EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 12
Issue: 11
Year: 2025

IN- VITRO PROPAGATION OF ENDANGER PLANT BRYOPHYLLUM PINNATUM UNDER DIFFERENT CONCENTRATION OF GROWTH HORMONE

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DOI: https://doi.org/10.5281/zenodo.17539492

How to cite this Article: *Sabiha Kaifi, Richa Verma Sondhiyan. (2025). IN- VITRO PROPAGATION OF ENDANGER PLANT BRYOPHYLLUM PINNATUM UNDER DIFFERENT CONCENTRATION OF GROWTH HORMONE. European Journal of Biomedical and Pharmaceutical Sciences, 12(11), 239–242.

Article Received on 05/10/2025 **Article Revised on 25/10/2025** **Article Published on 01/11/2025**

ABSTARCT

To establish a standardized protocol for in vitro propagation of endangered plant *Bryophyllum pinnatum* using leaf explants. The highest rate of shoot proliferation (85%) and number of shoots per explant 10 was obtained on media BAP 2.5mg/l + Kn0.5mg/l with average shoot length 2.0±0.66 The maximum shoot regeneration showed 80±1.45 in BAP 1.5mg/l + NAA 0.5mg/l + IBA 0.5mg/l .The largest effect on root formation occurred in BAP 0.5mg/l+ IBA 0.5mg/l +NAA 2.0 mg/l and 45±0.0 percentage of rooted explants with 9±11.5 number of roots produced. The plants were maintained at about 70% relative humidity in the greenhouse with 75% shading with 98% of survival rate was achieved after 6 weeks. The significance of the protocol is the formulation of growth regulators which affected very fast multiplication of the plant in less time that is, one-third time less of another methods.

KEYWORDS: Auxin, Calli, Cytokinine, Growth regulators, Micropropagation, Murashige and Skoog.

I. INTRODUCTION

Plants are essential source of medicines and play a key role in world health (**Constebel, 2000**). Among them ***Bryophillum pinnatum*** is one. Genus *Bryophyllum* of the family Crassulaceae is a valuable medicinal and ornamental plant. These plants are cultivated as ornamental house plants and rock or succulent garden plant **(Kulka, 2006**). It has a definite ornamental value **(Souza-Brito etal, 1993**) because of its beautiful inflorescence. It is a popular house plant and become naturalized in temperate region of Asia, the Pasific, Caribbean, Austrelia, Newzeland, West Indies, Mascarenes, Galapagoes, Polynesia And Hawai **(Zamore etal, 1998).** In South Eastern Nigeria, this herb is used to facilitate the dropping of the placenta of a newly born baby (**Daziel, 1995**). In India, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. So this plant is used in ethno medicine for treatment of earache, burns, abscesses, ulcer, insect bites, diarrhea and Lithiasis (**Chopra et al, 1956**).

Tissue culture methods are recognized as excellent tools for medicinal plants propagation, allowing the production of pathogen-free plants, under controlled conditions and with independence of climatic factors. Among them, micropropagation protocol is a necessary step for conservation and development **(Sajc *et al.*, 2000)**. It has many advantages over conventional methods of propagation, which suffers from several limitations. These plants are also used by the tribals of Kerela for treating Cancer symptoms (**Paranjpe, 2005), (Mathew, Unithan 1992).** This plant induce the typical symptoms of cardiac poising but repeted small doses also cause cotyledons, an intoxication affecting the nervous and muscular systems of small animals, particularly sheep in the karoo area of South Africa ***Bryophillum pinnatum*** is rich in alkaloids, terpines, glycosides, flavonoids, steroids and lipids (**Marriage and Wilson, 1971**). The leaves contains a group of chemicals called bufadianelids which are very active (**Franke etal, 1987**) and very similar in structure and activity as two other cardiac glycosides digoxin and digotoxin which possesses antibacterial, antitumorus cancer preventive

and insecticidal actions **(Chang etal, 1989**). Chromatography techniques are extensively used in bio analysis for the separation, isolation and purification of drugs and their metabolites. Due to its medicinal importance this plant is overexploited and there is a decline in the population of ***Bryophillum pinnatum***.

The aim of this study is invitro micropropagation through various growth hormones.

MATERIAL AND METHODS

2.1. Collection of Explants

Actively growing, healthy and disease free young shoots with green leaves of ***Bryophillum pinattum*** collected.

2.2. Surface Sterilization

The younger and clean leaves of Bryophillum pinnatum have been washed with 2% (v/v) detergent Teepol (Qualigen, India) and rinsed numerous times with jogging tap water. The explants were surface sterilized in 0.1% (w/v) aqueous mercury chloride for 3-4 minutes accompanied by means of 4 washing with sterile distilled water.

2.3 Inoculation of Explants, Shoot bud Initiation & Multiplication

As it's an instantaneous micro propagation procedure all sterilized leaves explants had been belittled and dipped in the one of a kind concentration of PGRs, BAP (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l) with particular extent of Kin (0.5mg/l) at the MS supplemented medium. The cultures were maintained at 25±2ºC less than 16h photoperiod light at cool, white fluorescent lamps. After remark for 4-5 weeks the shoot buds were transferred without delay again to MS medium supplemented with various concentrations of BAP (0.5, 1.0, 1.5, 2.0, 2.5mg/l) and Kn (0.5mg/l) respectively in mixture for shoot regeneration and multiplication. The cultures had been maintained with the aid of normal subcultures at 1-2 week intervals on fresh medium with the same compositions.

2.4 Root Induction & Acclimatization

All shoots were transferred to rooting medium, which consisted of MS supplemented with exceptional concentrations of IBA (0.5-2.5 mg/l and NAA (0.5, 1.0mg/l) and mixture of IBA (0.5 - 2.5mg/l), NAA (0.5-2.5 mg/l) and BAP (0.5-2.5 mg/l). All the cultures had been incubated in culture room at 25 ± 2°C, light depth (3000lux) with a photoperiod of 16 hours with 60-70% relative humidity. The cultures were monitored and the statistics have been recorded at each week interval. After 15-20 days of way of life, the sufficient rooted plantlets had been dipped in bavistin solution for approx 2-3 mins and planted cautiously inside the poly luggage containing soil mixtures (organic soil blended with lawn soil 1:1). They were maintained at about 70% relative humidity in the greenhouse with 75% shading to supply newer leaves / roots. Plants are maintained underneath shade with controlled temperature and humidity to produce more modern leaves/roots. They are ready to be transferred in open nursery.

3. STATISTICAL ANALYSIS

Data were collected on shoot and root regeneration. The experiments were laid out in completely randomized design (CRD). Each treatment was replicated thrice and 10 test tubes were used per replication. The data collected was analyzed by SPSS software (Version 13.00) and the means were compared by one way ANOVA.

4. RESULT AND DISCUSSION

4.1 Clonal Propagation

4.1.1 Shoot Initiation

An effective protocol was developed for the *in vitro* clonal propagation of *Bryophyllum pinnatum*. Although it was a direct regeneration technique, leaf explants were cultured on MS medium supplemented with different concentration of BAP and Kn respectively. But the best result was observed in BAP 1.0mg/l+ Kn 0.5mg/l with 70% of shoot initiation within 15-20 days and average shoot length were 1.5±0.15 (Table -1). A cytokinin supplement to MS was essential to induce shoot proliferation. A combination of cytokinin and auxin improves the percentage of shoot regeneration as well as the shoot number and shoot length. *Bryophyllum pinnatum* produced 2.8 times more shoots in 10-6M BAP as comparedto water (**Duen and Riserberg**).

Table 1: Effect of PGRs on shoot induction of *Bryophyllum pinnatum.*

Sl No	Concentration of PGR	No of shoots per plant	Percentage of Shoot Initiation	Average shoot length
I	BAP0.5+Kn0.5	1-2	64%	1.2±0.20
II	BAP1.0+ Kn0.5	1	70%	1.5±0.15
III	BAP1.5+Kn0.5	2	68%	2.2±0.52
IV	BAP2.0+Kn0.5	1-2	60%	1.5±0.32
V	BAP2.5+Kn0.5	1	48%	2.0±0.22
VI	BAP3.0+Kn0.5	1	40%	1.0±0.10

4.1.2 Multiplication

Different configuration of BAP supplemented with Kn (0.5mg/l) added with MS medium. In which the highest rate of shoot proliferation was 85% followed by 10 numbers of shoots per explants on the media supplemented with BAP 2.5mg/l + Kn 0.5mg/l.(**Table-2**). On the other hand, least shoots were recorded in MS medium that contained BAP 0.5mg/l+ Kn 0.5mg/l and

number of shoot per explants were 6.

S. Baghel studied High frequency of shoot regeneration was achieved on MS medium with BAP (1 mg/l).

Table 2: Effect of PGR on shoot multiplication in *Bryophyllum pinnatum*.

Sl No	Concentration of PGR	No of shoots per plant	Percentage of Shoot Initiation	Average shoot length
I	BAP0.5+Kn0.5	6	50%	1.7±0.80
II	BAP1.0+ Kn0.5	9	72%	2.8±0.23
III	BAP1.5+Kn0.5	6	69%	2.2±0.18
IV	BAP2.0+Kn0.5	8	70%	1.5±0.35
V	BAP2.5+Kn0.5	10	85%	2.0±0.66
VI	BAP3.0+Kn0.5	8	80%	2.0±0.58

4.1.3 Rooting

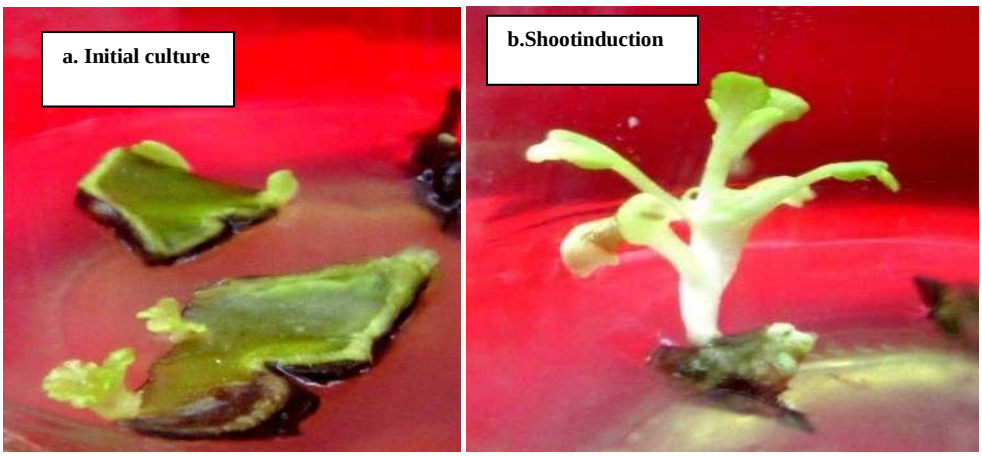
Elongated shoots were excised from parent culture and transfer onto full strength basal MS medium with growth regulators. Inclusion of BAP (0.5–2.5 mg/l), NAA and IBA (0.5 mg/l) with 2 % sucrose induced the rooting within 3 weeks of culture. The highest percentage of rooting was observed 32% with MS medium supplemented with BAP (1.5 mg/L) in combination with NAA (0.5mg/l) and IBA (0.5mg/l) with 5 no. of roots and with maximum 6.3 cm length in 22 days. Hakan study show the auxin group hormones (IAA, IBA, and NAA) do not have an apparent effect on rooting percentage, these hormones were detected to affect the morphological characteristics of the newly generated plants, especially root generation. Different concentrations of BAP, IBA and NAA had highly significant effect on the shoot and root regeneration.

Table 3: Effects of PGRs on Rooting.

Sl.No.	Concentration of PGRs (Mg/l)			Number of roots	Root length (cm)	Percent of rooting (Mean ±SE)*
	BAP	NAA	IBA			
I	0.5	0.5	0.5	3	2.02±0.46	10±0.66
II	1.0			4	3.12±0.93	20±0.33
III	1.5			5	4.86±0.13	32±1.52
IV	2.0			3	3.25±0.02	25±2.33
V	2.5			2	2.15±0.05	10±0.57

5.1.1. Acclimatization & Hardening

The acclimatization of rooted plants in ex vitro conditions was carried out with the plants bearing well-developed roots transferred to small pots containing soil mixtures (organic soil mixed with garden soil 1:1). They were maintained at about 70% relative humidity in the greenhouse with 75% shading. A survival rate 98% was achieved after 6 weeks. About 80 % of the plantlets established within 15 days of transfer. The plants grew well and attained 4–5 cm height within 1 months of transfer.



CONCLUSION

In conclusion it was clearly observed that rapid in vitro propagation of *Bryophillum pinatum* can be obtained by proper manipulation of leaf explants and concentration of plant growth regulators. Although it was a direct micropropagation technique so leaf explants were cultured on MS medium supplemented with different concentration of BAP and Kn respectively. But the best result was observed in BAP 1.0mg/l + Kn 0.5mg/l with 70% of shoot initiation within 15-20 days with average shoot length of 1.5±0.15 c.m. The highest rate of shoot proliferation was 85% with 10 numbers of shoots per explants on the media supplemented with BAP 2.5mg/l + Kn 0.5mg/l. Elongated shoots were excised from parent culture and transfer onto full strength basal MS medium with inclusion of BAP (1.5 mg/l), NAA (0.5 mg/l) and IBA (0.5 mg/l) with 2 % sucrose induced the rooting within 3 weeks of culture in which 32% of rooting observed with 5 numbers of roots and 6.3 cm of maximum root length. Micro propagated plantlets were hardened, acclimatized and transferred to the field. These results can be further used for clonal propagation.

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