International Journal of Research in Biosciences
Vol. 5 Issue 2, pp. (60-63), April 2016
Available online at http://www.ijrbs.in
ISSN 2319-2844

Research Paper

Initiation and establishment of callus for *in vitro* studies in *M. emerginata*

***Shweta Mathur, Asha Goswami**
Department of Botany, Plant tissue culture and Biotechnology Lab, Govt. Dungar College, Bikaner (Rajasthan), INDIA

(Received January 30, 2016, Accepted March 09, 2016)

Abstract

In present study unorganized tissues of *M. emerginata* were established on MS medium to determine the most suitable conditions for production of maximum biomass in cultures. Leaves shoot apices and flowers of *M. emerginata* used as explants showed poor response towards callus formation, but germinating seeds were used as explants for initiation and establishment of callus. Tissues were harvested at the age of 2, 4, 6, 8, 10 and 12 weeks and their growth indices were calculated on fresh weight basis. Best callusing was obtained on MS medium supplemented with 1.5 mg/L BAP + 1 mg/L NAA in *M. emerginata*, maximum GI was calculated at eight week callus (3.24).Main objective was to identify, isolate and estimate primary as well as medicinally and economically important secondary metabolites qualitatively and quantitatively to compare with *in vivo* studies.

Keywords: *M. emerginata*, GI, Unorganized callus, Germinating seeds, *in vitro*.

Introduction

Maytenus emerginata (Willd.) is an ever green tree that tolerates various types of stresses of the desert and is found in drier parts of central, south –western and north western India. *Maytenus* plant parts have been used for fever, asthma, rheumatism and gastrointestinal disorders, carcenoma and leukemia, gastrointestinal troubles etc. Medicinal plants are rich source of secondary metabolites, biosynthetically derived from primary metabolites but restricted to specific taxonomic genera of plant kingdom and specific part of plant body. Secondary plant products are of major interest because of their biological activities ranging from antibacterial, antibiotic, insecticidal, hormonal, pharmacological, pharmaceutical. Influence of growth regulators on unorganized cultures, were reported by Heble *et al*.[1] effect of hormones on growth regulations of *Solanum xanthocarpum* tissue cultures. Sairam and Khanna [2] effect of nicotinic acid on growth of *Trigonella foenum-graecum* culture. Khanna and Jain [3] and Khanna and Nag [4] observed the effect of tyrosine and phenylalanine on growth of *Datura tatula*, *Datura metel* and *Emblica officinalis* tissue cultures respectively. Nag *et al.* [5], Jain *et al.* [6] estimated ascorbic acid metabolism and growth in *Datura* tissue culture and ascorbic acid metabolism and growth in plant tissue cultures respectively. Estimation of free ascorbic acid from plant tissue cultures of *Solanum nigrum* was also reported. Main objective was to identify, isolate and estimate primary as well as medicinally and economically important secondary metabolites qualitatively and quantitatively to compare with *in vivo* studies.

Collection of plant material: Different fresh plant parts of *M. emerginata* like leaves, aerial roots, inflorescence, shoot apices, nodal segments, inflorescence and fruits were collected from local area. Seeds were taken out from fruits for use as explants.

Explant Preparation: Plant parts were washed thoroughly with 50% solution of liquid detergent (Teepol of Tween-80) in glass jars, after washing; these plant parts were kept under running water to remove remains of detergent completely. All the cleaned plant parts were cut into small pieces (1 cm) separately for the use as explants. Some of the seeds were first germinated in sterilized test tubes. The sterilized test tubes were

prepared with paper bridges of blotting paper strips, their ends were soaked in distilled water. Test tubes were plugged with cotton and autoclaved at 1.05 kg/cm^2 pressure for 15-20 minutes. Seeds were then sterilized with 0.1% $HgCl_2$ for 5 minutes and washed with sterilized distilled water thrice, and then they were inoculated on paper bridges. One to two seeds were inoculated on each paper bridge. Inoculated test tubes were then kept in culture chamber for germination of seeds.

Media Preparation: MS medium (Murashige and Skoog's 1962) [7] of Hi Media Company was used for establishment of callus. For preparation of medium readymade dry powder available in bottle (Hi Media PT-011 for one litre medium) was dissolved in nearly 500 ml of distilled water. The powder contain all essential minerals, sucrose, vitamins and solidifying agent i.e. agar-agar. Growth hormones and $CaCl_2$ were added according to requirements. Stock solution of growth hormones like auxins and cytokinins were prepared separately by dissolving their weighed amount in 1 N NaOH/C_2H_5OH and 1 N HCl respectively and making upto proportionate volume by adding distilled water (stored in refrigerator). Different concentrations and various combinations of growth hormones were supplemented in MS medium to select best combination for establishment of unorganized tissues. The volume of the medium was made upto 1 litre by adding distilled water. Medium was heated to dissolve Agar-Agar and stirred thoroughly. The pH of medium was adjusted at 5.8 by using 1N NaOH or HCl. The media was then poured into washed, rinsed and dried 100 ml flasks. Nearly 10 ml medium was poured in to each flask. Flasks were plugged tightly with non absorbent cotton. Plug was further wrapped by paper and tied with threads to protect from condensation of water during autoclaving. Combinations and concentrations of hormones were written on flasks with marking pencil. Flasks containing medium were sterilized at 1.05 kg/cm^2 pressure for 15-20 minutes. Autoclaved flasks were taken out, cooled at room temperature and kept in culture chamber, at least for five to ten days before inoculation.

Inoculation: Inoculation of explants was carried out in horizontal type of laminar air flow. Equipment was thoroughly cleaned with the help of cotton soaked in rectified spirit. Flasks containing culture medium were untied, papers were removed and kept in laminar air flow along with sterilized distilled water flask, 0.1% $HgCl_2$ solution, sterilized petridishes, and sterilized instruments like big forceps, spatula, and scalpel dipped in rectified spirit and one spirit lamp. In laminar air flow UV radiations were switched on for 40-45 minutes and complete sterilization was done, after sterilization inoculation was carried out in presence of air flow. Explants like pieces of shoot, leaves, aerial roots, nodal segments, inflorescence and intact mature seeds were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) solution for 3-5 minutes and then washed thrice with sterilized distilled water. Each explant was transferred to separate flask, containing culture media. Cotyledons, hypocotyle and radicles, formed by germinating seeds in sterilized test tubes were directly used as explants and transferred into separate flasks for initiation of unorganized tissue.

Incubation Conditions: Flasks containing explants and cultures were maintained under controlled conditions of sterilized culture chamber.

Callus Initiation, Its Growth Observation: Comparative initiation and growth of unorganized tissue was observed in all the flasks containing different explants of various combinations and concentrations of growth hormones up to thirty days. After these observations best suitable medium and explants were selected for further establishment and multiplication of tissue (callus). Multiplied callus was subcultured on fresh MS medium with selected concentration and combination of growth regulators after every 6-8 weeks. Sub culturing was done under sterilized conditions in laminar air flow chamber.

Growth Index: Tissues were harvested at the age of 2, 4, 6, 8, 10 and 12 weeks and their growth indices were calculated on fresh weight basis.

Results and Discussion

Leaves shoot apices and flowers of *M. emerginata* were used as explants and showed poor response towards callus formation. Best results were received by germinating seeds (cotyledons, plumule and radical), finally germinating seeds were used as explants for initiation and establishment of callus for further *in vitro* studies of *M. emerginata*. All explants were separately tried on MS medium supplemented with various combinations of growth hormones (BAP, IAA, NAA and 2, 4-D) with various concentrations ranging from 0.2 mg/L to 2.5 mg/L separately. Best callusing was obtained on MS medium supplemented

with 1.5 mg/L BAP + 1 mg/L NAA in *M. emerginata*. Callus was creamish and compact with fast multiplication up to eight weeks period. After that callus started becoming brownish black in tenth week which may be due to accumulation of phenolic compounds. Growth index was calculated at the age of 2, 4, 6, 8, 10 and 12 weeks, which showed increase from second (0.26) to eighth week (3.24) and after that declined in tenth (3.03) and twelvth week (2.11) in *M. emerginata.*

Table 1: Growth indices of static cultures of *M. emarginata*

Growth Indices at the age of					
2 Weeks	**4 Weeks**	**6 Weeks**	**8 Weeks**	**10 Weeks**	**12 Weeks**
0.26 ± 0.24	0.49 ±0.19	1.89 ±0.20	3.24 ±0.33	3.03 ±0.17	2.11 ±0.23

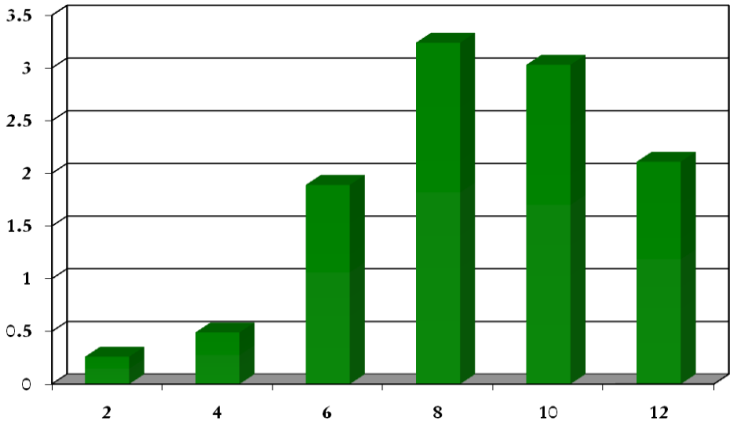

Figure 1: Growth indices of static cultures of *M. emarginata*

Figure 2: Unorganized callus in 4 week and 12 weeks

Conclusion

Callus of ***M. emerginata*** was harvested at their maximum growth indices i.e. eight weeks and analyzed for primary and secondary metabolites for comparison with *in vivo* studies.

Acknowledgement

I thank my guide Dr. Asha Goswami without whom this study would not have been possible. I also thank all those are directly or indirectly involved and who helped me to perform this study.

References

1. Heble M.R. Narayanaswamy S. and Chadha M.S., Hormonal control of steroid synthesis in *Solanum xanthocarpum* tissue cultures, Phytochemistry 10, 2393-2394 **(1971)**

2. Sairam T.V. and Khanna P., Effect of tyrosine and phenylalanine on growth and production of alkaloids in *Daturatatula* tissue cultures, Lloydia 34, 170-171 **(1971**)

3. Khanna P. and Jain S.C., Effect of nicotinic acid on growth and production of trigonelline in *Trigonellafoenum- graecum* tissue cultures, Ind. J. Exp. Biol., 10, 248-249 **(1972**)

4. Khanna P. and Nag T., Effects of phenylalanine and tyrosine on growth and alkaloid production in *Dalurametel* tissue cultures, Ind. J. Pharm., 34, 42-44 **(1972**)

5. Nag T.N., *Datura* tissue culture: A source of sterols, Ind. J. Pharm., 38, 80-81**(1976)**

6. Jain S.C., Khanna R. and Khanna P., Quercetin from the seeds and *in vitro* cultures of *Datura* spp., Ind. J. Exp. Biol., 13, 83-84 **(1975)**

7. Murashige T. and Skoog F.A., revised medium for rapid growth and bioassays for tobacco tissue cultures, Physiol. Plant, 15, 431-491 **(1962).**