ENVIRONMENTAL REPORTS

Original Research Article
ISSN: 3041-556X
Journal homepage: https://er.researchfloor.org/

First Report on Somatic Embryo and Shoot Induction in *Lindernia manilaliana* Sivar. (RET): A Step Toward Sustainable Reintroduction

Smitha R B*[1], Deepak Jyothi[2] and Krishnan P N[3]

[1]*Department of Botany, St. Dominic's College, Kanjirapally, Kottyam, 686512, India*
[2]*Kallala Estate Kalady Plantation, Ernakulam, Plantation Corporation of Kerala Ltd., Kerala, 683581, India,*
[3]*Biodiversity and Ecology, CISSA, Thiruvanathapuram, Kerala, India*

Citation: Smitha R B, Deepak Jyothi and Krishnan P N (2025). First Report on Somatic Embryo and Shoot Induction in *Lindernia manilaliana* Sivar. (RET): A Step Toward Sustainable Reintroduction. *Environmental Reports; an International Journal*.
DOI: https://doi.org/10.51470/ER.2025.7.2.124

Corresponding Author: **Smitha R B** | E-Mail: **(rbsmitha@gmail.com)**

Received 18 June 2025 | Revised 17 July 2025 | Accepted August 21 2025 | Available Online September 22 2025

ABSTRACT

Lindernia manilaliana Sivar., a rare, endangered and threatened (RET) aquatic plant included in red list category of IUCN and facing the threat of extinction, is reported to be endemic to Kerala. The leaf and nodal explants showed direct regeneration on MS media and developed multiple shoots. Nodal explants from basal medium when sub-cultured onto MS medium supplemented with BAP and KN alone or in combination with BAP, KN and NAA showed multiple shoot proliferation of 81.5±7.5 plantlets from single explants within 4 weeks. Tender leaves from in vitro-grown plants induced direct somatic embryos, which subsequently developed into normal seedlings, similar to those derived from zygotic embryos, at a rate of 50.5 ± 8.6 onto MS medium supplemented with BAP (0.5 mg/L). For rooting, MS medium supplemented with IBA (0.5 mg/L) was found to be the most suitable. Anatomical studies revealed the differentiation of somatic embryos from the leaf midrib and lamina, supported by well-developed vascular structures. In vitro propagation has proven to be a potential technique for large-scale production of planting material for the conservation and reintroduction of the aquatic RET species Lindernia manilaliana.

Keywords: *Lindernia manilaliana, RET, aquatic plants, clonal multiplication, Conservation*

INTRODUCTION

One of the 34 global biodiversity 'hotspots', the Western Ghats, situated in the west coast of India is an evergreen tropical evergreen forest. It is one of the best representatives of non-equatorial tropical evergreen forests in the world. Floristically, the Western Ghats is one of the richest areas in the country. A total of about 4000 plant species recorded from Western Ghats in which 27% approximately 51 genera and 1,600 species are endemic to the region [1]. Aquatic plants are highly valued for their nutritious and medicinal values, and are key species in the provision of wetland ecosystem services, such as water filtration and nutrient recycling [2]. However, there is little published information specifically on these aquatic species. *Lindernia manilaliana* Sivar., an endangered plant of the Western Ghats, is endemic to Kerala. Conservation protocols for this species have not been initiated to date, and it continues to face the threat of extinction. *Lindernia manilaliana* Sivar. (Family: Scrophulariaceae; Vernacular: Manilal Pushpam) is distributed in South India, specifically in Kozhikode, Kerala. Flowering and fruiting occur from August to December. *L. manilaliana* is used as an ornamental plant [3] and is typically found growing on moist grounds, swampy lowlands, and occasionally in wetlands, with an erect stem. The population of this plant is declining due to urbanization and habitat conversion for developmental activities, and it has been assessed as Endangered [3]. Currently, no conservation measures have been reported for this species. Further research is needed to confirm its distribution in ecologically similar habitats and to enable effective monitoring of its habitats and subpopulations. For the conservation of germplasm of medicinal plants considered rare, endangered, or threatened, various strategies have been employed. However, conservation through seeds or cuttings is not applicable to all species. In this context, tissue culture techniques and in vitro multiplication are gaining significant importance for the mass production of planting material in various plant species, including medicinal plants [4, 5].
Moreover, the in vitro multiplication of the selected plant species has not been studied previously.

Though the micropropagation of aquatic plants are difficult due to the infection rate, *in vitro* propagation protocols for some aquatic plants, namely *Aponogeton* sp. [6], *Cryptocoryne lucens* [7], *Anubias barteri* [8], *Cryptocorine wendtii* [9 & 10], *Ludwigia repens* [11], *Cryptocorine becketti, Cryptocorine lutea* and *Rotala rotundifolia* [12] have been reported. However, there have been no reports on the micropropagation and shoot proliferation of *Lindernia manilaliana* in the context of conservation through micropropagation.

MATERIALS AND METHODS

Source of plant material

Lindernia manilaliana Sivar. (Scrophularaceae), an an aquatic plant belonging to RET category (Fig. 1a) is endemic to Calicut, Kerala, India. It is found growing on moist grounds, swampy lowlands and occasionally in wetlands. This species is known from two adjacent sites in Calicut and is threatened by habitat degradation caused by urbanization, industrial development, and tourism-related activities. The live germplasm of the species is preserved in the Aquagene section (germplasm collection of aquatic plants) at the KSCSTE-Malabar Botanical Garden and Institute of Plant Sciences, Calicut, Kerala.
Nodal explants from the field as well as nodal and tender leaves from *in vitro* grown plants were selected as explants for culture initiation, multiple shoot proliferation and somatic embryogenesis, respectively. Basal MS medium with different hormone combinations were prepared and pH was adjusted to 5.8. The boiled media after adding 0.7% agar were decanted to 25 ml culture tubes/bottles or flasks as the case may be, plugged and sterilized at 121°C for 20 minutes at 15 psi pressure was used for the whole study.

Explants selection and sterilization

The disease disease-free, young and healthy nodal explants were selected. They were then washed under running tap water for 30 minutes in order to wash off the sticking external contaminants.
Then the explants were soaked in an aqueous solution containing 2 or 3 drops of detergent Tween 20 and washed thoroughly for 20 minutes.

This was followed by gentle wash in sterile double distilled water for 5 minutes for two cycles. Later the explants were sterilized with aqueous solution of 0.01% Mercuric chloride (Ranbaxy) for 5 minutes in a pre-sterilized culture bottle followed by three rinses with sterile double double-distilled water.

Establishment of in vitro culture

After sterilization the explants were inoculated onto the MS basal medium under a laminar airflow clean bench and incubated in the temperature (25± 2°C) and 16/18 hrs of day/night-controlled culture room. After bud proliferation the cultures were transferred fresh medium for further proliferation.

Multiplication

Nodal explants from plants proliferated on MS basal medium were sub-cultured onto MS medium supplemented with varying concentrations of BAP (0.1, 0.2, 0.5, and 1.0 mg/L), Kinetin (0.1, 0.2, 0.5, and 1.0 mg/L), either alone or in combination (BAP + Kinetin, BAP + NAA, or Kinetin + NAA), for multiple shoots induction.

Direct shoot regeneration from leaf

Leaves from in vitro grown plants were inoculated onto MS basal as well as MS with BAP (0.5 and 1.0 mg^{-1}l) for direct shoot initiation. In this case both direct as well as indirect organogenesis was developed in cultures. The initiated shoot cultures were multiplied through subculture and the plantlets developed were subsequently transferred to rooting media contain IBA.

Rooting

Although root formation occurred spontaneously in both the shoot initiation and multiplication media, the roots were slender and thin. Therefore, the plantlets were transferred to MS medium supplemented with different concentrations of IBA.

Hardening and acclimatization of plantlets

The rooted plantlets were removed from the culture bottles with the help of forceps and washed thoroughly with water to remove any sticking medium. They were then washed with Bavistin (0.1%) in order to protect them from fungal attack in the near future and successfully transferred to hardening pots. Sterile coir pith was used for hardening. The rooted plantlets were transplanted to hardening pots and incubated under a shade net. After hardening and establishment, the plants were carefully transferred in concrete pots with clayish soil. They were then reintroduced in their native habitat after 25-30 days.

RESULTS

Direct regeneration from nodes

Nodes of *Lindernia manilaliana* were inoculated onto basal MS media (pH-5.8) (Fig. 1b). Bud proliferation was noticed from nodal explants on 4th day after inoculation on basal media (Fig. 1 c) and leaf induction was noticed on 6th day of inoculation (Fig. 1d). Second leaf initiation was noticed on 8th day of inoculation and stem elongation was noticed on 10th day (Fig. 1e). Within one month after inoculation the plant became healthy with four branched multiple shoots and roots (Fig.1f) and roots were minute, thin and not healthy (Table 1).

Table 1: Initiation of shoot buds in Basal MS Media

Sl. No.	Time period (inoculation)	No. of shoot initiated	No. of leaves	No. of nodes
1	1 Week	2.4±0.6	1.1 ± 0.8	1
2	2 Week	3.5±1.5	2.5 ± 0.5	2
3	3 Week	4.8±1.2	3.5 ±1.5	2
4	4 Week	4.6±1.0	4.0±1.2	4
5	5 weeks	4.4±0.6	4.0± 1.5	4

Table 2: Table showing the development of multiple shoots from nodal buds cultured on MS basal medium, and the number of shoots produced upon subculturing onto MS medium supplemented with different concentrations of BAP, Kn and NAA after 4 weeks

Sl. No.	BAP mg^{-1} l	Kinetin mg^{-1} l	NAA mg^{-1} l	Number of multiple shoots produced Mean±sd	Average shoot length cm Mean±sd	Remarks
1	0	0		4.5±1.6^{f}	3.6±1.2^{bc}	Slender
2	0.1	0		15.3±3.5de	3.5±1.3^{bc}	Normal
3	0.2	0		50.6±4.4b	5.6±1.0^{b}	Normal
4	0.5	0		81.5±7.5a	7.70±1.7^{a}	Normal
5	1.0	0		71.5±7.5ab	7.42±1.5 a	Stunted
6	0	0.1		5.26±0.7f	2.9±0.8c	Normal
7	0	0.2		8.45±1.2f	3.9±0.9^{b}	Normal
8	0	0.5		10.35±1.5f	3.7±1.0^{bc}	Normal
9	0	1.0		15.39±4.4de	2.9±0.5^{cd}	Thick
10	0.5	0.5		18.47±3.0d	1.6±0.3^{d}	Stunted
11	1.0	1.0		20.45±2.9d	1.3±0.2^{de}	Stunted
12	0.5		0.1	28.90±3.8c	1.0±0.4^{de}	Base Callus
13	1.0		0.2	31.50±4.5c	2.7±0.4^{cd}	Base Callus
14		0.5	0.1	13.56±2.4e	2.4±0.3^{d}	Base Callus
15		1.0	0.2	15.50±1.4de	1.5±0.2^{de}	Base Callus

The data were analysed statically by ANOVA followed by Duncan's multiple range test at 0.5% level. The average followed by different letters are significantly different at 0.5%p level.

Effect of BAP on nodal explant

The nodal explants developed into seedlings on the basal medium (Fig. 1f), and when sub cultured onto MS medium supplemented with BAP and KN, either individually or in combination with BAP, KN and NAA exhibited multiple shoot proliferation (Table 2). BAP at different concentrations (0.1, 0.2, 0.5, and 1.0 mg l^{-1}) induced an average of 15.3 ± 3.5, 50.6 ± 4.4, 81.5 ± 7.5, and 71.5 ± 7.5 shoots per node, respectively, by the fourth week after inoculation. Although KN also induced multiple shoots, its effect was less pronounced compared to BAP, with shoot proliferation averaging 5.26 ± 0.7, 8.45 ± 1.2, 10.35 ± 1.5, and 15.39 ± 4.4 shoots per bud after 4 weeks (Table 2). While the combination of BAP and KN at both 0.5 and 1.0 mg l^{-1} induced multiple shoots, the shoots were stunted and abnormal. Similarly, the combination of BAP and KN with NAA led to callus formation at the base of the nodes, and the resulting shoots were also stunted. The optimum and maximum number of shoots was observed with BAP at 0.5 mg l^{-1}, producing 81.5 ± 7.5 shoots per node, followed by BAP at 1.0 mg l^{-1}, which produced 71.5 ± 7.5 shoots per node (Table 2 & Fig. 1). Rhizogenesis was observed three weeks after inoculation; however, the developed roots were very thin, unbranched, and unhealthy. Therefore, the in vitro plants were transferred to MS medium supplemented with IBA (0.5 mg/l) to promote the development of healthy roots.

Regeneration from leaf explants

Leaf segments from in vitro-grown plants were inoculated onto MS basal medium as well as onto MS basal medium supplemented with 0.5 mg/l BAP. Leaf explants (Fig. 2a) cultured onto MS basal medium developed roots from the midrib region by the fourth day of inoculation (Fig. 2B). The first leaf and shoot induction was observed on the 12th day of inoculation (Fig. 2C), followed by the second shoot on the 14th day (Fig. 2D & E), and the third shoot on the 18th day (Fig. 2F). The colour of the explant changed from green to white after three weeks of inoculation (Fig. 2G). On the 22nd day, the fourth shoot was induced, and the explant turned black in colour (Fig. 2H).

Multiple shoot induction through Callogenesis

In vitro-developed leaves inoculated onto MS medium supplemented with BAP alone, as well as with a combination of BAP and NAA, showed the initiation of multiple shoots through indirect organogenesis via callogenesis (Fig. 3A). Initially, embryoid-like globular structures appeared at the cut ends, margins, and sub-marginal regions on the upper surface of the leaves. These structures gradually increased in number and later developed into seedlings (Fig. 3B–D). Rapid shoot proliferation was observed, with an average of 50.5 ± 8.6 shoots per explant after three weeks of inoculation (Fig. 3E–H).

Although the combination of BAP and NAA also induced callus-like and globular structures, as well as plantlets, the number of shoots produced was less, and the developed plants were stunted. Additionally, the Calli formed in the BAP + NAA treatment were thicker compared to those induced by BAP alone. The responses of leaf explants on MS basal medium and MS supplemented with BAP are summarized in Table 3.

Table 3: Table showing number of shoots on Basal MS and MS+BAP from leaf explant

SI. No.	BAP mg^{-1} l	NAA mg^{-1} l	Number of multiple shoots after 4 weeks	Remarks
1	0	0	4.5±1.5	Globules/Shoot slender
2	0.1	0	14.8±6.2	Globules and organogenesis
3	0.2	0	35.6±5.3	Globules with organogenesis
4	0.5	0	50.5±8.6	Globules, good shoots
5	1.0	0	40.2±5.9	Globules with Thick shoots
6	0.2	0.1	12.9±2.7	Thick Calli and shoots stunted
7	0.5	0.2	10.5±3.9	Thick Calli and shoots stunted
8	1.0	0.5	13.7±2.9	Thick Calli and shoots stunted
9	1.0	1.0	8.35±2.0	Thick Calli and shoots stunted

To confirm indirect organogenesis, leaf explants were subjected to microscopic observation at various stages of culture. White globular structures developed on the margins and sub-marginal regions of the upper surface of the leaves, from which multiple shoots emerged (Fig. 3A–D). Microscopic examination of the leaf tissue containing these globules revealed that they consisted of homogeneous cells, likely representing friable callus-like structures, from which shoots developed through globular somatic embryos (Fig. 3).

The presence of vascular tissues within the developing structures confirmed their identity as somatic embryos, a key characteristic of true somatic embryogenesis (Fig. 3E–H). Initially, a single shoot emerged from each somatic embryo (Fig. 3E), which subsequently elongated (Fig. 3F) and developed into a leafy shoot with roots. In a similar mannerSimilarly, numerous somatic embryos formed and further developed into seedlings directly from the leaf lamina explant (Fig. 3).

Rooting

For rooting, healthy seedlings extracted from the cultures were transferred to MS medium supplemented with IBA (0.5 mg/l). Root induction was observed within one week of inoculation. The number of roots increased progressively over time (Fig. 8C), and the initially formed roots became thicker and healthier (Fig. 8D). The number of roots formed in the IBA-supplemented medium was significantly higher compared to those in the basal MS medium (Fig. 8E).

Hardening and acclimatization

In vitro-developed rooted plantlets, measuring 3–4 cm in height with 12–15 fully expanded leaves and well-developed roots, were successfully transferred to glass cups filled with sterile coir pith for hardening (Fig. 9A & B). Normal growth of the plantlets was observed within two weeks of transfer. The healthy plants were then transplanted into larger pots containing clay-rich soil and relocated to the Aquagene section (aquatic plants conservatory) of the KSCSTE-Malabar Botanical Garden and Institute of Plant Sciences, which is recognized as a Lead Garden in aquatic biodiversity by Botanic Gardens Conservation International (BGCI).

DISCUSSION

Aquatic ecosystems around the world are under serious threat, highlighting the urgent need for effective strategies to conserve and protect rare, endangered, and threatened (RET) aquatic plant species.

One of the most promising biotechnological approaches for their conservation is tissue culture, which enables the rapid propagation and preservation of these valuable plant resources [13]. In vitro techniques offer a promising approach to overcoming the significant challenges associated with biodiversity conservation [14]. One of the major issues in the tissue culture of aquatic plants is the high rate of culture contamination. However, the present study demonstrates that contamination can be effectively managed by standardizing protocols. Furthermore, explants derived from in vitro-grown plants can subsequently be used for the in vitro conservation of aquatic species. Hygienic conditions and proper care can reduce the level of contamination to a minimum extent.

Leaf and nodal explants were collected from field-established plants of *Lindernia manilaliana* (Fig. 1A). Aquatic plants, being mostly herbaceous and in constant contact with soil and water, are more prone to contamination due to the close association of microbes with their tissues. These microbes can easily enter the culture medium through the explants. However, in this study, the rate of contamination was found to be low. Tween 20 and 0.01% $HgCl_2$ were found to be the most effective surface sterilant used throughout the study. The sterilization procedure involved washing the explants in Tween 20 for 20 minutes and later rinsing with distilled water. This was then followed by treatment with a 0.01% aqueous solution of mercuric chloride, including an intermediate step of washing with sterile water. A sterilization protocol was reported on *Bacopa*, a herbaceous aquatic plant, which involved treatment with 0.1% mercuric chloride (w/v) for 2 minutes, followed by thorough rinsing with sterile distilled water [15]. For shoot proliferation, growth regulators-, particularly cytokinins - are among the most important factors influencing the response [16,17,18 19]. A range of cytokinins - including Kinetin, BAP, 2-iP, and Zeatin-has been used in micropropagation studies [20]. The present study demonstrated that BAP is the most reliable and effective hormone for shoot proliferation. The results clearly showed that a significant number of plants were successfully multiplied on media containing BAP.

At higher concentrations, cytokinins tend to induce adventitious bud formation [20 & 22]. In our study as well, shoot proliferation occurred only in the presence of cytokinin (Fig. 4, 5 & 7). Somatic embryos were formed on MS medium supplemented with BAP (Fig. 6). These results are supported by some other works [23], who reported the development of somatic embryos in *Pimpinella tirapataensis* using MS medium supplemented with TDS."

Micropropagation studies in *Lindernia* species are very limited, and no reports exist for *Lindernia manilaliana*. The present study revealed that MS medium with BAP is highly effective for inducing multiple shoots through the formation of somatic embryos (Table 3 & 4; Fig. 6). Somatic embryogenesis and encapsulation of somatic embryos from internode and leaf explants of *Rotula aquatica* is also reported [24]. Their study showed that half-strength MS medium with 2,4-D was more effective for embryogenic callus formation. In contrast, the present study demonstrated that embryogenic callus was formed on MS medium supplemented with BAP (Fig. 6) and with BAP and NAA but the former induced maximum embryos. These somatic embryos developed into multiple shoots within 2–3 days (Fig. 6). In the present study, embryogenesis was confirmed through anatomical evaluation, which revealed the vascular nature of the somatic embryos.

The results discussed above clearly indicate that vegetatively propagating aquatic plants can be efficiently propagated in vitro and successfully acclimatized under natural climatic conditions using nodal and leaf explants, with an appropriate medium that supports both callogenesis and direct regeneration (Fig. 9). MS medium with BAP was found to be the most suitable for multiple shoot induction in *Lindernia manilaliana* through embryogenic callus formation (Fig. 6).

Based on the above-mentioned results, it is evident that tissue culture plays a significant pivotal role in biodiversity conservation, enabling the rapid propagation and in vitro conservation of aquatic plants within a short period of time. Modern biotechnological approaches thus offer valuable tools for both the conservation and sustainable utilization of biodiversity. These techniques are particularly effective in conserving a wide range of plant species, especially aquatic plants categorized as endangered or threatened on the Red List.

ACKNOWLEDGEMENTS

First author is grateful to the Department of Science and Technology (DST-SERB), Government of India, for providing the financial support.

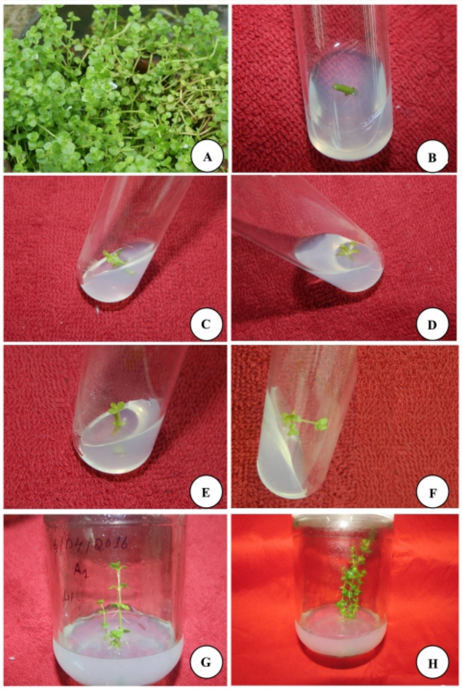

Figure 1: The plant selected for the study and direct regeneration from nodes. (A). Habit of *Lindernia manilaliana* Sivar., (B). Nodes inoculated on basal media, (C). Bud proliferation on 4th day, (D). Leaf induction on 6th day, (E). Second leaf initiation on 8th day, (F). Stem elongation on 10th day, (F& G), Root initiation and (H). Healthy plant with multiple shoot and root.

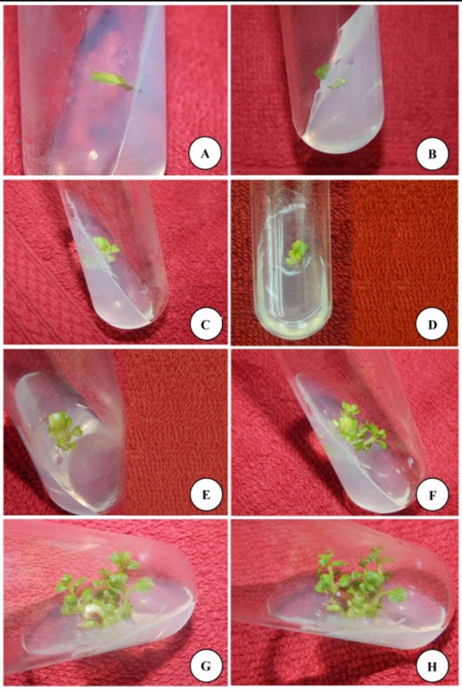

Figure 2: Direct regeneration of *Lindernia manilaliana* from leaf as explant. (A). Leaf explant, (B). Single root from midrib portion on 4th day, (C). First shoot with leaf induction on 12th day, (D & E). Second shoot induction on 14th day, (F). Third shoot induction on 18th day, (G). Decolorization of explant and (H). Healthy plant with multiple shoot on 22nd day.

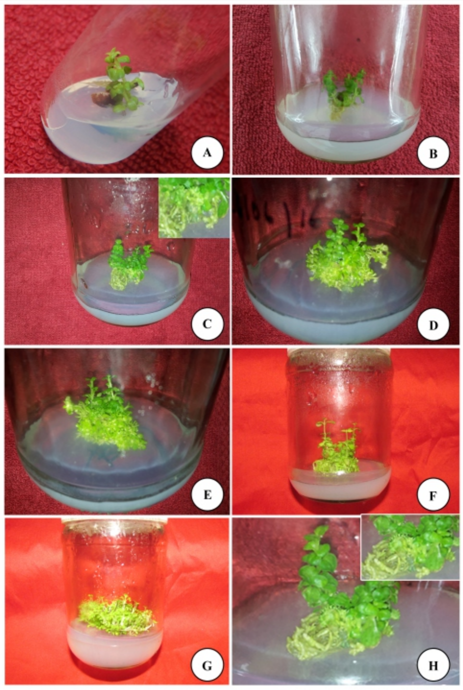

Figure 3: Callogenesis of *Lindernia manilaliana* by adding 0.5mg/l BAP. (A). In vitro developed plant as explant, (B). Muliple shoot induction, (C). Callus formation from roots and globular callus on the inset, (D). Development of mumerous multiple shoots, (E). Leaf induction, (F). Elongation of multiple shoot and (G & H). Development of multiple shoot from in vitro developed leaves.

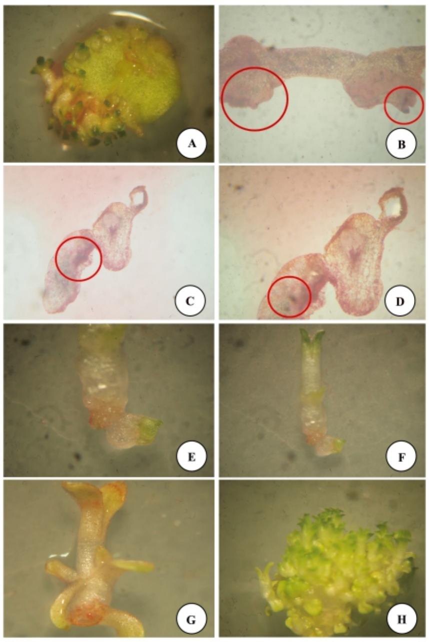

Figure 4: Microscopic observation of in vitro developed leaves. (A). In vitro developed leaf with white globules, (B, C & D). Anatomy of leaf showing somatic embryos, (E). Single shoot, (F). Elongation of single shoot, (G). Leafy stage and (H). Development of multiple shoot.

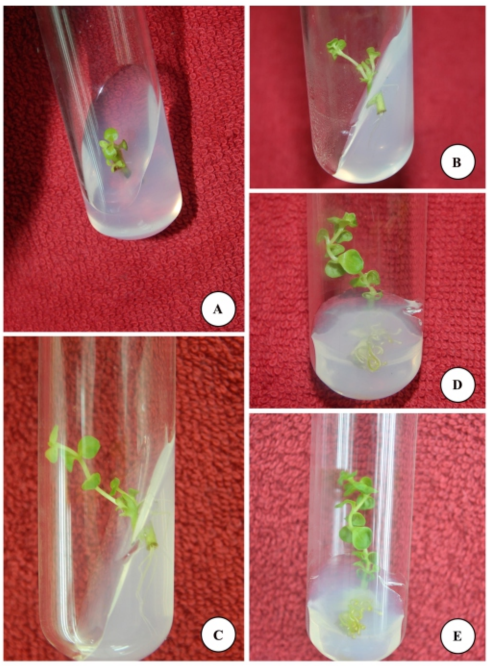

Figure 5: Effect of IBA on *Lindernia manilaliana*. (A). Nodal explant, (B). Development of three long slender roots, (C). Development of six roots, (D). Helathier root and (E). Development of numerous roots.

Figure 6: Hardening of *Lindernia manilaliana*. (A & B). In vitro plants transferred to glass cups filled with sterile coir pith.

REFERENCES

1. Nayar, M. P. 1996. Hotspots of Endemic Plants of India, Nepal and Bhutan. Tropical Botanical Garden & Research Institute, Palode, Trivandrum, India.

2. Brawin Kumar R , K. Anitha, Aparna Watve, S. Mani, Shiny Rehel and W. Arisdason 2011 Chapter 6 73-86. The status and distribution of aquatic plants of the Western Ghats In: The status and distribution of freshwater biodiversity in the Western Ghats, India. Molur, S., Smith, K.G., Daniel, B.A. and Darwall, W.R.T.

3. Prakashkumar R, Madhusoodhanan PV, Rajashekaran PE, Ansari R and Anoop KP. Aquatic RET plants under ex-situ conservation at Malabar Botanical Garden Institute of Plant Sciences. MBGIPS, Kerala, 2015, 21-38.

4. Krishnan PN, Decruse S W and Radha R K (2011) Conservation of medicinal plants of Western Ghats, India and its sustainable utilization through in vitro technology. In Vitro Cellular & Developmental Biology - Plant 47 (1): 110-122.

5. P.N. Krishnan, Dhanya B. Pillai and K. Satheeshkumar (2013) “Conservation of Tuberous Medicinal Plants of Western Ghats through Biotechnological Approach. Bulbous Plants: Biotechnology, Edited By Kishan Gopal Ramawat, Jean-Michel Merillon, Imprint CRC Press Boca Raton. Chapter 17: pp 370-418.

6. Kukulczanka K., Klimaszewska K., and Slawinski S., 1980, In vitro culture of Aponogeton, Tropical Fish Hobbyist, 29:36–39

7. Huang L.C., Chang Y.H., and Chang C.L., 1994, Rapid in vitro multiplication of the aquatic angiosperm, *Anubias barteri* var. undulate, Aquatic Botany, 47:77–83

8. Kane M.E., Davis G.L., Mc Connell D.B. and Gargiulo, J.A., 1999, In vitro propagation of *Cryptocoryne wendtii*, Aquatic Botany, 63:197–202

9. Kane M.E., Gilman E.F., Jenks, M.A., and Sheehan T.J., 1990, Micropropagation of the aquatic plant *Cryptocoryne lucens*, Horticulture Sciences, 25: 687–689

10. Mo X., and Jiang Q., 2003, Study on rapid proliferation and color appearing of tropical aquatic grass *Cryptocoryne wendtii*. Acta Agric Zhejiangensis, 15(2):91–94

11. Ozturk M., Khawar K.M., Atar H.H., Sancak C., and Ozcan S., 2004, In vitro micro-propagation of aquarium plant *Ludwigia repens*, Asia Pacific Journal of Molecular Biology and Biotechnology, 12(1&2):21–27

12. Micheli M., De Gasperis A., Prosperi F., and Standardi A., 2006, Micro-propagation of three species of aquatic plants, *Agricoltura mediterranea* (Italy), 131(1):46–51, 17: pp 370-418

13. Fay MF. Conservation of rare and endangered plant using in vitro methods. In vitro Cellular and Developmental Biology, 1992. 28: 1-4.

14. Withers LA. Free preservation of somatic embryos and clonal plantlets. Journal of Plant Physiology, 1979. 63: 460-467.

15. Shrivastava N and Rajani M. Multiple shoot regeneration and tissue culture studies on *Bacopa monnieri* (L) Pennell. Plant Cell Reports .1999. 18(11): 919-923.

16. Lane WD. In vitro propagation of *Spirea bumalda* and *Prunus cistena* from shoot apices. Canada Journal of Plant Science, 1979. 59: 1025-1029.

17. Stoltz LP. In vitro propagation of *Acalypha wilkesiana*. Horticultural Science, 1979. 14: 702-703.

18. Bhojwani SS. In vitro propagation of Garlic by shoot proliferation. Journal of Science Horticulture, 1980. 13: 47-52.

19. Garland P and Stoltz LP. Micropropagation of *Pissardi plum*. Journal of Annual Botany, 1981. 48: 387-389.

20. Bhojwani SS and Razdan MK. Plant tissue culture: theory and practice. Journal of Science, 1996. 6(3): 767-789.

21. McComb JA. Clonal propagation of woody plant species with special reference to apples. Journal of International Plant Propagators Society, 1978. 28: 413-426.

22. Zimmerman RH and Broome OC. Blueberry micro propagation in proceedings of the conference on nursery production of fruit plants through tissue culture- Application and Feasibility. Journal of Agricultural Research Science, 1980. 44-48.

23. Prakash E, Khan SV, Meru E and Rao KR. Somatic embryogenesis in *Pimpinella thirupathiensis*- An endangered medicinal plant of Thirumala hills. Journal of Current Science, 2001. 81(9): 1239-1242.

24. Chitra M, Martin KP, Sunandakumari C and Madhusoodanan PV. Somatic embryogenesis, encapsulation and plant regeneration of *Rotula aquatica* Lour. In vitro Cellular and Developmental Biology –Plant, 2005. 41(1): 28-31.