Proc. Indian natn. Sci. Acad. **B55** Nos. 5 & 6 pp. 469-472 (1989)

Regeneration of Plantlets from the Callus of Stem Segments of Mature Plants of *Morus alba* L.

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(Received 10 April 1989; Accepted 25 September 1989)

Callus cultures were raised from internodal segments of mature plants of *Morus alba* on Murashige and Skoog's medium supplemented with 1.0 mg/l 2,4-D and 0.5mg/l BAP. Shoots were regenerated when the induced calli were transferred to medium containing 0.5 to 3.0 mg/l BAP. Callus-derived shoots produced roots and developed into plantlets when transferred to medium supplemented with 0.5 mg/l NAA.

Key Words : *Morus alba* L., Internodal segments, Plantlet regeneration

Introduction

Tissue culture technology promises great impact in woody tree improvement (Jones 1983, Bajaj 1986). Improvement in trees by conventional methods of selection, breeding and progeny testing is very slow and difficult. The problem is due to the highly heterozygous nature and long life cycles of trees. Regeneration from cell and tissue culture could reduce the time for selection and propagation of desirable traits in trees within limited space (Gupta 1988).

Mulberry trees are valued for their foliage, as the chief feed for mulberry silkworms (*Bombyx mori* L.). It is conventionally propagated through cuttings. However, many desired cultivar do not root easily or have low rooting ability. Also, propagation via cuttings is restricted to only certain months of the year. Tissue culture methods therefore have an inherent advantage for plant multiplication throughout the year of elite strains as well as strains with difficult rooting. Differentiation of shoots and/or roots from the tissue culture of several mulberry species has been reported (Oka & Ohyama 1981, Kim et al. 1985, Mhatre et al. 1985, Bapat et al. 1987, Oka & Ohyama 1986, Yakuwa & Oka 1988, Tsuneyama et al. 1988, Saito & Katagiri 1989). However, in the majority of reports the explants used have been embryo, seedlings meristem, axillary buds and leaf but the stem explants produced only callus without further differentiation. In the present investigation, scope of large scale propagation of mulberry from the callus of internodal segments by using tissue culture techniques was examined.

Materials and Methods

About 3 cm long segments were taken from young branches of an approximately 5 years old tree of *Morus alba* L. var. S_1 from Germ Plasm Bank of the Central Sericultural Research and Training Institute, Berhampore. They were washed in running tap water for about 30 min, soaked 20 sec.in 95% ethanol and then treated with 1% solution of Cetavlon (20% w/v cetrimide, an antispectic and detergent) for 5 min. After rinsing with distilled water several times, the segments were surface sterilized with 0.1% (w/v) mercuric chloride solution for 5 min, followed by thorough washing with sterilized double distilled water. Internodal segments (8-10 mm) were aseptically excised and pretreated in

MS liquid medium (Murashige & Skoog 1962) supplemented with 0.5 mg/l benzylaminopurine (BAP) in 250 ml Erlenmeyer flask. The flasks were kept on gyratory shaker for 36 hr at 25 ± 2°C. Following treatment, the segments were inoculated on agar = based MS medium supplemented with various concentrations (0.5 to 3 mg/l) and combinations of benzylaminopurine (BAP), naphthaleneacetic acid (NAA) and 2,4-dichlorophenozyacetic acid (2,4-D). Better callus formation was recorded on MS medium supplemented with 1.0 mg/l 2,4-D and 0.5 mg/l BAP. The cultures were maintained at 25 ± 2°C with 16h illumination at about 3000 lux. For each treatment 10 cultures were raised and all experiments were repeated twice

Table 1 *Effect of BAP on the regeneration frequency of shoots from internodal callus of* ***M. alba*** *Var. S_1. Each treatment consisted of 10 cultures and results were scored after 10 weeks of subculture*

MS medium + BAP (mg/l)	Percentage of culture showing multiple shoot formation ± SE	No. of shoots regenerated per culture
0	0	0
0.5	26.67 ± 3.33	4-6
1.0	33.33 ± 3.33	8-10
2.0	50.00 ± 5.77	10-20
3.0	20.00 ± 5.77	3-4

Results

The internodal segments cultured on MS medium containing 1.0 mg/l 2,4-D and 0.5 mg/l BAP, produced light green friable callus from cut ends of the segments after 4-5 weeks of culture (figure 1A). The calli were maintained in the proliferating medium by transferring routinely at 30 days intervals.

Five to six weeks old calli (400 ± 50 mg) were obtained from these cultures and subcultured on medium supplemented with 0.5, 1, 2 and 3 mg/l BAP. Rapid growth of the callus took place and formed a number of small light green nodular structures on their surfaces. After 4 - 5 weeks of subculture shoot buds regenerated from the green tissues and developed further into shoots (figure 1B & C). The percentage of cultures showing shoot regeneration was maximum (50 ± 5.77) at 2 mg/l BAP. Each responsive culture showed the production of 10-20 shoots at the upper surface of the callus after 10 weeks of subculture. However, BAP above 2 mg/l decreased the percentage of shoot forming cultures (table 1).

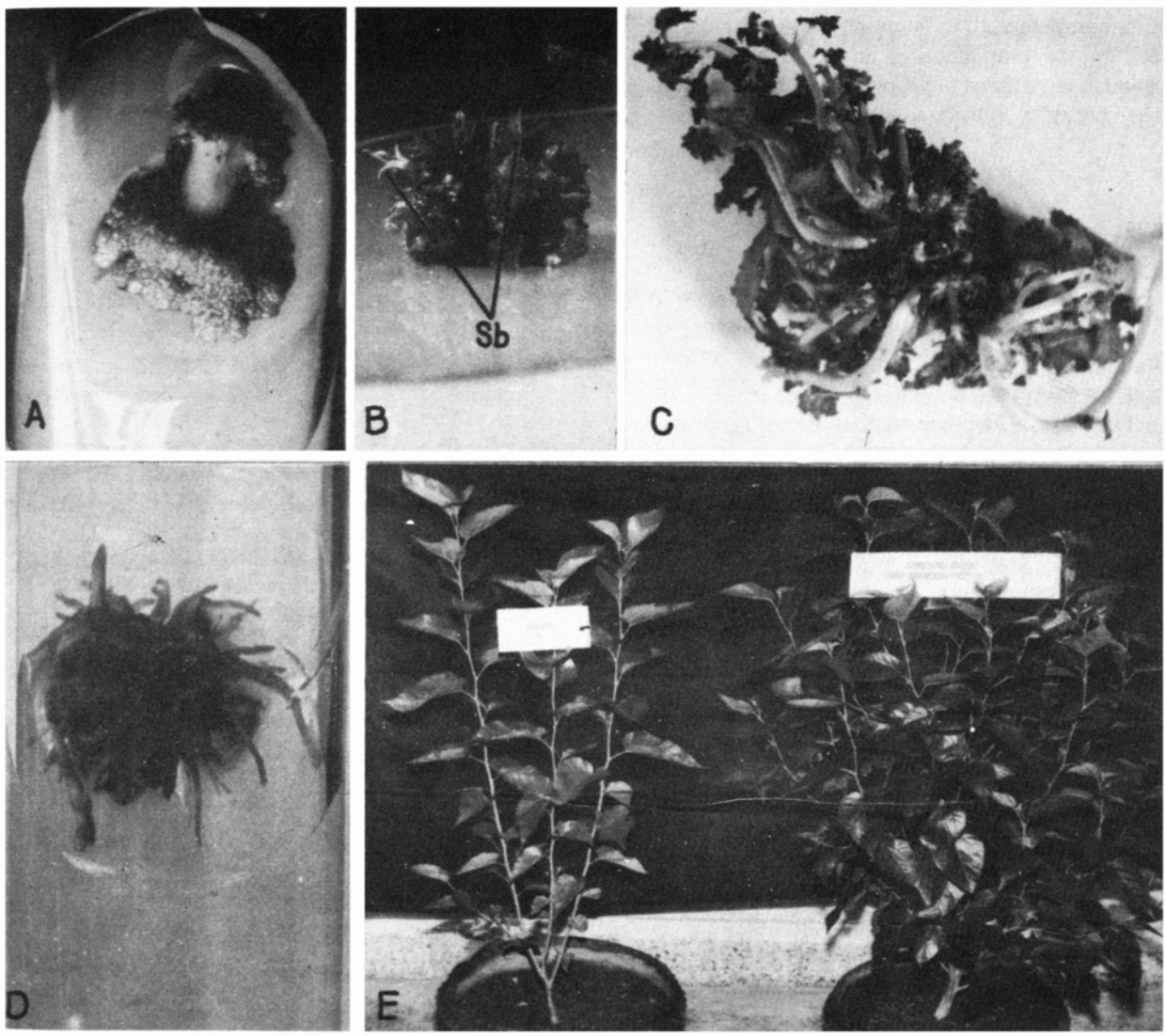
Application of 0.5 to 3 mg/l NAA alone stimulated only root regeneration from the subcultured calli (figure 1D). However, 0.5 mg/1 NAA in combination with 1 mg/l BAP induced regeneration of both shoots and roots after 7 weeks of subculture. Calli obtained from untreated segments did not show shoots bud regeneration.

The multiple shoots regenerated with BAP containing medium were excised and transferred to MS medium supplemented with 0.5 mg/1 NAA. About 95% of the shoots rooted within 3 weeks of transfer and gave rise to complete plantlets. Plantlets grew further and bore several leaves and attained a length of 6-8 cm after 6 weeks of incubation.

Six weeks old *in vitro* raised plantlets were transferred to pots containing mixture of sterile soil, sand and compost (3 : 3 : 1). Potted plantlets were incubated at 25 ± 2°C under fluorescent light (16hr photoperiod) covered with glass beakers to maintain high humidity around the plants. Pots were suitably irrigated with 1/20 strength of MS medium from time to time. The plantlets continued to grow and attain a length of 10 to 15 cm after 8 weeks of transplantation. The growth of plants continued successfully in last 6 months. Plants regenerated *in vitro* exhibited morphological variations such as thick leaves, short internodes and high branching pattern than those of normal plants (figure 1E).

Discussion

The present study clearly demonstrates the regeneration of shoot buds from the callus of internodal segments of *Morus alba* L. in presence of BAP. This finding is in conformity with the previous reports where BAP in the medium caused the multiple shoot proliferation in leaf tissue of *Morus alba* (Oka & Ohyama 1981) and *Morus indica* (Mhatre et al. 1985). Calli obtained from untreated segments are ineffective in inducing shoot bud regeneration and required the pre-treatment of segments prior to callus induction. The results indicate that there may be an existence of some specific potential for regeneration of shoots as well as complete plantlet from internodal callus produced from



Figures 1A-E Regeneration of plantlets from internodal callus of *Morus alba*. **A,** Proliferation of callus from internodal segment on MS + 2,4-D (1.0 mg/l) + BAP (0.5mg/l). Five-week-old culture; **B,** Regeneration of multiple shoot buds (sb) from callus subcultured on MS + BAP (2.0 mg/l). Six-week-old culture; **C,** Multiple shoots developing from callus subcultured on MS + BAP (2.0 mg/l). Ten-week-old culture; **D,** Induction of numerous roots from callus subcultured on MS + NAA (1.0 mg/l). Six-week-old culture; **E,** Normal and an *in vitro* grown plant showing high branching pattern. After six months of transplantation to the pot.

pre-treated segments. On the basis of differentiation of plantlets from internodal callus of mature plant, it is suggested that for regeneration, shoot of *Morus alba* requires pre-treatment of internodal segments prior of callus proliferation. The present findings show the potential for the production of large numbers of plants through rapid shoot proliferation in culture followed by the rooting of individual cultured shoots *in vitro*.

The investigation also reveals that the *in vitro* technique is of immense use for induction of morphological variation in *Morus* sp. Screening of such plants on the basis of their capacity to withstand drought, saline and leaf quality for selection of such phenotypes will be of practical significance.

Further studies are in progress to determine the cause of variability under *in vitro* conditions.

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