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Research Article

EFFECT OF DIFFERENT CULTURE MEDIA ON SHOOT INDUCTION OF *MALLOTUS PHILIPPENESIS* (LAM) M ARG

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ABSTRACT
A micropropagation protocol is presented for conservation of critically threatened woody tree species. The present work was carried out in different types of media and undertaken to develop a basic and simple protocol for shoot induction via micropropagation of *Mallotus Philippenesis*. Best result shown in different media in the order as WPM>MS>B5 media. The effect of various types of medium on the behavior of *in-vitro* consecutive micropropogation protocol was develoed.Many media were tested (MS, B5 and WPM) with different growth hormones for induction stage. WPM was found to be the best medium for shoot induction. High frequency of induced shoots was obtained on BAP (10mg/l), KN (2mg/l) and NAA (2mg/l). The low concentrations of growth hormones did no support in vitro shoot induction in *Mallotus Philippenesis.* The shoot induction protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plant. Further work for standardization of efficient *in-vitro* protocol for best shoot multiplication and *in-vitro* rooting is under progress in our laboratory.

Key Words: *M. Philippenesis, In-Vitro Propagation, Medicinal Plant Conservation, Different Type's Medium*
Abbreviation: *BAP: 6-Benzyllaminopurine, NAA: Naphthalene Acetic Acid, KN: Kinetin, MS: Murashige and Skoog Medium), Mg: Mile Gram and PGR: Plant Growth Regulators Composition*

INTRODUCTION
Mallotus philippiensis L. Locally known as kamala is a large woody multipurpose medicinal tree (wealth of India 2003) belongs to family Euphorbiaceae consisting of herbs, shrubs and trees. *M. philippenesis* is a medium sized much branched, tolerant and soil improving small tree. It is up to 10-12 meters in height and is widely distributed throughout tropical India along with the Himalaya from Kashmir east wards up to 5000 ft. all over the Punjab, Uttar-Pradesh, Bengal, Assam, Burma, Singapore, and from Sind south wards to Mumbai and Ceylon. The plants are a rich source of biologically active compounds and are used as a common dye yielding plant (Zafar and Yadav, 1993). 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, and antifungal tape worm eye-disease, cancer, diabetes, diarrhea, jaundice, and 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, insectidal/pestecidal, anti-microfilaria, antilithic, heptoprotective activities. (Z.R.S.Y.1993) *M.philippenesis* is highly cross-pollinated and variations among the same species are limited. However, it is now well documented that some selections are rare and possess beneficial characteristics such as high yield, high oil content, drought resistance, photoperiod insensitivity, resistance/tolerance to major insect pests and diseases. This opens up the opportunities of breeding for hybrids. The current requirement in our country is to mitigate fatty oil import and produce our own cosmetics and pant-varnish through large scale cultivation of crops like *Mallotus*. Limitations for such activity are non availability of quality planting materials seed, seedlings, bark and leaves (Sharma and Varma 2011). *M. philippenesis* consist of male and female plant. As the germination rate is often poor because of drought and insect attack and 6 months without losing viability. In Natural conditions seeds

germinate about 5% in 65-82 days (Jaya *et al.*, 2012). Because seed coat is very thick and stony while endosperms are soft and very small therefore this doesn't support quick and natural germination process thus propagation via natural reproduction is poor and its *in vitro* micro propagation is therefore very necessary. So the plant requires conservation to meet its demand in agriculture and medicine. The bio-technological approach such as plant tissue culture is an alternate and variable method for propagation and conservation of economically and medicinally important plants. 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 (Jaya *et al.*, 2012).

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. 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. Development of a protocol for micropropagetion of *M.philippenesis* is an important contribution of this could be applied for cloning plants selected for higher yield and medicinal of both and chemical content. There fore the objective of the study was developing a protocol for *in-vitro* propagation of *M.philippenesis* application. The present study was undertaken to effect of different types of media standardize a protocol for in-vitro shoot induction of *M. philippenesis* to regenerate plants by using different explants by tissue culture for Micro propagation to meet its demand in medicine and agriculture.

MATERIAL AND METHODS

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

Collection and Authentication of Plant Material

The shoots were collected in the month of November, 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.

Selection of Explants

Meristems were used as explants for this experiment. Explants were cut and reduced to length of 2 cm using surgical blade, retaining the apical dome (1 cm).

Surface Sterilization Procedure

Shoots were thoroughly washed under running tap water for 30 min to remove all the dirt and soil particles adhering to them, 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 20 minute's and rinsed 4-5 times tap water. Thereafter, again explants were kept immersed in distilled water with few drops of wetting agent, labolene for ten minutes. It was immediately followed by five time rinses in distilled water to remove traces of labolene. Further sterilization procedures were carried out inside laminar air flow chamber, where shoots were surface sterilization through 1 minute's treatment 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 10 minutes followed by four times rinsed in sterile distilled water. Thereafter shoots were carefully transferred to be placed over sterile Petri plats to remove excess water and were then and were then inoculated into the culture establishment medium (MS/B5/WM) using sterile forceps under aseptic conditions.

Chemicals and Glass Wear

Meristems induced from shoots were cultured on MS/B5/WM basal medium supplemented with 3 % (w/v) sucrose (Sd-fine Chemicals, India) for shoot induction. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.5-5.8 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 vertically 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/ m²/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/B5/WM (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 shoots, explants were cultured on MS/B5/WM medium supplemented with different concentration of cytokines, including BAP (0.5-15.0 mg/l), NAA (0.1-0.5.0 mg/l) and Kinetin (0.1-5.0 mg/l) 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 (Jiten Chandra *et al.*, 2011) 4 *M. philippenesis* is categorized as a rare and endangered species and is on the of Endangered species for central eco region.

Shoot Induction

Meristems node were excised and inoculated by vertical orientation on the culture medium containing different concentration of BAP (5.0mg/l-15.0mg/l), NAA (0.2mg/l-5.0mg/l), and KN (0.1mg/5.0mg/l). Ten single explants were assigned randomly to each treatment and culture were kept under 16 h light/day photoperiod at 25±2°C shoot induction the effects of different treatments were quantified and the data were subjected to presented. Medium lacking growth regulators served as control.

RESULTS AND DISCUSSION

MS medium supplemented with different levels of BAP, KN or NAA were tried to induce shoots from meristems explants of *M. philippenesis* nodal explants showed initiated on higher concentrations of BAP (10mg/l), KN (2mg/l), or lower levels of NAA (2mg/l) however these levels failed to induce shoot formation. Combined effect of cytokinins, KN in combination with BAP or NAA, was tested on *in vitro* nodal in. *M.philippenesis* interestingly, the above cytokinins when combined resulted in axillaries and maristem as well as bud brake. A combination of KN (2mg/l) plus BAP (10mg/l), NAA (2mg/l) showed maximum (80MS, 70B5, 90WPM %) And shoot induction (50MS, 70WPM, 50B5).

The purpose of this study was to develop an *in vitro* propagation method from mature nodes of *M.philippenesis* a medicinally important plant. In the present work we have, for the first time, established a rapid and reproducible method for high-frequency from mature node segments of *M.philippenesis.* Similar observations were previously reported with other species like *Feronia Limonia* (11 and 12). In this study, BAP (10mg/l) or KN (2mg/l), (NAA2mg/l) this concentration are show best result and other high concentrations failed to induce shoot in *M.philippenesis*.

Earlier studies have compared the effectiveness of different type's media. In the present investigation, interaction of KN with NAA or BAP with WPM basal media was established that resulted in efficient shoot regeneration. Further, the *in vitro* regenerated shoots are using further experiments. However, nodal segments incubated on medium supplemented with KN + NAA+BAP produced healthy shoots and overall shoot. Quality did not differ much when these media formulations were used. The synergistic action of a combination of two or more cytokinins resulting in to shoot induction from various explants has also been reported for *Gymnocladus dioicus* L. (Geneve, 2005); *Eclipta Alba* (Baskaran and

Jayabalan, 2005); *Feronia limonia* L. (*Vyas et al.*, 2005); *Albizia odoratissima* (Rajeswari and Paliwal, 2006) and *Momordica tuberose* Roxb (Aileni *et al.*, 2008).

Table 1: Effects of different type of media and concentration of plant growth regulators on *in-vitro* shoot initiation from meristems shoot of *Mallotus philippenesis* (Lam) M. Arg. after eight weeks of culture

Plant Growth Regulators mg/l	Media	Percentage Explants Bud Break	Percentage Explants Shoot Proliferation	Number of Shoots Produced/Explants ±se	Mean Length of Shoots in (cms) ±se
			MS		
5.0 BAP+0.2KN	**M1**	20%	10%	1.44 ±0.10	1.80 ± 0.04
6.0BAP+1.0KN+0.5NAA	**M2**	30%	10%	1.04 ±0.14	0.75 ± 0.06
8.0BAP+1.0KN+1.0NAA	**M3**	50%	30%	2.30 ±0.16	1.38 ± 0.06
10BAP+2.0KN+2.0NAA	**M4**	80%	50%	3.05±0.11	1.90 ± 0.10
12.BAP+3.0KN+3.0NAA	**M5**	50%	30%	2.00 ±0.20	1.68 ± 0.06
15BAP+5.0KN+5.0NAA	**M6**	10%	20%	2.05 ±0.16	0.75 ± 0.06
			WPM		
5.0 BAP+0.2KN	**W1**	30%	20%	1.20 ±0.10	1.80 ± 0.04
6.0BAP+1.0KN+0.5NAA	**W2**	40%	50%	1.40 ±0.14	2.20 ± 0.06
8.0BAP+1.0KN+1.0NAA	**W3**	50%	60%	2.00 ±0.16	1.30 ± 0.06
10BAP+2.0KN+2.0NAA	**W4**	90%	70%	3.40 ±0.11	2.01 ± 0.01
12BAP+3.0KN+3.0NAA	**W5**	50%	50%	2.00 ±0.20	1.67 ± 0.08
15BAP+5.0KN+5.0NAA	**W6**	40%	30%	2.00 ±0.16	1.80 ± 0.04
			B5		
5.0 BAP+0.2KN	**B1**	0%	0%	0.00 ±00.0	0.00 ± 0.00
6.0BAP+1.0KN+0.5NAA	**B2**	20%	0%	0.00 ±0.00	0.00 ± 0.00
8.0BAP+1.0KN+1.0NAA	**B3**	30%	20%	1.15 ±0.10	1.38 ± 0.06
10BAP+2.0KN+2.0NAA	**B4**	70%	50%	2.00 ±0.15	2.90 ± 0.10
12BAP+3.0KN+3.0NAA	**B5**	40%	20%	1.47 ±0.12	1.48 ± 0.06
15BAP+5.0KN+5.0NAA	**B6**	20%	20%	1.15 ±0.10	1.75 ± 0.06

Each value represents mean ± SE calculated from 10 separate experiments each with three replicates per treatment.

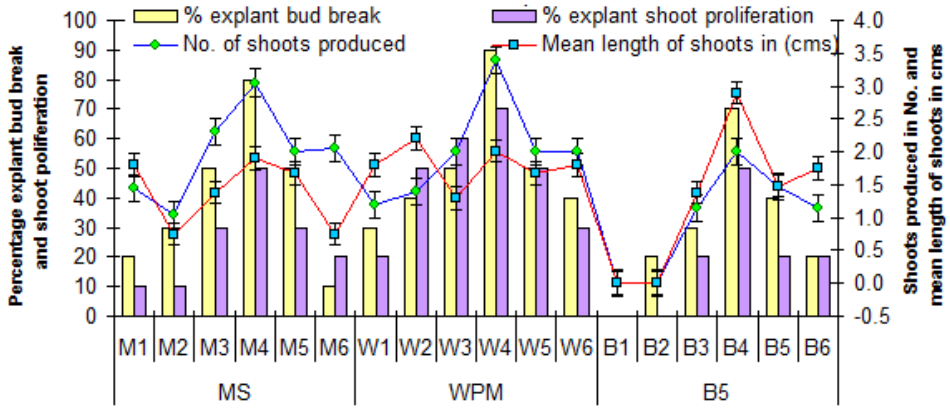

Figure 1: Effect of different concentration of PGR on shoot proliferation from meristems nodes of Mallotus philippenesis in different medium (MS, WPM.B5)

The role of cytokinins in shoot differentiation from nodal segments was reported in several woody species but only few reports were successful in inducing organogenesis from mature node explants (Ramesh *et al.*, 2002). Explants from juvenile plants were also used for raising *in vitro* cultures from several woody trees (Gurumurthi and Jagdees, 1992; Naomita *et al.*, 2004 and Ramesh *et al.*, 2005).

Here we report on shoot induction from mature node explants of *M.philippenesis.* In conclusion, *in vitro* growth and development from nodal explants of *M.philippenesis* was highly influenced by the type of BAP+NAA+KN+WPM basal medium combination used for propagation.. The results presented also demonstrate that mature nodal explants of *M.philippenesis* offer great potential as a source tissue for shoot induction. The procedure reported in this study may facilitate improvement, conservation, and mass propagation of this medicinally important tree species.

CONCLUSION

Different types of basal medium and plant growth regulators clearly effected shoot induction from meristum through of *in-vitro* micropropagation.

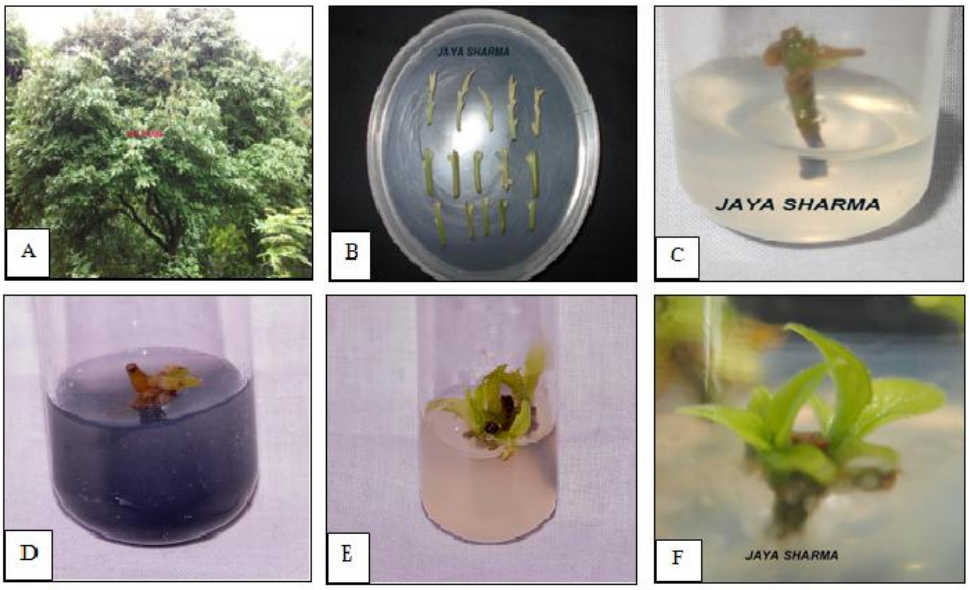

Figure 2: A - Mature Tree plant. **B** - Separation of x-plant. ***C*** **-** *In-vitro* Inoculated shoot. **D** - *In-vitro bud* sprouting. **E and F** - *In-vitro* initiation of meristems.

It can be concluded that the WPM showed high% of shoot induction as compare to other media. The protocol defined in this study as outlined below and is demonstrated in figers.The findings have several

implication for managing the diversity of this species as well as restoration of its degradation. The developed protocol can be used to produce uniform and desirable plants for plantation in order to reduce pressure on the wild population. It also affairs a potential system that should be used for improvement conservation and mass propagation of *M.philippenesis* conservation of this plant can be a challenge as well as a powerful tool to medicinal properties improve livelihoods and enhance biodiversity. The present experiment have show that it is possible to *in vitro* shoot induction and use for further experiments' and done by plantlets**.**

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