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Micropropagation of Mulberry (*Morus alba* L.) Through *In vitro* Culture of Shoot tip and Nodal Explants

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Abstract

A high frequency of sprouting (80%) from nodal- and (70%) from shoot tip explants and shoot differentiation was observed in the primary cultures of *Morus alba* L. on MS medium supplemented with BAP and Kn. *In vitro* proliferated shoots were multiplied rapidly by culture of shoot tips and nodal explants on MS with BAP (2 mg/l) and NAA (0.2 mg/l) as supplements. This combination proved best for multiple shoot formation. Multiplication was also achieved by culture of both the kinds of explants on MS fortified with BAP (2 mg/l) + NAA (0.2 mg/l) + aspargine (25 mg/l) + glutamine (1 mg/l). This medium facilitated the elongation of shoots and sprouting of axillary buds of *in vitro* grown shoots. About 80% rooting was obtained from shoots cultured on the MS supplemented with NAA (1.0 mg/l). Plants with well developed roots were transferred to soil with survival frequency of 70%.

Introduction

The foliage of mulberry (*Morus alba* L.), a woody perennial tree constitutes the main diet for the silk-worm (*Bombyx mori* L.). Conventionally, mulberry is propagated by cuttings as well as through seeds. Often cuttings prove difficult to root, thus posing problems for mulberry breeders. Propagation through seed is undesirable because of enormous heterozygosity in the plants resulting from cross pollination. Tissue culture techniques such as micropropagation provide a fast and dependable method for production of a large number of uniform plantlets in a short time. The *in vitro* production of plants from axillary buds has been reported by various workers in different species of *Morus* (Jain et al. 1990; Sharma and Thorpe 1990; Yadav et al. 1990; Rao and Bapat 193; Patnaik and Chand 1997; Chitra and Padmaja 1999). The present study was undertaken to determine the culture conditions for rapid induction, regeneration and proliferation of mulberry plants.

Materials and Methods

Shoot tips and nodal explants were collected from healthy growing shoots of mulberry (*Moras alb*a L.), growing in the Botanical Garden of the Aligargh Muslim University. The excised shoot tips and nodal explants were washed thoroughly under running tap water for 30 min and then with 5% teepol for 8 - 10 min and rinsed several times in sterile distilled water. Thereafter, the explants were surface sterilized in a 0.1% $HgCl_2$ solution for 5 - 7 min followed by thorough washing with sterile distilled water.

The sterilized single nodal and shoot tip explants were cultured on MS medium supplemented with various combinations and concentrations of auxin, cytokinin and two amino acids (glutamine and aspargine) for shoot differentiation. The pH of the media was adjusted between 5.6 and 5.8 before autoclaving at 15 lbs/cm^2 at 121_C for 20 min. Cultures after inoculation were incubated at 25 ± 2_C and 65 - 70% relative humidity with photoperiod of 16/8 h at 3000 lux intensity by florescent tubes.

Results and Discussion

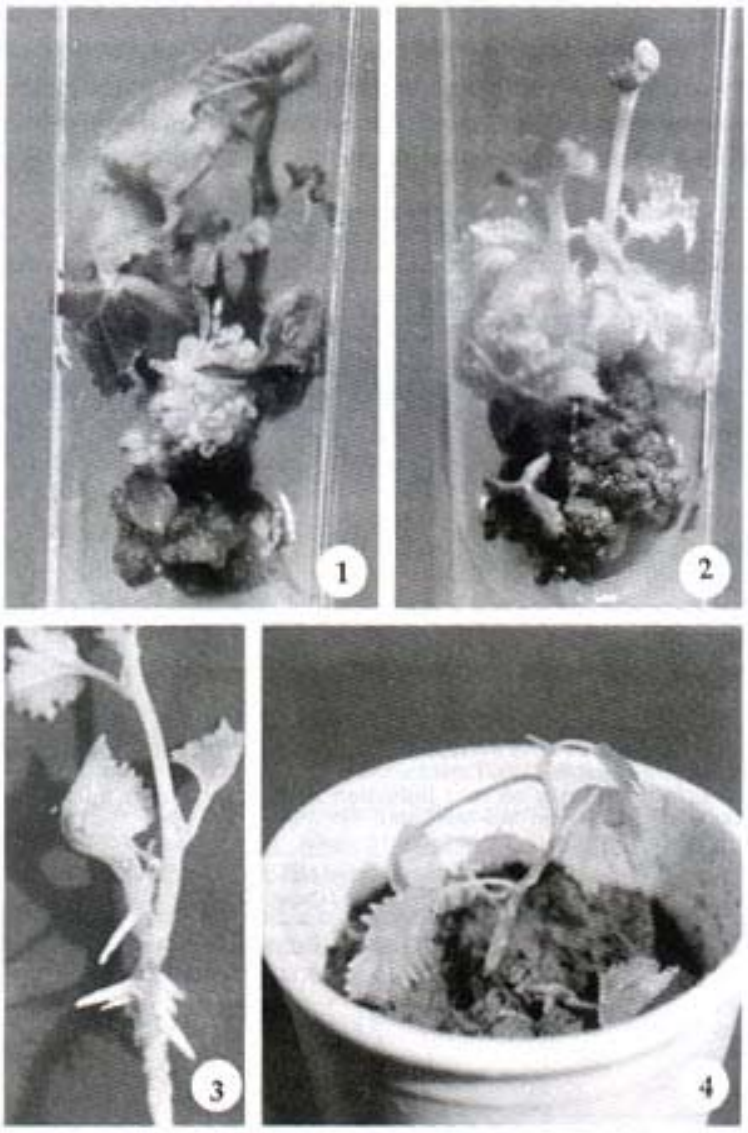
The present findings of *M. alba* demonstrate the possibility for mass propagation of mulberry through nodal and shoot tip culture. For successful micropropagation axillary buds or shoot tip cultures are preferred as pre-existing meristem easily develop into shoots while maintaining clonal fidelity.

Slightly tender nodal explants of medium thickness (0.5 - 0.6 cm) with emerging greenish axillary buds responded more favourably in terms of bud sprouting and shoot differentiation. The survival percentage and their subsequent development into shoots varied from 35 to 80% in nodal and 20 - 70% in shoot tip explants on MS supplemented with various plant growth regulators (Table 1). The frequency of sprouting was comparatively lower on Kn supplemented medium.

To obtain plantlets with uniform growth characteristics of the mother plant, the direct regeneration is essential.

Different combinations and concentrations of auxin and cytokinin with two amino acids (aspargine and glutamine) were used on MS medium for optimizing multiple shoot regeneration (Table 1). Among various combinations best response in terms of multiple shoot regeneration was observed on MS supplemented with 2 mg/l BAP + 0.2 mg/l NAA + 25 mg/l aspargine + 1 mg/l glutamine; an average of six - eight shoot buds regenerated from nodal segments

and four - six shoot buds regenerated from shoot tip explants. The lateral buds developed into shoots and inflorescence after 40 days of incubation (Fig. 1). Inflorescences were excised at an early stage to accelerate the development of shoots. Induction of inflorescence from cultured explants would be helpful in



Figs. 1 - 4: 1. Multiple shoots induced from axillary buds of nodal explants showing the emergence of inflorescence. 2. Four-week-old culture showing emergence of multiple shoots from shoot tip culture. 3. Root induction from microshoots of mulberry on MS medium with 1 mg/l NAA. 4. Regenerated plants in a pot four weeks after transfer.

anther culture studies as it does not demand sterilization as required in the inflorescence collected from field-grown plants. In *Morus australis*, most explants collected during the November - February produced inflorescence during the shoot elongation stage (Patnaik et al. 1996).

The frequency of shoot buds was low on medium containing Kn + IAA and slight callusing was also observed from the lower cut edge of explants (Fig. 2). Yadav et al. (1990); Patnaik and Chand (1997) and Chitra and Padmaja (1999) also observed that BAP was more effective than Kn in inducing shoot induction from both, shoot tip and nodal explants in the three different mulberry species.

Table 1. Effects of PGRs in *in vitro* micropropagation of mulberry on MS with 25 mg/l aspargine + 1 mg/l glutamine six weeks after culture.

Treatments (mg/l)	Nodal		Shoot tip	
	Response (%)	Average No. of shoots/ explant	Response (%)	Average No. of shoots/ explant
BAP + NAA				
0.5 + 0.2	50	3.4 ± 0.16	45	3.1 ± 0.60
1.0 + 0.2	70	5.6 ± 0.13	60	4.5 ± 0.16
2.0 + 0.2	80	6.4 ± 0.01	70	5.2 ± 0.45
5.0 + 0.2	35	2.8 ± 0.42	20	2.4 ± 0.32
BAP + IAA				
0.5 + 0.2	40	2.6 ± 0.10	25	2.0 ± 0.10
1.0 + 0.2	50	3.1 ± 0.55	30	2.7 ± 0.16
2.0 + 0.2	60	3.8 ± 0.19	50	3.2 ± 0.11
5.0+ 0.2	30	3.0 ± 0.12	15	1.5 ± 0.25

Values represent mean ± SE of ten replicates per treatment in three repeated experiments.

Table 2. Effects of auxin on root induction in *in vitro* grown microcuttings, four weeks after culture on MS medium.

Kinds of auxin (mg/l)	Response (%)	Average No. of roots/shoot
IBA (0.5	60	3.0 ± 0.02
IBA (1.0)	70	1.5 ± 0.14
IBA (2.0)	-	-
NAA (0.5)	65	3.4 ± 0.12
NAA (1.0)	80	5.0 ± 0.10
NAA (2.0)	20	2.0 ± 0.04

Values represent mean ± SE of ten replicates per treatment in three repeated experiments.

The elongated multiple shoots (2 - 3 cm) were clipped off and transferred to different rooting media (Table 2). The best root development was recorded on MS medium supplemented with 1 mg/l NAA within three weeks (Fig. 3).

Anuradha and Pullaiah (1992) reported that NAA was a more effective rooting agent for *M. alba*. On the other hand Chitra and Padmaja (1999) did not get any response with NAA as a rooting agent and reported 2,4-D to be more effective.

The plantlets with well developed shoot-roots were transferred to pots containing soilrite and the acclimatized plants were finally transferred to soil with 90% survival rate (Fig. 4). It is inferred that the technique described here provides a promising method for rapid propagation on a commercial scale of this horticulturally as well as economically important plant species. Induction of inflorescence from cultured axillary bud would be helpful for haploid production through anther culture.

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