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Research Paper

In vitro Culture of *Myrica esculenta* Buch.– Ham. ex D. Don Embryo from the Hilly Terrain of Meghalaya, North East India

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A B S T R A C T

A medicinal plant *Myrica esculenta* belonging to the family Myricaceae is utilized traditionally by the local medical practitioners in North East India for treatment of several ailments. The present investigation was undertaken to assess the suitability of different nutrient media and growth regulators for improving the propagation of *Myrica esculenta* zygotic embryos *in vitro*. The zygotic embryos were cultured in three different media namely, Murashige and Skoog (MS), B5 and Woody Pant Medium (WPM) in combination with three plant growth regulators to test their effect on callus formation and regeneration. Obtained results revealed that the cultured embryos of *M. esculenta* germinated only in MS medium and not in B5 and WPM media. MS media when combined with 20 µM BAP and 10 µM NAA showed maximum percentage of germination for the explant, followed by those when combined with 10 µM BAP and 20 µM NAA and the least response was represented by those cultured in 20 µM BAP with 30 µM NAA. The formed seedlings were acclimatized in sterile vermiculite: garden soil (1:1) and later shifted to field conditions. This technique can be utilized for multiplication and may help to promote conservation strategies of the species.

***Key words*:** *Myrica esculenta*, Zygotic embryos, Culture media, Growth regulators

Meghalaya lies in the Indo- Myanmar biodiversity hotspot harbors rich floral diversity. *Myrica esculenta* Buch.–Ham. ex D. Don belonging to the family Myricaceae, commonly known as "Kaphal", is also confined to this region [1]. This wild edible fruit species is a woody, evergreen, dioecious tree, medium to large in size. Like many other medicinal plants, different parts of *Myrica esculenta* is utilized traditionally by the local medical practitioners particularly for ailments such as dysentery, fever, diarrhoea, asthma, bronchitis, lung infections and skin diseases [2-3].

Myrica esculenta shows great variability among different populations owing to self-incompatibility of the species thus leaving outcrossing the only way to propagate this species. In addition, the impermeable nature of seed coats results in unpredictable germination. As vegetative propagation does not produce any progenies, micropropagation is the only technique to produce clones of the species [4]. An estimated quantity of 2,554 kg raw drug of *Myrica esculenta* is being consumed through folk healers in Meghalaya annually. However, lack of focus on sustainability and overexploitation of *Myrica esculenta* in the region poses a serious threat to its regeneration [5-7].

The clonal propagation technique of zygotic embryo culture is based on the totipotent nature of plant cells. This results in the development and regeneration of whole plants which eventually leads to its multiplication [8]. The relevance of this technique for micropropagation is in overcoming seed dormancy [9]. Available existing literatures revealed that no attempt has been made so far to develop a technique for the *in vitro* propagation of *Myrica esculenta* in the state of Meghalaya, North East India. Therefore, the present investigation was undertaken to assess the suitability of different nutrient media, and growth regulators for improving the propagation of *Myrica esculenta* zygotic embryos *in vitro*.

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MATERIALS AND METHODS

Myrica esculenta fruits were collected from Sohryngkham, Meghalaya, North East India (Altitude: 1609.6 m asl; Longitude: 91° 57' 27.3996" E; Latitude: 25° 32' 41.1000" N; recorded using Garmin etrex GPS). The fruits were stored in plastic containers and maintained at 4°C. Fruits were then soaked in 70% ethanol for half an hour followed by removal of the pulp. Further, the seeds were soaked in fresh 70% ethanol from which the zygotic embryos which served as explants were aseptically removed. Three tested media namely, Murashige and Skoog (MS), B5 and Woody Pant Medium (WPM) were utilized for assessment in the present investigation. Each medium contained 3% sucrose (W/V) and gelled with 0.8% agar (Himedia, India). Three plant growth regulators namely, 6-benzyl aminopurine (BAP), Naphthalene acetic acid (NAA), meta-Topolin were added to test their effect on callus formation and regeneration. All plant growth regulators were added to the respective media and the pH was adjusted to 5.8 prior to autoclaving at 1.06 kg cm^2 and 121°C for 15 min.

The temperature, light cycle and intensity of the growth room were 25 ± 2°C, 16 h light/8 h dark cycles and 2500 lux respectively. Cultures were observed at weekly intervals and the regeneration in the cultures is expressed as a per cent response. The data on zygotic embryo germination was evaluated after four weeks of culture. The plantlets with well - defined roots were transferred to thermocol pots containing vermiculite. At least 36 cultures were raised for each treatment and all experiments were repeated three times. Significance level was determined by calculating the mean and standard error.

RESULTS AND DISCUSSION

Obtained results revealed that zygotic embryo germination varied with the medium employed. It was observed that the cultured embryos of *M. esculenta* germinated only in MS medium and showed no significant morphological changes in WPM whereas showed callus formation in B5 media (Table 1, Fig 1). Further, at the end of the second week of culture in MS medium, the initially creamy white embryos appeared greenish in colour.

Table 1 Effect of four different media, Murashige and Skoog (MS), Woody Plant Medium (WPM) and B5 on germination of embryos of *Myrica esculenta*

Media	Response[a] (%)
MS	91.19 ± 0.75
WPM	No significant change
B5	Callus

[a]Values are mean ± Standard error

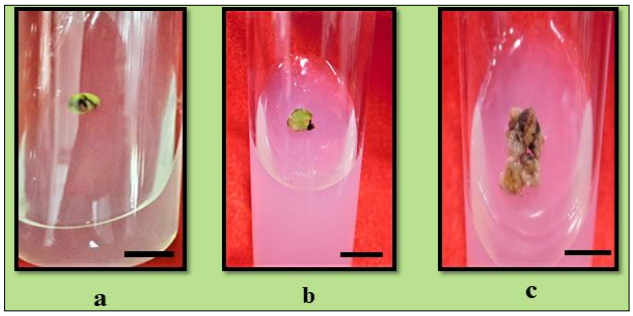

Fig 1 Response of *M. esculenta* embryos in (a). MS media, (b). WPM and (c). B5 media. The scale bar is equal to 1 cm

Zygotic embryos cultured on MS medium containing different concentrations of cytokinins (BAP, Metatopolin) and auxin (NAA), although showed slight response by a change in colour and texture of the explant yet had no significant effect in the further growth and development of the explant (Fig 2). Metatopolin when added individually at concentrations ranging from 10 to 40 µM showed callus formation of the explant with the highest response (100%) at 30 µM and 40 µM while lower concentrations of metatopolin (10 µM and 20 µM) showed a decrease in response. However, culture media supplemented with metatopolin did not lead to further development of the callus into organs.

The zygotic embryos of *M. esculenta* responded well in the medium containing BAP and NAA in combination and the mean shoot and root length (Mean cm ± SD; n=3) are shown in (Table 2). A high percentage of response (100%) of the explants was recorded for the explant cultured in MS media with 20 µM BAP and 10 µM NAA in combination. This was followed by those cultured in MS media with a combination of 10 µM BAP and 20 µM NAA at 67% response and a combination of 20 µM BAP with 30 µM NAA at 66% respectively. Seedlings formed from zygotic embryos were taken out from the culture vessels and washed with distilled water to remove traces of agar. These seedlings were then transferred to thermocol cups containing sterile vermiculate: garden soil (1:1) as shown in Figure 2(f) and 2(g). The plantlets were later shifted to earthenware pots and kept in the green house.

The present study is the first comprehensive attempt to ascertain the effects of different culture media and growth regulators on the zygotic embryo of *Myrica esculenta*. This technique provides an insight on the specific nutrient requirements of the developing zygotic embryo in culture. Zygotic embryo culture shortens the breeding cycle and helps in overcoming dormancy of seeds [10]. The culture media; a source of essential nutrients plays a crucial role in the *in vitro* growth and development of explants. The response shown by explants to different nutrient media varies considerably due to their interaction with the mineral as well as hormonal components of the media. In other words, the quantity and composition of various salts and ions differs among different nutrient media mainly [11]. The most commonly used culture media for in *vitro* propagation of plant species are MS and WPM medium. MS and WPM media is commonly used for herbaceous species and woody plants respectively [12].

Table 2 Effect of different plant growth regulators on zygotic embryos of *M. esculenta* cultured on MS medium

Growth regulator concentration (µM)		Percent of response	Time taken in days for emergence of embryonal axis	Mean shoot length (cm)[a]	Mean root length (cm)[a]
BAP	NAA				
10	10	30	9	4.16 ± 0.08	5.30 ± 0.33
	20	67	9	1.20 ± 0.20	3.66 ± 0.33
	30	33	15	5.50 ± 0.25	1.0 ± 0
20	10	100	9	4.46 ± 0.14	2.0 ±0
	20	33	14	3.10 ± 0.05	2.7 ± 0.67
	30	66	14	1.20 ± 0.12	NR
			mT		
10		25	Callus	-	-
20		40	Callus	-	-
30		100	Callus	-	-
40		100	Callus	-	-

[a]Mean ± Standard error

The present investigation revealed that MS media was found to be the most effective in the induction of development of *Myrica esculenta* zygotic embryo in comparison to WPM and B5 Media. Zygotic embryo culture shortens the breeding

cycle and helps in overcoming dormancy of seeds [13]. Similarly, [14] reported that MS media is a better culture media in comparison to WPM. It may be suggested that an increase in salt concentration is of critical importance for the development of young plants which is otherwise low in WPM. It is also known that the levels of inorganic nutrients such as ammonium nitrate is completely absent in B5 medium and this may have contributed to the low response rate of explants in this media. Other similar findings revealed that MS media was most effective in the *in vitro* micropropagation of a number of plant species [15-17]. The effectiveness of MS media in comparison with other plant tissue culture medium is probably due to the fact that, it possesses high ion concentration of nitrogen, potassium, zinc and chlorine [18].

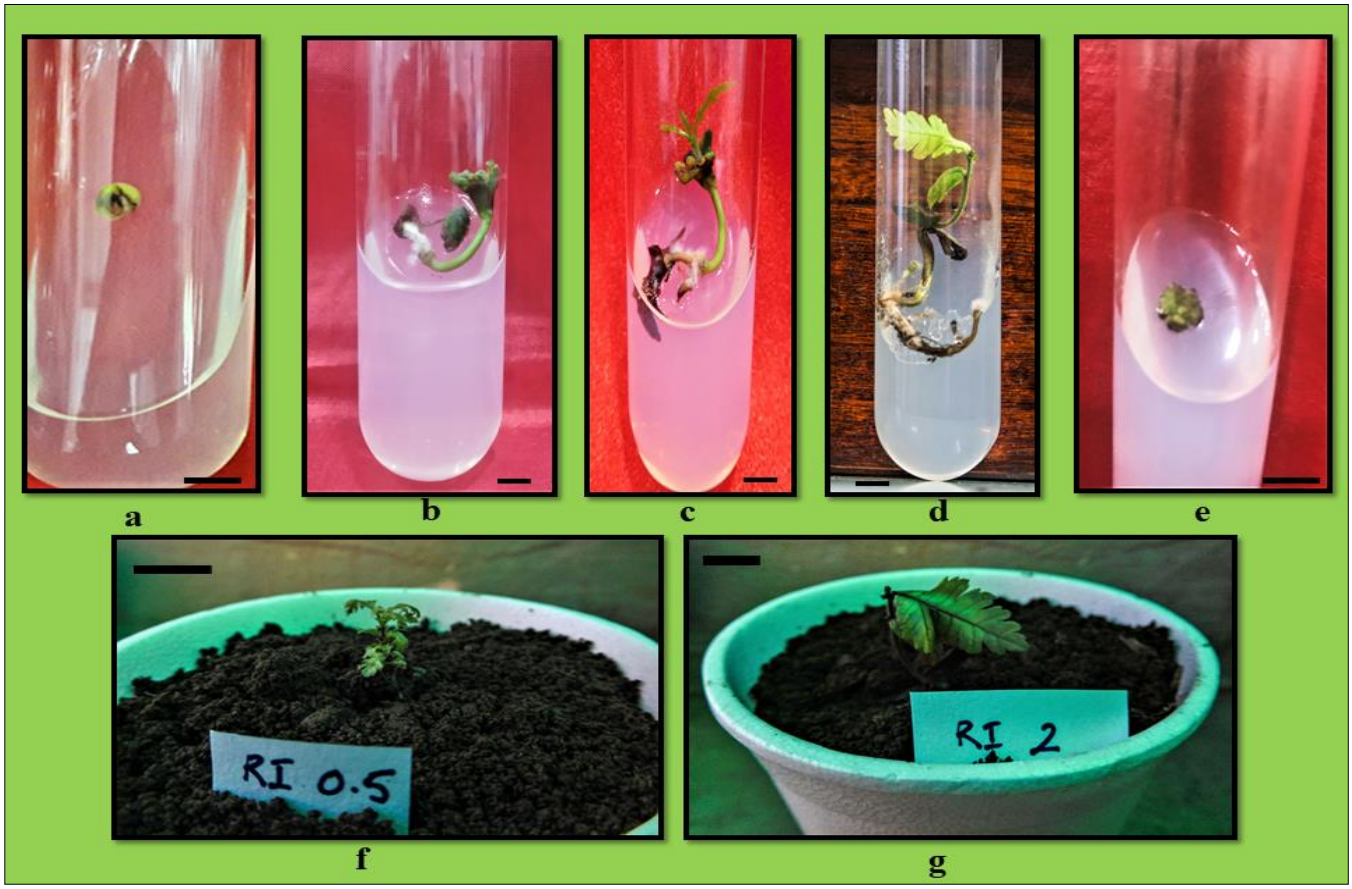

Fig 2 Zygotic embryo culture in *M. esculenta* (a). Zygotic embryo on full strength MS medium; (b). Emergence of shoot after two months of culture; (c) and (d) shoot elongation after three and four months of inoculation; (e). Callus formation on MS with meta-Topolin; (f) and (g) shows hardening of well rooted plantlets. The scale bar is equal to 1 cm

Meta-topolin (mT) an active aromatic cytokinin is considered as an alternate to other commonly used cytokinins in the micropropagation of plant species which are otherwise unresponsive to commonly used growth regulators [19]. *In vitro* shoot proliferation and improved quality of shoots in several plant species is triggered by mT [20]. Use of mT has been reported in micropropagation of herbaceous species such as *Aloe polyphylla* [21] *Spathiphyllum floribundum Shott cv. Petite* [22]. Limited available data on the use of mT for the *in vitro* propagation of woody species using zygotic embryos are available. However, [23] reported that 96.7% of cultured zygotic embryos of *Pinus pinaster* formed shoots using mT. On the contrary, [24] reported that although shoot organogenesis was obtained from zygotic embryos of *Franklinia alatamaha* when mT was added in combination with IBA, yet the shoot development was found to be abnormal. Besides these reports, Mt has been used to propagate other woody species using alternative explants. However, in the present investigation, callus formation was obtained in all the cultured embryos using mT at a concentration ranging from 10-40 µM. Therefore, callus formation varies considerably with the concentration of mT in media. Germination of zygotic embyos of *Myrica esculenta* is evident by root and shoot formation in MS media supplemented with BAP and NAA [25]. The effectiveness of the media containing NAA and BAP is evident as it maintains the general health, shoot growth and propagation of plants [26].

CONCLUSIONS

To summarize the present investigation, it may be concluded that MS medium supplemented with NAA and BAP was best for *in vitro* germination of zygotic embryo of *Myrica esculenta* in comparison to that of other media. Hence, the optimal conditions of the media and growth regulators suitable for propagation of the zygotic embryos of different plant species are of utmost importance. Therefore, this study may prove beneficial in highlighting the suitable germination media and growth regulators for *Myrica esculenta* embryos which may in turn enhance the large-scale multiplication and to formulate more realistic conservation strategies of the species.

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LITERATURE CITED

1. Nainwa P, Kalra K. 2009. Study on the wound activity potential on the aqueous extract of the bark of Myrica esculenta Buch. & Ham. *Int. Jr. Pharm. Clin. Research* 1: 85-87.
2. Kumar S. 2002. *The Medicinal Plants of North- East India*. Scientific Publishers, Jodhpur. pp 134.
3. Ahmed AA, Borthakur SK. 2005. Ethnobotanical Wisdom of the Khasis (Hynniew Treps) of Meghalaya. Bishen Singh, Mahendra Pal Singh, Editors, Dehradun. pp 114-47.
4. Hynniewta SR, Kumar Y. 2010. The lesser known medicine Ka Dawai Niangsohpet of the Khasis in Meghalaya, Northeast India. *Ind. Jr. of Trad. Knowledge* 9(3): 475-479.
5. Barik SK, Haridasan K, Lakadong NJ. 2007. Medicinal plant resources of Meghalaya: Endemism, Threat Status and Consumption Pattern. *ENVIS For. Bulletin* 7: 17-26.
6. Gusain YS, Khanduri VP. 2016. *Myrica esculenta* wild edible fruit of Indian Himalaya: need a sustainable approach for indigenous utilization. *Eco. Env. and Conservation* 22: 267-270.
7. Bhatt ID, Dhar U. 2004. Factors controlling micropropagation of *Myrica esculenta* buch. – Ham. ex D. Don: a high value wild edible of Kumaun Himalaya. *Afr. Jr. Biotechnology* 3(10): 534-540.
8. Talari S, Penchala S, Marka R, Rudroju, Swammy NR. 2013. Embryo culture an efficient tool for conservation of an endangered medicinally important forest tree *Oroxylum indicum* L. Kurx. *International Journal of Biotechnology and Research* 3(2): 45-52.
9. Kaveri S, Rao S. 2015. In vitro seed germination and Embryo Culture in *Nothapodytes foetida* (Wight) Sleumer. *Int. Letrs. of Nat. Science* 48: 23-31.
10. Karami S. 2016. Conservation efforts through embryo culture of a vulnerable plant Hemidesmus indicus L. *Int. Jr. of Plant, Animal and Env. Science* 6(1): 294-300.
11. George E, Hall M, De Klerk G. 2007. The components of plant tissue culture media I: Macro- and Micro-Nutrients. 10.1007/978-1-4020-5005-3_3.
12. Yeung ECT, Stasolla C, Sumner MJ, Huang BQ. 2015. *Plant Microtechniques and Protocols*. Nova York: Springer. pp 576. DOI: 10.1007/978-3-319-19944-3.
13. Comfort AC, Cyprian OU, Agaba OCE. 2018. In vitro plant regeneration from mature embryo explants of *Jatropha curcas* L. (A Biodiesel Plant) on two standard basal nutrient media. *American Journal of Plant Physiology* 13(1): 23-35.
14. Araruna EC, Ribeiro-Oliveira JP, Pereira VJ, Asmar SA, de Melo B. 2017. Salt concentrations in culture media for the development of *Dipteryx alata in vitro*. *Brasília* 52(12): 1295-1300.
15. Upadhyay S, Vijaya Koche V. 2015. Comparison of different medium and establishment of an efficient micropropagation technique of *Clerodendrum serratum* L. An endangered medicinal plant. *Journal of Environmental Science, Toxicology and Food Technology* 1(2): 27-35.
16. Pamulaparthi A, Banala M, Nanna RS. 2014. Optimization of culture media for in vitro Zygotic embryo culture of *Senna alata* L. Roxb –An Ethnomedicinal plant. *Journal of Pharmacy and Biological Sciences* 9(3): 01-06.
17. Pandey P, Mehta R, Upadhyay R. 2014. Effect of different media, photoperiod in seeds germination and effects of plant growth regulators on seedling growth of endangered medicinal plant *Psoralea corylifolia* Linn. *American Journal of Phytomedicine and Clinical Therapeutics* 2(1): 102-109.
18. Leifert C, Murphy KP, Lumsden PJ. 1995. Mineral and carbohydrate nutrition of plant cell and tissue cultures. *Critical Review in Plant Science* 14(2): 83-109. DOI: 10.1080/07352689509701923
19. Vijayakumar J, Ponmanickam P, Samuel P, Sudarmani DNP, Pandiarajan J. 2017. Influence of Meta-Topolin on efficient plant regeneration via micropropagation and organogenesis of safflower (*Carthamus tinctorius* L.) cv. NARI-H-15. *American Journal of Plant Science* 8: 688-705.
20. Aremu AO, Bairu MW, Doležal K. 2012. Topolins: A panacea to plant tissue culture challenges? *Plant Cell Tissue Organ Culture* 108: 1-16. https://doi.org/10.1007/s11240-011-0007-7.
21. Bairu MW, Stirk WA, Dolezal K, Staden JV. 2007. Optimizing the micropropagation protocol for the endangered Aloe polyphylla: can meta-topolin and its derivatives serve as replacement for benzyladenine and zeatin? *Plant Cell Tissue Organ Culture* 90: 15-23.
22. Werbrouck SPO, Strnad M, Onckelen HAV, Debergh PC. 1996. Meta-topolin, an alternative to benzyladenine in tissue culture? *Physiologia plantarum* 98(2): 291-297.
23. De-Diego N, Itziar M, Larrinoa, E, Moncaleán P. 2008. In vitro regeneration of Pinus pinaster adult trees. *Canadian Journal of Forest Research* 38: 2607-2615. 10.1139/X08-102.
24. Gladfelter HJ, Johnston J, Wilde HD, Merkle SA. 2021. Somatic embryogenesis and cryopreservation of Stewartia species. *Plant Cell Tissue Organ Culture* 144: 211-221. https://doi.org/10.1007/s11240-020-01834-1.
25. Pathi KM, Tula S, Huda KMK, Srivastava VK, Tuteja N. 2013. An efficient and rapid regeneration via multiple shoot induction from mature seed derived embryogenic and organogenic callus of Indian maize (*Zea mays* L.). *Plant Signaling and Behavior* 8(10): 1-6.
26. Paritala V, Mohammed A, Kayat F. 2017. In vitro plant regeneration of Andrographis Paniculata Nees using mature zygotic embryonic explants. *Agricultural Research and Technology* 5(5): 121-129.