

Development of Proliferation and Acclimatization of *Melia dubia*-Australian Teak Variety through Micropropagation

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ABSTRACT: *Melia dubia* is the Australian Teak wood variety or Forest neem. This study is aimed at the Standardization of *Invitro* propagation for the proliferation of *Melia dubia* for the production of saplings trailed with various explants. Bud break of *Melia dubia* was followed by multiplication, elongation, differentiation, shooting; Rooting and Hardening that resulted in the production of tissue culture *Melia dubia* plant. The initiated leaves explants were carried over to differentiation stage for the formation of shoots and the recorded response was about 84%. The multiplied plants were transferred to elongation phase for the nodal explants and the best response of the elongated shoot was found to be 88% unlike leaves explants whose elongation response was 82% followed by the Rooting stage which is important for the growth of any plant. It was observed that the activated charcoal addition to the media helped in the higher percentage in root induction of about 90% than 2,4-D of about 85% almost both in the leaves and nodes as explants. Hardening was the final trial done after *Invitro* technique which was observed for the acclimatization efficiency where the rooted plants were successfully acclimatized using the combination of several media trials and the recorded mortality was observed to be 3%. This proved the possibility of the production of *Melia dubia* as multipurpose plant through Micropropagation for the replacement of timber varieties and also for its valuable therapeutic properties.

KEYWORDS: Meliadubia, Differentiation, Shooting, Rooting, *Invitro*, Acclimatization.

I. INTRODUCTION

Melia dubia Cav. Family Meliaceae is an indigenous species to India, Southeast Asia and Australia. *Melia dubia* is the fastest growing tree and the wood from this tree is used in Plywood Industry [1]. 400 trees can be planted in an acre that fetches 15-20 lakhs in 6 - 7years.Meliadubia is a good energy source crop and allows more diversity than bamboo species [10].

Characteristics

- Height upto 25m.
- Leaves: Alternate, 20-40 cm long,
- bipinnate or occasionally tripinnate.
- Leaflets 3-11, with serrate edges.
- Seed viability: 1 to 3 years [2, 10]

Importance

It is a fast growing multipurpose tree species, which is in high demand in plywood industry and high-quality termite and fungal resistant timber and has potential to use in biomass power plants [3]

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Acclimatization challenges

The benefit of any micropropagation system can, however, only be fully realized by the successful transfer of plantlets from tissue-culture vessels to the ambient conditions found *ex vitro*. Most species grown *invitro* require an acclimatization process in order to ensure that sufficient number of plants survive and grow vigorously when transferred to soil [4, 10].

II. MATERIALS AND METHODS

Differentiation of Developed Callus

Explant sterilization and Bud break of the selected explants were already observed [10]. The Leaves explants after the initiation stage were differentiated as callus regeneration is Indirect organogenesis. In order to induce shoot formation from the callus, the callus must be differentiated [3]. The callus was inoculated in following MS basal media treatments + Sucrose 3% with various growth regulator concentrations [11].

COMBINATION OF GROWTH REGULATORS	CONCENTRATION (mg/l)
6BAP + IAA	1 + 1
	2 + 1
	3 + 1
	4 + 1
	5 + 1
	1 + 2
	2 + 2
	3 + 2
	4 + 2
	5 + 2

Table 1: DIFFERENTIATION MEDIA

Leaves which were observed for callus formation should be differentiated further and processed for Multiplication. The callus was sub-cultured in the above trialed media and the inoculated jars were incubated [12].

Multiplication

The shoots arising from the Nodal parts were further transferred to multiplication stage for rapid propagation [7]. The emerged shoots were properly trimmed and inoculated in the media consisted of basal MS media + 3% Sucrose with the following [7].

The leaves of *Meliadubia* which were initiated and gave rise to callus were then differentiated for the formation of shoots. The cultures raised shoots were then multiplied for the mass production using the MS media with several combination trials using growth regulators [8]

EXPLANTS	COMBINATION OF GROWTH REGULATORS	CONCENTRATION (mg/l)
Differentiated callus and Nodes	NAA + 6BAP + GA	0.1 + 0.2 + 1
		0.1 + 0.3 + 1
		0.1 + 0.4 + 1
		0.1 + 0.5 + 1
		0.2 + 0.2 + 2
		0.2 + 0.3 + 2
		0.2 + 0.4 + 2
		0.2 + 0.5 + 2

Table 2: MULTIPLICATION MEDIA

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This multiplication media was prepared with the combination of auxins and cytokinins for the shoot proliferation.

Culture Conditions

The inoculated jars were subjected to light intensity for 10-12 h in the growth room for rapid multiplication. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%. The observation for the shoot formation was monitored often [9].

Elongation of shoots

The nodal explants which had been multiplied have to be elongated to raise the shoots and the multiplied leaves explants should elongated which are very important for knowing the ability of the plants to grow longer before the induction of roots. It had been done by the preparation of media with various growth regulator combinations [8].

EXPLANTS	COMBINATION OF GROWTH REGULATORS	CONCENTRATION (mg/l)
Multiplied callus and Nodes	NAA + Kinetin + Adenine sulfate	0.05 + 1 + 30
		0.05 + 2 + 60
		0.05 + 3 + 90
		0.05 + 4 + 120
		0.1 + 1 + 30
		0.1 + 2 + 60
		0.1 + 3 + 90
		0.1 + 4 + 120

Table 3: ELONGATION MEDIA

Elongation media was trialled out with various combinations for the development of shoots in the leaves and nodes as explants.

Rooting

After the elongation of shoots in both nodal and leaves explants, the next stage was concentrated on the induction of roots in the elongated roots [11]. Roots are the heart of a plant, so it is very much necessary that the roots inducing by growth regulator combinations are very healthy. The following growth regulator combinations were trailed [6].

EXPLANTS	COMBINATION OF GROWTH REGULATORS	CONCENTRATION (mg/l)
Elongated callus shoots and Nodes	½ MS + Activated carbon + IBA	0.5 + 0.1
		0.5 + 0.3
		0.5 + 0.5
		0.5 + 0.7
		0.5 + 1
	½ MS + Activated carbon + 2, 4 - D	0.5 + 1
		0.5 + 2
		0.5 + 3
		0.5 + 4
		0.5 + 5

Table 4: ROOTING MEDIA

The rooting media was specialized for the induction of roots with excess concentration of auxins with half concentration of MS media [12].

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Culture Conditions

The explants are subjected under light intensity for 10-12 h in the growth room. Photoperiod provided by cool white fluorescent lamps of 1500-3000 lux, temperature of about 25 ± 2 °C and humidity of 35 - 40%. The observation for the root induction will be recorded after 4-5 weeks [10].

Acclimatization

Primary hardening:

- The rooted exagar *Melia dubia* plants were removed carefully.
- The plants were washed with running water without damaging roots
- The plants were rinsed thoroughly for the complete removal of medium and gelling agent traces.
- The plantlets were dipped in 2 gms /per liter of antifungal agent Carbendazim and planted in sterile coir pith medium having EC < 0.5 ms [3].
- The planted trays were kept in Hardening chambers maintaining the 5000-6000 lux light intensity and 60-75% RH and temperature between 25-38° C for 30 days.
- Periodic moisture check for 40 % was done and fertigation was given to ensure plant growth.

Secondary hardening:

- The rooted Primary hardened plants were removed from hardening trays.
- They were then planted in polybags (5 Cms x 7 Cms) containing soil: coir pith: sand in the ratio 1:1:1.
- The plants were maintained under the 75% green shade net maintaining the 12000-18000 lux light intensity and 40-50% RH and temperature between 25-40 ° C for 30 days.
- Periodic moisture check for 30 % was done and fertigation was given to ensure plant growth [4].

III. EXPERIMENTAL RESULTS

DIFFERENTIATION OF LEAVES:

The leaves were differentiated after its initiation [10] for the capability of the production of shoots out of the callus. The number of leaves produced from various trials also were observed and tabulated.

TRIAL MEDIA	MEAN SHOOT LENGTH FROM LEAVES (cm)	MEAN NUMBER OF LEAVES (cm)	RESPONSE %
DM1	1.51	1	20
DM2	1.71	4	84
DM3	1.22	2	52
DM4	1.19	1	47
DM5	1.17	1	44
DM6	0.98	2	41
DM7	0.84	1	38
DM8	0.61	1	34
DM9	0.43	2	31
DM10	0.21	1	24

*DM – Differentiation media

Table 5: DIFFERENTIATION RESPONSE BY LEAVES EXPLANT

It was recorded that the differentiation of callus was successful when 6BAP of 2 mg/l and IAA of 1 mg/l were used in the media which supported the growth of shoots and responded up to 84% proving the efficiency of the leaves raising to shoots through Indirect organogenesis.

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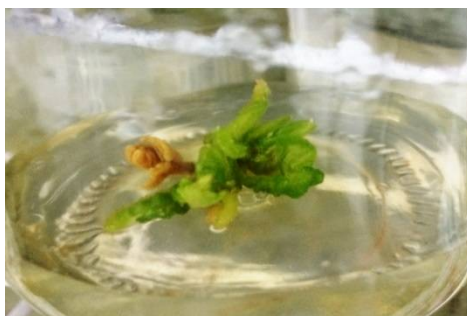


Figure 1: Differentiation of leaves

The image indicates the differentiation of leaves in the media with higher response which proves the ability of cytokinin in differentiation of cells.

MULTIPLICATION OF NODAL EXPLANTS AFTER DIFFERENTIATION:

The nodal explants were targeted for increasing its number by multiplying the single explant which was subcultured 4 times at an equal interval of time in order to observe the fold rate which increases gradually at each sub-culture.

TRIAL MEDIA	MEAN SHOOT LENGTH (cm)	MEAN NUMBER OF SHOOTS (cm)	MULTIPLICATION FOLD RATE			
			1 st SUB-CULTURE	2 nd SUB-CULTURE	3 rd SUB-CULTURE	4 th SUB-CULTURE
NM1	0.70	1	1	1.1	1.21	1.33
NM2	0.98	3	0.9	1.1	1.23	1.58
NM3	0.96	3	0.95	1.21	1.41	1.67
NM4	1.71	6	1.21	1.51	1.8	2.22
NM5	1.49	3	1.08	1.31	1.49	1.66
NM6	1.41	1	1	1.1	1.28	1.31
NM7	1.29	1	0.87	1.04	1.34	1.4
NM8	1.22	1	0.51	0.72	0.97	1.15

*NM – Nodal Multiplication

Table 6: MULTIPLICATION RESPONSE BY NODAL EXPLANTS

It was observed that the multiplication fold rate was extremely higher when the combinations of NAA of 0.1 mg/l, 6BAP of 0.5 mg/l and GA of 1 mg/l were used in the basal media which reached the fold rate 2.22 at the end of 4th sub-culturing.

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MULTIPLICATION OF LEAVES EXPLANTS AFTER DIFFERENTIATION:

Similar to the nodes, differentiated leaves were also multiplied for the assessment of fold rate increase at the end of 4 sub-cultures.

TRIAL MEDIA	MEAN SHOOT LENGTH (cm)	MEAN NUMBER OF SHOOTS (cm)	MULTIPLICATION RATIO			
			1 st SUB-CULTURE	2 nd SUB-CULTURE	3 rd SUB-CULTURE	4 th SUB-CULTURE
LM1	0.60	1	1	1.13	1.2	1.31
LM2	0.68	2	0.7	1.05	1.18	1.48
LM3	1.14	4	0.93	1.11	1.31	1.57
LM4	1.51	5	1.18	1.49	1.82	2.13
LM5	1.29	2	1.01	1.26	1.45	1.59
LM6	1.31	2	1.08	1.21	1.38	1.41
LM7	1.21	1	0.77	1.1	1.21	1.33
LM8	1.11	1	0.41	0.76	0.94	1.1

*LM – Leaves Multiplication

Table 7: MULTIPLICATION RESPONSE BY LEABES EXPLANTS

It was observed that the multiplication fold rate was extremely higher when the combinations of NAA of 0.1 mg/l, 6BAP of 0.5 mg/l and GA of 1 mg/l were used in the basal media which reached the fold rate 2.13 at the end of 4th sub-culturing. Nodal multiplication fold was higher than the leaves multiplication. Nodal multiplication was achieved through Direct organogenesis

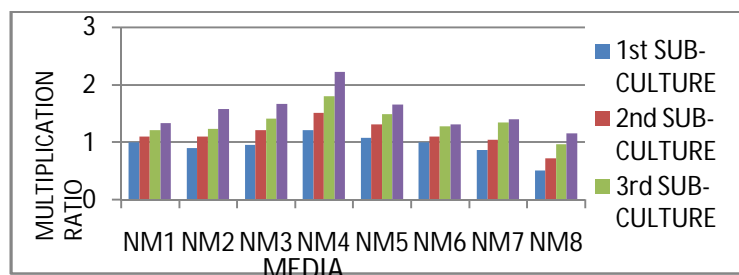


Figure 2: Graph showing Multiplication response by Nodal explants

The above graph shows the efficiency of media in proliferation up to 4 subcultures in increasing the fold rate in nodes which showed higher ratio of 2.22 at 4th sub-culture.

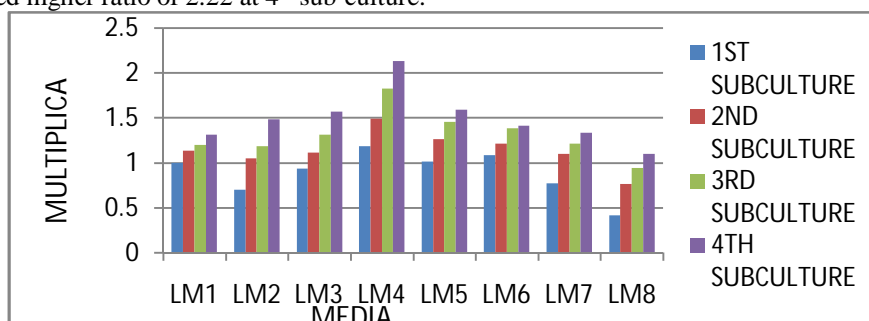


Figure 3: Graph showing Leaves Multiplication response

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The above graph shows the efficiency of media in proliferation upto 4 subcultures in increasing the fold rate in leaves as Indirect organogenesis which showed comparatively higher ratio of 2.13 at 4th sub-culture unlike nodes.

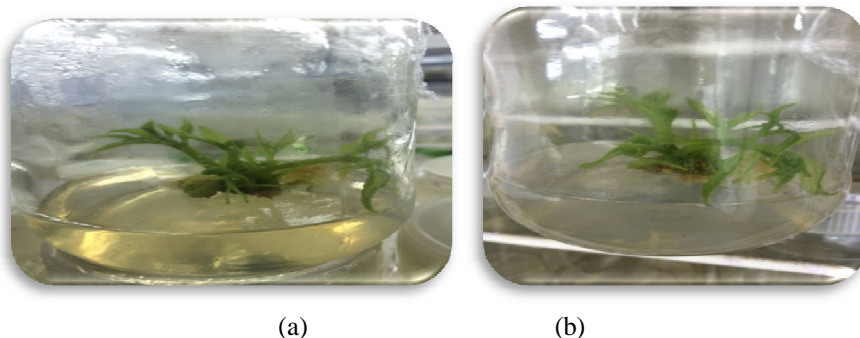


Figure 4: Multiplication response (a) by nodes (b) by Leaves

Multiplication stage of the nodal explants was found with the multiplication ratio of 2.22 folds at the end of 4th sub-culturing which showed constant increase in the ratio from the 1st sub-culturing. The mean shoot number from the trialed nodal explants was found as 6 and the mean shoot length was observed as 1.71 cm. the higher multiplication ratio was found with the combination of 6BAP – 0.5 mg/l + NAA – 0.1 mg/l + GA – 1 mg/l whereas the other combinations led to the low multiplication ratio that showed slow growth rate with less number of shoots and shoot length.

ELONGATION OF NODAL SHOOTS:

The multiplied nodal shoots were proceeded for Elongation to obtain the maximum shoot height. Various trials were done to compare the efficiency of the nodal shoots.

TRIAL MEDIA	MEAN SHOOT LENGTH (cm)		MEAN NUMBER OF SHOOTS (cm)		RESPONSE %	
	NODAL SHOOT	CALLUS SHOOT	NODAL SHOOT	CALLUS SHOOT	NODAL SHOOT	CALLUS SHOOT
EM1	1.15	1.08	4	3	20	18
EM2	1.11	1.01	3	2	24	23
EM3	1.54	1.13	4	3	26	27
EM4	1.61	1.18	3	3	38	32
EM5	3.56	2.65	6	4	88	78
EM6	3.01	2.4	4	3	69	70
EM7	2.41	2.19	2	2	45	33
EM8	2.07	1.98	2	3	40	21

*EM – Elongation Media **Table 8: Elongation response of Nodal shoots**

The elongation media was trialed out both in the callus and nodal shoots and the efficiency was compared by recording the shoot height and number of shoots produced by them.

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(a) (b)

Figure 5: Elongation response (a) By nodes (b) By Leaves

The elongation was successful in both the nodes and leaves as explants but the shoot length was higher with nodes as explants compared to leaves where the response reached 88% in nodes and 78% in leaves proving the fastest responding explant as nodes.

ROOTING:

Roots were induced for the plants after the elongation of shoots in both the nodes and leaves as explants

GROWTH REGULATORS TRIALED		NO OF SHOOT BOTTLES TAKEN	NO OF ROOTS		LENGTH OF ROOTS (cm)		MEAN ROOTING RESPONSE (%)	
IBA	2,4-D		IBA	2,4-D	IBA	2,4-D	IBA	2,4-D
0.1	1	20	6	7	1.5	1.53	30	35
0.3	2		18	17	2.91	2.93	90	85
0.5	3		10	11	2.09	2.14	50	55
0.7	4		9	10	1.63	1.84	45	50
1	5		8	9	1.57	1.71	40	45

Table 9: MEAN ROOT RESPONSE FROM NODES AS EXPLANTS

The Rooting efficiency was recorded in nodes and compared against the leaves as explants

GROWTH REGULATORS TRIALED		NO OF SHOOT BOTTLES TAKEN	NO OF ROOTS		LENGTH OF ROOTS (cm)		MEAN ROOTING RESPONSE (%)	
IBA	2,4-D		IBA	2,4-D	IBA	2,4-D	IBA	2,4-D
0.1	1	20	3	7	1.23	1.2	22	27
0.3	2		11	10	2.5	2.54	84	78
0.5	3		6	7	2.02	2.16	35	48
0.7	4		4	5	1.34	1.57	28	33
1	5		4	4	1.28	1.51	22	27

Table 10: MEAN ROOT RESPONSE FROM LEAVES AS EXPLANTS

According to Bhimi Ram et al., 2012, long roots were developed on MS/2 medium (Table 3, Figure 1G), which was distinctly found better than the MS/4 and full strength MS medium. IBA (0.3mg/l) in MS/2 medium was found to be most effective in term of rooting frequency (90%) with maximum root length (5.6 cm±0.48) which induced directly

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from the basal end of shoot, whereas, 0.5 mg/l IBA in full MS medium was foundless effective in terms of rooting frequency (30%) with minimum root length (0.5 cm±0.16).

In this study, rooting stage of the nodal explants was found highest with the Trial 1 which responded about 90% when compared to Trial 2 of 85% and the length of the roots was higher I Trial 2 with 2.93 cm. This stage was very successful which the end of tissue culture process is. After a period of 3-4 weeks, the rooted plants were hardened.



(a) (b)
Figure 6: Rooting Response (a) By Nodes (b) By leaves

Rooting response was clearly witnessed by the above image which was found to be thick and lengthy in nodes than in the leaves.

HARDENING:

Acclimatization is the final challenge to be faced with any plant after its growth in vitro. In this study, the primary hardening was achieved successfully with NPK solubilizing bacteria as treatment for the plants whose survival percentage was 93 better than the other treatment using 19:19:19 where the NPK did not reach plants because of the inability of plants to absorb chemical fertilizers easily. After a period of 45 days, the plants were transferred to secondary hardening stage for the better development of roots.

STAGE	SOIL TYPE	ROOTED PLANTLETS TAKEN (nos)	SURVIVAL UNDER EXVIVO CONDITIONS (%)	HEIGHT OF THE SURVIVED PLANT (cm)
PRIMARY HARDENING	Sterilized coir pith + NPK Solubilizing bacteria	50	93	2.21
	Sterilized coir pith + 19:19:19 fertilizer	50	69	1.5
PRIMARY HARDENED PLANTS TAKEN				

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SECONDARY HARDENING	Coir pith + 19:19:19	50	74	4.3
	Soil: Coir pith : Sand + 19:19:19	50	88	4.1

Table 11: ACCLIMATIZATION EFFICIENCY

The acclimatization protocol was standardized in *Meliadubia* using sterilized coir pith and the use of NPK solubilizing bacteria which was compared against the chemical fertilizer 19:19:19 using the 1:1:1 ratio of soil:sand: coir pith whose growth reached to 88% when NP solubilizing bacteria was used as a treatment.



Figure 7: Acclimatization efficiency of *Meliadubia*

The acclimatization efficiency in the above image shows the plantlet after the complete hardening with the healthy roots and thick stem and greener leaves which explains the healthiness of the plant through tissue culture.

IV. DISCUSSION

In the research study, after the bud break [10], the differentiation of leaves were continued with 10 trial media in which higher differentiation was observed as 84% with shoot length of 1.71 cm in DM2 unlike other trial media. Further, the process continued with its multiplication to record the multiplication ratio. It was observed as 2.13 as multiplication ratio in 4 sub cultures which was gradually increasing during the subculturing. For the nodes, the multiplied shoots were elongated in different trial media which showed the elongation efficiency as 88% in EM5. After the elongation, both explants were processed for Rooting stage in which rooting response was higher in Nodal explants with 90% and 2.91 cm in length in RM2 whereas the rooting response in Leaves as explants was found as 85% and 2.93 cm in length in RM2 through Indirect organogenesis. The rooted explants were acclimatized to the environment through Hardening process where Mortality observed was only 2% when soil, sand and coir pith were used as media in the equal ratio, death rate was 4% which was found as highest in red soil as media.

V. CONCLUSION

Direct organogenesis was succeeded till the hardening stage where we were able to produce mass number of *Meliadubia* plants through Tissue culture. The plants were able to acclimatize itself in the media given for it and found

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2% mortality only in HI media. In this study, Both Direct and Indirect organogenesis were achieved successfully which is ideally a fast approach today for the production of medicinal plants and economic plants in a shorter duration of time.

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REFERENCES

1. Hsu, Y., Wu,C.,Chem,J., YangY., Wang, "The presence of three isoflavonoid compounds in *Psoralea corylifolia*", Journal of Chromatographic Science, Vol.39,pp. 441- 444, 2001.
2. James,C.,"Global Status of Commercialized Biotech/ GM Crops", ISAAA Brief No. 39. Ithaca, NY.,Vol. 243, 2008
3. Kaufman, P., Duke, P., Briemann,J.,Boik, H., Hoit, J.,"A comparative survey of leguminous plants as a source of the isoflavones, genistein and daidzein: Implications for human nutrition and health", Journal of Alternative and Complementary Medicine, Vol.3,pp. 7-12, 1997.
4. Khan, M., Kihara, M., Omolosa,A., "Antimicrobial activity of *Horsfieldiahelwigii* and *Meliaazedarach*",Fitoterapia, Vol. 72, pp.423-427, 2001.
5. Kirtikar, K. R.,Basu,B. D., "Indian Medicinal Plants", Second edition (Published by Lalit Mohan Basu, Allahabad, India), Vol. 2, pp.126-127, 1935.
6. Kiritkar, K.R.,Basu,B.D., "Advanced Indian Medicinal Plants", International Book Distributors, Dehradun, India, Vol. 1,pp. 75-196, 1999.
7. Linnaeus and Carl Von, "Species Plantarum", Vol.1,pp. 384-385, 1753.
8. Manjunatha, K.B.,"Clonal propagation of *Meliadubia*(Cav)", Vol. 43,pp. 455-458, 2007.
9. Margarat E. Daup, "Tissue Culture and the Selection of Resistance to Pathogens", Annual Review of Phytopathology, Vol. 24,pp.159-186, 1986.
10. Aishwarya, R.,Saravana Kumar, V. G.," Standardization of Explant Bud Break in *Meliadubia*- Australian Teak Using Tissue Culture Techniques", International Journal of Science and Research, Vol.5, no. 6, pp.2383 – 2387, 2016
11. Pettit, G.R., "Anti-neoplastic agents:489, Isolation and structure of meliastatins and related euphanetriterpenes from tree *Meliadubia*",Journal of Natural Products, Vol 65, pp. 1886 1891, 2002.
12. Vijayan P., Raghu C., Ashok G., Dhanaraj S.A., Suresh B., "Antiviral activity of medicinal plants of Nilgiris", Indian Journal of Medicinal Research, Vol 120: 24, 2004