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IN-VITRO PROPAGATION BY DEVELOPED SEEDLING THAN CALLUS INDUCTION IN M. PHILIPPENESIS (LAM.) M. ARG

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ABSTRACT

This research work is on a method for in-vitro propagation by developed seedling than callus induction in M. philippenesis (lam.) M. Arg. which is a medicinal plant of high commercial value. For this experiment different explants such as leaves, cotyledons, root in vitro grown part were used for callus induction. Leaves and cotyledons found to best the explants for in vitro study.MS media a supplemented with 24D,BAP,KI responsed best of callus formation. The protocol developed here can be used for plant regeneration and chemical analysis experiments.

KEYWORDS : *M. philippenesis, callus formation, Medicinal plant, In-vitro study* ***Abbreviation:*** *MS- Murashige and Skoog media, KN-Kinetin, BAP-6-Benzyllaminopurine 24D-2, 4-dichlorophenoxy acetic acid L-Leaves, Cotyledons, R-Root*

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INTRODUCTION

The genus *Mallotus* commonly known as' Ba bet' in Vietnam is one of the most diverse and richest genera of the euphorbiaceae family is Vietnam where about 40 Mallotus species may be among which six species and one verity are endemic. The plant of the genus *Mallotus* are a rich source of biologically active compounds such as phloroglucinols,tannins,terpenoids,benzopyrans and chalcons.*Mallotus* or of individual chemical constituents isolated from these exracts have been reported by several authours. (1,2, 3,4,.5,6.7,8,9,10,11,12).

Mallotus philippenesis (Lam.) M.Arg.is one of the endangered plants of central eco region (13).It belong to the (euphorbiaceae) family. It is a commonly dye yielding plant locally known as kamala, mostly found in India subcontinent. Whole

parts of the plants are rich in secondary metabolites, which impart medicinal uses to the plant. Extract of fruits of kamala from the glands and hairs yielded a resin a wax and the crystalline compound rottlerin. Kamala also contains a minute amount of essential oil. Which when gently warmed emits a peculiar odour. The principal constituent, rottlerin, is found in the kamala resin. Its fruits contain Rottlerin (reddish-yellow resin) 47.80% fixed oil. 5.83-24% , citric acid , mallotoxin ,kamalin.The principal constituent , rottlerin, is from the kamala resin rottlerin.The seed contains a Fixed oil,camul oil and a bitter glucosidal .Betulin -3 acetate lupeol acetate , berginin acetylaleuritote acid,sitosterol,bergenin , rottlerin resin . Solid hydroxy acid, kamlonenic acid, linoleic. Oleic, lauric ,myristic, palmitic acid , stearic acid , crotoxigenin, rhamnoside , coroghcignin , octa cosanol,iso rottlerin ,rottlerin , homorottlerin, tannins , citric , oxalic acid.(30) The plants have found application in pharmaceuticals as it is one of the common plants used in Indian system of medicine. Various parts of the plant are used in the treatment of skin problem, bronchitis, antifungal, tape worm, eye-disease, cancer, diabetes, diarrhea, jaundice, malaria, urinogenital infection etc. In dispersing swellings of the joints from acute rheumatism and of the testes from suppressed gonorrhea. It also shows anti-oxidant, anti-bacterial, anti-fungal, anti-microbial insectidal /pesticide, anti-microfilaria, anti-lithic, heptoprotctive activities. Employment of techniques such as cell and tissue culture would provide means of rapid propagation and conservation of the plant species and from the point of view of phytochemistry give scope for enhancement of the quality and quantity of the bioactive secondary metabolites occurring in the plant (15).

Currently the plant is facing a threat of extinction due to destructive harvesting of plant parts for medicinal use as well as devastation of its natural habitat by deforestation. Besides, the conventional vegetative propagation methods for mass multiplication of this tree species are hampered due to low rates of seed germination (only-30%). Many rare and endangered plant species are propagated *in-vitro* because they do not respond well to conventional methods of propagation. The media composition and qualitative and quantitative aspects of plant growth regulators play a vital role in micro propagation. Therefore, optimization of these conditions is a prerequisite for *in-vitro* related work.(15)

There are no reports available on *in-vitro* propagation of *M. philippnesis* that made us interested to develop micro propagation protocol for this threatened and medicinally important woody plant species. Micro propagated plants can thus act as source for germplasm conservation for this important tree species.

Callus culture has made it possible to obtain plants because callus formation is wool part of plant is possibal and callus in presence of high chemical value compared by natural plant.

The present study was undertaken to standardize a protocol for in-vitro callus formation of *M. philippenesis* to regenerate plants by using different explants by tissue culture for Micro propagation to meet its demand in medicine.

MATERIALS AND METHODS

The research was conducted at the Department of Botany.Sarojini Naidu Gove. Girls Post Graduate (Autonomous) Collage Shiva ji Nagar, Bhopal. Madhya Pradesh (India).

Collection and authentication of plant material

The seeds were collected in the month of March, 2011 from mature tree growing inside the Botanical garden of BHEL College Bhopal and The plant were identified by Botanical survey of India, CRC (BSI) Allahabad, where voucher specimen code (1370-158-696) was deposited

Surface sterilization procedure

Seeds were thoroughly washed under running tap water for 30 min then treated with 5% tween-20 for 5 minutes with constant stirring followed by 3-4 rinses in sterile distilled water and further treated with an antifungal agent (Bavistin) for 2 hours and were further with detergent for 10 min. and rinsed 4-5 times tap water. Further sterilization procedures were carried out inside laminar air flow

chamber, where seeds were surface sterilization through single dip in 70% (v/v) for half minute followed by three times rinses in sterile distilled water. There after mercuric chloride (0.1%) treatment was given to explants for 8 minutes followed by four times rinsed in sterile distilled water. Thereafter seeds were carefully transferred to be placed over sterile petridish & were then inoculated into the culture establishment medium inoculated into the culture establishment medium (MS Medium; Murashige & Skoog 1962) using sterile forceps under aseptic conditions. The seeds placed horizontally on the culture medium.2 (MS Medium; Murashige & Skoog 1962) using sterile forceps under aseptic conditions. The seeds placed horizontally on the culture medium. The seedlings induced from the *in-vitro* cultures were used as explants for further experiment.

Selection of explants

Leaves,coteledons, ,root, of 25 day old in vitro raised seedlings were selected as explants for direct callus induction. The Leaves, cotyledons, root in small picas were excised aseptically.

Chemicals

Leaves, coteledons, root, induced from seedlings were cultured on MS basal medium supplemented with 3 % (w/v) sucrose (Sd-fine Chemicals, India) for callus induction. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.7 with 1N NaOH or 1N HCl before gelling with 0.8 % (w/v) agar. In all the experiments, the chemicals used were of analytical grade (Merck and SD-fine Chemicals, India). The medium was dispensed into culture vessels (Borosil, Mumbai, India) and autoclaved at 105 kPa at 125°C for 15 minute. The surface sterilized explants were placed horizontally on the culture medium. All the cultures were incubated at 25±2°C under 16h light/8h dark photoperiod with irradiance of 45 - 50 µ mol/ m^2/s photo synthetically active radiation (PAR) provided by cool white fluorescent tubes (Philip, India) and with 60 - 65 % relative humidity.

All subsequent subcultures were done at four weeks intervals. Culture media consisted of MS (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) was evaluated for their effects on *in-vitro* growth and development of *M. philippeneesis*. For induction of callus, explants were cultured on MS medium supplemented with different concentration of cytokines, including BAP, 24D and Kinetin either individually or in combination. Application of tissue culture to plant conservation in India has been largely restricted to economically important species However, the approach could usefully be extended to conserve all threatened plants so that vital biodiversity and the ecological network is sustains can be preserved (JitenChandra et al., 2011)4 .*Mallotus philippenesis* is categorized as a rare and endangered species and is on the of Endangered species for central eco region.

Callus induction

Leaves, cotyledons, root, were excised and inoculated by horizontal orientation on the culture medium containing different concentration of BAP (.0.5-7 mg/l.), 24D (0.5-7.0mg/l), and KN (0.1-3.0mg/l). Explants were assigned randomly to each treatment and culture were kept under 16 h light/day photoperiod at 25±2°C.callus induction was assessed after process same different media composition for further experiment.

Callus formation

After 25-30 days have shifted inciated callus in to fresh media of same concentration as old one media is used due to callus formation period after shifting in to fresh media and observed further callus growth.

RESULTS AND DISCUSSION

Different explants were cultured *in vitro* to find out the most suitable explants for callus induction Both leaves,cotiledons ,root explants showed callusing but the frequency was low (>20%), and the texture of the calli were non-organogenic(hard green), which on further sub culturing declined to grow. Among the different explants tested leaves explants was found to be best of (callus initiation (Table 1). The media found most suitable for callus induction and regeneration were MS media.

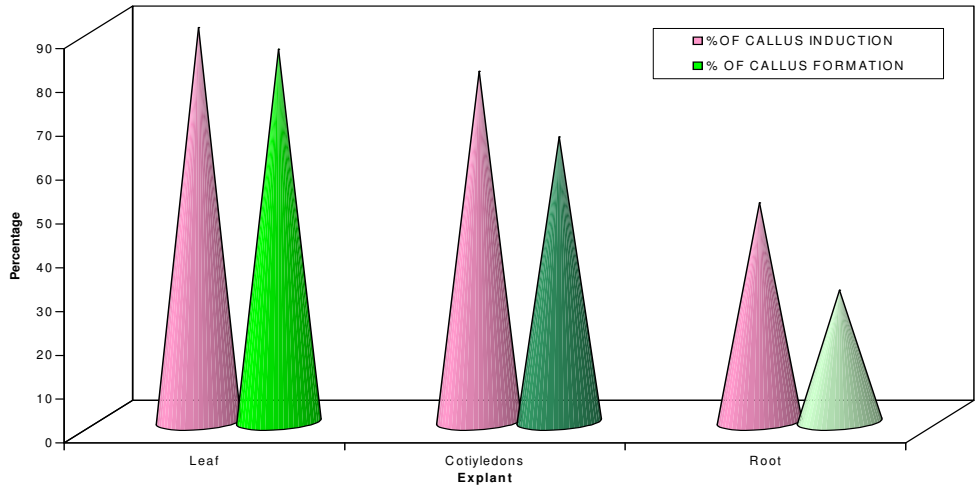
MS medium supplemented with different levels of BAP, KN or 24D were tried to induce callus

induction from in vitro seedling segments (leaves, cotyledons, and root) of *M. philippenesis.* Although leaves explants showed callus induction on higher concentrations of BAP (4BAP mg/l) or (224D mg/l) lower however these level lower and failed to induce callus formation.

Table 1. Morphological based Percentage of Callus induction and Callus formation from different explants.

S.NO.	EXPANTS	%OF CALLUS INDUCTION	% OF CALLUS FORMATION	COLOUR AND QUALITY OF CALLUS
1	L	90%	85%	Friable Grayish green fast growing callus
2	C	80%	65%	Hard green with white border.
3	R	50%	30%	White watery calli with watery exudates

Graph 1 : Morphological based Percentage of Callus induction and Callus formation from different explants



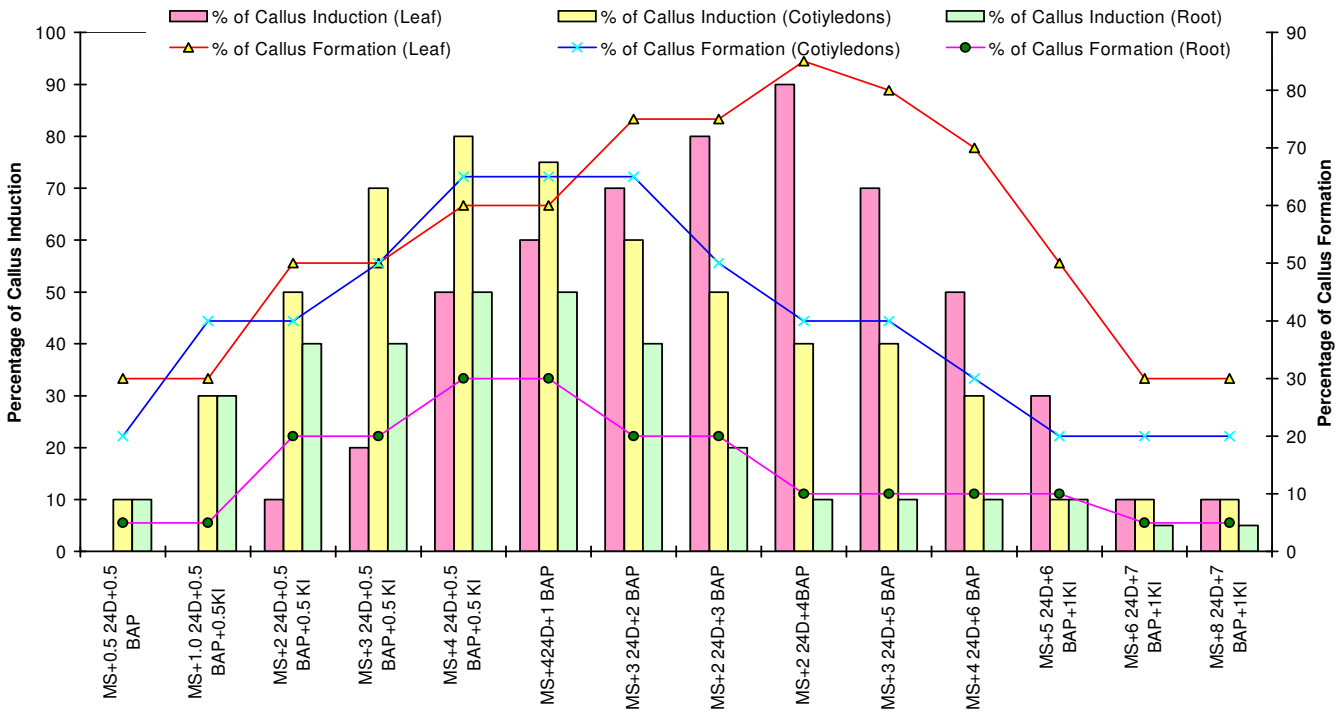
Combined effect of cytokinins, KI and in combination, was tested on *in-vitro callus induction* and callus formation in *M. philippenesis* interestingly; the above cytokinins when combined resulted in leaves as well as callus formation. A combination of (224D mg/l) plus BAP (4 mg/l) showed maximum (90%) formation).

Table 2-Percentage of Callus induction and Callus formation from different explants.

MEDIA _HORMONES	%OF CALLUS INDUCTION			%OF CALLUS FORMATION		
(mg/ml)	L	C	R	L	C	R
MS+0.5 24D+0.5 BAP	0	10	10	30	20	5
MS+1.0 24D+0.5 BAP+0.5KI	0	30	30	30	40	5
MS+2 24D+0.5 BAP+0.5 KI	10	50	40	50	40	20
MS+3 24D+0.5 BAP+0.5 KI	20	70	40	50	50	20
MS+4 24D+0.5 BAP+0.5 KI	50	80	50	60	65	30
MS+424D+1 BAP	60	75	50	60	65	30
MS+3 24D+2 BAP	70	60	40	75	65	20
MS+2 24D+3 BAP	80	50	20	75	50	20
MS+2 24D+4BAP	90	40	10	85	40	10
MS+3 24D+5 BAP	70	40	10	80	40	10
MS+4 24D+6 BAP	50	30	10	70	30	10
MS+5 24D+6 BAP+1KI	30	10	10	50	20	10
MS+6 24D+7 BAP+1KI	10	10	5	30	20	5
MS+8 24D+7 BAP+1KI	10	10	5	30	20	5

*Data represents mean of 10 replicates in 3 repeated experiments

Graph 2 : Percentage of Callus induction and Callus formation from different explants



Other combinations of 24D with BAP showed 65-75% of formation response and the average callus formation 50%. Well developed callus (25-30 days old) with primary condition cotton like stages. Were further cultures to obtain process. The purpose of this study was to develop an *in vitro* propagation method from callus of, *M.philippenesis* a medicinally important plant. In the present work we have, for the first time in M.P., established a rapid and reproducible method for high-frequency callus induction from in- vitro raised seedling segments of, *M. philippenesis* followed by establishment of regenerated plants in soil. Leaves, cotyledons, root was dependent on the interaction between plant growth regulator concentrations in the medium

The results showed that BAP alone or in combination with compared with BAP alone. Increasing the concentration of plant growth regulators (BAP +24D) in both explants (Leaves, cotyledons, root) enhanced the response of callus induction & best callus formation of leaves achieved at 4.0 mg/l BAP + 224D.0mg/l,cotyledons and root response of callus induction & best callus formation of achieved at 0.5 mg/l BAP + 424D.0mg/l+0.5mg/l KI. Further increase in concentration decrease the callus induction. 24D alone or in combination with BAP is not effective in callus formation and leaves, cotyledons; root is showed are best callus formation for same concentration of callus induction media.

CONCLUSION

The important part of present study was the preparation successful growth of induced callus of in *Mallotus philippenesis in-vitro* conditions. The results of this study shown that tissue culture techniques can play an important role in plant propagation of elite genotypes of. *Mallotus philippenesis* which has diverse medicinal applications and eventually due to over exploitation this plant is facing local extinction. It has been affirmed as endangered plant by central eco-region and hence there is pressing need to conserve this medicinal herb of high commercial value *Mallotus philippenesis* usually multiply by root secures and seeds but due to low germination capability it restricts for the regeneration. It seems likely that this protocol for callus induction, possibly with modification, can be used for *in-vitro*

plant induction of other species of the genus *Mallotus philippenesis* using seedling segments.

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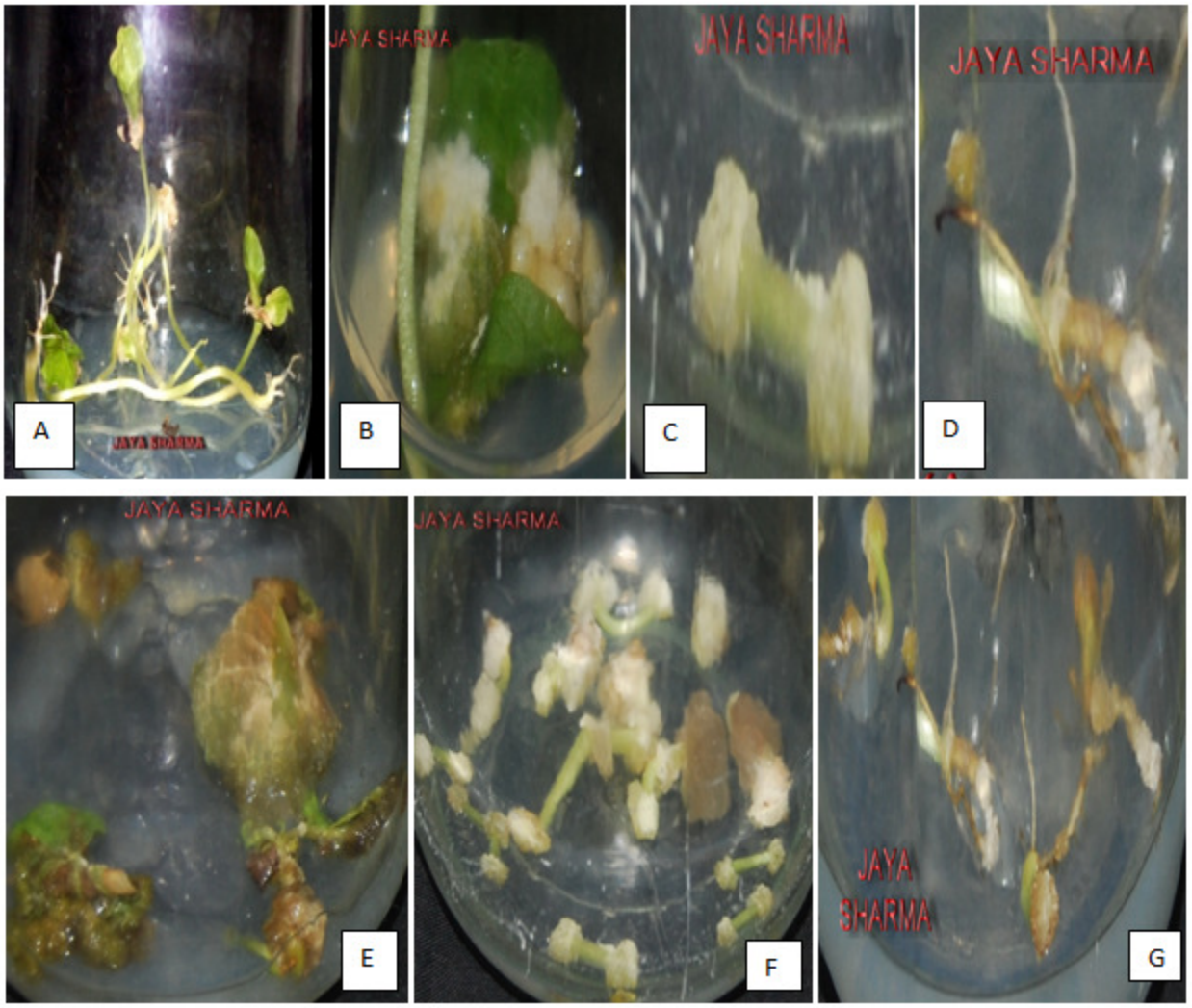
Figure-**A**-*In vitro* raised seedling. **B**.Callus induction through of leaf **C**.Callus induction through of cotyledons **D**. Callus induction through of root **E**. Callus formation through of leaf **F**.Callus formation through of cotyledons **G**.Callus formation through of root.



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