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Optimization of *in vitro* culture conditions for callogenesis in *Evolvulus alsinoides* (L.)

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Abstract
Evolvulus alsinoides L. is a medicinal plant of the family Convolvulaceae. It is used traditionally for its medicinal properties. The present investigation was carried out to standardize an efficient *in vitro* protocol for callus induction and plant regeneration using leaf, nodal and internodal explants. The explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of plant growth regulators like 2,4-D, NAA, BAP and IBA. Results showed that nodal explants gave the maximum 100 % callus induction on MS medium supplemented with 1.5 mg/L 2,4-D and 1.5 mg/L NAA, and showed the highest callogenic response. Callus initiation was observed after about 10 days. Substantial callus development occurred during subsequent culture. The results show the potential of *in vitro* tissue culture for propagation and conservation of E. alsinoides and as a basis for further studies on the production of its valuable secondary metabolites.

Keywords: Callogenesis, *Evolvulus alsinoides*, MS medium, plant tissue culture, medicinal plant, auxins, cytokinins

Introduction
Plant tissue culture is extensively utilized in botanical research and possesses various commercial uses. This encompasses:

- A plant breeder might employ tissue culture to evaluate cells instead of whole plants for beneficial traits, such as herbicide resistance or tolerance (Gill *et al*., 2008).
- The generation of uniform sterile hybrid species can be achieved (Srivastava *et al*., 2006).
- Extensive proliferation of plant cells in liquid culture within bioreactors for the synthesis of valuable substances, such as plant-derived secondary metabolites and recombinant proteins utilized as biopharmaceuticals (Georgiev *et al*., 2009) [13].
- To hybridize distantly related species through protoplast fusion and the subsequent regeneration of the novel hybrid (Manstein *et al*., 1880).
- To facilitate cross-pollination between distantly related species and subsequently perform tissue culture on the resulting embryo that would typically perish (Embryo Rescue) (George *et al*., 2008) [11, 12].
- To generate doubled monoploid (dihaploid) plants from haploid cultures for the expedited development of homozygous lines in breeding programs, colchicine treatment is typically employed to induce chromosome number doubling (Edwin *et al*., 2009).
- As a medium for alteration, succeeded by either brief evaluation of genetic constructs or the restoration of transgenic flora.
- Certain methodologies, including meristem tip culture, can be employed to generate pristine plant material from virus-free sources, such as potatoes and various soft fruit species.
- Micropropagation utilizing meristem and shoot culture to generate substantial quantities of uniform specimens.

Importance and scope of Medicinal Plant
More than seventy-five percent of the global population depends predominantly on botanical sources and plant derivatives for medical care. Over 30% of all plant species have been utilized for medicinal applications at various points in time (Kumar 1998) [19].
Researchers in biotechnology find medicinal plants highly intriguing, as a significant portion of the pharmaceutical industry relies on plants for the synthesis of medicinal compounds (Chand *et al*., 1997) [6].
The Ayurvedic medical system employs approximately 70 species, Unani utilizes 700, Siddha incorporates 600, while contemporary medicine relies on around 30 species. The pharmaceuticals are obtained from either the entire plant or various parts, such as leaves, stems, bark, roots, flowers, and seeds. The investigation of traditional medicines, the extraction of pharmaceuticals via bioprospecting, and the organized preservation of relevant medicinal flora are consequently of significant value (Joy *et al*., 1998).

Botanical description of plant
Evolvulus alsinoides (L.) is classified within the Convolvulaceae family (Ahmad *et al*., 2022) [2]. The organism was initially classified as *Convolvulus alsinoides* and subsequently reassigned to the genus Evolvulus. It is recognized by common names such as Vishnukranti, Sankhavalli, Vishnukraandi, and Sankhaholi (Ahmad *et al*., 2022) [2].

Habit and stem
The flora is a diminutive perennial herb, typically sprawling, slender, and wiry. The stems are adorned with elongated trichomes (Ahmad *et al*., 2022) [2]. Anatomically, the stem comprises a solitary epidermal layer, roughly one to four layers of chlorenchyma, and between one to seven layers of cortical cells. It encompasses a bicollateral vascular bundle along with a central pith (Ketjarun *et al*., 2016).

Leaves and micromorphology

The leaves have a single epidermal layer on both the upper and lower surfaces and an isobilateral mesophyll. The stomata may be anomocytic, paracytic, or laterocytic, and capitate glandular trichomes occur on the leaf surfaces. The anticlinal epidermal cell walls are sinuous. Y-shaped hairs have also been reported as a diagnostic feature in *Evolvulus* species, including *E. alsinoides* (Ketjarun *et al*., 2016).

Flowers and reproductive characters

The flowers are small. The corolla is reported to be approximately 7–9 mm long, a character used to distinguish *E. alsinoides* from related species such as *E. filipes* (Moreira *et al*.,). The pollen grains are spheroidal monads, approximately 28–47 µm in diameter, with 15-pantocolpate apertures and microechinate ornamentation (Ketjarun *et al*., 2016). The seed-coat epidermal cells are irregular or polygonal, with raised and undulated anticlinal boundaries; their periclinal walls may be flattened to concave or folded (Ketjarun *et al*., 2016).

Distribution

Evolvulus alsinoides occurs in tropical and subtropical regions. Historical records include Malabar, Sri Lanka, and the Bahamas. The species is also widely recognized and used in traditional medicine in India and other tropical regions, including parts of Africa and the Philippines (Singh, 2008).

Traditional medicinal uses

The plant, known in different traditions as Shankhpushpi, Vishnukranti, and Sankhaholi, is traditionally used to support memory, intellect, and cognitive function (R *et al*., 2021; Ahmad *et al*., 2022; Agrahari *et al*., 2026) [1, 2]. Reported traditional applications include:

- **Neurological uses:** management of amnesia, epilepsy, anxiety, neurosis, insomnia, and other cerebral disorders (R *et al*., 2021; Ahmad *et al*., 2022) [2].
- **Metabolic and systemic uses:** use as an adaptogenic, antihypertensive, hepatoprotective, and aphrodisiac herb (R *et al*., 2021; Ahmad *et al*., 2022; Isa & Garba, 2022) [2, 17].
- **Other traditional indications:** asthma, fever, inflammation, haemorrhoids, diabetes, flatulence, and urinary disorders (R *et al*., 2021; Ahmad *et al*., 2022; Agrahari *et al*., 2026) [1, 2].
- **External and general applications:** wound healing, hair-fall management, skin infections, and antiseptic preparations (R *et al*., 2021; Drvinay, 2022; Agrahari *et al*., 2026) [1, 7].

These uses represent ethnomedicinal knowledge and should not be interpreted as proof that the plant treats these conditions in humans.

Important phytochemical constituents

Phytochemical investigations have reported several classes of secondary metabolites in *E. alsinoides*. These include β-sitosterol, β-sitosterol-3-O-glucoside, ursolic acid, kaempferol 3,7-di-O-rhamnoside, tannins, phenolic compounds, betaine, scopolin, scopoletin, umbelliferone, triacontane, pentatriacontane, and shankhpushpine (Ahmad *et al*.,2022; Nithya *et al*.,2023) [2, 22]. Carbohydrates, cardiac glycosides, and saponins have also been reported in extracts of the plant.

These compounds may contribute to antioxidant, enzyme-inhibitory, anti inflammatory, or cytotoxic effects, but the presence of a compound alone does not demonstrate therapeutic effectiveness in humans.

Reported pharmacological activities

1. Neuroprotective and cognitive effects

In a rat model of streptozotocin-induced cognitive impairment, *E. alsinoides* extract improved cognitive performance and was associated with effects on cholinergic function and oxidative stress (Mehla *et al*., 2012). Leaf extracts have also shown in-*vitro* inhibitory activity against acetylcholinesterase and butyrylcholinesterase, enzymes relevant to cholinergic signaling and Alzheimer's disease research (Sundaramoorthy & Packiam, 2020) [24]. These findings are preclinical and do not demonstrate efficacy for dementia or memory disorders in humans.

2. Antioxidant and possible antidiabetic activity

Extracts of *E. alsinoides* have shown antioxidant activity in laboratory assays such as DPPH and FRAP. In-*vitro* studies have also reported inhibition of α-amylase and α-glucosidase, enzymes involved in carbohydrate digestion (Sundaramoorthy & Packiam, 2020; Nithya *et al*., 2023) [22, 24]. These results suggest possible antidiabetic potential, but clinical trials are required before any therapeutic conclusion can be made.

3. Blood and immune-related effects

In rats, administration of an aqueous extract affected blood parameters, including red and white blood-cell counts. The study also examined spleen histology and reported tissue changes at tested doses (Isa & Garba, 2022) [17]. These findings are useful for evaluating biological activity and safety, but they do not establish a human haematinic or immunostimulatory effect.

4. Antileukaemic and cytotoxic activity

Extracts or isolated constituents of *E. alsinoides*, including kaempferol 3,7-di-O-rhamnoside, have been investigated for cytotoxic or antileukaemic activity against cultured human leukemic cell lines such as U937, K562, and HL60 (Mulukuri, 2017). Such cell-culture results may support further anticancer research, but they are not evidence that the plant is an effective or safe cancer treatment in patients.

Materials and Methods

1. Collection of Plant Material

The plants of *Evolvulus alsinoides* were collected from the forest area of Sant Gadge Baba Amravati University and subsequently established in the garden of the Department of Botany at the same institution. A variety of explants, including nodal explants, shoot tips, and leaves from plantlets of *Evolvulus alsinoides*, were employed in the present study.

The entire experiment was conducted in the plant tissue culture laboratory of the Department of Botany at Sant Gadge Baba Amravati University, Amravati, under aseptic and controlled conditions.

2. Preparation of Stock Solutions of Plant Growth Regulators

Stock solutions of plant growth regulators (PGRs), such as auxins and cytokinins, were prepared in accordance with the standard protocols employed in plant tissue culture. To facilitate the dissolution of growth regulators before dilution with sterile double distilled water, appropriate solvents such as ethanol, sodium hydroxide (NaOH), and hydrochloric acid (HCl) were employed (Murashige and Skoog, 1962; George *et al*., 2008) [11, 12, 21].

a. Preparation of Auxin Stock Solutions

- **2,4-Dichlorophenoxyacetic Acid (2,4-D)**

A stock solution of 2,4-D was prepared by dissolving 20 mg of the compound in 3 mL of ethanol to ensure complete solubility. The volume was then adjusted to 20 mL using sterile double distilled water under aseptic conditions (George *et al*., 2008) [11, 12].

- **α-Naphthalene Acetic Acid (NAA)**

For NAA, 20 mg of the compound was dissolved in 3 mL of 1N NaOH, as auxins are more soluble in alkaline solutions. The final volume was adjusted to 20 mL with sterile double distilled water (Bhojwani and Razdan, 1996) [5].

- **Indole-3-Acetic Acid (IAA)**

Similarly, 20 mg of IAA was dissolved in 3 mL of 1N NaOH, followed by volume adjustment to 20 mL with sterile double distilled water. The alkaline medium aids in complete dissolution of IAA (George *et al*., 2008) [11, 12].

b. Preparation of Cytokinin Stock Solutions

- **6-Benzylaminopurine (BAP)**

A stock solution of BAP was prepared by dissolving 20 mg in 1 mL of 1N NaOH, followed by dilution up to 20 mL with sterile double distilled water. Cytokinins like BAP require slight alkalinity for proper solubilization (Murashige and Skoog, 1962) [21].

- **Kinetin**

For kinetin, 20 mg of the compound was dissolved in 1 mL of 1N HCl to enhance solubility, and the volume was made up to 20 mL using sterile double distilled water (Bhojwani and Razdan, 1996) [5].

c. Storage of Stock Solutions

All prepared stock solutions were stored in sterile, labeled containers and kept at 4°C until further use. Light-sensitive compounds such as IAA were stored in amber-colored bottles to prevent degradation (George *et al*., 2008) [11, 12]

d. Excision and surface sterilization of explant:

Strict aseptic conditions are necessary for the successful establishment and growth of explants in *in vitro* plant tissue culture techniques. Surface sterilization of explants is an essential procedure that ensures the preservation of tissue viability while eliminating microbial contamination (Murashige and Skoog, 1962; George *et al*., 2008) [11, 12, 21]. Proper sterilization protocols, which include detergents, alcohol, and chemical sterilants like mercuric chloride ($HgCl_2$), are frequently implemented in plant tissue culture research (Bhojwani and Razdan, 1996) [5].

The successful induction of calluses and the regeneration of plants are contingent upon the selection of healthy and disease-free explants, followed by sterilization and inoculation on nutrient media that contain plant growth regulators (George *et al*., 2008) [11, 12]. *In vitro* growth is additionally influenced by controlled environmental conditions, including temperature, photoperiod, and humidity.

Selection of healthy plant material
↓
Excision of explants (internodal / nodal / leaf)
↓
Washing with running tap water
↓
Rinsing with sterile distilled water
↓
Treatment with soap solution (Labolin)
↓
Rinsing with sterile distilled water
↓
Surface sterilization with 0.1% $HgCl_2$ (2–3 min)
↓
Rinsing with sterile distilled water (3–4 times)
↓
Treatment with 70% ethanol (2–3 min)
↓
Final washing with sterile distilled water
↓
Drying on sterile tissue paper
↓
Trimming edges using sterile scalpel
↓
Inoculation on MS medium + PGRs
↓
Incubation under controlled conditions

Results and Discussion

Callus Initiation and Growth Response

In the present investigation, different explants (leaf, nodal, and internodal) of *Evolvulus alsinoides* were cultured on Murashige and Skoog (MS) medium supplemented with various concentrations and combinations of plant growth regulators (PGRs). Callus initiation was observed within 9–12 days of inoculation across all treatments. The initiation response varied significantly depending on:

- Type of explant
- Concentration of growth regulators

In the present investigation which was carried out in the well-equipped plant tissue culture laboratory. Murashige and Skoog's media supplemented with combination of different growth regulators including auxins and cytokinins i.e. 2,4-D. IAA, NAA, and BAP, kinetin respectively. Leaf, Node and Internodes were used as explants.

Different concentrations of plant growth regulators had a significant effect on callus regeneration. During the present investigation Leaf, nodal and internodal explants of *Evolvulus alsinoides* were cultured on MS media fortified with 2, 4-D, 2,4- D-NAA, NAA-BAP, and BAP +IBA in different concentrations to test the efficiency of sprouting of various explants. It was observed that after 9-12 days of inoculation, the explants showed callus initiation in all the concentrations.

The nodal explants were the first to show callus initiation. The callogenic response showed variations among the hormonal combinations/concentrations and types of explants used. The callus response varied from 50 to 80% in nodal explants, 60 to 100% in leaf explants and 50 to 90% in internodal explants.

Among different types of explants used, nodal segments proved to be best for callus induction. Highest callogenic response, i.e. 100%, was noticed with nodal explants at 1.5 mg/L. 2, 4-D+1.5 mg/L NAA and lowest callogenic response i.e. 50% was observed in leaf explants at 2, 4-D 3.0 mg/l . A total of 14 combinations of auxins and cytokinins were tried for callus formation. The degree of callus formation varies from very little in leaf explants at 2,4-D (1.5mg/l) and large amount of callus in nodal explants at 2,4-D+NAA(1.5+1.5mg/l) denoted by + and +++ respectively. (Table 1, graph 1).

Table 1: Callus induction from Nodal and leaf explants at different concentrations and combinations of plant growth regulators.

Leaf explant			Degree of callus formations	Nodal explants	Degree of callus formations	Internodal explants	Degree of callus formations
PGR	Conc.(mg/l)	RP (%)		RP (%)		RP (%)	
2, 4 D	1.5	60	+	90	+++	70	++
	2.0	75	++	80	++	80	++
	2.5	50	+	70	++	60	+
	3.0	50	+	70	++	60	+
2,4 D + NAA	1.0 + 1.0	50	+	70	++	60	+
	1.5 + 1.5	70	++	100	+++	90	+++
	1.5 + 1.0	70	++	90	+++	80	++
NAA + BAP	1.5 + 2.5	60	+	90	+++	70	++
	2.0 + 2.0	70	++	90	+++	70	++
	2.5 + 1.5	80	++	90	+++	80	++
	2.5 + 2.0	60	+	90	+++	70	++
BAP + IBA	1.0 + 0.5	50	+	80	++	70	++
	1.0 + 2.0	70	++	90	+++	80	++
	2.0 + 1.0	50	+	60	+	50	+

+ = Little Callus,
++ = Average Callus,
+++ = Large amount of callus.

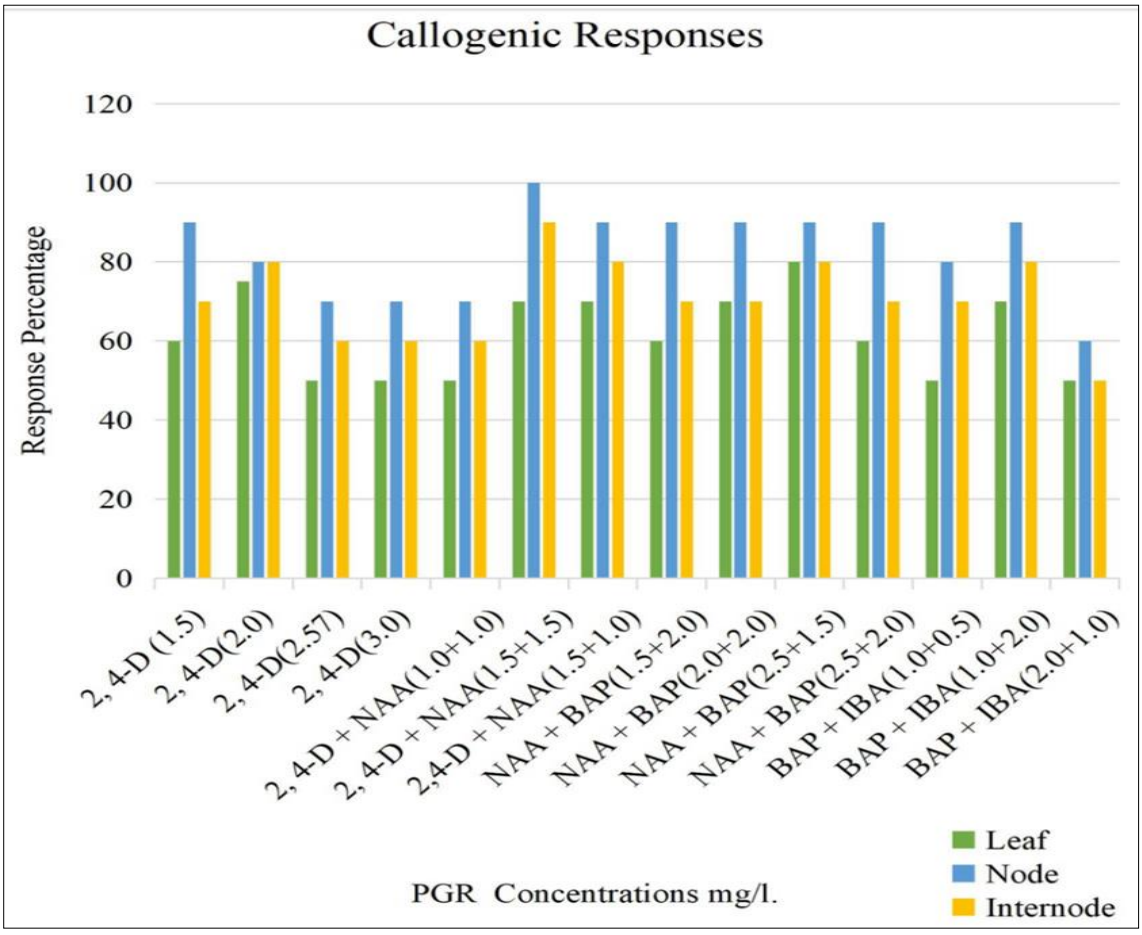


Fig 2: Callogenic response from Leaf, Nodal and Internodal Explants

Table 2: Response of the explants for callusing to different combinations of growth regulators used at different concentrations.

Sr.No.	Explant used	Growth Regulators	Concentrations mg/lit	Time taken by explants to form callus (days)	Result	Colour & Morphology
1.	Node	2,4-D	1.5	18	+	White & Friable
2.	Node	2,4-D + NAA	1.5 +1.5	16	++	Brown & Comapct
3.	Node	NAA+BAP	2.0 + 2.0	13	++	Greenish White & Friable
4.	Leaf	2,4-D + NAA	1.5 + 1.5	20	+	Green & Friable

Callus formation in nodal explants began earlier in MS medium containing 2, 4 D (1.5 ~mg/lit), 2, 4-D + NAA (1.5 + 1.5 ~mg/lit), (1.5 + 1.0 ~mg/lit), and NAA + BAP (2.0 + 2.0 ~mg/lit) (Table-1). After the second week, nodal explants begin initiation for callus induction. After the third week, the inoculated tissue showed signs of callusing in MS medium containing 2, 4 D (1.5 ~mg/lit), 2, 4-D + NAA (1.5 + 1.5 ~mg/lit), (1.5 + 1.0 ~mg/lit), and NAA + BAP (2.0 + 2.0 ~mg/lit). The current study found that nodal explants responded better to higher doses of 2, 4-D + NAA (1.5 + 1.5 ~mg/lit), NAA + BAP (2.0 + 2.0 ~mg/lit), and NAA + BAP (2.0 + 2.0 ~mg/lit). However, nodal explants show a response to all concentrations. Leaf explants also responded to 2, 4-D + NAA concentrations (1.5 + 1.5 ~mg/lit). Morphologically, the callus appeared green and friable after 15-20 days from leaf explants, etc. (Table 2).

Discussion

The study confirms that plant growth regulators play a critical role in callogenesis. Auxins, particularly 2,4-D in combination with NAA, significantly enhanced callus formation. Similar findings have been reported in other medicinal plants.

As per the results obtained, it is confirmed that nodal explants of *Evolvulus alsinoides* gave extremely best response to callus formation on Murashige and Skoog's media containing different concentrations of cytokinins and Auxins. The callus formation from nodal explants required 2,4-D and NAA in range of 1.50-2.50 mg/lit. Cultures supplemented with this combination showed initiation of callus after 10 days of inoculation while as full-grown callus was formed after 4 weeks of interval.

The maximum callus response was observed from nodal segments at 2,4-D+NAA (1.5+1.5) mg/l. Besides Nodal explants, leaf and internodal explants have also responded well for callus formation

Callus culture provides a platform for:

- Secondary metabolite production
- Genetic improvement
- Mass propagation

Figures



Fig.1: Habit

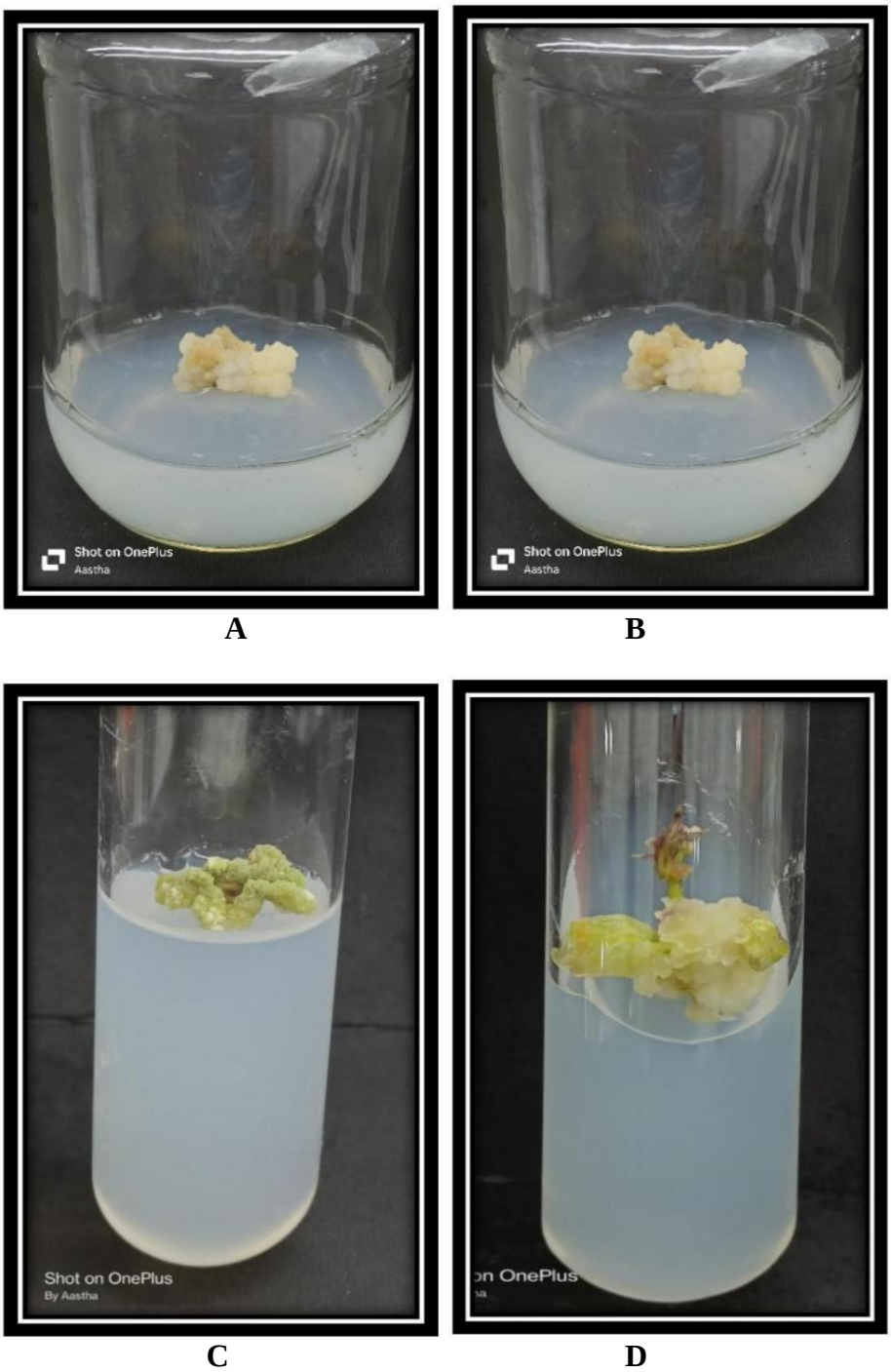


Fig.2: Callus induction from Nodal and leaf explants at different concentrations and combinations of plant growth regulators.

A = Callogenic response from nodal explants at 2, 4 – D (1.5) mg / l.
B = Callogenic response from nodal explants at 2, 4 - D + NAA (1.5 + 1.5) mg /l
C = Callogenic response from nodal explants at NAA + BAP (2.0 + 2.0) mg /l.
D = Callogenic response from leaf explants at 2, 4 - D + NAA (1.5 + 1.5) mg /l, respectively.

Conclusion

The study presented a protocol for successfully establishing callogenesis and organogenesis in *Evolvulus alsinoides* L. using callus, leaf, node, and internodal explants. Callus formation may facilitate secondary metabolite extraction compared to the intact plant.

Callus induction varies based on hormonal combination, concentration, and type of explant used. Combining NAA with 2,4-D improved callus induction in leaf, nodal, and internodal explants of *Evolvulus alsinoides* L. Nodal explants exhibited a 100% callogenic response at 2,4-D+NAA (1.5+1.5) mg/l. Explants of *Evolvulus alsinoides* showed varying levels of callogenic induction based on their order (node, internode, and leaf). Nodal explants showed a 100% callogenic response at 1.5mg/l of 2,4-D in combination with NAA (1.5mg/l). The callus obtained was white and friable.

Manipulating the culture medium's nutrient and hormonal constituents can regenerate a large number of plants from callus tissue. This callus can produce bioactive secondary metabolites and enhance plant resistance to salt, draft, diseases, and pests. The callus can also be used for

cytological purposes. This study suggests that enhancing biomass growth can help conserve *Evolvulus alsinoides* L. and produce bioactive compounds on a large scale.

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References

1. Agrahari D, Mishra AK, Tripathi G, Lyngdoh D, Sahu BK, Kumar S. *Evolvulus alsinoides* (L.) L. (Vishnukranti): An ethnomedicinally important herb of India. Zenodo, 2026.
2. Ahmad A, Kalam MA, Hussain Z. Sankhaholi (*Evolvulus alsinoides* Linn.): A valuable herb of Unani medicine. Journal of Biological and Scientific Opinion,2022:10(4):45–48.
3. pharmacology of Shankapushpi—four varieties. Ancient Sci Life,1988:7:149–156 Austin DF. *Evolvulus alsinoides* (Convolvulaceae). An American herb in the old world. J Ethnopharmacol,2008:117:185–198.
4. Balachandran SM, Bhat SR, Chandel KPS. Clonal multiplication of turmeric (*Curcuma* spp.) and ginger (*Zingiber officinale* Rosc.). Plant Cell Reports,1990:8:521–524.
5. Bhojwani SS, Razdan MK. Plant tissue culture: Theory and practice. Elsevier, 1996.
6. Chand S, *et al*. Role of plant tissue culture in medicinal plants, 1997.
7. Drvinay VP. Pharmacognostic evaluation and investigation of revitalizer herb *Evolvulus alsinoides* Linn. International Journal of Pharmacognosy and Chemistry, 2022, 53–63.
8. Faisal M, Singh M, Anis M. *In vitro* regeneration and plant establishment of *Tylophora indica*. *In vitro* Cellular & Developmental Biology - Plant,2005:41(4):511–515.
9. Flick CE, Evans DA, Sharp WR. Organogenesis. In D. A. Evans *et al*. (Eds.), Handbook of plant cell culture. Macmillan, 1983, 13–81.
10. Gautheret RJ. History of plant tissue and cell culture: A personal account. In Cell culture and somatic cell genetics of plants. Academic Press,1985:2:1–59.
11. George EF, Hall MA, De Klerk GJ. Plant propagation by tissue culture. Springer, 2008.
12. George EF, Debergh PC. Micropropagation: Uses and methods. In Plant propagation by tissue culture (3rd ed., Vol. 1). Springer, 2008.
13. Georgiev MI, Weber JM. Bioprocessing of plant cell cultures for mass production of targeted compounds. AppliedMicrobiologyand Biotechnology,2009:83(5):809–823.
14. Ghatge S, Dixit GB. Somatic embryogenesis and plant regeneration from leaf cultures of *Hemidesmus indicus* R. Br. In Proceedings of International Symposium on Frontiers in Genetics and Biotechnology, 2006, 179.
15. Guha S, Maheshwari SC. Cell division and differentiation of embryos in the pollen grains of *Datura in vitro*. Nature,1966:212:97–98.
16. Haberlandt G. Culture experiments with isolated plant cells. Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften, Wien,1902:111:69–92.
17. Isa ZA, Garba SH. Effect of aqueous extract of *Evolvulus alsinoides* Linn. on some blood parameters and the histology of the spleen in rats. African Journal of Biomedical Research,2022:25(2):229–233.
18. Kumar B, *et al*. Wound healing activity of *Hemidesmus indicus*, 2007.
19. Kumar S. Medicinal plants and their role in healthcare, 1998.
20. Miller CO. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symposia of the Society for Experimental Biology,1957:11:118–130.
21. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum,1962:15:473–497.
22. Nithya K, Siddaraman R, Sheelajoice PP, Rajarathinam M, Chandra VB. Biochemical analysis of methanolic extract from *Evolvulus alsinoides*. Bioinformation,2023:19:1173–1178.
23. R HK, Srinivasan J, Sundaramoorthy PMK, Packiam KK. *Evolvulus alsinoides* (Linn.) Linn.: A revitalizer. IntechOpen, 2021.
24. Sundaramoorthy PMK, Packiam KK. *In vitro* enzyme inhibitory and cytotoxic studies with *Evolvulus alsinoides* (Linn.) Linn. leaf extract: A plant from Ayurveda recognized as Dasapushpam for the management of Alzheimer's disease and diabetes mellitus. BMC Complementary Medicine and Therapies,2020:20(1):1–12.
25. Phytochemical and pharmacological review of *Evolvulus alsinoides* (Linn.). Zenodo, 2025.
26. Skoog F, Miller CO. Chemical regulation of growth and organ formation in plant tissues cultured *in vitro*. Symposia of the Society for Experimental Biology,1957:11:118–131.