



# *In vitro* plant regeneration through somatic embryogenesis in *Anaphyllum wightii* Schott

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## Abstract

*Anaphyllum wightii* Schott. is an ethnomedicinally significant plant endemic to the southern region of Western Ghats. The present study aimed to develop an efficient protocol for the *in vitro* propagation of the plant through somatic embryogenesis. Fresh petioles were selected as explants for the experiment. The medium used was half-strength Murashige and Skoog (MS) medium supplemented with different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic acid (NAA). Both 2,4-D and NAA showed embryogenic callus induction from petiole explants after up to 4 wk of culture. NAA at 2 mg L<sup>-1</sup> showed the highest percentage of embryogenic callus induction (83.33 ± 8.80%). Histological and stereomicroscopic observations of the embryogenic callus revealed various stages of somatic embryos indicating an asynchronous type of embryogenesis. Full-strength MS medium containing 3 mg L<sup>-1</sup> 6-benzylaminopurine induced the maximum number of shoots per callus (6.00 ± 0.58) after 30 d of culture for NAA-induced calluses. The highest rooting response was obtained with half-strength MS medium fortified with 0.5 mg L<sup>-1</sup> indole-3-butyric acid. The *in vitro* rooted plantlets were hardened by transferring to small plastic pots containing sand and garden soil (1:1) and showed a 76% survival rate after 4 wk. Thus, the present work developed an efficient *in vitro* protocol for the conservation of this plant species and also contributes to the study of the embryonic development.

**Keywords** *Anaphyllum wightii* · Somatic embryogenesis · *In vitro* propagation · Embryogenic callus · Asynchronous

## Introduction

The plant *Anaphyllum wightii* Schott., commonly known as ‘Wight’s twisted arum’ (‘Keerikizhangu’ in Malayalam), is an endemic and threatened genus of South India (Ahmedullah and Nayar 1986). It is included in the ‘Vulnerable’ category of IUCN (Rajasekharan and Wani 2020). The plant is a tall herb belonging to the family ‘Araceae’ and is usually found in the Western Ghats region at an altitude of 650 to 1000 m. The plant has a rhizomatous stem and pinnately compound leaves. The inflorescence is a spadix with a once or twice twisted spathe.

The tribal communities in Kerala, including the Kani tribes of Kottoor reserve forest and the Agasthyavanam, Thiruvananthapuram, and Malapandaram tribes of Achankovil forest, Kollam, use fresh tubers of *A. wightii* as an antidote

against snake bite and also as food (Ramachandran 2007; Mathew and George 2013). The rhizome of this plant is used by the Kanikkar tribal community of Tamil Nadu to treat skin diseases like eczema and scabies (Kunjumon *et al.* 2016). Reports suggest that the rhizome of *A. wightii* possesses antimicrobial, antioxidant, hepatoprotective, anthelmintic, anti-inflammatory, and antidiabetic pharmacological activities (Udayan *et al.* 2007).

Even though vegetative propagation through tuber is an effective plant regeneration method, the overexploitation of tubers for their medicinal and food value limits their use as a propagule. The seeds of this plant can also germinate into plantlets; however, flowering and seed setting usually occur once a year, and the propagation through seeds alone is not sufficient for the conservation of such an endemic plant species. Hence, it is necessary to develop an efficient protocol for the *in vitro* propagation of this ethnomedicinally significant plant. Somatic embryogenesis is one of the efficient methods of *in vitro* plant propagation where a bipolar structure, similar to a zygotic embryo, develops from a non-zygotic cell having independent vasculature without

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connection to that of the original tissue (Arnold *et al.* 2002). Somatic embryogenesis is a powerful tool for the study of plant developmental processes since it involves most of the developmental stages, the same as that of zygotic embryos (Elhiti *et al.* 2013). Since there are no previous reports regarding somatic embryogenesis in this endemic plant, the present study aimed to develop a protocol for the *in vitro* propagation of the plant through somatic embryogenesis.

## Materials and Methods

**Explant source** The plant materials of *A. wightii* Schott. were collected from the Kallar region of Thiruvananthapuram District, Kerala, and maintained in the garden of the Department of Botany, University of Kerala, Thiruvananthapuram. Fresh and healthy petiole explants, obtained from approximately 6- to 8-mo-old field-grown plants, were used for the *in vitro* culture experiments.

**Explant preparation and sterilization** The petiole explants were cut into pieces and washed under running tap water for 15 min. The explants were then washed with diluted Tween™ 20 detergent solution (1 drop per 100 mL) (HiMedia Laboratories Pvt. Ltd., Mumbai, India) for 10 min, followed by washing under running tap water for about 30 min. The explants were subsequently rinsed with sterile distilled water three times and surface sterilized by immersing in 70% ethanol for 30 s, followed by washing with 0.1% (*w/v*) mercuric chloride (HiMedia Laboratories Pvt. Ltd.) for 4 min. Again, the explants were vigorously rinsed with sterile distilled water 3 to 5 times to remove the traces of mercuric chloride solution.

**Embryogenic callus induction and proliferation** After surface sterilization, the petiole explants were cut into pieces of smaller size (0.5 to 1 cm) and inoculated onto half-strength basal Murashige and Skoog (MS; Murashige and Skoog 1962) medium (HiMedia Laboratories Pvt. Ltd.) (pH 5.8) supplemented with 0.5 mg L<sup>-1</sup>, 1.0 mg L<sup>-1</sup>, 2.0 mg L<sup>-1</sup>, 3.0 mg L<sup>-1</sup>, 4.0 mg L<sup>-1</sup>, or 5.0 mg L<sup>-1</sup> 2,4-D or 0.5 mg L<sup>-1</sup>, 1.0 mg L<sup>-1</sup>, 2.0 mg L<sup>-1</sup>, 4.0 mg L<sup>-1</sup>, 6.0 mg L<sup>-1</sup>, or 8.0 mg L<sup>-1</sup> naphthaleneacetic acid (NAA) (Sigma-Aldrich, Bangalore, India). The experimental design used was a completely randomized block design. Each treatment consisted of 10 explants, and the experiment was conducted in triplicate. The cultures were maintained at 25 ± 1 °C with alternate light (12-h) and dark (12-h) conditions, and the percentage of embryogenic callus induction was recorded after 45 d of incubation. Subculturing was done at an interval of 30 d on the same medium composition, in which they induced, for the proliferation of embryogenic callus cultures.

**Shoot regeneration from embryogenic callus cultures** The embryogenic calluses were inoculated on full-strength MS medium fortified with 1 to 5 mg L<sup>-1</sup> 6-benzylaminopurine (BAP) (Sigma-Aldrich) with regular subculturing after 30 d of incubation. The percentage of shoot induction, shoot number, and shoot length were recorded separately for 2,4-D- and NAA-induced calluses.

**Histological analysis** The embryogenic calluses and embryoids were subjected to free-hand sectioning followed by staining with safranin (HiMedia Laboratories Pvt. Ltd.) and were observed under a Leica EZ4E stereomicroscope and image analyzer (Leica DM2000 microscope) (Leica Microsystems; Schweiz AG, Heerbrugg, Switzerland) to study the anatomical features of somatic embryos.

***In vitro* root induction** The individual shoots formed from embryogenic callus cultures were transferred to half-strength MS medium supplemented with 0.2 to 2.0 mg L<sup>-1</sup> indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), or NAA (Sigma-Aldrich) for *in vitro* root induction. The percentage of rooting response, the number of roots per shoot, and root length were recorded for all three hormones after 45 d of incubation.

**Hardening and acclimatization** After 45 d, the *in vitro* rooted plantlets were removed from the culture bottles (400-mL glass bottles with plastic screw caps) (Abroach Exim Pvt. Ltd., Ahmedabad, India) containing medium, washed in tap water to remove traces of medium, and planted in small pots containing sand and garden soil (1:1) and covered with a transparent polythene bag with small pores to maintain humidity. The plantlets were watered once a week. After 4 wk, the polythene bags were removed and transferred to greenhouse conditions.

**Statistical analysis** Statistical analysis was performed using the software SPSS/PC version 22 (SPSS Inc., Chicago, IL). Mean values and standard error were calculated by one-way ANOVA, and the means were compared by Duncan's multiple range test at the level of  $P \leq 0.05$  (Duncan 1955).

## Results

**Embryogenic callus induction** Properly sterilized fresh petiole explants were used for the induction of somatic embryogenesis. The half-strength MS medium fortified with 0.5 to 5.0 mg L<sup>-1</sup> 2,4-D or 0.5 to 8.0 mg L<sup>-1</sup> NAA was used for the experiment. Off-white or cream-colored compact calluses were obtained from the cut edges of horizontally inoculated petiole explants after about 4 wk of culture in

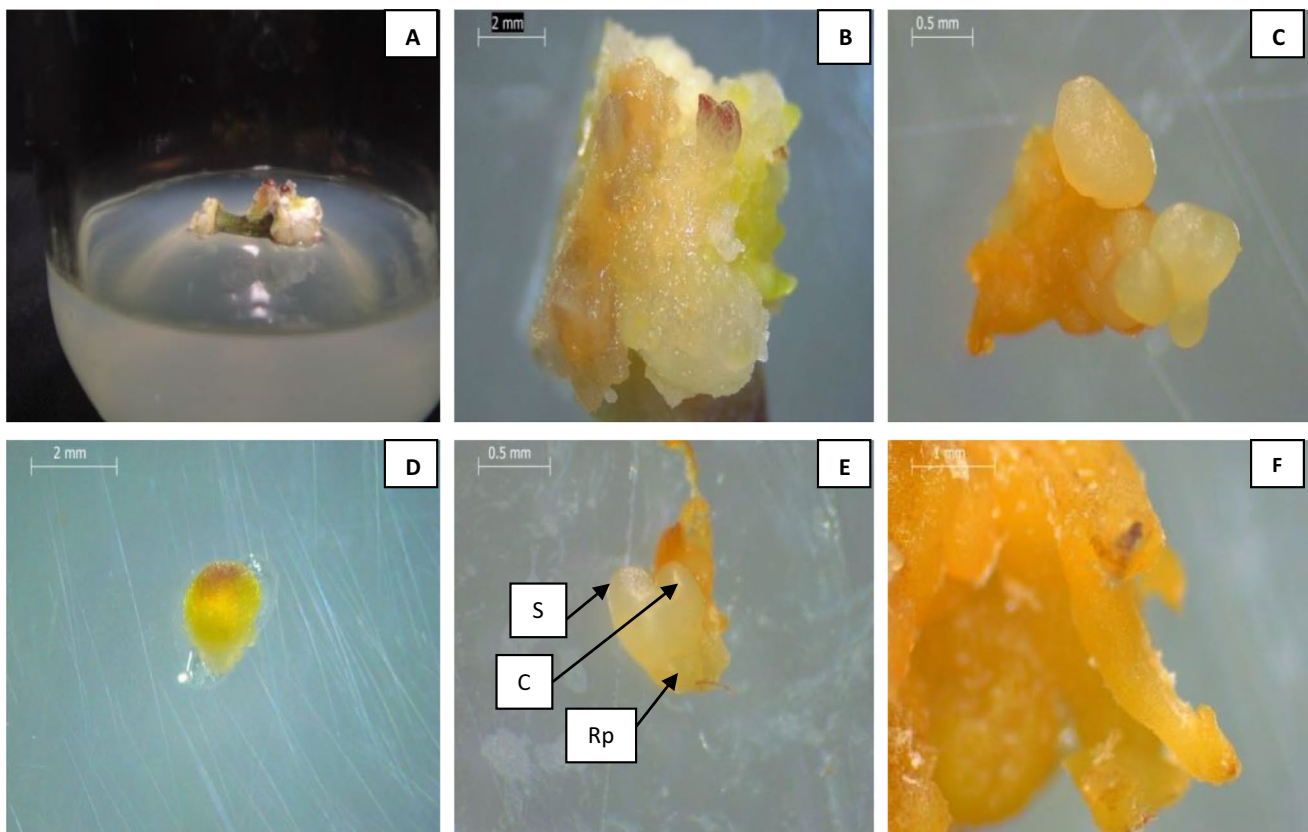
the half-strength MS medium supplemented with 1 mg L<sup>-1</sup> 2,4-D and 2 mg L<sup>-1</sup> NAA (Fig. 1).

The calluses were subcultured on the same medium composition in which they were induced and were turned embryogenic after 2 wk of subculturing. The percentage of embryogenic callus induction from petiole explants was recorded for 2,4-D and NAA-supplemented medium after 45 d of culture (Table 1). The results showed that petiole explants exhibited the highest percentage of embryogenic callus induction (83.33 ± 8.80%) on half-strength MS medium containing 2 mg L<sup>-1</sup> NAA. In the case of 2,4-D, used in half-strength MS medium, the highest percentage of embryogenic callus induction was observed at 1 mg L<sup>-1</sup> (76.67 ± 8.82%). The embryogenic calluses obtained were repeatedly subcultured every 30 d for proliferation and maintenance of embryogenic cultures. Thus, the results indicated that NAA is superior to 2,4-D for embryogenic callus induction and maintenance. The various developmental stages of somatic embryos, such as globular, scutellar, and coleoptilar embryos, were observed under a stereomicroscope (Fig. 1).

**Table 1.** Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) and naphthaleneacetic acid (NAA) on *Anaphyllum wightii* Schott. embryogenic callus induction after 45 d

Plant growth regulators	Concentration (mg L <sup>-1</sup> )	Percentage of embryogenic callus induction (%)
2,4-D	0.5	50.00 ± 5.77 <sup>b</sup>
	1	76.67 ± 8.82 <sup>c</sup>
	2	40.00 ± 5.77 <sup>b</sup>
	3	0.00
	4	0.00
NAA	0.5	0.00
	1	66.67 ± 8.82 <sup>ab</sup>
	2	83.33 ± 8.80 <sup>a</sup>
	4	50.00 ± 5.77 <sup>b</sup>
	6	30.00 ± 5.37 <sup>c</sup>
	8	0.00

Values represent the mean ± SE ( $n=3$ ). Mean values in the same column followed by the same superscript are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$



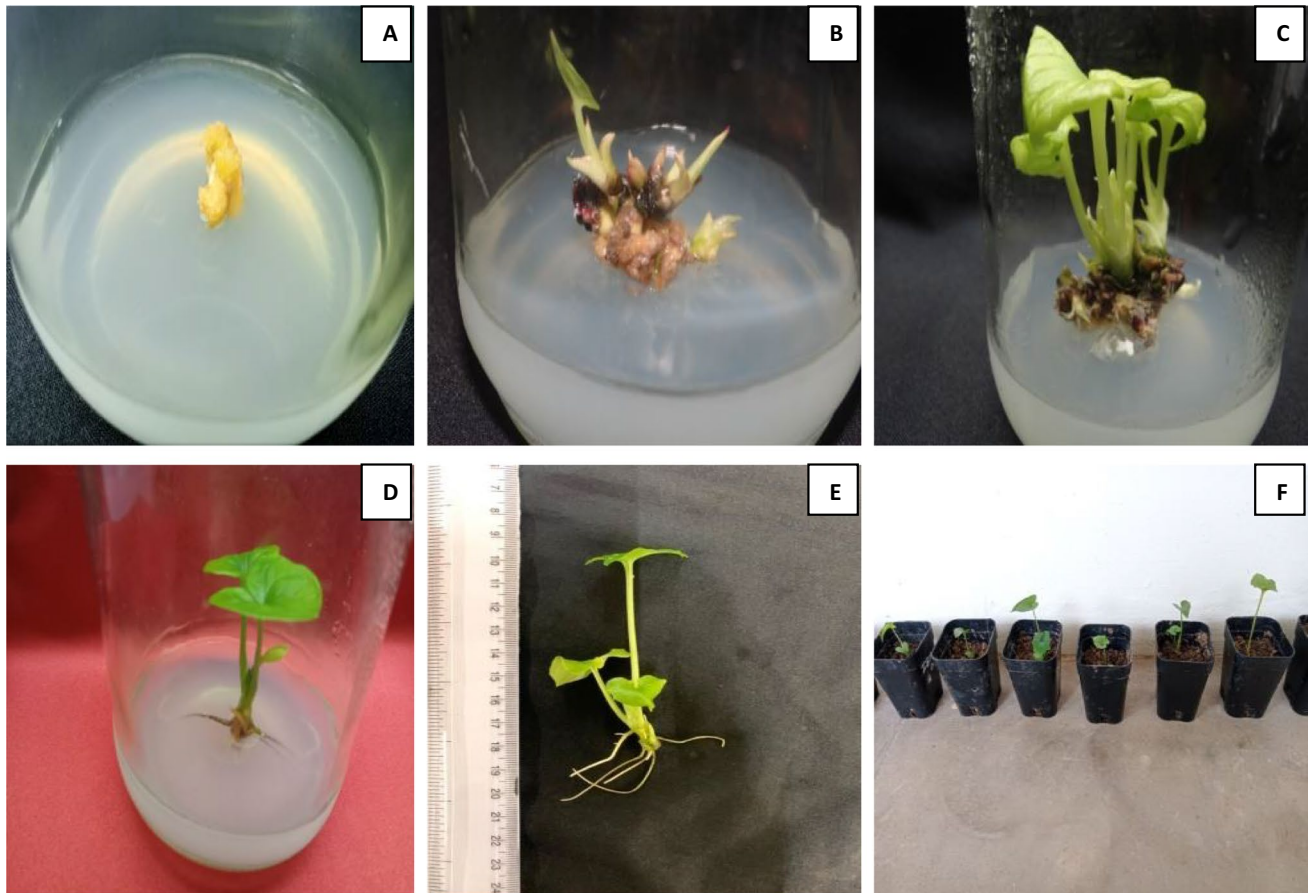
**Figure 1.** Embryogenic callus induction from petiole explants of *Anaphyllum wightii* Schott. (A) Embryogenic callus (naphthaleneacetic acid 2 mg L<sup>-1</sup>). (B, C) Stereomicroscopic images of callus with globular and scutellar embryos ( $bar=2$  mm and 0.5 mm,

respectively). (D) Isolated globular embryo ( $bar=2$  mm). (E) Scutellar embryo with scutellum (S), coleoptile (C), and root pole (Rp) ( $bar=0.5$  mm). (F) Coleoptilar embryo ( $bar=1$  mm).

**Shoot regeneration from embryogenic callus cultures** After proliferation, the NAA- and 2,4-D-induced embryogenic calluses were transferred to full-strength MS medium containing 1 to 5 mg L<sup>-1</sup> BAP separately for the maturation of embryos and shoot regeneration from the embryogenic calluses. The embryogenic callus cultures exhibited a good

percentage of multiple shoot induction from the calluses within 2 wk (Fig. 2). The shooting response shown by BAP on 2,4-D- and NAA-induced embryogenic calluses are represented in Tables 2 and 3, respectively.

For the 2,4-D-induced callus cultures, the maximum percentage of regeneration (100%), the highest number of



**Figure 2.** Multiple shoot regeneration from embryogenic callus of *Anaphyllum wightii* Schott. (A) Embryogenic callus. (B) Multiple shoot regeneration after 45 d on Murashige and Skoog medium (MS) containing 3 mg L<sup>-1</sup> 6-benzylaminopurine. (C) After 90 d. (D) *In vitro*

root induction from shoot explants on half-strength MS medium containing 0.5 mg L<sup>-1</sup> indole-3-butyric acid after 30 d. (E) *In vitro* rooted plantlet after 45 d. (F) Acclimatized plants after 4 wk.

**Table 2.** Effect of 6-benzylaminopurine (BAP) on 2,4-dichlorophenoxyacetic acid (2,4-D)-induced embryogenic callus of *Anaphyllum wightii* Schott

BAP (mg L <sup>-1</sup> )	After 45 d			After 90 d	
	Percentage of regeneration (%)	Number of shoots per callus	Shoot length (cm)	Number of shoots per callus	Shoot length (cm)
1	86.67 ± 3.33 <sup>b</sup>	3.67 ± 0.33 <sup>a</sup>	1.01 ± 0.15 <sup>b</sup>	5.33 ± 0.30 <sup>b</sup>	2.56 ± 0.17 <sup>b</sup>
2	96.67 ± 3.30 <sup>a</sup>	4.00 ± 0.58 <sup>a</sup>	1.11 ± 0.05 <sup>b</sup>	5.67 ± 0.23 <sup>b</sup>	2.60 ± 0.15 <sup>b</sup>
3	100.00 ± 0.00 <sup>a</sup>	4.67 ± 0.30 <sup>a</sup>	2.23 ± 0.25 <sup>a</sup>	7.33 ± 0.33 <sup>a</sup>	3.66 ± 0.05 <sup>a</sup>
4	100.00 ± 0.00 <sup>a</sup>	4.00 ± 0.57 <sup>a</sup>	1.16 ± 0.11 <sup>b</sup>	6.00 ± 0.58 <sup>b</sup>	3.35 ± 0.08 <sup>a</sup>
5	100.00 ± 0.00 <sup>a</sup>	4.33 ± 0.33 <sup>a</sup>	0.97 ± 0.04 <sup>b</sup>	5.66 ± 0.33 <sup>b</sup>	3.29 ± 0.09 <sup>a</sup>

Values represent the mean ± SE ( $n=3$ ). Mean values in the same column followed by the same superscript are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$

**Table 3.** Effect of 6-benzylaminopurine (BAP) on naphthaleneacetic acid (NAA)-induced embryogenic callus of *Anaphyllum wightii* Schott. after 30 d

BAP (mg L <sup>-1</sup> )	Percentage of regeneration (%)	Number of shoots per callus	Shoot length (cm)
1	100.00±0.00	3.67±0.32 <sup>b</sup>	1.81±0.16 <sup>c</sup>
2	100.00±0.00	4.33±0.33 <sup>b</sup>	3.11±0.15 <sup>b</sup>
3	100.00±0.00	6.00±0.58 <sup>a</sup>	4.80±0.11 <sup>a</sup>
4	100.00±0.00	4.67±0.33 <sup>b</sup>	4.98±0.22 <sup>a</sup>
5	100.00±0.00	4.33±0.30 <sup>b</sup>	5.34±0.67 <sup>a</sup>

Values represent the mean ± SE ( $n=3$ ). Mean values in the same column followed by the same superscript are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$

shoots per callus, and maximum shoot length were recorded on full-strength MS medium containing 3 mg L<sup>-1</sup> BAP after 90 d of culture. The maximum shoot number and shoot length obtained were  $7.33 \pm 0.33$  and  $3.66 \pm 0.05$  cm, respectively.

Full-strength MS medium containing 3 mg L<sup>-1</sup> BAP gave the highest number of shoots per callus ( $6.00 \pm 0.58$ ), and 5 mg L<sup>-1</sup> BAP showed a maximum shoot length of  $5.34 \pm 0.67$  cm after 30 d of culture for NAA-induced calluses. The NAA-induced calluses were capable of 100% regeneration on all five concentrations of BAP. While comparing the responses obtained from 2,4-D- and NAA-induced callus cultures on medium supplemented with 1 to 5 mg L<sup>-1</sup> BAP, it can be seen that NAA-induced calluses showed a higher number of shoots per callus and maximum shoot length in a short period of culture. However, the shoots

that arose from 2,4-D-induced calluses were seen healthier with comparatively thicker petioles and wider leaf blades than those from NAA-induced callus cultures.

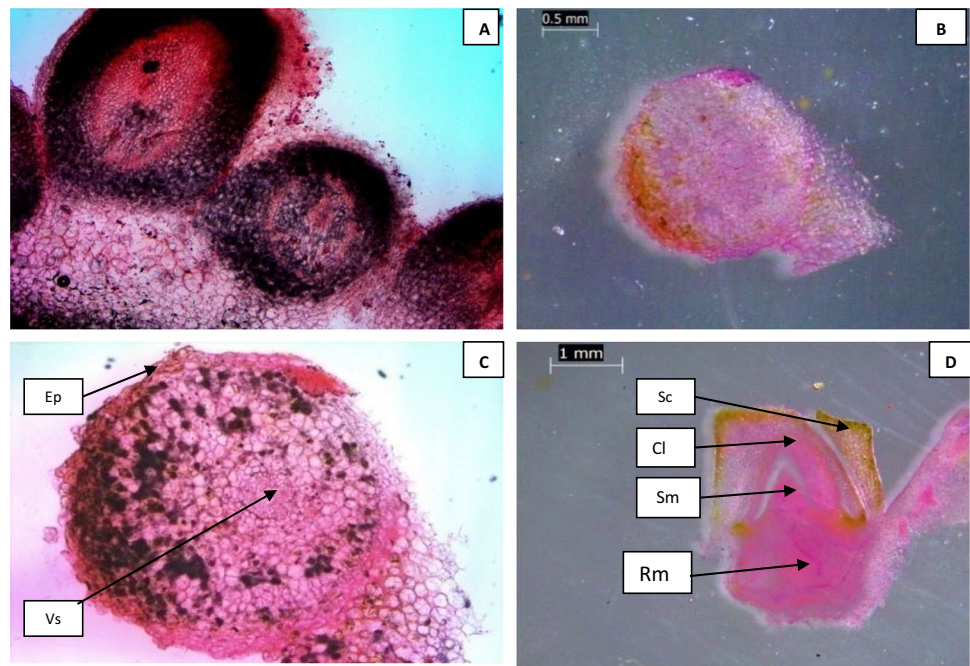
**Histological analysis of embryogenic callus and embryos** Histological analysis of embryogenic callus revealed the presence of globular and coleoptilar embryos. Globular embryo consisted of an epidermis that differentiated it from the callus tissue and an independent vascular system without any connection to that of the callus. The coleoptilar stage of the embryo consisted of scutellum, coleoptile, shoot meristem, and root meristem (Fig. 3).

**In vitro root induction** *In vitro* raised individual shoot explants were inoculated on half-strength MS medium containing 0.2 to 2 mg L<sup>-1</sup> IAA, IBA, or NAA for root induction. The results obtained from *in vitro* rooting experiments are mentioned in Table 4.

Among the three auxins, IAA and IBA showed root induction after about 3 wk of culture, whereas NAA showed no response. IBA at 0.5 mg L<sup>-1</sup> induced a better rooting response, after 45 d, in terms of the percentage of root induction ( $83.30 \pm 3.30\%$ ), root number per shoot ( $4.33 \pm 0.31$ ), and root length ( $5.83 \pm 0.93$  cm) compared to other hormone concentrations used (Fig. 2).

**Hardening and acclimatization** The hardening stage is an important step involved in every micropropagation experiment before introducing the *in vitro* regenerated plants to the natural environment. Since the tissue culture plants are grown under controlled temperature and humidity conditions

**Figure 3.** Histological observations of embryogenic callus and somatic embryos of *Anaphyllum wightii* Schott. (A) Longitudinal section of callus with globular embryos. (B, C) Longitudinal section of a globular embryo with the epidermis (Ep) and independent vascular system (Vs) viewed under a stereomicroscope ( $bar=0.5$  mm) and image analyzer. (D) Longitudinal section of coleoptilar embryo with scutellum (Sc), coleoptile (Cl), shoot meristem (Sm), and root meristem (Rm) ( $bar=1$  mm).



**Table 4.** Effect of auxins on *in vitro* root induction in *Anaphyllum wightii* Schott. using half-strength Murashige and Skoog medium after 45 d

Growth regulators	Concentration (mg L <sup>-1</sup> )	Percentage of root induction (%)	Number of roots per shoot	Root length (cm)
Indole-3-acetic acid (IAA)	0.2	0 ± 0.00	0 ± 0.00	0 ± 0.00
	0.5	66.67 ± 3.33 <sup>b</sup>	2.33 ± 0.33 <sup>cde</sup>	3.77 ± 0.12 <sup>bc</sup>
	1	73.33 ± 6.67 <sup>ab</sup>	4.33 ± 0.30 <sup>c</sup>	4.93 ± 0.48 <sup>ab</sup>
	1.5	70.00 ± 5.77 <sup>ab</sup>	3.33 ± 0.33 <sup>abc</sup>	3.83 ± 0.44 <sup>bc</sup>
	2	0 ± 0.00	0 ± 0.00	0 ± 0.00
Indole-3-butyric acid (IBA)	0.2	70.00 ± 5.80 <sup>ab</sup>	1.67 ± 0.33 <sup>de</sup>	5.10 ± 0.10 <sup>ab</sup>
	0.5	83.30 ± 3.30 <sup>a</sup>	4.33 ± 0.31 <sup>c</sup>	5.83 ± 0.93 <sup>a</sup>
	1	63.33 ± 3.33 <sup>b</sup>	3.67 ± 0.29 <sup>ab</sup>	4.90 ± 0.21 <sup>ab</sup>
	1.5	46.67 ± 3.32 <sup>c</sup>	2.67 ± 0.33 <sup>bcd</sup>	3.17 ± 0.16 <sup>c</sup>
	2	40.00 ± 5.73 <sup>c</sup>	1.33 ± 0.26 <sup>e</sup>	2.50 ± 0.29 <sup>e</sup>
Naphthaleneacetic acid (NAA)	0.2	0 ± 0.00	0 ± 0.00	0 ± 0.00
	0.5	0 ± 0.00	0 ± 0.00	0 ± 0.00
	1	0 ± 0.00	0 ± 0.00	0 ± 0.00
	1.5	0 ± 0.00	0 ± 0.00	0 ± 0.00
	2	0 ± 0.00	0 ± 0.00	0 ± 0.00

Values represent the mean ± SE ( $n=3$ ). Mean values in the same *column* followed by the same *superscript* are not significantly different according to Duncan's multiple range test at  $P \leq 0.05$

in an aseptic environment, they will have a tendency of rapid water loss and wilting outside the culture room. Hence, the *in vitro* regenerated plantlets were initially covered with transparent polythene bags in order to maintain optimum humidity and watered once a week. After 4 wk, the polythene bags were removed, and the plants were transferred to greenhouse conditions. The plants were watered three times a week and were acclimatized with a survival rate of 76% (Fig. 2).

## Discussion

Somatic embryogenesis is an efficient method for the mass propagation of many plant species and is also suitable for the regeneration of transgenic plants (Bhattacharyya *et al.* 2016). Plant regeneration through somatic embryogenesis is affected by various factors such as the genetic composition of plant material, the treatment of primary explants, and culture conditions (Mikuła and Rybczyński 2001). The culture medium has been reported to exert a more substantial effect on embryogenesis than other factors (Pavlović *et al.* 2013). Somatic embryos may arise either directly from the epidermal or sub-epidermal cells of explants (Moradi *et al.* 2017) or indirectly from an intervening callus phase induced from explants (Niknejad *et al.* 2011). The plant growth regulators and their concentration also influence the induction of somatic embryogenesis. Higher concentrations of auxins, especially 2,4-D, are used for inducing somatic embryos in some plant species (Raghavan 2004; Chen *et*

*al.* 2010), whereas cytokinins at high concentrations are effective in other species (Kim *et al.* 2003).

Among the aroid species, plant regeneration through somatic embryogenesis was first reported in *Anthurium andraeanum* (Kuehnle *et al.* 1992). Later, several other aroids, including *Colocasia esculenta* (Deo *et al.* 2009), *Dieffenbachia* sp. (Shen and Lee 2009), and *Spathiphyllum* sp. (Zhao *et al.* 2012), were reported to be competent for induction of somatic embryogenesis. However, there were no previous reports of somatic embryogenesis induction in the genus *Anaphyllum*. Thus, the present work developed an easy and efficient protocol for inducing somatic embryogenesis in *A. wightii* for the first time. Embryogenic callus cultures possess the potential to proliferate for many generations and to develop somatic embryos (Hu *et al.* 2017; Thorat *et al.* 2017).

Blessy *et al.* (2021) reported the use of nodal portions of the rhizome as the explants for micropropagation of *A. wightii*. Since the overharvesting of the rhizome of this vulnerable species for its edible and medicinal purposes limits its use as planting material or explant, hence in the present study, the fresh petiole explants were used for the mass propagation of *A. wightii* through somatic embryogenesis. Hu *et al.* (2005) also reported the use of petiole explants for the induction of embryogenic calluses in the aroid *Amorphophallus konjac*. Even though the petiole explants exhibited embryogenic callus induction on half-strength MS medium containing both 2,4-D and NAA, the latter was observed to be superior for embryogenic callus induction and maintenance. Here, the type of embryogenesis observed is indirect somatic embryogenesis since an

intervening callus phase is involved in the process. Similar results were obtained in the case of *Anthurium andraeanum*, where NAA was found to be more effective than other auxins, including 2,4-D, for the induction of embryogenic callus cultures from petiole explants of *in vitro* established plantlets (Pinheiro *et al.* 2014).

In the present work, the embryogenic callus was observed to contain developing somatic embryos at the globular, scutellar, and coleoptilar stages. This agrees with the general pattern of somatic embryogenesis in monocots, where the process of somatic embryo differentiation involves three major stages, namely globular, scutellar, and coleoptilar embryos (Burrieza *et al.* 2012). These stages of somatic embryos are also observed in other monocot species like *Crocus sativus* (Devi *et al.* 2014), *Hordeum vulgare* (Kachhwaha *et al.* 1997), and *Phalaenopsis amabilis* (Mose *et al.* 2017). Here, the formation of globular proembryos was observed as embryonic masses consisting of small, deeply stained meristematic cells resulting from repeated cell division. The proembryos later differentiated into large globular-shaped embryos with a protoderm differentiating it from the callus tissue which is a characteristic feature of somatic embryo development (Yeung 1995; Arnold *et al.* 2002).

In the next stage of development, the globular embryo forms a notch at the terminal position, similar to that reported in ginger (Lincy *et al.* 2009), leading to the scutellar stage (de Alcantara *et al.* 2014). The scutellar embryo consisted of two protrusions at the apical part and a notch between them. The shoot meristem develops from the scutellar notch and becomes enclosed by the coleoptile. The root meristem will also be formed at this stage in the basal part of the embryo (Mose *et al.* 2017). The matured coleoptile embryo was characterized by the development of a scutellum, coleoptile, and shoot and root meristems. Here, the scutellum was found to be slightly greenish color, which was similar to that in the case of *Hordeum vulgare* (Kachhwaha *et al.* 1997).

The cytokinin BAP was found to be effective for multiple shoot induction, as reported previously in the same species. IBA at 0.5 mg L<sup>-1</sup> induced a better rooting response compared to other auxins used, which also agrees with the findings of Blessy *et al.* (2021). The *in vitro* regenerated plants were successfully acclimatized with a good survival rate of 76%, which was better than that obtained in a previous study (65%) on the same species (Blessy *et al.* 2021).

## Conclusions

Somatic embryogenesis serves as a tool for the large-scale propagation of plants and is also an effective method for studying the regulation of embryo development. The present study developed a successful protocol for the *in*

*vitro* plant regeneration of the endemic and ethnomedicinal plant *Anaphyllum wightii*. Here, the somatic embryos were obtained by the process of indirect somatic embryogenesis due to the presence of an intervening callus phase. In *A. wightii*, the process of embryo development was asynchronous since the embryogenic callus consisted of embryos at various stages of development adjacent to each other. Hence, it can be concluded that the auxins 2,4-D and NAA are effective in the induction of somatic embryogenesis using the petiole explants of *A. wightii*, which may lead to the conservation of this endemic plant. The present study may also be helpful in studying the pattern of embryo development in this species since the developmental stages of somatic embryos are often similar to that of zygotic embryos.

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