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Micropropagation of *Madhuca latifolia* Macb. through nodal culture

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Abstract

Micropropagation of mahua, *Madhuca latifolia* Macb. (Sapotaceae) was achieved by culturing excised nodes on WPM supplemented with different plant growth regulators. A combination of IBA with BA or kinetin was found suitable for axillary shoot initiation within a week of culture. Excised microshoots treated with IBA produced roots. Best regenerative response was observed from explants collected in May.

1. Introduction

Madhuca latifolia Macb. (Syn. *M. indica* JF Gmel: Syn *Bassia latifolia* Roxb; Vern mahua family Sapotaceae) is a multipurpose, deciduous, hardwood, tree species (Venkatesh 1988) valued for its flowers, seeds and wood (Sastri 1962). Mahua flowers are a rich source of carbohydrates, proteins, minerals and vitamins and are eaten raw. Mahua seeds are the source of oil commonly known as mahua butter variously used in Central State of Madhya Pradesh in India. It has a great potential to be used for its wood (Maithani 1990) states (Kuruvilla 1989, Prasad 1993).

Despite its significance mahua has remained non-conserved and often cut to meet the growing demand of constructional timber and fuelwood (Prasad 1991). This plant is mainly seed propagated. However, the seeds are recalcitrant and susceptible to fungal attack (Prasad 1993). As a result, opportunities for its propagation and improvement are limited. Micropropagation has a great potential in cloning of genetically improved strains for raising new plantations to increase forest productivity (Dunstan and Thorpe 1986, Boulay 1987). Reports on the regeneration of *M. latifolia* plantlets are few (Singh and Bansal 1994). The present study deals with the micropropagation of *M. latifolia* through nodal culture.

2. Materials and Methods

Nodal segments (length 10 × diameter 3-5 mm) were collected from physiologically mature shoots from higher crown of 15-20 years old phenotypically superior (plus) trees around the Rani Durgavati University Campus, Jabalpur (Madhya Pradesh) India situated between 22°49' and 24°8' North latitude and 78°21' and 80°58' East longitude. After thorough wash with running tap water, Teepol (2%) (Labolene, Glindia, India) (7-10 min) and distilled water the explants were immersed in 70% ethanol (3 min) and rinsed with distilled water (3-4 time). The explants were finally surface disinfected with 0.1% mercuric chloride (10 min) and rinsed in distilled water 4-5 times. The explants were dried between pieces of sterile filter paper and placed both vertically as well as horizontally on solidified WPM (Woody Plant Medium) (Lloyd and McCown 1980) supplemented with different concentrations of auxins (2,4-D, IBA and NAA) alone or cytokinins (kinetin and BA) alone as well as auxin and cytokinins combined together in Latin square design.

The pH of the medium was abjusted to 5.6-5.8 before adding 8 gl^{-1} agar (Qualigen, Bombay). The medium was sterilized by autoclaving at 1.1 $kg.cm^{-2}$ for 15 min. The cultures were kept under controlled conditions of temperature (23-27°C) and light provided by white fluorescent tubes during a 16 h photoperiod (40-50 μ mol m^{-2} s^{-1}) / 8h dark and 60-70% relative humidity (RH).

For each experiment a minimum of 15 cultures were raised and each experiment was conducted three times. Observations were recorded after an interval of 4 weeks when subcultures were made. Once culture conditions for optimum shoot formation from the explants were established, the individual shoots (1.5-2 cm long) were cultured for elongation on WPM supplemented with kinetin (1.66-46.4 μM), BA (0.44, 2.2, 44.4 μM), GA_3 (0.3-3.0 μM) or phloroglucinol (100-200 mgl^{-1}). The root induction media employed were half, quarter

Table 1. Effect of kinetin on shoot regeneration from *Madhuca latifolia* nodes cultured on WPM after 4 weeks of culture. Values are mean ± SE. Three replicates of 15 explants per treatment. Entries with the same letters are not significantly different at 5% level, according to Duncan's Multiple Range Test.

Kinetin (μM)	Percentage of culture forming	No. of shoots per explant	Shoot length (cm)
0.46	24.4 ± 3.81a	2.2 ± 0.10a	5.0 ± 0.44cd
1.66	38.2 ± 2.98abc	3.1 ± 0.22ab	5.8 ± 0.46e
2.32	35.5 ± 3.86ab	3.2 ± 0.31b	5.4 ± 0.46de
4.64	57.7 ± 3.86d	6.0 ± 0.34d	4.8 ± 0.42bc
23.20	48.8 ± 3.86cd	4.2 ± 0.42cd	4.0 ± 0.40a
46.40	42.2 ± 3.8bcd	3.2 ± 0.30bc	4.2 ± 0.42ab

strength, and full WPM both without as well as with activated charcoal (10 gml^{-1}) and with various concentrations of IBA (0.49, 2.46, 24.6, 49.2 μM) and NAA (0.53, 2.68, 5.37, 26.8, 53.7 μM). Duncan's multiple range test was performed to determine the difference between the various treatments.

3. Results and Discussion

3.1 Shoot Differentiation

Nodal explants did not elicit any morphogenic response on media lacking growth regulators. Of the cytokinins used, BA when used alone, failed to induce bud break from the explants but kinetin readily did it in separate treatment with all concentrations (**Table 1, Fig. 1**) within 1-2 weeks of inoculation. Kinetin was most suitable at 4.64 μM concentration. This observation agrees with the past findings (Jones 1983, Dodds 1983) that axillary shooting occurs as a result of release of axillary buds from apical dominance, i. e., the correlative inhibition of axillary bud out growth (Suzuki 1990). Cytokinins employed break dormancy of axillary buds present in the axis of each leaf.

The position and orientation of the explant on agar medium also influenced the growth response. Vertically placed nodal explants differentiated more shoots than explants placed horizontally (**Table 2**). This could possible be due to the influence on polarity of growth regulator transport and directed supply of nutrients (Durzan 1984).

Nodes failed the respond to different auxins viz. NAA, IBA and 2,4-D used alone. However, IBA (0.49 μ M) when used in combination with kinetin (0.464 μ M) or BA (2.2 μM) induced the growth of the axillary buds (12.1% and 9.6% respectively) within a week of culture.

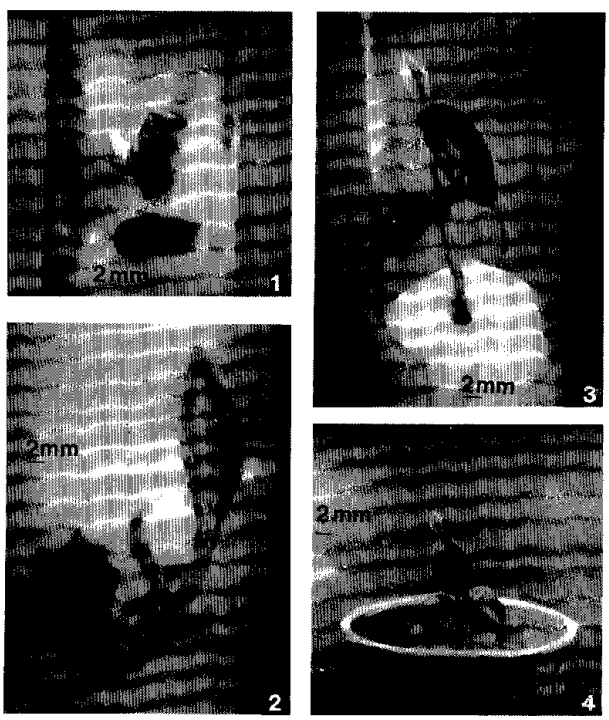

Fig. 1 Shoot differentiation on nodal explants with kinetin (4.64 μM) (2 weeks old).

Fig. 2 Axillary microshoot on IBA (2.46 μM)+BA (4.44 μM) (6 weeks old).

Fig. 3 Vigorously growing shoot on filter bridges (2.46 μM IBA) (7 weeks old).

Fig. 4 Plantlet transferred to soil (8 weeks old).

3.2 Elongation of Microshoots

Experiments were conducted to achieve elongation of the axillary shoots on various media at the time of first or second subculture. The best shoot elongation was achieved on WPM supplemented with kinetin (1.66-2.32 μM) and shoots attained a length of 5-6 cms bearing 2-3 leaflets within 7-8 weeks (data not shown) (**Fig 2**).

Table 2. Effect of explant orientation on shoot differentiation from the node. Values are mean ± SE. Three replicates of 15 explants per treatment. Entries with the same letters are not significantly different at 5% level, according to Duncan's Multiple Range Test.

Kinetin (μM)	% Shoot differentiation		No. of shoots/explant	
	Vertically oriented explant	Horizontally oriented explant	Vertically oriented explant	Horizontally oriented explant
0.46	24.4 ± 38.1a	10.2 ± 1.00cd	2.2 ± 0.12a	1.1 ± 0.11ae
2.32	38.2 ± 3.86ab	12.4 ± 1.20d	3.2 ± 0.31b	1.8 ± 0.20d
4.64	57.8 ± 3.86d	8.2 ± 0.80bc	6.0 ± 0.34d	1.6 ± 0.20cd
23.20	48.8 ± 3.80cd	6.6 ± 0.38ab	4.2 ± 0.42cd	1.4 ± 0.16bce
46.40	42.2 ± 3.81bc	6.0 ± 0.26a	3.2 ± 0.30bc	1.2 ± 0.10ab

3.3 Rooting of Microshoots

The shoots regenerated from the axillary buds were excised at the base and transferred to rooting media for root induction. Woody Plant Media containing IBA (0.49-49.2 μM) initiated roots more readily from the axillary shoots **(Fig 3)** supported on a filter bridge than the media containing NAA (0.53-53.7 μM) (data not shown). Roots were produced in a single step as observed in *Paulonia tomentosa* (Burger 1989) with activated charcoal supporting pre-rooting elongation of the shoots in *Commiphora wightii* (Barve and Mehta 1993). The suitability of IBA for rooting of *in vitro* regenerated shoots has been discussed in past (Manzanera and Pardos 1990).

3.4 Effect of Seasonal Variation

In order to study the effect of the season of explant collection on the shoot induction frequency the explants were taken in three seasons of four months each: spring season S_1 (February-May), monsoon season S_2 (June-September) and autumn season S_3 (October-January). Experiments were carried out employing the most effective kinetin concentration (4.64 μM) for shoot induction. Best regenerative response was observed in May of season S_1. In season S_3 explants there was no bud break **(Table 3)**.

Physiological state of the tree has been known to influence the behaviour of explant in culture (Thorpe and Biondi 1984). And seasonal changes have a marked effect on the concentration of various plant growth regulators in the cambial zone (Savidge and Wareing 1983). Excellent regeneration has been reported in plant species during spring season (March-May) when reserve food material (carbohydrate) is made available and helps plants sprout and bloom (Bhatt and Todaria 1990). Similar seasonal effect has been clearly visible in the present study.

Table 3. Seasonal variation in bud break from explants of *M. latifolia* as WPM + Kinetin (4.68 μM). Values are mean ± SE. Three replicates of 15 explants per treatment. Entries with the same letters are not significantly different at 5% level, according to Duncan's Multiple Range Test.

Season	Month	%Bud break
S_1	May	62.2 ± 3.81e
S_1	Apr.	57.7 ± 3.86d
S_2	June	53.3 ± 3.86d
S_2	July	48.8 ± 3.86abcd
S_2	Aug.	37.7 ± 3.86abc
S_1	Mar.	28.8 ± 3.8ab
S_1	Feb.	24.4 ± 3.81a
S_2	Sep.	–
S_3	Oct.	–
S_3	Nov.	–
S_3	Dec.	–
S_3	Jan.	–

3.5 Acclimatization of Plantlets

In vitro regenerated plantlets were transferred to filter paper bridges successively in full and half strength of WPM liquid media after 8 weeks of growth. After 3 weeks of growth plantlets were carefully transferred to presterilized paper cups with a mixture of sterilized sand and soil (1:1) **(Fig 4)**. The potted plants were enclosed inside polythene bags and irrigated with sterile distilled water. The polythene covers were periodically withdrawn once or twice a day to acclimatize the plants and after 3-

4 weeks of transplantation covers were fully removed.

The present protocol describes that mahua (*M. latifolia*) possesses much higher efficiency of propagation through axillary branching from the nodes as compared to the adventitious organ formation from the cotyledon as reported earlier (Singh and Bansal 1994). The present method can be envisaged to be more suitable than the earlier one both in terms of the number of shoots produced as well as the length of shoots reached. Besides, the cloning technique through axillary branching allows mature genotype to be cloned true to type in large numbers and maintained indefinitely.

The present study concludes that axillary buds of *M. latifolia* can be elongated by kinetin (4.64 μM) inducing the maximum number of shoot forming cultures, multiple (6) shoots and rapid growth of the shoots (4.8 cm in 4 weeks).

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