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# STANDARDIZATION OF TISSUE CULTURE MEDIA AND CONDITIONS FOR PROPAGATION OF CALYCOPTERIS FLORIBUNDA LAM

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## ABSTRACT

In the present research it was aimed to the development and standardization of an effective media and culture conditions for regeneration technique for Calycopteris floribunda. Nodal explants from juvenile plants were cultured on Murashige and Skoog's (MS) media with an array of different combinations of Cytokines and Auxins. Among the various protocols tested for standardization of media, MS media was found to be the best, for nodal segments. Maximum growth was obtained from the MS mediacontaining IAA and kinetin. The optimum temp for tissue culture was found to be $25^0$Cwhich was kept in BOD incubator. Findings of this study have to be used for development of an effective protocol for the establishment of callus from nodal explants. Sprout formation was observed from nodal explants and were used for further studies.

## INTRODUCTION

Plant tissue culture is a collection of *invitro* technique used to maintain or grow plant cells, tissues or organs under sterile condition on a nutrient medium of known composition. Tissue culturing is the most common form of plant reproduction and cloning.[1]

Different techniques in plant tissue culture may offer certain advantages over traditional methods of propagation, including the production of exact copies of plants that produce particularly good flowers, fruits, or have other desirable traits. It can be used to quickly produce mature plants. The production of multiples of plants in the

Absence of seeds or necessary pollinators to produce seeds can be done by tissue culture.It can be used for the regeneration of whole plants from plant cells that have been genetically modified.[2]

Invitro studies have been carried out on calycopteris floribunda for its antibacterial, antimicrobial, anthelmintic, hypolipidemic, hypoglycemic, anticancer, antioxidant activities. The plant is commonly known as 'PULLANI' in Malayalam,' KOKKARAI' in Hindi, and 'MINNARAKOTI' in Tamil. It is found extensively in the low tropical evergreen forests of Western Ghats and also found in 'kavus' or sacred grooves of kerala.[3]

Invitro propagation has been developed for plant species in the combretaceae family include Terminalia Bellerica, Terminalia Arjuna, Terminalia Catappa, Quisqualisindica, Laguncularia Racemosa.

A plant growth regulator plays an important role in growth and differentiation of cultured cells and tissues. They include auxins, cytokines, indole acetic acid, indole-3- butyric acid, zeatin etc. Auxins and cytokines plays major role in morphogenesis of culture system. When the ratio of auxins to cytokines is high, embryogenesis, callus initiation and root initiation occurs and ratio to be kept low for shoots proliferation. When living cells are isolated from living plant and cultured in a medium containing cytokines and auxin, cell division proceed, forming a mass of undifferentiated cells called callus. The callus is able to differentiate and produce an entire organism.[4]

The composition of the medium, particularly the plant hormones and the nitrogen source (nitrate versus ammonium salts or amino acids) have profound effects on the morphology of the tissues that grow from the initial explant. An excess of auxin will often result in a proliferation of roots, while an excess of cytokine may yield shoots. A balance of both auxin and cytokinin will often produce an unorganized growth of cells, or callus, but the morphology of the outgrowth will depend on the plant species as wellas the medium composition. As cultures grow, pieces are typically sliced off and sub cultured onto new media to allow for growth or to alter the morphology of the culture. The skill and experience of the tissue culturist are important in judging which pieces toculture and which to discard.[5]

As shoots emerge from a culture, they may be sliced off and rooted with auxin to produce plantlets which, when mature, can be transferred to potting soil for further

growth in the greenhouse as normal plants.

The present researches was carried out to screen the free radical scavenging and anticancer activities of various extracts of whole plant of calycopteris floribunda, and was found to be effective.

Plant tissue culture technology could be a potential alternative approach for bioproduction of phytoconstituents of therapeutic value and might be attractive under certain conditions.

**AIM AND OBJECTIVE**

To standardize and assess the preparation of tissue culture media and to optimize the conditions for propagation of ***calycopteris floribunda lam*** and perform the following studies.

- Collection of pant material
- Authentication of plant material
- Preparation of plant explant
- Preparation of different medias
- Inoculation of sterilized explant into culture media
- Incubation of plant explant

**MATERIALS AND MEDIA USED**

Murashige and skoog medium. Major salts (macronutrients)/ 1l ammonium nitrate ($nh_4no_3$) 1,650 mg/l calcium chloride ($cacl_2 \cdot 2h_2o$) 440 mg/l, Coconut water media, Mixture of coconut water and murashige skoog media.

**METHODOLOGY**

**Plant collection**

Calycopteris Floribunda was collected in the month of January 2021 from the areas of Chalavara, Kayiliyad, Palakkad (Dist), Kerala.

**Sterilization**

**Fumigation of laboratory**

- Used safety goggles and hand gloves during fumigation
- Put off the A/c unit
- Taken potassium permanganate about 15g in a petridish and kept in the areawhere fumigation is to be carried out
- Placed the petridish on a polythene bag
- Added about 25 ml of 35 formaldehyde solution to potassium permanganate
- Immediately closed the area
- Labelled the area as “Area under fumigation” so that nobody enters the room
- Kept the room under fumigation for 8-12 hours
- Defumigated the room by putting on the A/c unit[23]

**Sterilization of equipments**

- Firstly the glass wares were thoroughly washed with water, followed by washing with suitable detergents
- Glass wares should be free from the microbiological organism after thefollowing rinsing
- Then the glass wares were rinsed with proper solvent
- Then finished up with a couple of rinses with distilled water
- All the glass wares used for tissue culture was sterilized by autoclaving.[24]
- All glass wares are covered with cotton plugs made up to non-absorbent cotton, which in turn is covered with aluminum foil during autoclaving
- All the instruments that are needed during tissue culture like forceps and scalps are also sterilized by autoclaving
- All the sterilized instruments and glass wares were opened for use whenever required only inside the laminar air flow to prevent contamination.[25]

**Preparation of culture media**

**Coconut water as medium**

Coconut water was collected from tender coconut and filtered. Accurately measured 30ml of coconut water and added 1gm agar to it, then heated. Transferred 10ml of mediato each test tube and autoclaved.

**MS media, Mixed media**

Take 15ml of coconut water and 15 ml of MS media in a conical flask. Add 1gm of agarto it and heated. Transfer it to each test tube and autoclaved$_2$ sterilization of culture media.

The Prepared media was autoclaved at 121$^o$c at 105 kpa for 20mins. The duration of autoclaving is increased for larger volumes of media.

**Preparation of explant**

- The plant material was collected and washed thoroughly.
- The plant material may also carry certain bacterial / fungus infections which are not visible to naked eye, but may be prominent after few days of tissue culture. To avoid this problems, the plant material was properly disinfected.
- Firstly the explant material washed with distilled water.
- Then the plant material were sterilized by using 0.1% solution of mercuric chloride.[9]

**INOCCULATION OF STERILIZED EXPLANT INTO CULTURE MEDIA**

- The Important aspect of maintaining an aseptic environment for tissue culture is the use of sterile transfer area for all the operation of tissue culture
- All the cultured vessels containing autoclaved media

are opened inside the laminar flow hood and not outside it.[8]

- Then each explant such as shoot, leaf, flower were transferred into suitable culture media
- 12 test tubes were filled with 10ml of MS media. Out of these 4 tubes were inoculated with leaf explants, 4 were inoculated with shoot explant, and remaining with flower buds. These test tubes were kept in Bod incubator at 250c temperature for 4 weeks.
- The procedure was repeated and the test tubes were kept in room temp.
- 12 test tubes were filled with 10ml of coconut water as media. Out of these 4 tubeswere inoculated with leaf explants, 4 were inoculated with shoot explant, and remaining with flower buds. These test tubes were kept in Bod incubator at 250c temperature for 4 weeks.
- The procedure was repeated and the test tubes were kept in room temp.
- 12 test tubes were filled with 10ml of mixed media. Out of these 4 tubes were inoculated with leaf explants, 4 were inoculated with shoot explant, and remaining with flower buds. These test tubes were kept in Bod incubator at $25^0$C temperature for 4 weeks.
- The procedure was repeated and the test tubes were kept in room temp.

## RESULT AND DISCUSSION

The inoculated test tubes were kept for 1 month.

### Leaf culture

Sprout formation was found in the leaf culture which was kept in the BOD incubator, where the temperature was maintained as $25^0$C. Those test tubes which was kept in theroom temperature was found to show brownish color change indicating dryness and white patches were found which indicates infection by fungus. N- Not showing sprout,S- sprout

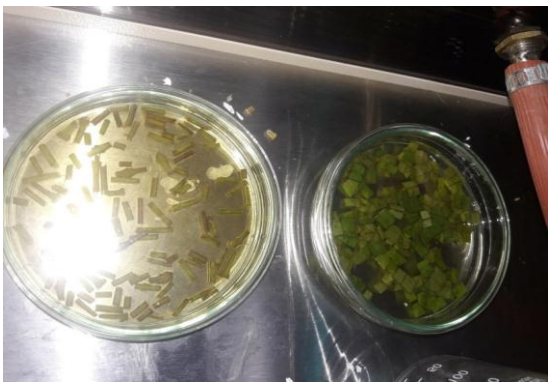

**Fig 2: leaf culture.**

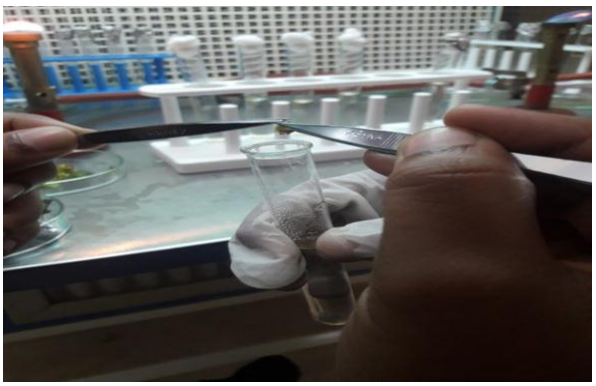

**Fig 3: spout formation.**

**Table 1: spout formation In leaf culture.**

| Sample | Temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Room temperature | | | | $25^0$C | | | |
| $L_1$ | N | N | N | N | N | N | N | S |
| $L_2$ | N | N | N | N | N | N | S | S |
| $L_3$ | N | N | N | N | N | N | N | S |
| $L_4$ | N | N | N | N | N | S | N | S |

### Shoot culture

Those culture which were kept in the BOD incubator was found to be not damaged but those kept at the room temperature shows brownish color change indicating dryness.

**Table 2: spout formation in shoot culture.**

| Sample | Temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Room temperature | | | | $25^0$C | | | |
| $S_1$ | N | N | N | N | N | N | N | N |
| $S_2$ | N | N | N | N | N | N | N | N |
| $S_3$ | N | N | N | N | N | N | N | N |
| $S_4$ | N | N | N | N | N | N | N | N |

**Root culture**

Those cultures which were kept in the bod incubator were found to be not damaged butthose kept at the room temperature shows brownish color change indicating dryness.

**Table 3: spout formation in root culture.**

| Sample | Temperature | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Room temperature | | | | $25^0$C | | | |
| $R_1$ | N | N | N | N | N | N | N | N |
| $R_2$ | N | N | N | N | N | N | N | N |
| $R_3$ | N | N | N | N | N | N | N | N |
| $R_4$ | N | N | N | N | N | N | N | N |

The surface sterilization was optimized that prevented the blackening of tissues.

The use of MS media proved a little bit successful procedure as maximum explants were undamaged and responded to tissue culture medium. Sprout formation was seen in leaf culture of MS media. Sprouts were not seen in shoot and root cultures.

Coconut water was used for tissue culture in some cases but it is not effective in case ofCalyptopteris floribunda. Even if it contains enough vitamins and nutrients it doesn't cause regeneration from leaf, shoot, and root plantlets. Many remained dried because plants produce a huge variety of secondary compounds as natural protection against microbial and insect attack and may have inhibited the initiation of cells.

Mixed media was also not found to be effective in any cases.

Temperature has an important role in the induction of callus. Those test tubes kept inthe atmospheric conditions didn't show any growth. $25^0$c was found to be the optimum temperature for callus initiation in Calyptopteris floribunda.

For the specified plant tissue culture with MS media under $25^0$c temperature was foundto be optimum.

## CONCLUSION

in vitro regeneration of plants has gained significance during recent years as thistechnique can be used for the rapid multiplication and ex situ conservation of some plants having threatened aspects. Selected media with different combinations of Auxins and Cytokines were analyzed to determine the best media composition for shoot induction in Calyptopteris floribunda. In the present study, in vitro regeneration was reported wherein nodes of in mother plant were utilized as initial explants for multiple shoot formation, sterilization of explants, followed by rooting of individual micro shoots. The findings of this study have resulted in development of an effective protocol for preparation of media and establishment of culture conditions for regeneration from nodalexplants. This method of micro propagation can be used for the development large scale plantlets, establishment of nurseries of Calyptopteris floribunda and also be used for themedicinal purposes.

## BIBILIOGRAPHY


1. Yen G, Duh P, Su H. Antioxidant properties of lotus seed and its effect on DNA damage in human lymphocytes. Food Chemistry, 2005; 89: 379-385.
2. Elmastas M, Gulcin I, Isildak O, Kufrevioglu OI, Ibaoglu K and Aboul-Enein HY.Radical scavenging activity and antioxidant capacity of Bay leaf extracts. Journal of Iranian Chemical Society, 2016; 3(3): 258-266.
3. Dixit P, Ghaskadbi S, Mohan H and Devasagayam TPA. Antioxidant properties ofgerminated fenugreek seeds.Phytotherapy Research, 2015; 19: 977-983.
4. Chatterjee S, Poduval TB, Tilak JC and Devasagayam TPA. A modified, economic,sensitive method for measuring total antioxidant capacities of human plasma and natural compounds using Indian saffron (Crocus sativus).Clinica Chimica Acta, 2015; 352: 155-163.
5. Devasagayam TPA, Boloor KK, Mishra KP. Some new methods for free radical research. SFRR-India Bulletin, 2013; 2(2): 20-28.
6. Tilak JC, Adhikari S, Devasagayam TPA. Antioxidant properties of Plumbagozeylanica, and Indian medicinal plant and its active ingredient, plumbagin.Redox Report, 2004; 9(4): 220-227.
7. Dasgupta N, De B. Antioxidant activity of Piper betle L. leaf extract in vitro. Food Chemistry, 2004; 88: 219-224.
8. Chung Y, Chien C, Teng K, Chou S. Antioxidative and mutagenic properties of Zanthoxylumailanthoides Sieb & zucc. Food Chemistry, 2006; 97: 418-425.
9. Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarshini KI. In vitro studies on antiradical and antioxidant activities of fenugreek (Trigonellafoenumgraecum) seeds. Food Chemistry, 2007; 103: 31-37.
10. Kaisoon O, Siriamornpun S, Weerapreeyakul N, Meeso N. Phenolic compounds and antioxidant activities of edible flowers from Thailand. Journal of Functional Foods, 2011; 3: 88-99.
11. Cowan MM. Plant products as antimicrobial agents. Clinical Microbiology Reviews, 2019; 12(4): 564-582.
12. Demain AL, Sanchez S. Microbial drug discovery: 80 years of progress. Journal of Antibiotics, 2019;

62: 5-16.
13. Davies J, Davies D. Origins and evolutions of antibiotic resistance. Microbiology and Molecular Biology Reviews, 2019; 74(3): 417-433.
14. Rop O, Mlcek J, Jurikova T, Neugebauerova J, Vabkova J. Edible flowers- A newpromising source of mineral elements in human nutrition. Molecules, 2021; 17: 6672- 6683.
15. Mohan P, Chakraborthy M, Bambawale OM, Raj S. Evaluation of antimicrobial properties of some indigenous plant species against cotton pathogens. Journal of Cotton Research and Development, 2014; 8(2): 142-148.
16. Bhukya B, Anreddy RNR, William CM, Gottumukkala KM. Analgesic and antiinflammatory activities of leaf extract of KydiacalycinaRoxb. Bangladesh Journal of Pharmacology, 2019; 4: 101-104.
17. Parameshwar H, Rao BB, Kumar BR, Reddy YN, Mohan GK. Hepatoprotective effect of methanolic extract of the leaves of Kydiacalycina on carbon tetrachloride induced hepatotoxicity in albino rats. African Journal of Pharmacy and Pharmacology , 2021; 5(16): 1920-1924.
18. Shrisha DL, Raveesha KA, Nagabhushan. Bioprospecting of selected medicinal plants for antibacterial activity against some pathogenic bacteria. Journal of MedicinalPlants Research, 2021; 5(17): 4087-4093.
19. Palanisamy P, Srinath KR, Kumar DY, Chowdary PC. Protective efficacy of Humboldtiabrunonis Wall on Doxorubicin induced oxidative damage. International Research Journal of Pharmacy, 2019; 3(2): 125-128.
20. Wall ME, Wani MC, Fullas F, Oswald JB, Brown DM, Santisuk T, Reutrakul V, McPhail AT, Farnsworth NR. Plant Antitumor Agents. 31.1 The Calycopterones, a New Class of Biflavonoids with Novel Cytotoxicity in a Diverse Panel of Human Tumor Cell Lines. Journal of Medicinal Chemistry, 1994; 37(10): 1465-1470.
21. Thalla S, Tammu J, Delhiraj N, Kumar KS. Hypoglycemic Activity of Hydro- alcoholic Extract of Calycopteris floribunda Induced by Streptozotocin in Rats. International Journal of Pharmaceutical Sciences and Drug Research, 2012; 4(4): 250-252.
22. Eswaraiah MC, Satyanarayana T. Hepatoprotective activity of extracts from stem of Calycopteris floribunda Lam. against carbon tetrachloride induced toxicity in rats. International Journal of Pharmacognosy and Phytochemical Research, 2010; 2(3): 53-57.
23. Thalla S, Pentela B. Hepatoprotective Effect of hydroalcoholic extract of Calycopteris floribunda leaves on Rifampicin-Isoniazid induced rats. International Journal of Chemical and Pharmaceutical Sciences, 2011; 2(3): 15-21.