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Dr. Samiksha Dwivedi
Guest Lecturer, Department of Botany, Govt. College Churahat Sidhi, Madhya Pradesh, India

Dr. Ashish Patel
Assistant Professor, Department of Botany, Govt. Model Science College Rewa, Madhya Pradesh, India

Corresponding Author:
Dr. Samiksha Dwivedi
Guest Lecturer, Department of Botany, Govt. College Churahat Sidhi, Madhya Pradesh, India

Effect of Plant Growth Regulators on *In vitro* Shoot Multiplication and Rooting in *Bacopa monnieri*

Samiksha Dwivedi and Ashish Patel

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Abstract
Bacopa monnieri (L.) Pennell, commonly known as Brahmi, is a medicinally important herb widely used in traditional medicine for cognitive enhancement. Due to overexploitation and poor natural regeneration, there is an urgent need for efficient propagation techniques. The present study evaluates the effect of plant growth regulators (PGRs) on *in vitro* shoot multiplication and rooting of *Bacopa monnieri*. Nodal explants were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of cytokinins (BAP, Kinetin) and auxins (IAA, IBA, NAA). Maximum shoot multiplication was observed on MS medium supplemented with BAP (1.5 mg/L) in combination with IAA (0.5 mg/L). Rooting was most effective on MS medium supplemented with IBA (1.0 mg/L). The study demonstrates that optimized PGR combinations significantly enhance micropropagation efficiency and can be used for large-scale production and conservation of *Bacopa monnieri*.

Keywords: *Bacopa monnieri*, Micropropagation, Plant Growth Regulators, BAP, IBA and Shoot multiplication

1. Introduction
Bacopa monnieri (L.) Pennell, belonging to the family Plantaginaceae, is an important medicinal plant known for its neuropharmacological properties, particularly in enhancing memory and treating neurological disorders. It is widely distributed in wetlands and marshy areas of tropical and subtropical regions.

The increasing demand for herbal medicines has led to excessive harvesting of natural populations, resulting in depletion of this valuable species. Conventional propagation through seeds is limited due to poor germination rates and slow growth. Therefore, plant tissue culture techniques offer a promising alternative for rapid and large-scale propagation.

Plant Growth Regulators (PGRs), including cytokinins and auxins, play a vital role in regulating plant growth and morphogenesis in tissue culture. Cytokinins are primarily responsible for shoot proliferation, whereas auxins promote root formation. The present study aims to investigate the effect of different PGRs on *in vitro* shoot multiplication and rooting in *Bacopa monnieri*.

2. Materials and Methods
2.1 Plant Material and Explant Preparation
Healthy and disease-free plants of *Bacopa monnieri* were collected from natural wetland habitats and used as the source material for *in vitro* studies. Nodal segments measuring approximately 1-2 cm in length were excised from actively growing shoots and selected as explants due to their high regenerative potential and ability to produce multiple shoots under controlled culture conditions.

2.2 Surface Sterilization
The explants were first washed thoroughly under running tap water for 15-20 minutes to remove surface contaminants such as dust and debris. This was followed by treatment with 1-2% Teepol (a liquid detergent) for 5 minutes to eliminate adhering impurities, after which the explants were rinsed with distilled water. Subsequently, surface sterilization was carried out under aseptic conditions using 70% ethanol for 30 seconds, followed by 0.1% mercuric

chloride ($HgCl_2$) treatment for 2-3 minutes. Finally, the explants were rinsed 3-4 times with sterile distilled water to remove any residual traces of sterilizing agents.

2.3 Culture Medium
Murashige and Skoog (MS) basal medium was used as the nutrient medium for culture establishment and growth. The medium was supplemented with 3% (w/v) sucrose as a carbon source and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.8 prior to autoclaving at 121°C and 15 psi pressure for 15-20 minutes to ensure sterility.

2.4 Shoot Multiplication
For shoot induction and multiplication, the sterilized nodal explants were inoculated onto MS medium supplemented with varying concentrations of cytokinins such as 6-benzylaminopurine (BAP) ranging from 0.5 to 2.0 mg/L and kinetin from 0.5 to 1.5 mg/L, either alone or in combination with auxins like indole-3-acetic acid (IAA) or naphthalene acetic acid (NAA). The cultures were incubated in a growth room maintained at 25±2°C under a photoperiod of 16 hours light and 8 hours dark. Observations on shoot initiation, number of shoots per explant, and shoot length were recorded at regular intervals.

2.5 Root Induction
Well-developed shoots obtained from multiplication cultures were carefully excised and transferred to fresh MS medium supplemented with different concentrations of auxins for root induction. Auxins such as indole-3-acetic acid (IAA), indole-3-butyric acid (IBA), and naphthalene acetic acid (NAA) were used in the range of 0.5-1.5 mg/L. The cultures were maintained under the same environmental conditions, and observations on root number, length, and percentage of rooting were recorded.

2.6 Acclimatization
The rooted plantlets were gently removed from the culture vessels, washed thoroughly to remove adhering agar, and transferred to small pots containing a sterilized mixture of soil, sand, and vermiculite in the ratio of 1:1:1. The plantlets were initially maintained under high humidity conditions, often by covering with transparent polyethylene bags, and gradually acclimatized to ambient environmental conditions. After successful hardening, the plants were transferred to field conditions for further growth and development.

3. Results
3.1 Effect of Cytokinins on Shoot Multiplication
BAP was found to be more effective than kinetin in inducing shoot proliferation.

Table 1: Effect of PGRs on Shoot Multiplication

Treatment	No. of Shoots/Explant	Shoot Length (cm)	Response (%)
BAP (0.5 mg/L)	8.2±0.5	3.1	80
BAP (1.0 mg/L)	15.6±0.7	4.2	95
BAP (1.5 mg/L)	22.4±1.2	5.3	100
BAP (2.0 mg/L)	18.2±1.0	4.8	90
Kinetin (1.0 mg/L)	10.3±0.6	3.5	85
BAP (1.5) + IAA (0.5)	25.8±1.4	5.6	100

Maximum shoot multiplication was observed in MS medium supplemented with BAP (1.5 mg/L) + IAA (0.5 mg/L).

3.2 Effect of Auxins on Rooting

Table 2: Effect of Auxins on Root Induction

Treatment	No. of Roots	Root Length (cm)	Response (%)
IAA (0.5 mg/L)	4.2±0.4	2.8	80
IAA (1.0 mg/L)	7.1±0.6	3.5	95
IBA (1.0 mg/L)	6.5±0.5	4.2	100
IBA (1.5 mg/L)	6.8±0.6	4.0	100
NAA (1.0 mg/L)	5.0±0.5	3.0	85

IBA was found to be most effective for root elongation, while IAA produced maximum root numbers.

3.3 Acclimatization
Approximately 80% of plantlets survived after transfer to soil under greenhouse conditions.

4. Discussion
The results clearly indicate that cytokinins, particularly BAP, play a crucial role in shoot multiplication. The synergistic effect of BAP and IAA significantly enhanced shoot proliferation, which is consistent with earlier findings. Auxins were essential for root induction, with IBA being more effective for root elongation and IAA for root initiation. The variation in response may be attributed to differential uptake and metabolism of plant growth regulators.

The success of acclimatization demonstrates the practical applicability of the protocol for large-scale propagation.

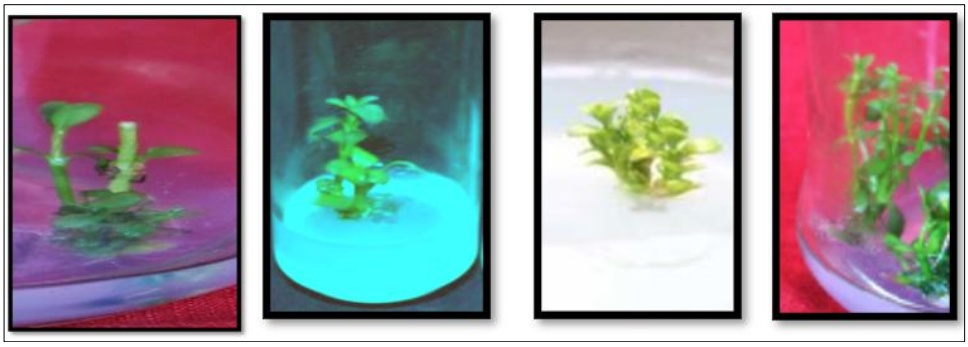

Fig 1: Shoot initiation from axillary meristem

Fig 2: Multiplication of shoots

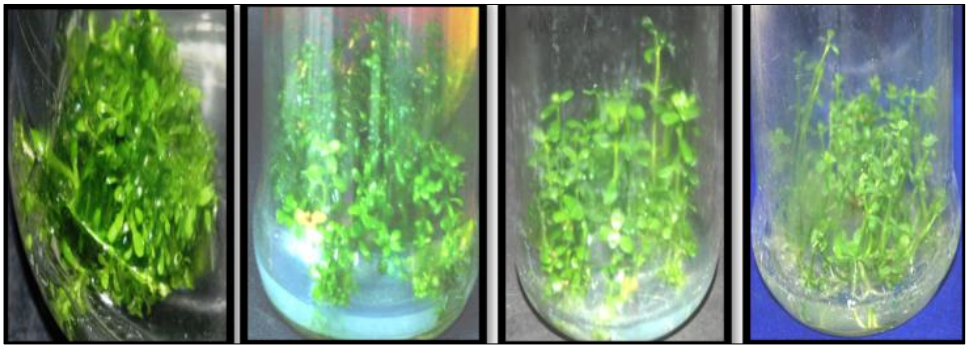

Fig 3: Rapid multiplication of shoots

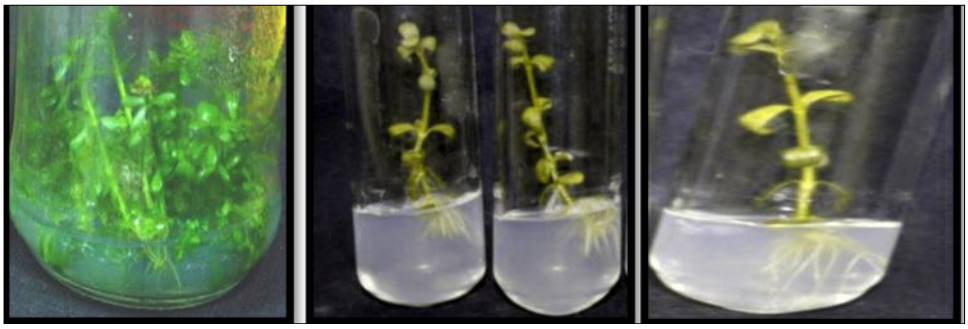

Fig 4: Root induction in MS 1/2 Strength + 1mg/l IB

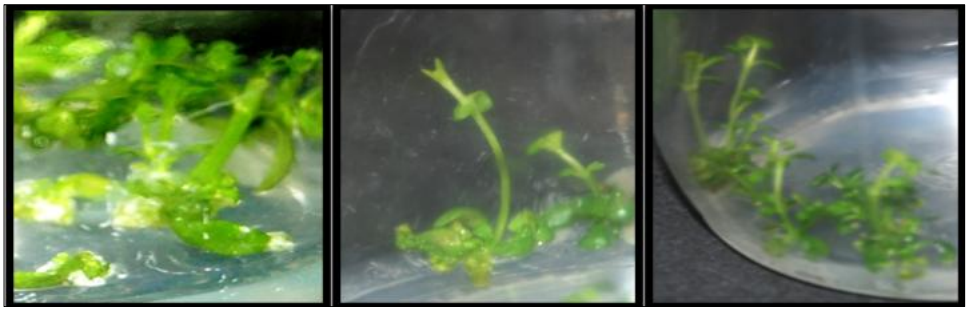

Fig 5: Shoot Production In salt stress

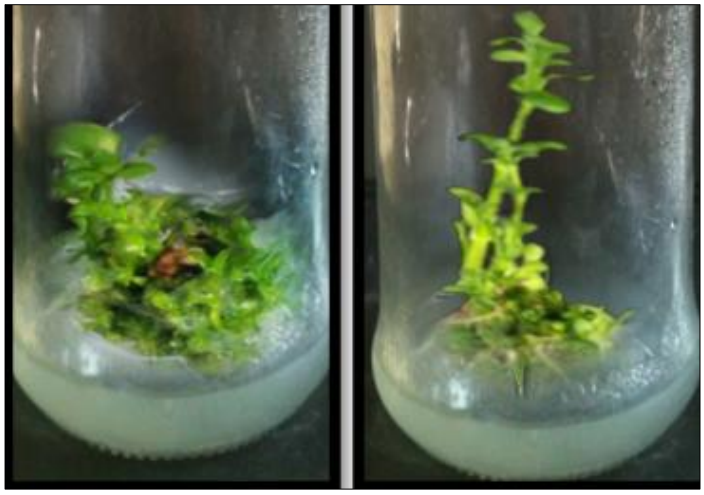

Fig 6: Shoot Production with callus In salt stress

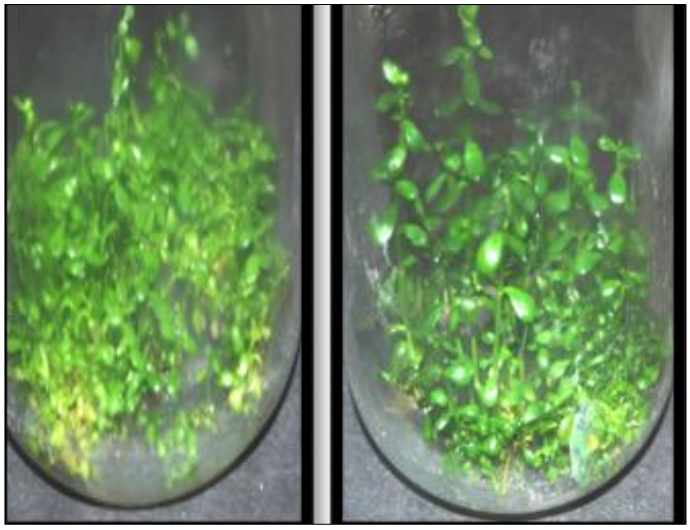

Fig 7: Shoot multiplication and elongation In salt stress

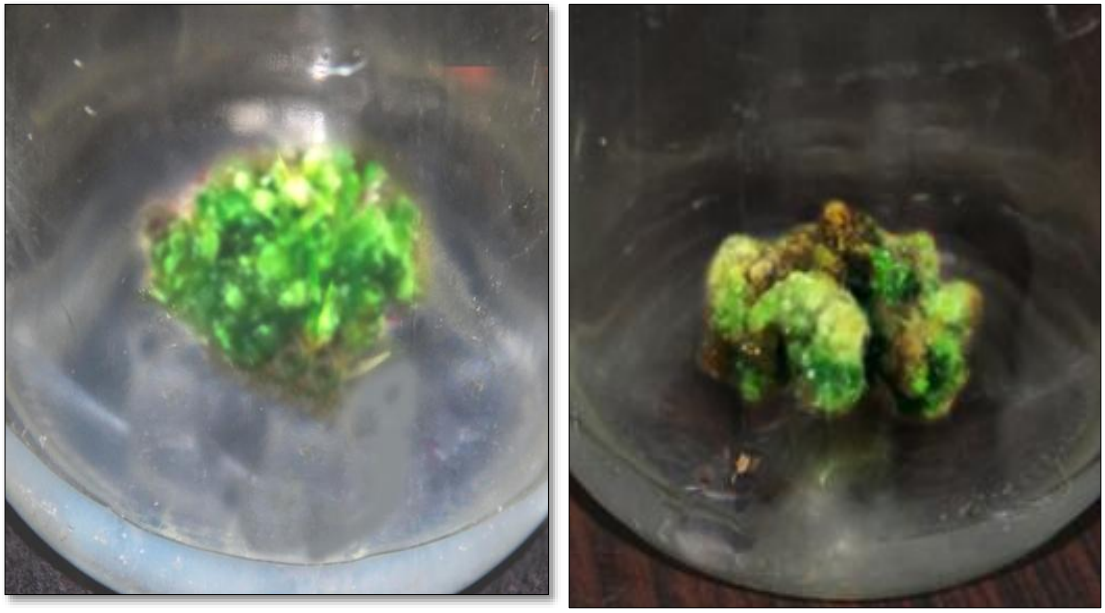

Fig 8: Callus formation of *Bacopa monnieri* under higher salt stress concentration.

5. Conclusion

The study concludes that:

- BAP (1.5 mg/L) is optimal for shoot multiplication
- Combination of BAP + IAA enhances shoot proliferation
- IBA (1.0 mg/L) is best for root development

- The developed protocol is efficient for mass propagation

This protocol can be effectively used for conservation and commercial cultivation of *Bacopa monnieri*.

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