In-vitro endosperm culture and seedling growth of *Mallotus philippienesis. (Lam) M. Arg.*

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ABSTRACT: An efficient protocol has been developed for in-vitro seed (endosperm) germination and seedling growth of a woody multipurpose medicinal plant M. philippinesis. The explants used for the present experiment are seeds which were cultured on MS media supplemented with 3% sucrose and 10% agar with or without any growth hormone. Seeds of different age groups like 20, 40, 60, 80 and 100 days were taken. Best seed germination (94%) is observed in MS medium (half concentration) while maximum seedling growth were observed in the MS media without any growth hormone. The seedlings developed were further used for the multiplication of shoots from different parts, for the regeneration of plants of *Mallotus phillipienesis.*

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Key words - in-vitro endosperm culture, *M. phillipiensis*.

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. Mallotus 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, R.S.Yadav K.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, 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, insectidal /pestecidal, anti-microflarial, anti-lithic, heptoprotective activities (Z.R.S.Y.1993). Mallotus 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. (A.F.D.B. 2009).

Mallotus 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.(AFTD)because seed coat is very thick and stony while endosperms are soft and very small so not be observed natural growing condition then natural reproduction are poor and in vitro micro propagation is 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. In-vitro propagation of *Mallotus philippensis* has been achieved by (Abbas, 1993) by obtaining a continuously growing callus on MS + 2,4-D (5.78µM)+ Kn (2.5µM). Shoot regeneration occurred in callus when sub cultured on MS + BA (13.3µM) +NAA (1.1µM). Abbas (1996) induced

triploid plants from the endosperm cultures of *Mallotus philippensis*. Endosperm culture has made it possible to obtain plants that otherwise would not have been recovered. Endosperm culture involves the isolation of the endosperm from the seed and subsequent growth *in vitro* until the developing plant can be transplanted to the soil and grown to maturity (Smith M.K. Drew RA.1990).

The present study was undertaken to standardize a protocol for in-vitro seed germination and seedling growth of M. philippenesis to regenerate plants by using different explants by tissue culture for Micropropagation to meet its demand in medicine and agriculture.

MATERIALS AND METHODS

Fruits were collected from the plants from Botanical garden of BHEL College Bhopal after different stages of their maturity i.e. 20 days, 40 days, 60 days, 80 days, and 100 days. Seeds were isolated from the fruits and were washed thoroughly in running tap water for 30 minutes were further treated with an antifungal agent (Bavistin) for 2 hours and were further with detergent for 10 min. and finally surface sterilization with 0.1% $HgCl_2$ in laminar airflow chamber.

The seed coat is carefully dissected in laminar airflow chamber without causing any injury to the endosperm. This was one of the methods adopted in the present study to dissect out the seed under *in vitro* conditions. The whole endosperm inoculated into each of glass bottles containing the initiation medium. The endosperms were incubated in the culture growth room at a temperature of 25±2°C, with16 photoperiod, with light intensity of 2,500 flux from cool fluorescent tubes.

Different types of media (MS, Whites and B5) have been used for seed germination (Murashige and Skoogs 1962), with or without any growth hormone for in-vitro seed germination and seedling growth of *M.philippenesis.* The Basal medium was amended with 3 % (w/v) sucrose and 0.10% agar. The pH was adjusted between 5.5-5.9. The medium was autoclaved at a temperature of 125ºC and a pressure of 15 psi for 15-20 minutes.

RESULTS AND CONCLUSION

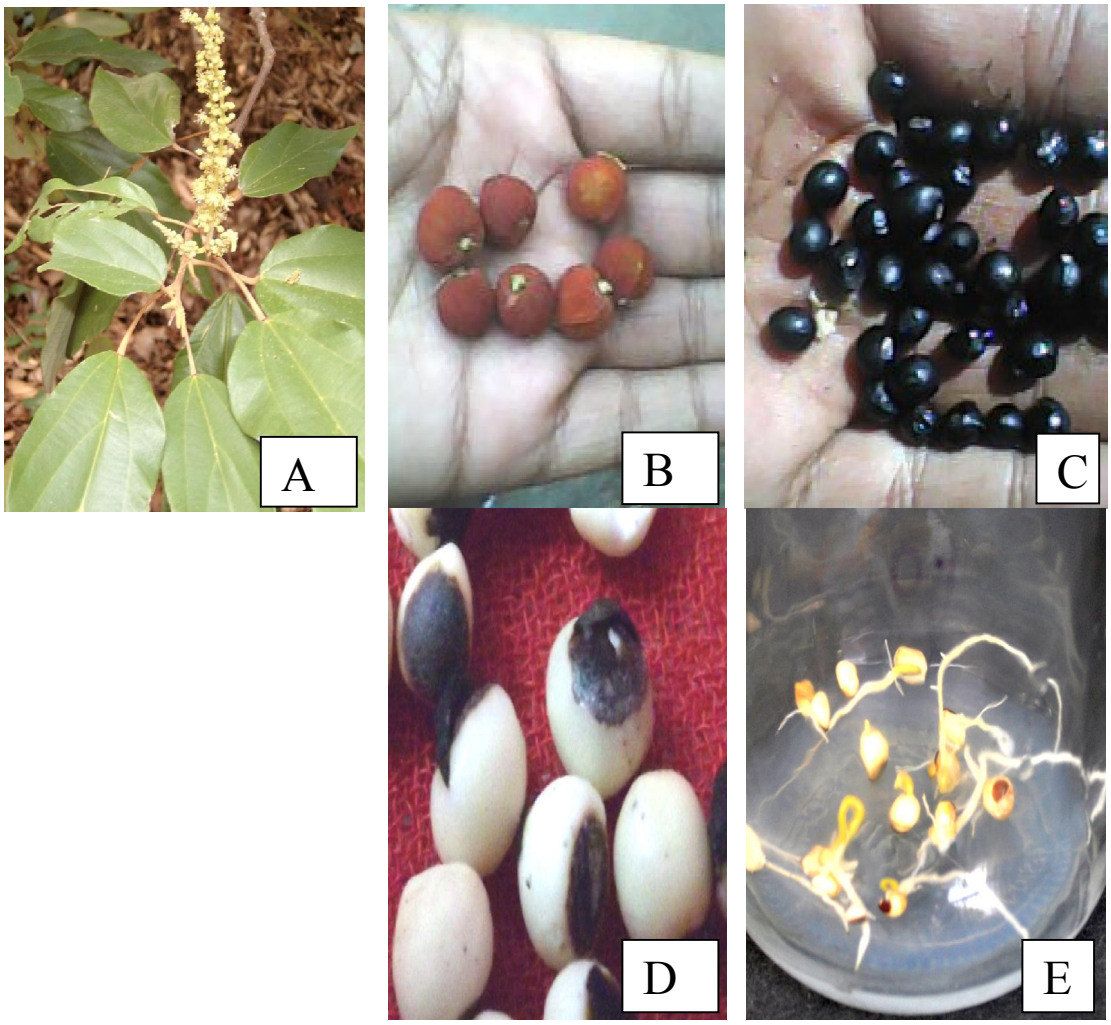
The mature seeds of *Mallotus philippenesis* are globular and are similar to that of castor seed. The mature seeds are of 4mm in.diameter. The seeds have a hard seed coat (SC) that is black in color and encloses the endosperm and the cotyledons. In between the black seed coat and the endosperm/cotyledon complex there is a thick protective seed membrane (SM) that tightly binds to the inner complex. This kind of seed structure is unusual in dicotyledons. However, it is a common feature of members Euphorbiaceae and this kind of seed structure is similar to that of *Ricinus*. The hard seed coat that encloses the endosperm and the cotyledons. Induces seed dormancy in *M.philippenesis*.

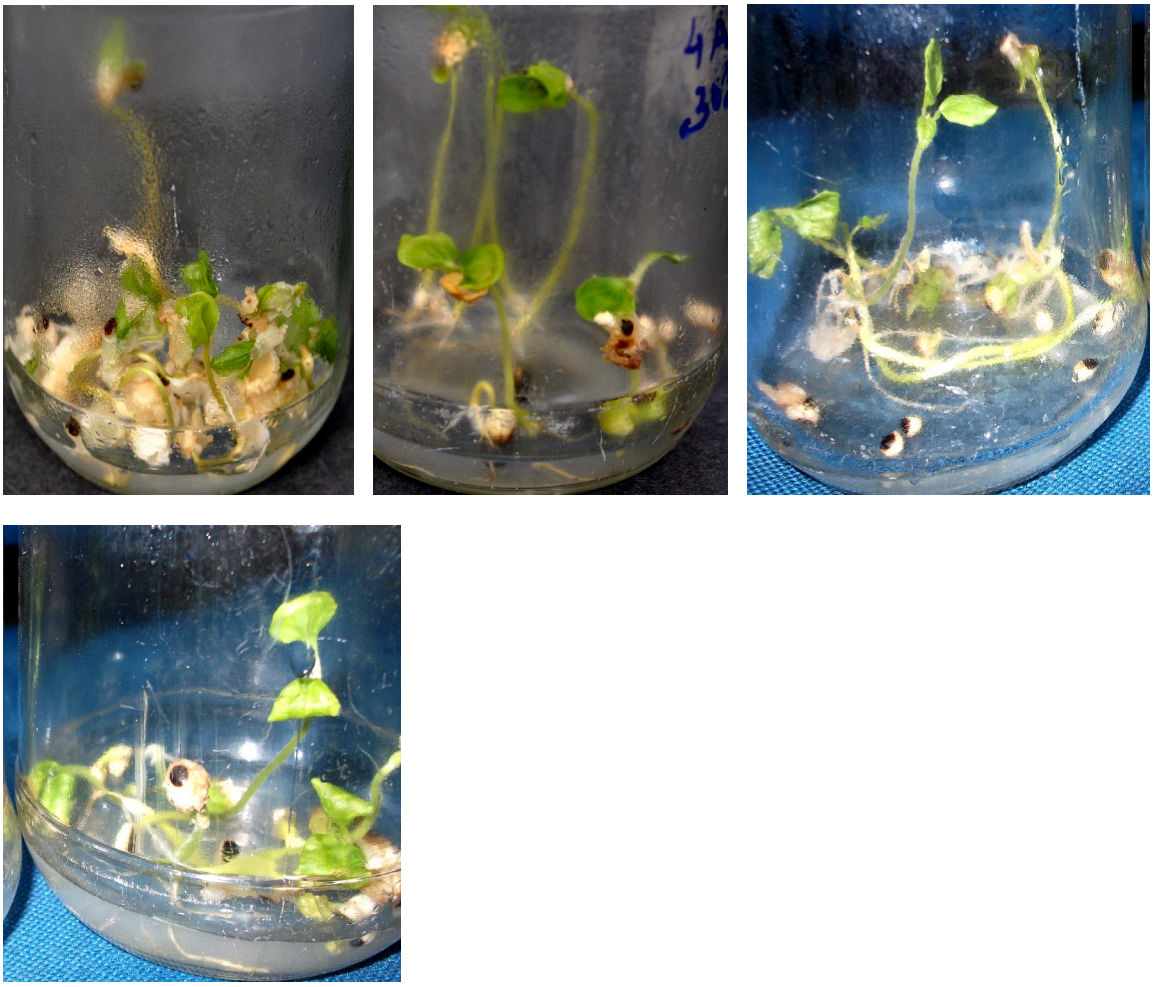
It is observed in other studies, that the seed dormancy prevents quick germination of seeds and the viability of seeds is lost quickly within a period of 6 months.(AFTD 2009).In addition, it has been observed that the seed membrane and the endosperm complex together carries microorganisms consisting of bacteria and/or fungi that hamper the normal germination with naturally growing seedlings (Deshmukh S.D. and Borle, M.N. 1975) thus in he present study it has been investigated that if seed are collected in immature condition & their endosperm r are grown/cultured in-vitro condition highest percentage of seedlings are obtained. These seedlings will be further cultured to obtain plantlets.

It was observed after 5 days of inoculation that the embryos swell up to 7mm size approximately from 3mm in initial condition. After 4 day primary root appear, followed by the appearance of cotyledons after 3-4 days and elongation of green, thick hypocotyls (HP) and a short, white radical with secondary roots growing in the medium. Now full plantlets are ready within 10-12 days. Thus within 20-25 days full plantlets are ready from. Endosperm By the fourth day, green cotyledons emerged out of the central part of the seed. The cotyledons opened fully with elongation of hypocotyls and full growth of the radical with secondary roots into the medium, by cotyledons alone emerged out leaving the endosperm in the medium. The maximum size of plantlet obtained was 9-10 cm. Similar results in Chinese Leymus embryos have been reported by Liu et al (Liu GS, Qi DM, Zhang WD, et al. *In-Vitro* Cell. Develop. Biol - Plant 2004) which has a time-consuming breeding cycle and high level of seed dormancy. In this case, to improve the breeding process, a simple technique was established by them that shorten the breeding cycle by culturing immature embryos *in vitro* to produce plantlets immediately. This technique could potentially save one year in each sexual breeding cycle. Sanchez-Zamora Sanchez-Zamora (M, Jose CT, Diego FT, Roberto GL. Sci. Hort. 2006) also achieved success in *in-vitro* germination for *Juglans regia* L. root stock c v. Peralta. Arbeloa (Sanchez-Zamora M, Jose CT, Diego FT, Roberto GL. Sci. Hort. 2006), obtained clonal plants to shorten crossing programs in Prunus who have reported seedling germination, multiplication, and successful establishment of *in-*

vitro cultures of hybrid seedlings obtained from inter-specific hybrid seeds after cross-pollination between myrobalan x apricot (*Prunus cerasifera* x *P. armeniaca*).

In the present study, 40 day old embryos exhibited germination as early as 7-8 days. This was followed by germination of 60 and 80 days old endosperm which was observed in seven days. The 60 day old endosperm took about 10-12 days for their germination, endosperm of 60 days old gave the highest percentage of results (94+_0.20).In the present study, endosperm of different ages like, 20, 40, 60, 80 and 100 days after pollination were chosen to analyze the minimum number of days required for successful germination it was noticed that embryos of 60 days gave the best results with (94+_0.20) germination. In *M. phlippenersis* immature endosperm of 60 days were found to be successful for the germination and greenhouse establishment. Further, simple medium like MS1/2 basal, without any growth hormone was sufficient to generate the plantlets.





Figute 1: (A).Matoure flowring plant (B). Pigment contening fruits (C).Seeds (D) Isolated endosperm (E) Endosperm germition after 15 days (F) Seedling growth after 25 days (E1 E2 E3 E4).

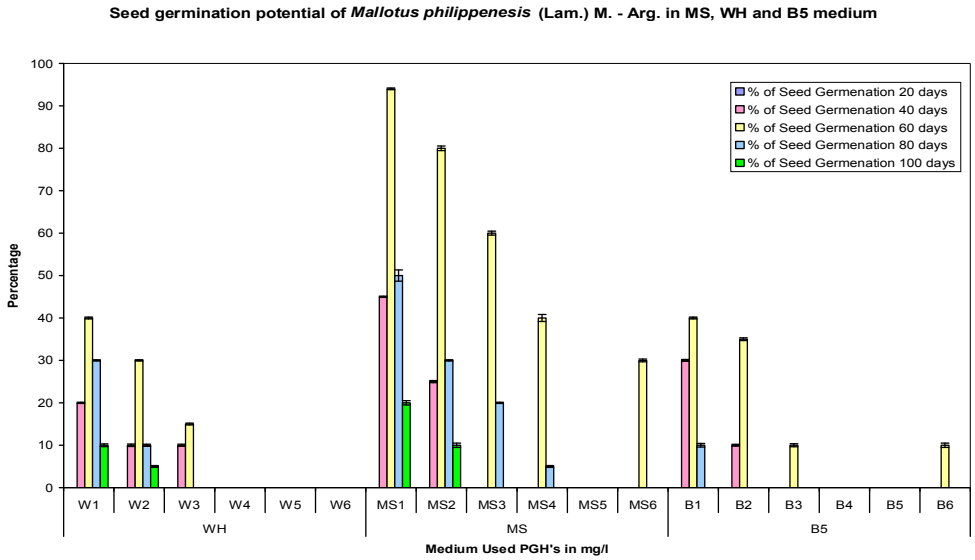


Figure 2. Showing seed germination of Mallotus phillippenesis (Lam.) M. Arg.

Table 1. Seed germination potential of *Mallotus philippenesis* (Lam.) M. - Arg. in MS, WH and B5 medium

Medium Used PGH's in mg/l			% of germination ± S.E. after 15 days					% of Seedling Growth (cm) after 25 days	Callus induction intensity
			20 days	40 days	60 days	80 days	100 days		
WH	W1	1/2 con.	0.0 ± 0.0	20 ± 0.14	40 ± 0.20	30 ± 0.14	10 ± 0.33	6± 1.34	-
	W2	Full con.	0.0 ± 0.0	10 ± 0.28	30 ± 0.14	10 ± 0.24	5 ± 0.21	5 ± 1.14	-
	W3	C 0.1 BAP	0.0 ± 0.0	10 ± 0.24	15 ± 0.21	0.0 ± 0.0	0.0 ± 0.0	5± 1.28	-
	W4	C 0.2 BAP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	W5	C 0.1 BAP + 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	W6	C 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	+
MS	MS1	1/2 con.	0.0 ± 0.0	45 ± 0.15	94 ± 0.20	50 ± 1.32	20 ± 0.49	10 ±1.48	-
	MS2	Full con.	0.0 ± 0.0	25 ± 0.25	80 ± 0.54	30 ± 0.14	10 ± 0.49	8 ± 0.97	-
	MS3	C 0.1 BAP	0.0 ± 0.0	0.0 ± 0.0	60 ± 0.45	20 ± 0.14	0.0 ± 0.0	6 ± 0.56	-
	MS4	C 0.2 BAP	0.0 ± 0.0	0.0 ± 0.0	40 ± 0.83	5 ± 0.21	0.0 ± 0.0	6 ± 0.41	-
	MS5	C 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	MS6	C 0.2 BAP + 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	30 ± 0.31	0.0 ± 0.0	0.0 ± 0.0	4± 0.49	++
B5	B1	1/2 con.	0.0 ± 0.0	30 ± 0.20	40 ± 0.20	10 ± 0.44	0.0 ± 0.0	6 ± 0.38	-
	B2	Full con.	0.0 ± 0.0	10 ± 0.20	35 ± 0.32	0.0 ± 0.0	0.0 ± 0.0	4 ± 0.48	-
	B3	C 0.1 BAP	0.0 ± 0.0	0.0 ± 0.0	10 ± 0.37	0.0 ± 0.0	0.0 ± 0.0	4± 0.24	-
	B4	C 0.2 BAP	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	-
	B5	C 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0± 0.0	-
	B6	C 0.2 BAP + 0.1 NAA	0.0 ± 0.0	0.0 ± 0.0	10 ± 0.51	0.0 ± 0.0	0.0 ± 0.0	4 ± 0.37	+

Mean ± S.E. of 3 replications of 10 cultures

Media used: MS basal medium + 3% sucrose + 0.48% agar culture
Condition: 16h photoperiod and 25± 2C

The results of the present study on endosperm culture of M, phillipiensis investigates that why the plants of mallotus are going to be extinct in the natural habitat. Thus a simple and efficient method has been developed for plant formation from immature seeds of M.phillipienesis to increase the production of the plant which is suitable for the conservation of germplasm and to meet its demand in medicine and agriculture**.**

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