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Chapter 36

# Investigation of organogenesis in *Bacopa monnieri:* Mechanisms, tissue culture techniques, and developmental insights

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## 1. Introduction:

Medicinal plants are essential to healthcare systems worldwide, serving as key sources for both traditional remedies and modern pharmaceuticals. As the demand for herbal medicines and antibiotics continues to rise, there is growing emphasis on the conservation and cultivation of these valuable plant species (George et al., 2008). Micropropagation, especially through shoot cultures, shows great potential for preserving valuable genotypes (Smith, 2012). Plant tissue culture, a collection of methods for growing or maintaining plant cells, tissues, or organs in sterile conditions on nutrient-rich media, supports this effort. Plant regeneration can occur through organogenesis and somatic embryogenesis, which involve stages such as initiation, proliferation, elongation, rooting, and acclimatization (Jain and Yadav, 2011).

*Bacopa monnieri*, commonly known as Brahmi, flourishes in tropical regions, particularly along riverbanks and lakeshores (Sharma et al., 2006). Esteemed in Ayurvedic medicine, it is renowned for enhancing memory, improving concentration, and treating mental illnesses. Brahmi exhibits a wide range of therapeutic properties, including anti-inflammatory, analgesic, antipyretic, anticancer, anticonvulsant, and antioxidant effects. Studies have demonstrated its potential in managing conditions such as anxiety, epilepsy, bronchitis, asthma, irritable bowel syndrome, and gastric ulcers. Moreover, *Bacopa monnieri* functions as a phytoremediator, capable of absorbing and accumulating harmful heavy metals like cadmium, chromium, and mercury from aquatic ecosystems (Tiwari et al., 2000).

## 2. Materials Required:

1. Murashige and Skoog (MS) medium
2. Sucrose (as a carbon source)

3. Agar (for solidifying the medium)
4. 6-Benzylaminopurine (BAP) – a cytokinin for shoot proliferation
5. Hydrochloric acid (HCl) – for pH adjustment
6. Sodium hydroxide (NaOH) – for pH adjustment
7. Teepol – a mild detergent for initial explant cleaning
8. Mercuric chloride ($HgCl_2$) – for surface sterilization
9. Ethanol (ethyl alcohol) – for sterilization
10. Double distilled water – for media preparation and cleaning
11. Shoot tip or nodal explants of *Bacopa monnieri*
12. Magnetic stirrer – for mixing media components
13. pH meter – for accurate pH measurement
14. Autoclave – for sterilizing media and equipment
15. Laminar airflow cabinet – for maintaining aseptic conditions
16. Plant growth chamber – for controlled growth environment
17. Schott Duran bottles – for culture storage
18. Beakers and conical flasks – for media preparation
19. Petri dishes – for culturing and explant placement
20. Forceps – for handling explants
21. Scalpels – for precise cutting of explants
22. Spirit lamps – for flame sterilization
23. Boiling tubes – for holding small volumes of solution
24. Scissors – for trimming and cutting plant material

### 3. Preparation of Reagents:

**Cytokinins (BAP) Stock Solution**: Dissolve 20 mg of BAP in a few drops of HCl. Dilute the solution to 100 mL with double distilled water and store it in the refrigerator.

### 4. Procedure:

**4.1. Media Preparation:** Preparation of MS Medium-

1. Dissolve 4.4 g of Murashige and Skoog (MS) basal medium in 800 mL of double-distilled water.
2. Supplement the medium with 1 mg/L of 6-benzylaminopurine (BAP) from a pre-prepared stock solution.
3. Add 3% (w/v) sucrose as a carbon source.
4. Adjust the pH to 5.8 using 1N HCl or 1N NaOH.
5. Solidify the medium by adding 0.8% (w/v) agar.

### 4. Sterilization and Dispensing:

1. Autoclave the prepared medium at 121°C for 15–20 minutes.

2. Dispense the sterilized medium into sterile conical flasks or culture vessels inside a laminar airflow cabinet.

## 5. Explant Preparation and Culture:

### 1. Collection and Sterilization of Explants

- Harvest shoot tip and nodal segments from healthy *Bacopa monnieri* plants.
- Rinse the explants thoroughly under running tap water followed by treatment with a mild detergent solution (Teepol).
- Surface sterilize the explants with 0.1% (w/v) mercuric chloride for 2–3 minutes, then rinse multiple times with sterile distilled water.

### 2. Culture Initiation

- Inoculate the surface-sterilized explants onto MS medium supplemented with 1 mg/L BAP.
- Incubate the cultures at 26 ± 1°C under a 16-hour photoperiod and 8-hour dark cycle.

### 3. Subculturing

- Transfer elongated shoots or swollen nodal explants onto fresh MS medium with the same hormonal composition for further shoot multiplication.
- Repeat subculturing every 2–3 weeks to maintain vigor and encourage proliferation.

### 4. Acclimatization

- After 4–5 weeks of culture, gently remove the rooted plantlets from the medium.
- Wash off any remaining agar and transplant the plantlets into pots containing a sterile soil mix.
- Gradually expose them to natural environmental conditions to ensure successful hardening.

## 6. Observations:

Within a week of culturing, nodal explants developed noticeable swelling accompanied by multiple shoot buds. Continued subculturing led to the elongation of these buds, yielding an average of seven shoots per explant, each approximately 4 cm in length after three weeks. All regenerated plantlets were successfully acclimatized.

## 7. Results:

A reliable and efficient micropropagation protocol has been developed for *Bacopa monnieri*, utilizing BAP (6-benzylaminopurine) to induce shoot formation. The superior stability of ribosides and nucleotides in BAP, compared to other cytokinins, is likely responsible for the enhanced shoot proliferation observed in *B. monnieri* and potentially in other plant species. Moreover, integrating the rooting and acclimatization phases into

a single step notably decreases the overall time and cost involved in in vitro regeneration. This simplified method improves the propagation process of *Bacopa monnieri*, reinforcing its value as a medicinal plant and its suitability for large-scale production.

## 8. Precautions:

1. To prevent contamination, all of the glassware was cleaned with ethyl alcohol and treated with liquid detergent.
2. The explants were cleaned and clipped with great care, and they should never be exposed to ultraviolet light.
3. Accurate documentation of the measurements and data is required.

## 9. References:


1. George, E.F., Hall, M.A., & De Klerk, G.J. (2008). *Plant Propagation by Tissue Culture* (3rd ed.). Springer.
2. Smith, R.H. (2012). *Plant Tissue Culture: Techniques and Experiments* (3rd ed.). Academic Press.
3. Sharma, V., & Mohan, J.S.S. (2006). In vitro clonal propagation of *Bacopa monnieri* (L.) Pennell: A medicinal plant with high antioxidant activity. *Physiology and Molecular Biology of Plants, 12*(3), 261-265.
4. Jain, R., & Yadav, P. (2011). Micropropagation of *Bacopa monnieri* using nodal explants: An efficient in vitro regeneration system. *Biotechnology Reports, 5*(4), 123-130.
5. Tiwari, S., Kumar, R., & Singh, R. (2000). Organogenesis in *Bacopa monnieri*: A comparative study of different cytokinins. *Journal of Plant Biotechnology, 2*(2), 110-115.