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IN VITRO MICROPROPAGATION OF *MORUS ALBA* L. (VARIETIES S36 AND S54)

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Shoot proliferation was obtained from nodal bud explants of *Morus alba* L. (varieties S36 and S54) on modified Murashigae and Skoogs medium supplemented with Benzyl adenine (BA) singly or incombination with 1-naphthalene acetic acid (NAA) and Indole-3-acetic acid (IAA). Excised shoots were rooted on 1/2 strength MS medium without or with IAA or NAA (either separately or together) after 14 d of culture period. Regenerated plantlets were acclimatized and successfully transferred to soil.

Keywords: Micropropagation; *Morus alba* L; Nodal explants.

Introduction

Mulberry *(Morus alba L.)* is the most important crop in sericulture industry because silkworms *(Bombyx mori L.)* feed on its leaves. Since cross pollination is the rule rather than an exception, enormous heterozygosity occurs in this plant.[1] Propagation through cuttings was also found unreliable because of poor rooting ability.[2] Micropropagation, therefore, is an ideal propagation method, especially for woody species, that are beset with conventional problems. Considerable progress has been achieved over recent years in the *in vitro* propagation of *M. nigra*[3],. *M. indica*[4] *M.laevigata*[5]. However, there are few reports on *M. alba* planlet regeneration from leaf, seedling and stem explants[6-8], but their results do not entail large scale cloning of superior genotypes. We report an *in vitro* micrpropagation method for *Morus alba L* two suitable sericultural varities S36 and S54 using nodal explants from mature trees.

Materials and Methods

Stem cuttings of*Morus alba* two varieties S36 and S54 were procured from central silk board nursery,Palamaner, Chittoor District, A.P. The cuttings were planted in the field as well as in the pots in the Botanical Garden, Department of Botany, Sri Venkateswara University, Tirupati, A.P. Fresh young shoots each having 5-7 nodes were collected from the above source and cut into nodal bud explants of 2-3 cm for shoot proliferation. The nodal bud explants were thoroughly washed with running tap water and then treated with 70% Ethanol for 1 min. Subsequently, surface sterilizied with 0.1% (w/v) aqueous mercuric chloride for 8 min., followed by thorough washing with sterilized double distilled water. The cut edges of explants were trimmed and planted vertically with nodal buds facing upwards in culture medium.

Table 1. Effect of BA and auxins on shoot proliferation from nodal explants of M. alba variety S36.

Hormonal concentration (mg/l)	Days taken to shoot proliferation	% of shoot proliferation	Number of shoots per culture	Shoot length (cm)	Callus formation at the explant base
Basal	—a	—	—	—	—
1.0 BA	20	42.6±1.4	1.2±0.05	4.76±0.08	—
1.0 IAA Plus					
1.0 BA	20	54.6±2.8	2.33±0.2	4.7±0.5	Less
2.0 BA	20	64.0±2.0	2.72±0.5	4.2±0.2	Medium
3.0 BA	10	76.6±1.7	10.9±0.5	2.6±0.2	Medium
4.0 BA	10	73.0±2.0	10.5±0.5	2.3±0.2	Exuberant
1.0 NAA Plus					
1.0 BA	20	58.3±2.3	2.55±0.2	5.3±0.1	Less
2.0 BA	15	65.3±4.4	3.7±0.1	5.1±0.1	Less
3.0 BA	10	79.0±3.7	12.05±0.8	2.7±0.2	More
4.0 BA	10	75.3±4.3	11.05±0.4	2.4±0.3	Exuberant

1. Each traeatment consisted of 12 replicates and the experiment was performed thrice.
2. Data ($\bar{X}$±S.E) were recorded after 6 weeks of culture.
3. _a indicates no response.

Table 2. Effect of BA and auxins on shoot proliferation from nodal explants of *M. alba variety* S54.

Hormonal concentration (mg/l)	Days taken to shoot proliferation	% of shoot proliferation	Number of shoots per culture	Shoot length (cm)	Callus formation at the explant base
Basal	—a	—	—	—	—
1.0 BA	20	43.3±2.6	1.3±0.05	4.8±0.5	—
1.0 IAA Plus					
1.0 BA	20	51.6±3.7	2.8±0.1	4.06±0.2	Less
2.0 BA	20	60.3±3.7	3.52±0.1	4.9±0.05	Medium
3.0 BA	10	63.3±1.6	6.9±0.2	3.6±0.1	More
4.0 BA	10	73.6±1.7	16.8±0.3	2.8±0.1	More
1.0 NAA Plus					
1.0 BA	15	58.6±1.7	2.2±0.03	5.2±0.1	Less
2.0 BA	15	75.0±1.7	4.7±0.1	5.6±0.2	Medium
3.0 BA	10	76.3±0.5	7.8±0.3	3.9±0.1	More
4.0 BA	10	82.3±4.3	17.5±0.3	3.5±0.1	Exuberant

1. Each traeatment consisted of 12 replicates and the experiment was performed thrice.
2. Data (X±S.E) were recorded after 6 weeks of culture.
3. _a indicates no response.

Murashigae and Skoog[9] basal medium was variously supplemented with BA (1 to 4 mg/l) and IAA or NAA (1 mg/l each). All media were adjusted to pH 5.7 using 0.1 N HC1 or 0.1 N NaOH before autoclaving. The medium was gelled with 0.8% (w/v) agar (Qualigan, India). Routinely, 20ml of molten medium was dispensed into a culture tube (25x150mm) and plugged with non-absorbent cotton wrapped in one layer of cheese cloth. The culture tubes were steam sterilized at 1.06 kg cm^2 for 15min.

All cultures were maintained at 16h light/8h dark photoperiod under light intensity of about 2000 lux provided by cool white fluorescent lamps in combination with incandescent bulbs (Phillips, India) at 25 ± 2^0C with 55% relative humidity. For each treatment 12 explants were used and all experiments were performed thrice.

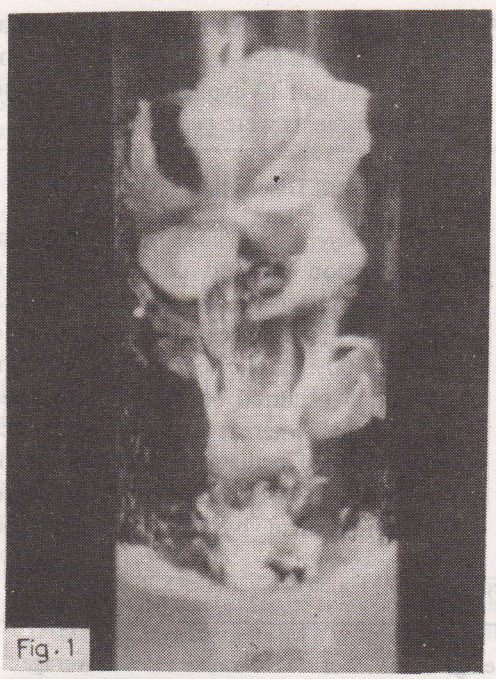
Results and Discussion

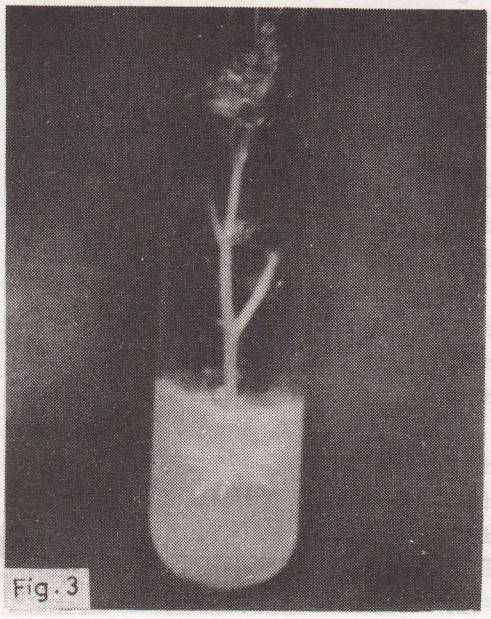
Shoot proliferation: The explant preparation, disinfection method described above prevented extensive exudation of a latex like milky substances from explants and yielded 100% aseptic bud cultures. No shoot formation occurred on growth hormones free medium whereas on growth hormones supplemented media nodal bud explants of *M.alba* L two varieties S36 and S54 showed their response by enlargement and

Table 3. Effect of auxins on rooting of invitro formed shoots of M. alba two varieties S36 amd S54.

1/2 MS+auxin (mg/l)	M. alba variety S36			M. alba variety S 54		
	% of rooted shoots	Number of roots per shoot	Average length of root (cm)	%of rooted shoots	Number of roots per shoot	Average length of root (cm)
Basal	18.3±1.6	5.3±0.3	2.7±0.1	16.6±1.6	5.6±0.3	1.6±0.2
IAA						
1.0	46.6±6.6	2.7±0.1	9.1±0.5	47.3±2.1	10.7±0.6	2.1±0.2
2.0	49.3±6.6	2.2±0.2	9.9±0.3	52.6±7.8	11.2±0.2	2.4±0.2
3.0	50.0±3.0	1.7±0.2	10.1±0.6	59.3±4.6	12.0±1.0	2.1±0.1
NAA						
1.0	56.6±2.6	11.8±0.1	2.4±0.2	53.3±3.7	11.2±0.7	2.3±0.3
2.0	56.0±6.4	10.9±0.7	2.6±0.2	63.3±5.6	12.2±0.6	2.2±0.2
3.0	63.0±4.5	11.4±1.5	1.6±0.2	64.0±6.0	12.6±0.9	1.8±0.4
1.0 IAA Plus						
1.0 NAA	64.0±5.0	12.0±1.2	2.4±0.2	68.0±6.9	12.7±0.7	2.7±0.1
2.0 NAA	88.6±2.3	12.1±1.6	2.7±0.2	85.3±2.9	13.1±0.1	2.8±0.4
3.0 NAA	86.0±2.3	14.7±1.8	1.9±0.1	70.6±3.3	14.9±0.6	1.6±0.2

1. Each treatment consisted of 20 replicates and the experiment was pereformed thrice.
2. Date ($\bar{X}$+S.E) were recorded after 6 weeks of culture.





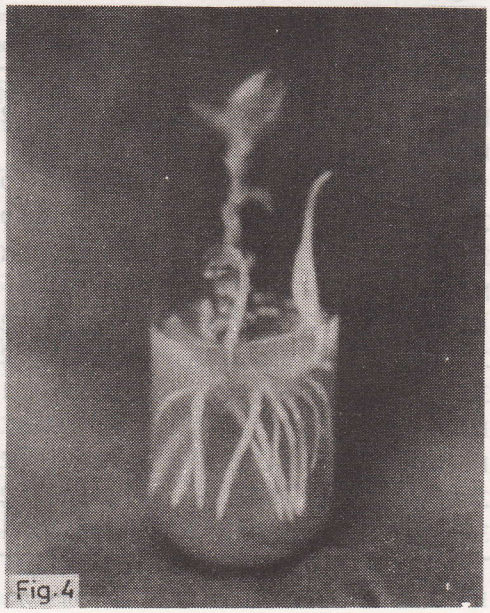


Figure 1. Shoot proliferation from nodal bud explants of S36 (3 mg/l BA and 1mg/l NAA); 2. Shoot proliferation from nodal bud explants of S54 (4 mg/l BA and 1mg/l NAA); 3 & 4. Rooting of S36 and S54 shoots *in vitro* (2 mg/l NAA and 1 mg/l IAA), 5 & 6. S36 and S54 Transplanted plantets in plastic pots.


Fig. 5

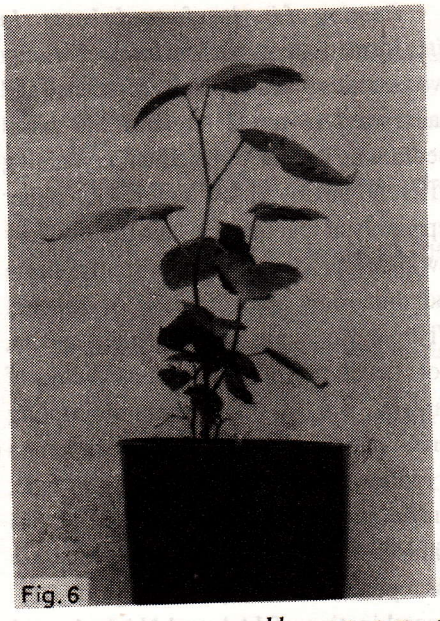


Fig. 6

adventitious shoot proliferation. The time taken for shoot proliferation, percentage of cultures showing shoot proliferation, number and length of shoots varied according to BA concentration and type of auxin combination used (Table 1 & 2). The use of single BA (1mg/1) was not effective. But combination of BA (1 to 4mg/1) in combination with IAA or NAA (1mg/1 each) proved to be best for shoot proliferation. Previously, similar results have been reported by Kim *et al.*[6] and Jain *et al.*[10] Except shoot length, overall shooting response increased with the increase of BA from 1mg/l to 4mg/l. Higher concentrations of BA i.e. 3 and 4mg/1, produced more shoots, but did not permit their length. At these concentrations the extent of callusing from the base of explant was also more than that of lower concentrations. The callus obtained was initially loose and yellowish, but it turned dark brown within 3-4 weeks of culture period. Similar observations were made in *Pterocarpus santalinus*[11] and Jack *Fruit*[12]. With regard to shoot proliferation, nodal bud explants of both S36 and S54 varieties responded well in the presence of BA and NAA than BA and IAA combination. These results were in accordance with that of *Dalbergia latifolia*[13] *Madhuca longifolia*[14]. Following 6 weeks of culture period, the highest number of shoots each having 3-4 nodes were recorded at 3mg/1 BA ard 1mg/1 NAA for S36 (Fig.1) and 4mg/1 BA and 1mg/1 NAA for S54 (Fig. 2). Maximum shoot length (5 to 6 cm) was obtained at 1mg /l BA and 1mg/l NAA for S36 and 2mg/l BA and 1mg/l NAA for S54. The nodal explants of S54 exhibited better response of shoot proliferation compared to that of S36 in all concentrations of growth hormones tested. This probably is due to differences between the endongenous hormone levels or genomic differences of the explants of the two varieties.

Rooting of in vitro formed shoots : In vitro formed shoots (ca 3cm long) of *M.alba* two varieties S36 and S54 were isolated and transferred to a 1/2 strength MS basal medium as well as the medium containing different auxins (IAA and NAA) either separately or together. On basal media, very few shoots produced less number of delicate and thin roots after 20 days of transfer. Whereas on auxin supplemented media, roots developed on shoots after 14 days of transfer. But the differences in rooting percentage, rooting quality, number, length of roots were observed according to type, concentration and combination of auxins (Table 3). Similarly, easy rooting response was reported in other species of *Morus*[5,10]. A combination of IAA and NAA showed best rooting response compared to that of individual treatment of auxins.Similarly a combination of two auxins showed good response of rooting in *Syzygium cumini*.[15] Higher concentrations of auxins in the media i. e. 3mg/l of IAA or NAA, 3mg/l NAA and 1mg/l IAA resulted in more number of roots and inhibited their growth. These levels also favoured callus formation between roots and excised shoots. This phenomenon was also reported by Kim *et al*[6] and pointed out as a disadvantage for field survival because of poor vascular connection. However, in presence of 2mg/l NAA and 1mg/l IAA, thick, healthy and more vigorous roots were formed directly from the base of shoots of two varieties S36 and S 54 without any callus formation (Fig.3 &4). These roots attained a length of 2-3 cm after 6 weeks of culture period.

Transfer of plantlets into soil: In vitro formed plantlets were removed from the culture vessels, washed thoroughly with sterile water to remove the trace of nutrient culture medium and transplanted to plastic pots (Fig.5&6) containing autoclaved sand and soil (1:1). Plantlets were covered with glass beaker to maintain humidity and kept in culture room conditions. These plantlets were irrigated two times daily with sterilized 1/2 strength MS basal medium for 6 days. After 10 days plantlets were transferred to soil where they continued to grow normally.About 65% of the plantlets were successfully established in soil and no variation was observed among the plantlets suggesting the stability of phenotypes or genotypes.

This study illustrates a successful micrporpagation system for *Morus alba* L. two varieties S36 and S54, which will be helpful in the mass production of these economically important sericultural varieties.

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