand for this potential medicinal plant. Based on the above facts, this study was conducted to develop an effective *in vitro* protocol for its mass cultivation.

MATERIALS AND METHODS

The young and healthy plants of *Arisaema leschenaultii* were collected from the study area, Kolli Hills, in Namakkal District and maintained under greenhouse conditions. The explants were collected and thoroughly washed with tap water three times. The washed explants were treated with 5% tween-80 solutions for 5 minutes and then thoroughly washed with tap water three times. Further, the explants were treated with 5% Amphicillin and Rifampicin (antibiotics) for 30 minutes and a 2% (w/v) Bavistin solution for 5 minutes, and then three times rinsed with sterile distilled water. For surface sterilization, explants were dipped in 0.1% $HgCl_2$ for 35 minutes. Before inoculating into the nutrient medium, these explants were washed three to four times in sterile distilled water. The MS (Murashige & Skoog, 1962) medium was prepared by using 3% sucrose solidified with 8 g of agar. Before autoclaving the agar medium at 1210 °C for 15 minutes, the pH was adjusted to 5.7. All the cultures were maintained in the culture chamber with a 16/8-hour photoperiod and maintained at a temperature of 25± 20 °C with 60-65% relative humidity.

The leaf explants were inoculated in MS medium supplemented with various concentrations and combinations of BAP and NAA for callus initiation. MS medium fortified with various concentrations and combinations of BAP and GA_3 was used for shooting attributes. After regeneration, the shoots (5-6 cm long) were carefully excised and washed with sterile distilled water and then transferred to MS medium containing NAA

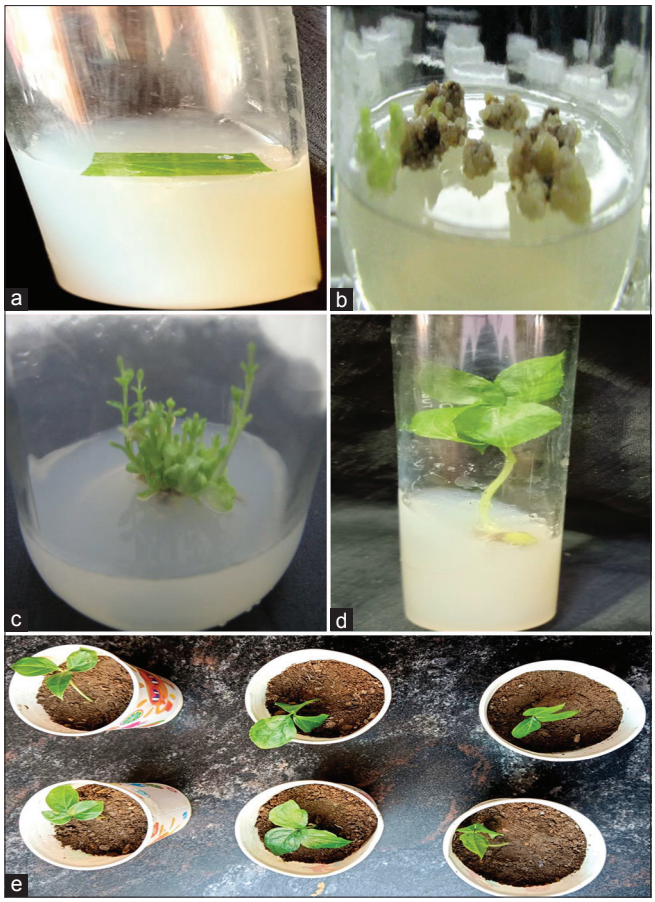

Figure 1: *In vitro* regeneration of *Arisaema leschenaultii*. a) inoculation of leaf explant, b) Callus induction, c) Multiple shoot induction, d) Root induction and e) Acclimatized plantlets

and IBA for root induction. After proper development of roots in the plantlets, plantlets with 3-5 leaves were transferred to plastic pots containing garden soil, sand, and vermicompost in the ratio of 1:1:1 and maintained under greenhouse conditions for the determination of the survivability rate of the plantlets. The average number and length of shoots per explant were recorded after 35 days of inoculation of leaf explants in the MS medium. The average number and length of roots per explant and the percentage of roots produced by shoots were recorded. All the experiments were repeated two times. The statistical analysis was done, and Duncan's multiple range test ($P<0.05$) was used to compare the mean values.

RESULTS AND DISCUSSION

The regeneration potential of the leaf explants was explored on MS medium with various concentrations and combinations of Benzyl Amino Purine (BAP) and Indole-3-acetic acid (IAA), and the results are summarized in Tables 1 to 4 (Figure 1a). Callus initiation was observed after 26 days of inoculation of leaf explants. The best response to callus formation (78%) was achieved by using MS medium supplemented with synergistic influences of 1.5 mg/L of BAP and 0.9 mg/L NAA (Table 1 & Figure 1b). A similar kind of callus initiation was observed

Table 1: Effect of growth regulators on callus induction from leaf explants of the species *Arisaema leschenaultii*

Growth regulators (mg/L)				Days required for callus formation after inoculation	Callus formation (%)
BAP	2, 4-D	NAA	Kn	Leaf Explant	Leaf Explant
0.5	0.0	0.0	0.0	14	30.25 ± 1.14^{a}
1.0	0.0	0.0	0.0	17	37.76 ± 0.82^{c}
1.5	0.0	0.0	0.0	18	46.86 ± 1.23^{e}
2.0	0.0	0.0	0.0	20	54.35 ± 1.45^{g}
2.5	0.0	0.0	0.0	21	48.53 ± 0.82^{i}
3.0	0.0	0.0	0.0	19	40.84 ± 0.31^{f}
0.0	0.5	0.4	0.0	16	48.57 ± 1.32^{c}
0.0	1.0	0.4	0.0	15	55.17 ± 1.63^{e}
0.0	1.5	0.4	0.0	20	59.36 ± 0.82^{f}
0.0	2.0	0.4	0.0	23	62.58 ± 1.67^{i}
0.0	2.5	0.4	0.0	18	57.97 ± 1.21^{a}
0.5	0.0	0.3	0.0	15	56.00 ± 0.42^{cd}
1.0	0.0	0.6	0.0	16	67.35 ± 1.21^{e}
1.5	0.0	0.9	0.0	26	78.15 ± 0.42^{i}
2.0	0.0	1.2	0.0	24	65.13 ± 1.45^{j}
2.5	0.0	1.5	0.0	21	62.25 ± 1.32^{h}
3.0	0.0	1.8	0.0	18	54.87 ± 1.21^{b}
0.0	0.3	0.0	0.2	15	36.48 ± 1.41^{c}
0.0	0.6	0.0	0.4	17	41.59 ± 0.82^{d}
0.0	0.9	0.0	0.6	16	50.32 ± 1.63^{e}
0.0	1.2	0.0	0.8	18	40.21 ± 0.42^{f}
0.0	1.5	0.0	1.0	14	47.00 ± 0.82^{cd}

Means in columns followed by different letter(s) are significant to each other at 5% level according to DMRT

by Thambiraj and Paulsamy (2012) and Magendiran *et al.* (2022b). In the shoot regeneration experiment, different concentrations and combinations of BAP (2.0 mg/L) and GA_3 (0.5 mg/L) were used to observe their effect on multiple shoot formations, and the percentage of shoot regeneration was 75% (Figure 1c). The maximum number of shoots/callus (8.65 ± 0.21^{i}) and shoot length (6.4 ± 1.63^{e}) were observed in MS medium fortified with the same concentrations of BAP and GA_3 (Table 2). The best response of BAP towards shoot proliferation of several medicinal plants compared to the other cytokinins has been reported by Jebakumar and Jayabalan (2000), Faisal & Anis (2003), Husain and Anis (2006), Raja and Arockiasamy (2008) and Magendiran *et al.* (2022a).

After the successful multiplication of shoots from the leaf-derived callus, the shoots were carefully excised, washed with sterile distilled water, and then transferred to MS medium containing NAA and IBA for root induction. The root induction attributes are summarized in Table 3. The highest rooting percentage (70%), maximum number of roots/shoot (7.81 ± 1.13^{f}), and root length (6.2 ± 1.32^{f} cm) were achieved in MS medium fortified with 1.5 mg/L of NAA and 0.6 mg/L of IBA (Figure 1d). The same results were confirmed with other species like *Aegle marmelos* (Ramanathan *et al.*, 2010) and *Ricinus communis* (Sujatha & Reddy, 1998; Ahn *et al.*, 2007; Alam *et al.*, 2010). After 15 days, the *in vitro* plantlets were transferred to plastic pots containing garden soil, sand, and vermicompost in the ratio of 1:1:1 and maintained under greenhouse conditions for the determination of the survivability rate of the plantlets (Figure 1e). The survivability rate of the leaf-derived callus plantlets in plastic pots under green house

Table 2: Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length the after subculturing of leaf derived callus of the species *Arisaema leschenaultii*

Growth regulators (mg/L)				Culture response (%)	No. of shoots/ callus	Shoot length (cm)
BAP	NAA	GA_3	IAA			
0.3	0.0	0.0	0.0	31.23 ± 0.82^{d}	3.14 ± 0.82^{ab}	2.9 ± 0.42^{ab}
0.3	0.0	0.0	0.0	42.64 ± 0.82^{hi}	4.74 ± 0.34^{bcd}	3.3 ± 1.41^{abc}
0.3	0.0	0.0	0.0	45.78 ± 1.63^{j}	4.98 ± 1.47^{def}	4.0 ± 1.63^{abc}
0.3	0.0	0.0	0.0	38.37 ± 0.82^{fg}	4.00 ± 1.43^{abc}	4.4 ± 0.26^{bc}
0.3	0.0	0.0	0.0	32.64 ± 1.63^{gh}	3.38 ± 1.21^{fgh}	4.6 ± 0.82^{abc}
0.3	0.0	0.0	0.0	33.53 ± 1.63^{kl}	4.64 ± 0.42^{fgh}	4.7 ± 1.23^{c}
0.5	0.0	0.5	0.0	51.27 ± 0.82^{k}	5.75 ± 0.16^{hi}	4.3 ± 0.34^{abc}
1.0	0.0	0.5	0.0	62.00 ± 1.31^{m}	6.35 ± 0.82^{ghi}	4.9 ± 0.62^{abc}
1.5	0.0	0.5	0.0	69.58 ± 0.42^{n}	7.17 ± 0.42^{i}	5.8 ± 1.32^{c}
2.0	0.0	0.5	0.0	75.18 ± 0.42^{n}	8.65 ± 0.21^{i}	6.4 ± 1.63^{c}
2.5	0.0	0.5	0.0	63.58 ± 1.63^{b}	7.07 ± 0.31^{efg}	5.5 ± 0.81^{abc}
3.0	0.0	0.5	0.0	50.36 ± 1.63^{a}	5.12 ± 0.61^{bcd}	4.5 ± 0.42^{ab}
0.0	0.5	0.0	0.3	45.26 ± 0.82^{de}	4.41 ± 0.26^{ghi}	3.5 ± 1.34^{abc}
0.0	0.5	0.0	0.3	41.12 ± 0.82^{a}	5.67 ± 0.82^{fgh}	4.3 ± 0.82^{bc}
0.0	0.5	0.0	0.3	49.11 ± 0.82^{c}	5.52 ± 1.21^{cde}	4.9 ± 1.41^{bc}
0.0	0.5	0.0	0.3	51.85 ± 1.63^{fg}	4.43 ± 1.63^{ab}	4.7 ± 1.63^{abc}
0.0	0.5	0.0	0.3	54.26 ± 0.82^{ij}	4.28 ± 0.21^{bcd}	3.3 ± 0.32^{abc}
0.0	0.5	0.0	0.3	50.16 ± 1.63^{i}	4.07 ± 1.32^{efg}	3.8 ± 0.41^{ab}
0.5	0.0	0.0	0.2	25.49 ± 0.82^{d}	3.90 ± 0.12^{def}	3.7 ± 0.26^{abc}
1.0	0.0	0.0	0.4	49.00 ± 1.63^{k}	3.38 ± 0.24^{ghi}	3.9 ± 0.82^{a}
1.5	0.0	0.0	0.6	59.45 ± 1.63^{ef}	4.15 ± 1.31^{abc}	4.4 ± 0.65^{abc}
2.0	0.0	0.0	0.8	62.75 ± 0.82^{hi}	4.47 ± 0.62^{a}	4.5 ± 1.63^{abc}
2.5	0.0	0.0	1.0	63.32 ± 0.82^{fg}	5.74 ± 1.23^{ab}	3.7 ± 0.82^{a}
3.0	0.0	0.0	1.2	64.21 ± 1.63^{ij}	4.00 ± 0.4^{fgh}	3.5 ± 0.41^{ab}

Means in columns followed by different letter(s) are significant to each other at 5% level according to DMRT

Table 3: Effect of different concentrations of growth regulators on root number, rooting percentage and root length after the subculturing of leaf callus derived *in vitro* produced shoots of the species *Arisaema leschenaultii*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/ shoot	Root length (cm)
NAA	IAA	IBA			
0.5	0.0	0.2	50.30 ± 0.41^{j}	5.67 ± 0.82^{abc}	4.4 ± 0.22^{a-d}
1.0	0.0	0.4	64.25 ± 0.82^{k}	6.39 ± 1.63^{def}	5.5 ± 0.42^{ef}
1.5	0.0	0.6	70.29 ± 0.41^{m}	7.81 ± 1.13^{f}	6.2 ± 1.32^{f}
2.0	0.0	0.8	67.65 ± 0.42^{l}	6.78 ± 0.41^{ef}	5.2 ± 1.63^{ef}
2.5	0.0	1.0	59.54 ± 1.23^{h}	5.98 ± 0.82^{abc}	4.8 ± 0.41^{de}
3.0	0.0	1.2	50.45 ± 0.82^{g}	4.56 ± 0.16^{ab}	4.2 ± 0.16^{a-d}
0.5	0.2	0.0	42.65 ± 0.41^{e}	3.26 ± 1.63^{bcd}	3.1 ± 0.82^{bcd}
1.0	0.4	0.0	49.32 ± 0.82^{d}	4.19 ± 1.23^{ab}	3.9 ± 0.33^{a-d}
1.5	0.6	0.0	55.54 ± 1.63^{c}	5.64 ± 0.82^{abc}	4.8 ± 0.49^{abc}
2.0	0.8	0.0	60.24 ± 0.32^{b}	6.38 ± 0.33^{abc}	5.5 ± 0.41^{a-d}
2.5	1.0	0.0	57.17 ± 0.41^{a}	5.59 ± 0.21^{a}	4.6 ± 0.82^{ab}
3.0	1.2	0.0	48.98 ± 0.67^{a}	4.38 ± 0.82^{ab}	4.2 ± 0.16^{a}
0.5	0.0	0.0	34.87 ± 0.82^{c}	3.29 ± 1.13^{ab}	3.8 ± 0.26^{a-d}
1.0	0.0	0.0	35.67 ± 0.26^{f}	4.48 ± 0.49^{bcd}	4.3 ± 0.24^{abc}
1.5	0.0	0.0	42.54 ± 1.63^{h}	5.76 ± 1.23^{cde}	4.8 ± 0.82^{bcd}
2.0	0.0	0.0	47.87 ± 0.42^{h}	5.53 ± 0.42^{bcd}	4.5 ± 0.41^{cde}
2.5	0.0	0.0	40.88 ± 0.31^{g}	4.68 ± 0.21^{abc}	3.9 ± 0.31^{a-d}
3.0	0.0	0.0	33.34 ± 0.62^{i}	4.58 ± 0.82^{bcd}	3.8 ± 0.24^{de}

Means in columns followed by different letter(s) are significant to each other at 5% level according to DMRT

conditions was approximately 70% (Table 4). After three months, the *in vitro* regenerated plants were transplanted into the soil.

Table 4: Effect of different composition of hardening medium on survivability rate of leaf callus derived *in vitro* rooted plantlets of the species *Arisaema leschenaultii*

Hardening medium composition (V/V)	No. of plantlets under hardening	No. of plantlets survived	Survivability (%)
Red soil+sand (1:1)	50	24	49 ± 0.82^{a}
Garden soil+sand+ vermicompost (1:1:1)	50	44	70 ± 0.41^{d}
Decomposed coir waste+perlite +compost (1:1:1)	50	38	66 ± 1.21^{c}
Vermicompost+soil (1:1)	50	32	63 ± 0.64^{b}
Red soil+sand+vermicompost (1:1:1)	50	25	52 ± 0.32^{a}

Means in column followed by different letter(s) are significant to each other at 5% level according to DMRT

CONCLUSION

The results of the present study showed that the use of leaf explants is an effective, alternately reproducible, and reliable method for micropropagation of the medicinal plant A. *leschenaultii*. The high percentage of callus formation, shoot and root initiations, and high rate of survivability of the species also indicate that this protocol could be used for large-scale commercial cultivation of A. *leschenaultii*.

REFERENCES

Ahn, Y. J., Vang, L., McKeon, T. A., & Chen, G. Q. (2007). High - frequency plant regeneration through adventitious shoot formation in castor (*Ricinus communis* L.). *In Vitro Cellular & Developmental Biology - Plant, 43*, 9-15. https://doi.org/10.1007/s11627-006-9009-2

Alam, I., Sharmin, S. A., Mondal, S. C., Alam, M. J., Khalekuzzaman, M., Anisuzzaman, M., & Alam, M. F. (2010). *In vitro* micropropagation through cotyledonary node culture of castor bean (*Ricinus communis* L.). *Australian Journal of Crop Science, 4*(2), 81-84.

Boro, P., Sarma, S., Deka, A. C., & Kalita, M. C. (1998). Clonal propagation of *Alternanthera sessilis* - A biopharmaceutically potent herbal medicinal plant. *Journal of Phytological Research, 11*(2), 103-106.

Faisal, M., & Anis, M. (2003). Rapid mass propagation of *Tylophora indica* Merrill via leaf callus culture. *Plant Cell, Tissue and Organ Culture, 75*, 125-129. https://doi.org/10.1023/A:1025084203958

Gamborg, O. L., & Phillips, G. C. (1995). Laboratory facilities, operation and management. In O. L. Gamborg & G. C. Phillips (Eds.), *Fundamental methods of plant cell, tissue and organ culture* (pp. 3-20) Berlin, New York: Springer.

Husain, M. K., & Anis, M. (2006). Rapid *in vitro* propagation of *Eclipta alba* (L.) Hassk. through high frequency axillary shoot proliferation. *Acta Physiologiae Plantarum, 28*, 325-330. https://doi.org/10.1007/s11738-006-0028-8

Jebakumar, M., & Jayabalan, M. (2000). An efficient method for regeneration of plantlets from nodal explants of *Prosalea corydifolia* Linn. *Plant Cell Biotechnology and Molecular Biology, 1*, 37-40.

Magendiran, M., Vijayakumar, K. K., Thambiraj, J., & Mahendran, S. (2022a). Germplasm conservation of *Phyllanthus virgatus* G. Forst. by encapsulation of *in vitro* derived leaf segments. *Current Botany, 13*, 4-7. https://doi.org/10.25081/cb.2022.v13.7303

Magendiran, M., Vijayakumar, K. K., Thambiraj, J., & Mahendran, S., (2022b). Rapid *in vitro* regeneration of medicinal plant *Achyranthes bidentata* Blume from the leaf explant through callus culture. NOVY 1 *MIR Research Journal, 7*(6), 1-12.

Mathew, K. M. (1999). *The flora of the Palani hills (Monocotyledons)* (Vol. 3, pp. 1368-1371) Tiruchirapalli, Tamil Nadu: Rapinat Herbarium.

Murashige, T., & Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiologia Plantarum, 15*(3),

473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Raja, H. D., & Arockiasamy, D. I. (2008). In vitro Propagation of *Mentha viridis* L. from Nodal and Shoot tip Explants. *Plant Tissue Culture and Biotechnology, 18*(1), 1-6. https://doi.org/10.3329/ptcb.v18i1.3243

Ramanathan, T., Satyavani, K., & Gurudeeban, S. (2010). *In vitro* plant regeneration from leaf primordia of gum-bearing tree *Aegle marmelos*. *Research Journal of Forestry, 4*(4), 208-212.

Sujatha, M., & Reddy, T. P. (1998). Differential Cytokinin effects on the stimulation of *in vitro* shoot proliferation from meristematic explants of castor (*Ricinus Communis* L.). *Plant Cell Reports, 17*, 561-566. https://doi.org/10.1007/s002990050442

Thambiraj, J., & Paulsamy, S. (2012). Rapid *in vitro* multiplication of the ethnomedicinal shrub, *Acacia caesia* (L.) Willd. (Mimosaceae) from leaf explants. *Asian Pacific Journal of Tropical Biomedicine, 2*(S2), S618-S622. https://doi.org/10.1016/S2221-1691(12)60284-6