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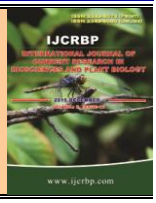

Original Research Article

In Vitro Propagation of *Litsea glutinosa* (Lour) C.B. Robinson - An Endangered Medicinal Tree in Madhya Pradesh, India

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Abstract

Litsea glutinosa (Lour) C.B. Robinson is a member of family Lauraceae and it is small tree commonly known as Maida lakdi. It is found through in India such as Andhra Pradesh, Madhya Pradesh, Chhitgarh, Orissa, Western ghat and outer Himalayas. In Madhya Pradesh this species has been reported in Hosangabad, Chhindawara, Anuppur, Mandla Balaghat Satna and Rewa districts in mixed forest areas, along with streams and hilly slopes. It was distributed throughout Madhya Pradesh but due to over exploitation it becomes endangered now. *In vitro* propagation of the species has been achieved through nodal explants. The nodal explants of 1-2 cm length were inoculated on to MS medium with supplemented plant growth regulators, IAA and BAP for multiplication and IBA and NAA for rooting. Multiple shoots (3.5 shoots per explants with 5.07 cm length) were recorded after 50 days in the MS+IAA+BAP (2:3mg/lit.). Multiple roots (4.50 roots per explants with 4.50 cm length) were recorded after 70 days in the MS+IAA+IBA (0.1:2 mg/lit.). The plant survival rate was found 15% during hardening.

Keywords

Growth regulators

Litsea glutinosa

Propagation

Introduction

Litsea glutinosa (Lour) C.B. Robinson is a member of family Lauraceae and is well known for the evergreen small tree commonly known as Maida lakdi, and found at tropical and sub-tropical Asia (CSIR, 1998). *Litsea glutinosa* is found through in India such as Andhra Pradesh, Madhya Pradesh, Chhitgarh, Orissa, Western Ghats and outer Himalayas. In Madhya Pradesh this species has been reported from mixed forest areas, along with streams and hilly slopes. It was distributed throughout Madhya Pradesh but due to over exploitation it becomes endangered now. It is found in mixed primary and secondary forest and thickets throughout India and in the outer Himalayas' (Kirtikar and Basu1981). As per the IUCN category the species is listed in endangered category (Mudgal et.al., 1997).

Litsea glutinosa (Lour.) C. B. Rob., an evergreen or deciduous tree that reaches 3-15 m. It is a polymorphic species. Its leaves are alternate and elliptical to oblong-elliptical 3.5–10 × 1.5–11 cm, velvety (particularly when young) or glabrous. Umbels contain many small yellowish flowers, with 8-20 stamens for male flowers.

Flowering occurs between March and June and fruits appear in September-October. Fruits are round and about 8 mm or less in diameter. The tree is able to reproduce vegetatively: over half of the stems are produced by vegetative reproduction, mostly root-suckering (Rabena, 2010).

Litsea glutinosa is a multipurpose, fast-growing tree. The leaves are chopped and soaked in water to make plaster in the Northern Philippines. While *Litsea glutinosa* gives a poor timber due to a low wood density, it is often used as fuel (Rabena, 2008). The species is highly recalcitrant in nature as the seeds are very small in size and the seed germination is also very poor (Soerianegara, 1995). It is a good coppicer and new shoots sprouting from damaged adventitious buds (called coppice shoots) are mainly used for propagation (Training manual, 2002).

The conventional propagation is hampered due to low seed viability and no rooting of vegetative cuttings (Rabena, 2010). Thus there is need for alternative in vitro propagation method for large scale multiplication, improvement and conservation of the species. The objective of the study was to develop a procedure for its micropropagation.

Materials and methods

The study was carried out in the plant tissue culture laboratory at State forest research, Jabalpur, India.

Planting material

The planting material cuttings were collected from different agro climatic zone of M.P. and planted in mist chamber. Single nodal segment of 1.5-2.5 cm length were collected from cutting and used as explants. The explants were washed with 2% liquid detergent Extran (MERCK) for 5-6 min solution to removing dust particles. This was followed by the treatment of 1% solution of Bavistin (BASF, Mumbai) broad spectrum fungicides for 5-10 min to minimize fungal contamination and then, these were washed 3-4 time distilled water. The washed explants were sterilized for 1-9 min. with mercuric chlorite (Hi-Media, Mumbai, India). After the HgCl2 treatment explants were washed with sterilized distilled water for 3-4 times under laminar air flow cabinet. Explants were inoculated in culture tube containing Murashige and Skoog (1962) with supplemented auxin and cytokinin. Culture was kept in culture room at 25±2^{o} and light for 16 h photo period. Observation recorded every 10 days.

Results and discussion

The data presented in Table 1 indicated that $HgCl_2$ concentration 0.1% and timing of the concentration is important factor for reducing contamination. The percentage of contamination–free explants varied from 30-100%. The lowest value (30%) was recorded with 4 min. treatment (0.1%) whereas the highest value (100%) was obtained with 7 min. treatment.

Table 1. Effect of $HgCl_2$ (0.1%) treatment period on sterilization of nodal segments of *Litsea glutinosa*.

Treatment duration (min.)	No. of explants	Rate of contamination (After days of treatment)					Contamination free explants after ten days (%)
		2nd	4th	6th	8th	10th	
1	10	-	6	9	10	10	0
2	10	-	5	10	10	10	0
3	10	-	3	6	9	10	0
4	10	-	2	5	7	7	30
5	10	-	-	1	4	5	50
6	10	-	-	-	1	1	90
7	10	-	-	-	-	-	100
8	10	-	-	-	-	-	100*
9	10	-	-	-	-	-	100**

--Indicate no contamination; *Indicate culture death due to tissue killing; (* 60-80%)(**80-100%)

Shoot multiplication

Shoot induction was recorded after 10 days of fresh culturing. But Multiple shoots (3.60 shoots per explants with 5.12 cm length) were recorded after 50 days (Table 2) in the MS+IAA+BAP (2:3mg/lit.). The shoot multiplication stage of *Litsea glutinosa* is shown in Fig. 1(A).

In vitro rooting

Root induction was recorded after 20 days of sub culturing. But Multiple roots (4.50 roots per explants with 4.50 cm length) were recorded after 70 days (Table 3) in the MS+IAA+IBA (0.1:2 mg/lit.). The rooting stage of *Litsea glutinosa* is shown in Fig. 1(B).

Table 2A. *In vitro* multiplication of *Litsea glutinosa* (Oneway ANOVA).

Parameter	Treatments/ PGRs combination	No.	Mean	Std. Deviation	Std. Error	95% Confidence interval for Mean		Minimum	Maximum
	IAA+BAP					Lower Bound	Upper Bound		
Shoot no.	T1 (2:3 mg/lit)	3	3.5700	0.03000	0.01732	3.4955	3.6445	3.54	3.60
	T2 (2:4mg/lit)	3	2.0000	0.10000	0.05774	1.7516	2.2484	1.90	2.10
	T3 (2:5 mg/lit)	3	1.5200	0.02000	0.01155	1.4703	1.5697	1.50	1.54
	Total	9	2.3633	0.93008	0.31003	1.6484	3.0783	1.50	3.60
Length	T1 (2:3 mg/lit)	3	5.0700	0.07000	0.04041	4.8961	5.2439	4.99	5.12
	T2 (2:4mg/lit)	3	5.2100	0.01000	0.00577	5.1852	5.2348	5.20	5.22
	T3(2:5 mg/lit)	3	3.8700	0.01000	0.00577	3.8452	3.8948	3.86	3.88
	Total	9	4.7167	0.63889	0.21296	4.2256	5.2078	3.86	5.22

Table 2B. *In vitro* multiplication of *Litsea glutinosa* (Post Hoc Tests, LSD).

Dependent Variable	(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Shoot no.	T1	T2	1.57000 (*)	0.05011	0.000	1.4474	1.6926
		T3	2.05000 (*)	0.05011	0.000	1.9274	2.1726
	T2	T1	-1.57000 (*)	0.05011	0.000	-1.6926	-1.4474
		T3	0.48000 (*)	0.05011	0.000	0.3574	0.6026
	T3	T1	-2.05000 (*)	0.05011	0.000	-2.1726	-1.9274
		T2	-0.48000 (*)	0.05011	0.000	-0.6026	-0.3574
Length	T1	T2	-0.14000 (*)	0.03367	0.006	-0.2224	-0.0576
		T3	1.20000 (*)	0.03367	0.000	1.1176	1.2824
	T2	T1	0.14000 (*)	0.03367	0.006	0.0576	0.2224
		T3	1.34000 (*)	0.03367	0.000	1.2576	1.4224
	T3	T1	-1.20000 (*)	0.03367	0.000	-1.2824	-1.1176
		T2	-1.34000 (*)	0.03367	0.000	-1.4224	-1.2576
* The mean difference is significant at the .05 level.							

Table 3A. *In vitro* rooting response in *Litsea glutinosa* (Oneway ANOVA).

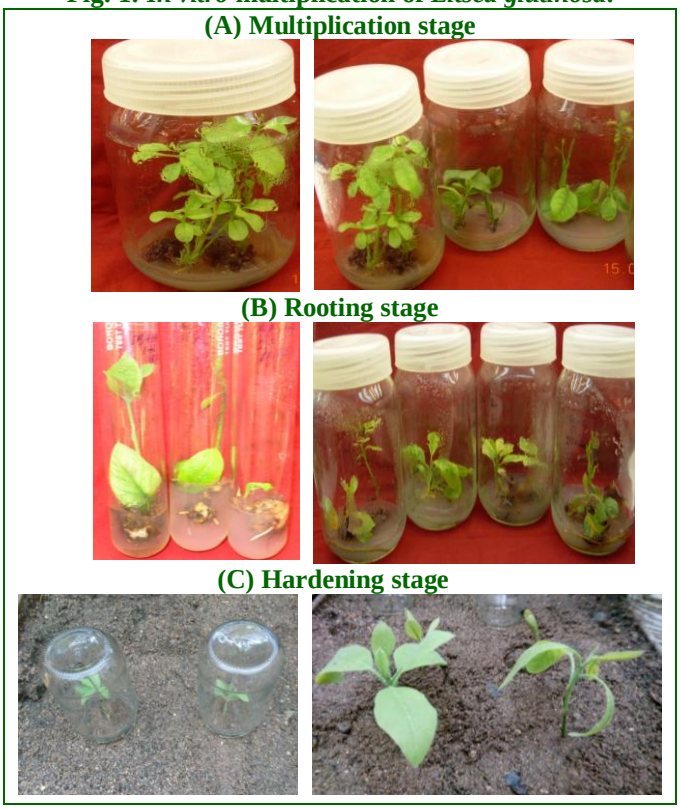
Parameter	Treatments/ PGRs combination	No.	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
						Lower Bound	Upper Bound		
Root No.	t1= IBA 1mg/lit.	3	2.0000	0.50000	0.28868	0.7579	3.2421	1.50	2.50
	t2= IBA 2mg/lit.	3	4.0000	0.50000	0.28868	2.7579	5.2421	3.50	4.50
	t3= IBA 3mg/lit.	3	3.0000	0.50000	0.28868	1.7579	4.2421	2.50	3.50
	Total	9	3.0000	0.96825	0.32275	2.2557	3.7443	1.50	4.50
Root length	t1= IBA 1mg/lit.	3	2.0000	0.50000	0.28868	0.7579	3.2421	1.50	2.50
	t2= IBA 2mg/lit.	3	4.0000	0.50000	0.28868	2.7579	5.2421	3.50	4.50
	t3= IBA 3mg/lit.	3	3.0000	0.50000	0.28868	1.7579	4.2421	2.50	3.50
	Total	9	3.0000	0.96825	0.32275	2.2557	3.7443	1.50	4.50
Shoot length	t1= IBA 1mg/lit.	3	3.6500	0.05196	0.03000	3.5209	3.7791	3.62	3.71
	t2= IBA 2mg/lit.	3	4.6133	1.62562	0.93855	0.5751	8.6516	3.64	6.49
	t3= IBA 3mg/lit.	3	6.2100	0.45211	0.26102	5.0869	7.3331	5.69	6.51
	Total	9	4.8244	1.40225	0.46742	3.7466	5.9023	3.62	6.51

Table 3B. *In vitro* rooting response in *Litsea glutinosa* (Post Hoc Tests LSD).

Dependent Variable	(I) Treatments	(J) Treatments	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Root no.	t1= IBA 1mg/lit.	t2= IBA 2mg/lit.	-2.00000(*)	0.40825	0.003	-2.9989	-1.0011
		t3= IBA 3mg/lit.	-1.00000(*)	0.40825	0.050	-1.9989	-0.0011
	t2= IBA 2mg/lit.	t1= IBA 1mg/lit.	2.00000(*)	0.40825	0.003	1.0011	2.9989
		t3= IBA 3mg/lit.	1.00000(*)	0.40825	0.050	0.0011	1.9989
	t3= IBA 3mg/lit.	t1= IBA 1mg/lit.	1.00000(*)	0.40825	0.050	0.0011	1.9989
		t2= IBA 2mg/lit.	-1.00000(*)	0.40825	0.050	-1.9989	-0.0011
Root length	t1= IBA 1mg/lit.	t2= IBA 2mg/lit.	-2.00000(*)	0.40825	0.003	-2.9989	-1.0011
		t3= IBA 3mg/lit.	-1.00000(*)	0.40825	0.050	-1.9989	-0.0011
	t2= IBA 2mg/lit.	t1= IBA 1mg/lit.	2.00000(*)	0.40825	0.003	1.0011	2.9989
		t3= IBA 3mg/lit.	1.00000(*)	0.40825	0.050	0.0011	1.9989
	t3= IBA 3mg/lit.	t1= IBA 1mg/lit.	1.00000(*)	0.40825	0.050	0.0011	1.9989
		t2= IBA 2mg/lit.	-1.00000(*)	0.40825	0.050	-1.9989	-0.0011
Shoot length	t1= IBA 1mg/lit.	t2= IBA 2mg/lit.	-0.96333	0.79579	0.272	-2.9105	0.9839
		t3= IBA 3mg/lit.	-2.56000(*)	0.79579	0.018	-4.5072	-0.6128
	t2= IBA 2mg/lit.	t1= IBA 1mg/lit.	0.96333	0.79579	0.272	-0.9839	2.9105
		t3= IBA 3mg/lit.	-1.59667	0.79579	0.092	-3.5439	0.3505
	t3= IBA 3mg/lit.	t1= IBA 1mg/lit.	2.56000(*)	0.79579	0.018	0.6128	4.5072
		t2= IBA 2mg/lit.	1.59667	0.79579	0.092	-0.3505	3.5439

* The mean difference is significant at the .05 level.

Fig. 1: *In vitro* multiplication of *Litsea glutinosa*.



Hardening of *in vitro* regenerated shoots

The *in vitro* regenerated shoots were carefully washed in running tap water, so as to remove media from the surface of the roots. Plantlet placed on mixture sand, soil and FYM (1:1:1) were used for the hardening and covered with bottles for 2-3 days for maintaining humidity. The plant survival rate was found 15% during hardening. The temperature of the mist chamber was ranged between 35 ±5ºC with relative humidity of 80 to 90% (Fig. 1C).

Conclusion

The present study, *in vitro* micropropagation protocol was developed through nodal explants of *Litsea glutinosa*. High frequency of multiplication of shoot after 50 days (3.5 shoots per explants with 5.07 cm length) was obtained from nodal shoot segment in MS medium with IAA+BAP (2:3mg/lit.).

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