BIOLOGIA PLANTARUM 46 (3): 349-355, 2003

Rapid micropropagation of five cultivars of mulberry

B.S. BHAU* and A.K. WAKHLU

Plant Tissue Culture Laboratory, Department of Botany, University of Jammu, Jammu 180 006, India

Abstract

Multiple shoots were initiated from nodal and shoot tip explants collected from mature trees of *Morus alba* L. cultivars Chinese White, Kokuso-27 and Ichinose, and *M. multicaulis* Perr. cultivars Goshoerami and Rokokuyaso after 2 weeks of culture. Nodal explants were more responsive than shoot tip explants. Murashige and Skoog basal medium was found to be most suitable medium and 6-benzylaminopurine was the most effective cytokinin for shoot induction. Explants collected between April and September evoked better response than the explants collected between October and March. Shoots were multiplied by transferring nodal explants excised from *in vitro* raised shoots onto a medium containing cytokinins. Sucrose was the most suitable carbon source examined for shoot multiplication. An increase in shoot multiplication rate was noticed upto 4 - 5 subcultures. Nodal explants rooted on an auxin-supplemented medium. The acclimatized plants were successfully transplanted in the field.

Additional key words: axillary shoot proliferation, carbon source, *Morus alba*, *Morus multicaulis*, subculture.

Introduction

The genus *Morus* belonging to the family *Moraceae* comprises nearly 35 species. Many members of this genus are cultivated on commercial scale. *Morus alba* L. cvs. Chinese White, Kokuso-27, Ichinose and *Morus multicaulis* Perr. cvs. Goshoerami and Rokokuyaso are promising cultivars for sericulture industry in temperate regions of the world. Their foliage is used as a source of feed for rearing silkworms (*Bombyx mori* L.). Low rooting potential of cuttings from these cultivars is a serious bottleneck to their large-scale propagation. Mulberry plants are out breeder and as a result their progeny show genetic variability, which makes them unsuitable for the commercial purpose. Methods of conventional vegetative propagation like production of plants through grafting is not economically viable because it involves lot of skilled manpower, expensive nursery facilities and a long wait of 4 - 5 years to obtain plants ready for harvest (Bhau 1999, Bhau and Wakhlu 2001). Propagation of plants through cuttings is also not viable for these cultivars due to their extremely low rooting ability. An attempt made in the past to induce rooting in stem cuttings of these cultivars by the use of auxins has not yielded encouraging results (Fotadar *et al.* 1990).

Micropropagation provides an alternative method for mass clonal propagation. The successful regeneration of plants *in vitro* has been achieved in several mulberry species by axillary shoot proliferation (for review see Wakhlu and Bhau 2000). These studies have revealed that *in vitro* micropropagation in mulberry is dependent on the growth regulator combinations, explant type and the genotype. New mulberry cultivars require development of successful *in vitro* regeneration system. The objectives of this study were to establish an efficient protocol for micropropagation of *M. alba* cvs. Chinese White, Kokuso-27, Ichinose and *M. multicaulis* cvs. Rokokuyaso and Goshoerami and optimize conditions for acclimatization and transplantation of plants to field.

Received 24 July 2001, *accepted* 4 March 2002.

Abbreviations: BAP - 6-benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; B_5 - Gamborg's medium; IAA - indole-3-acetic acid; IBA - indole-3-butyric acid; Kn - kinetin; MS - Murashige and Skoog; NAA - α-napthaleneacetic acid; WPM - woody plant medium.

Acknowledgements: Authors are grateful to Professors N. S. Rangaswamy and S. S. Bhojwani, Department of Botany, University of Delhi, Delhi, for helpful discussions and Drs. B. B. Bindroo and R. K. Fotedar (CSRTI, Jammu) and Dr. A. Koul (RHRS, Jammu) for providing the plant material. AKW is thankful to the Central Silk Board and Department of Biotechnology (Govt. of India) for providing the financial support and BSB is grateful to UGC for the award of NET fellowship.

*Corresponding author; present address: Plant Molecular Biology Laboratory, Tata Energy Research Institute, India Habitat Center, Lodhi Road, New Delhi 110 003, India; fax: (+91) 11 4682144, e-mail: bhau-bs@hotmail.com

Materials and methods

The explants were collected from 10-year-old mature trees maintained in the Mulberry Germplasm of the Sericulture Division, Regional Horticulture Research Station, Sher-e-Kashmir University of Agricultural Sciences and Technology, Udheywalla, Jammu, India. For each cultivar, the explants were collected from a single tree between February and October, 1993 - 1997 unless otherwise stated. Nodal segments (1 - 2 cm long) and shoot tips (1 cm long) were excised from current branches. The explants were washed with detergent under running tap water for 1 h, then immersed in 0.5 % *Bavistin* solution (*BASF India Ltd.*, Mumbai, India) for 30 min, rinsed 3 - 4 times with sterile distilled water. These explants were surface-sterilized with 70 % ethanol for 1 min, followed by a dip in 4.6 % sodium hypochlorite solution containing 2 - 3 drops of *Tween-80* (*Loba Chemie*, Mumbai, India) for 20 min. The explants were finally rinsed 4 - 5 times with sterilized distilled water.

Three basal media, MS (Murashige and Skoog 1962), B_5 (Gamborg *et al.* 1968) and WPM (Lloyd and McCown 1980) media were used in this study. Each medium was fortified with 3 % sucrose and 0.8 % agar unless otherwise stated. The pH of the media was adjusted to 5.8 prior to autoclaving. Cultures were maintained at 28 ± 2 °C and 16-h photoperiod with a irradiance of 30 μmol $m^{-2} s^{-1}$ provided by cool-white fluorescent tubes.

The effects of different growth regulators (0.5 - 10 mg dm^{-3} BAP or Kn and 0.1 - 0.5 mg dm^{-3} IBA, IAA, or NAA), basal media (MS, B_5, WPM), explant type (nodal segments, shoot tips) and explanting period were tested for establishment of cultures. Shoots formed *in vitro* were cut into single nodes and used for shoot proliferation. The effects of above mentioned growth regulators and carbon source (sucrose, fructose, sorbitol, and mannitol in 3 % concentration) were tested for proliferation of shoots. Multiplication rate was evaluated after a regular gap of 4 weeks for 6 sequential subcultures on a medium supplemented with 1.5 mg dm^{-3} BAP. Data were taken in terms of the number of shoots formed per explant and the shoot length.

Nodal explants (1 cm) excised from *in vitro* raised shoots were used for rooting. The effect of growth regulators (0.1 - 2 mg dm^{-3} NAA, IBA, or IAA, and 0.1 - 1.0 mg dm^{-3} BAP or Kn) was tested for root formation. Data were taken in terms of the percentage of rooted explants, the average number of roots formed per explant and the root length. The regenerated plantlets were transferred to plastic cups (8-cm diameter) containing sand and vermiculite (1:1). The plantlets were covered with a polythene bag in order to maintain high humidity and were placed in a greenhouse. Plantlets were watered every 2 d with Knop's solution for 4 weeks. Hardened plantlets were then transplanted in polythene bags (16 × 13 cm) containing garden soil, sand and farmyard manure (1:1:1). These plants were transplanted in the experimental plots of Department of Botany, University of Jammu and Regional Sericulture Research Station, Miranshaib, Jammu, India.

Ten replicates with 50 explants were used for each treatment and repeated 3 times. The results were recorded at a regular interval of 4 weeks of culture and analyzed by analysis of variance using randomized block design method. Data taken in percentage was subjected to arcsin transformation for proportions before analysis and converted back to percentages for presentation in tables. Means were compared using Duncan's new multiple range test (Duncan 1955).

Results

Multiple shoot induction: At the beginning of the experiment Kokuso-27, Ichinose and Rokokuyaso cultivars grew well, while Chinese White and Goshoerami required an adaptation period to the *in vitro* conditions. Multiple shoots were initiated from nodal and shoot tip explants after 2 weeks of culture on a growth regulator supplemented medium (Fig. 1). The greatest numbers of shoots were regenerated on a medium with BAP. The most effective concentration of BAP was from 1 - 2.5 mg dm^{-3} for Kokuso-27, Ichinose and Rokokuyaso and 4 mg dm^{-3} in Chinese White and Goshoerami. Nodal segments were more responsive than shoot tip explants. Maximum number of shoots formed per nodal explant was higher than the number of shoots formed per shoot tip explant. Auxin type and concentration had no significant effect on multiple shoot induction and favored callus formation at the cut ends of the explants (data not presented).

MS basal medium was more suitable than B_5 and WPM medium for shoot induction from nodal explants (Table 1). The type of basal medium did not influence multiple shoot induction from shoot tip explants. The season of explant collection had a significant influence on multiple shoot initiation. Maximum number of shoots per explant was obtained from nodal explants collected between April and September than from explants collected between October and March (Fig. 2).

Table 1. Effect of different basal media supplemented with 1.5 mg dm^3 BAP on number of shoots formed per nodal explant of different cultivars of mulberry after 4 weeks of culture. Means in a column with the same superscript are not significantly different from each other at a 5% level according to Duncan's new multiple range test.

	Number of shoots [explant^{-1}]				
	Chinese White	Kokuso-27	Ichinose	Goshoerami	Rokokuyaso
MS	7.6^{c}	6.2^{b}	9.5^{c}	9.5^{c}	6.5^{c}
B_5	4.2^{b}	5.4^{ab}	6.3^{b}	6.3^{b}	4.1^{b}
WPM	2.2^{a}	5.2^{a}	4.3^{a}	4.3^{a}	2.5^{a}

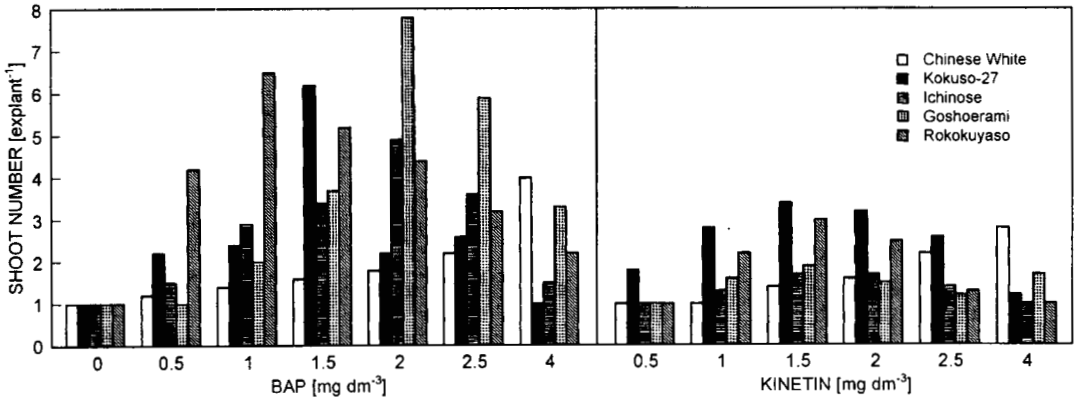

Fig. 1. Effect of different concentrations of BAP and kinetin on multiple shoot induction in different cultivars of mulberry.

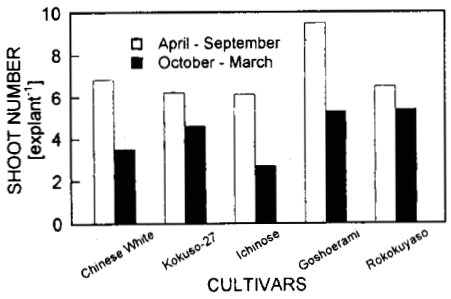

Fig. 2. Effect of explanting season on multiple shoot induction in different cultivars of mulberry.

Multiple shoot proliferation: Shoot tip explants were not used for further multiplication because of their comparatively poor response during multiple shoot induction. The effect of cytokinin on shoot proliferation was significant (Table 2). For all cultivars, the higher BAP concentration (> 2 mg dm^{-3}) decreased the multiplication rate. The media containing 1.5 mg dm^{-3} BAP gave maximum multiplication in Chinese White and Ichinose, whereas the medium containing 1 mg dm^{-3} BAP gave maximum multiplication rate in Kokuso-27, Rokokuyaso and Goshoerami.

Carbon source had a significant influence on shoot proliferation. Sucrose and fructose were more suitable than mannitol and sorbitol. The number of shoot formed on each nodal explant and shoot length was highest on a medium supplemented with sucrose (Fig. 3). The presence of mannitol in the medium was noticed to completely restrain shoot multiplication. The effect of

Table 2. Effect of BAP concentrations on shoot multiplication from *in vitro* raised nodal explants of different cultivars of mulberry after 4 weeks of culture. Means in a column with the same superscript are not significantly different from each other at a 5 % level according to Duncan's new multiple range test.

BAP	Number of shoots [explant^{-1}]				
[mg dm^{-3}]	Chinese White	Kokuso-27	Ichinose	Goshoerami	Rokokuyaso
0.0	1.8^{a}	1.0^{a}	1.0^{a}	1.0^{a}	1.0^{a}
0.5	2.9^{b}	1.7^{ab}	1.5^{ab}	1.7^{ab}	4.2^{b}
1.0	5.6^{d}	4.1^{d}	2.9^{bc}	4.1^{c}	6.5^{c}
1.5	7.0^{e}	3.3^{c}	4.9^{d}	3.3^{b}	4.4^{b}
2.0	5.8^{d}	2.1^{b}	3.6^{c}	2.1^{ab}	2.0^{a}
2.5	4.6^{c}	1.5^{ab}	1.1^{ab}	1.5^{a}	1.0^{a}

Table 3. Effect of sequential subculture (4 week culture period each) on multiple shoot formation (number of shoots per explant and shoot length) in different cultivars of mulberry cultured on MS medium supplemented with 1 mg dm^{-3} BAP. Means in a column with the same superscript are not significantly different from each other at a 5 % level according to Duncan's new multiple range test.

Subculture	Chinese White		Kokuso-27		Ichinose		Goshoerami		Rokokuyaso	
	number [explant^{-1}]	length [cm]	number [explant^{-1}]	length [cm]	number [explant^{-1}]	length [cm]	number [explant^{-1}]	length [cm]	number [explant^{-1}]	length [cm]
1st	7.8^{a}	5.3^{a}	6.7^{c}	3.9^{b}	5.3^{b}	4.2^{a}	6.1^{a}	4.1^{a}	8.0^{a}	3.9^{a}
2nd	11.0^{c}	5.1^{a}	8.3^{d}	3.7^{b}	5.7^{bc}	3.9^{a}	5.6^{a}	4.5^{ab}	8.0^{a}	3.9^{a}
3rd	11.0^{c}	5.5^{a}	9.0^{d}	3.5^{b}	8.8^{d}	4.1^{a}	6.3^{a}	4.5^{ab}	10.6^{c}	4.1^{a}
4th	14.0^{d}	5.1^{a}	5.5^{b}	3.2^{ab}	6.4^{c}	3.6^{a}	6.2^{a}	4.9^{ab}	10.4^{c}	4.3^{a}
5th	9.0^{b}	4.8^{a}	3.8^{a}	2.9^{ab}	5.2^{b}	3.8^{a}	6.3^{a}	5.1^{b}	12.8^{d}	3.7^{a}
6th	7.0^{a}	5.0^{a}	3.3^{a}	2.5^{a}	4.0^{a}	3.6^{a}	6.6^{a}	5.3^{b}	9.0^{b}	3.5^{a}

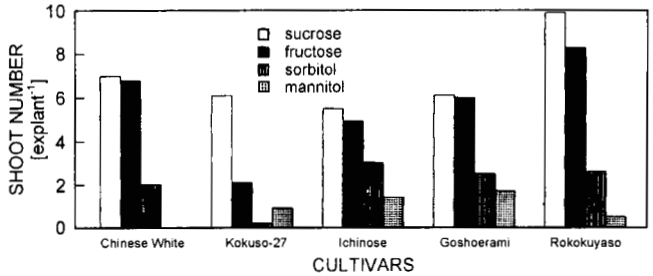

Fig. 3. Effect of different carbon sources on multiple shoot proliferation in different mulberry cultivars after 4 weeks of culture.

Table 4. Effect of IBA, IAA or NAA in different concentration [mg dm^{-3}] on rooting of nodal segments (percentage of rooting explants, number of roots and root length) of different mulberry cultivars on MS medium supplemented with different concentrations of auxins after 4-weeks of culture. Means in a column with the same superscript are not significantly different from each other at a 5 % level according to Duncan's new multiple range test.

Auxin	Conc. [mg dm^{-3}]	Chinese White rooting [%]	number [exp.$^{-1}$]	length [cm]	Kokuso-27 rooting [%]	number [exp.$^{-1}$]	length [cm]	Ichinose rooting [%]	number [exp.$^{-1}$]	length [cm]	Goshoerami rooting [%]	number [exp.$^{-1}$]	length [cm]	Rokokuyaso rooting [%]	number [exp.$^{-1}$]	length [cm]
None	0.0	0^{a}	0.0^{a}	0.0^{a}	0	0.0^{a}	0.0^{a}	10^{a}	2.3^{a}	1.4^{a}	0^{a}	0.0^{a}	0.0^{a}	0^{a}	0.0^{a}	0.0^{a}
IBA	0.1	10^{b}	3.4^{c}	3.9^{cd}	45	2.1^{b}	2.9^{bc}	75^{cd}	3.6^{ab}	4.3^{c}	15^{b}	1.3^{b}	1.7^{b}	10^{b}	1.5^{b}	2.2^{b}
	0.5	25^{c}	4.6^{d}	4.5^{cd}	50	2.1^{b}	3.7^{c}	85^{d}	6.9^{c}	8.5^{e}	40^{c}	2.4^{b}	2.9^{bc}	15^{bc}	1.9^{b}	2.7^{b}
	1.0	30^{c}	5.2^{de}	4.8^{d}	65	2.7^{bc}	3.5^{c}	80^{cd}	4.4^{b}	6.2^{d}	50^{d}	2.9^{c}	3.4^{c}	30^{d}	2.4^{bc}	3.2^{c}
	2.0	15^{b}	3.0^{c}	3.3^{bc}	65	2.5^{b}	4.4^{cd}	55^{b}	3.3^{a}	3.4^{bc}	55^{d}	2.5^{bc}	3.1^{c}	35de	1.6^{b}	2.5^{b}
IAA	0.1	5^{b}	1.0^{b}	0.4^{a}	45	2.2^{b}	3.2^{bc}	55^{b}	3.9^{ab}	2.8^{b}	45^{cd}	4.2^{d}	3.2^{c}	15^{bc}	1.9^{b}	2.5^{b}
	0.5	15^{b}	2.4^{bc}	2.9^{b}	60	2.8^{bc}	4.1^{cd}	65^{bc}	4.8^{b}	4.5^{c}	65de	5.4^{e}	4.1^{d}	30^{d}	2.8^{c}	2.9^{bc}
	1.0	30^{cd}	3.0^{c}	3.3^{bc}	90	3.6^{c}	5.2^{d}	70^{c}	5.2^{b}	4.5^{c}	55^{d}	3.9^{cd}	3.5^{c}	40^{e}	2.5^{bc}	3.1^{bc}
	2.0	20^{bc}	1.6^{b}	2.6^{b}	70	2.4^{b}	4.2^{cd}	60^{bc}	4.7^{b}	3.2^{bc}	40^{cd}	3.6^{c}	2.9^{bc}	40^{e}	1.9^{b}	3.3^{c}
NAA	0.1	20^{bc}	5.2^{de}	7.1^{f}	40	2.0^{b}	2.7^{bc}	50^{b}	3.0^{a}	2.5^{b}	45^{cd}	2.7^{bc}	3.8^{cd}	20^{c}	2.2^{bc}	2.5^{b}
	0.5	70^{e}	7.8^{f}	8.0^{g}	50	1.9^{b}	3.1^{bc}	55^{b}	3.0^{a}	2.9^{b}	25^{bc}	1.8^{b}	2.2^{b}	45^{e}	2.5^{bc}	3.4^{c}
	1.0	50^{d}	5.6^{e}	6.1^{e}	50	2.5^{b}	2.4^{b}	50^{b}	4.5^{b}	3.4^{bc}	20^{b}	1.8^{b}	2.5^{b}	80^{g}	3.8^{cd}	4.6^{d}
	2.0	10^{b}	2.4^{bc}	2.9^{b}	55	1.0^{b}	2.3^{b}	55^{b}	4.9^{b}	3.9^{c}	15^{b}	1.5^{b}	2.1^{b}	65^{i}	3.5^{cd}	3.5^{c}

repeated subculturing on the number of shoots formed per explant was significant. The shoot number increases upto 4 - 5 subcultures but thereafter the multiplication rate was gradually declined in all the 5 cultivars (Table 3).

Rooting: Auxins had a significant influence on root formation (Table 4). Rooting was successfully induced within 15 d of culture. The percentage of nodal explants forming roots, the number of roots per explant and the root length was highest on a medium containing 0.5 mg dm^{-3} and 1.0 mg dm^{-3} NAA in Chinese White and Rokokuyaso respectively. IBA at concentration of 0.5 and 1.0 mg dm^{-3} was most effective for rooting in Ichinose and Kokuso-27. Goshoerami explants rooted best on the MS medium supplemented 1.0 mg dm^{-3} IAA. High concentrations of auxins (≥ 1.0 mg dm^{-3}) stimulated

callus formation at the cut ends of the explants in all the cultivars.

Regenerated plants were not able to survive when transferred directly to field. Regenerated plantlets with 3 - 5 fully expanded leaves and well-developed roots were successfully hardened during July and August months. The hardened plants were transferred to field after 2 months.

Efficiency: One explant upon culture resulted in the large number of shoots at the end of 2nd subculture (12 weeks). These nodes after rooting (100 %) in the next 4 weeks produced 441, 324, 272, 341 and 900 plantlets in Chinese White, Kokuso-27, Ichinose, Goshoerami and Rokokuyaso, respectively. Chinese White was the most fast multiplying cultivar among the 5 cultivars studied.

Discussion

The present study indicates that multiple shoots can be induced from shoot tip and nodal explants in all cultivars of mulberry on a medium containing cytokinin. Compared to Kn, BAP (1 - 2 mg dm^3) induced the formation of higher number of shoots per explant. The promotive effect of BAP for inducing multiple shoots has been previously reported in several mulberry species (Enomoto 1987, Yadav *et al.* 1990, Pattnaik and Chand 1997). Superiority of BAP for shoot induction may be attributed to the ability of plant tissues to metabolize BAP more readily than other synthetic growth regulators or to the ability of BAP to induce production of natural hormones such as zeatin within the tissue (Zaerr and Mapes 1982). The level of BAP used in the medium significantly influenced the multiple shoot formation. Maximum numbers of shoots per explant were produced with low concentrations of BAP (1 - 2 mg dm^3) in Kokuso-27, Ichinose and Rokokuyaso. Low BAP concentrations have also encouraged shoot induction in *Morus alba* (Chattopadhyay and Datta 1990), *M. nigra* (Yadav *et al.* 1990, Vijaya Chitra and Padmaja 1999) and *M. indica* (Mhatre *et al.* 1985, Patel *et al.* 1983). An increase in BAP concentration (> 2.5 mg dm^3) was inhibitory to the formation of shoots. Similar results have been obtained in *M. australis* (Pattnaik *et al.* 1996), *M. cathayana, M. ihou* and *M. serrata* (Pattnaik and Chand 1997) and *M. laevigata* (Islam *et al.* 1993, Hossain *et al.* 1992). However high concentrations of BAP (4 mg dm^3) promoted shoot formation in cv. Chinese White. This observation agrees with earlier reports on multiple shoot formation in *Prosopis chilensis* and *P. juliflora* (Yao *et al.* 1989) and *Fraxinus excelsior* (Hammatt and Ridout 1992). Incorporation of auxins in the medium did not improve the response of shoot formation and encouraged formation of callus. Similar effect of auxins has been earlier reported in *M. laevigata* (Islam *et al.* 1993). This inhibition of shoot formation may be due to the action of accumulated auxins at the basal end of the explants (Marks and Simpson 1994).

Among the basal media tested for shoot induction, MS basal medium was the most effective for all mulberries investigated in this study. The effectiveness of MS basal medium for shoot formation has been previously observed in *M. australis* (Pattnaik *et al.* 1996), and *M. alba* (Oka and Ohyama 1986). Oka and Ohyama (1986) have reported that both dilution and strengthening of the original MS salt concentration decreased the growth of buds in *M. alba*.

Nodal explants gave better shoot regeneration response than shoot tip explants. Superiority of nodal explants in terms of the number of shoots formed per explant has also been reported in *M. australis* (Pattnaik *et al.* 1996), *M. cathayana, M. ihou* and *M. serrata* (Pattnaik and Chand 1997) and *M. nigra* (Yadav *et al.* 1990). This differential response of nodal and shoot tip explants has been attributed to the differences between the physiological states of the buds on different regions of a stem (Vieitez *et al.* 1985), and shoot tips exert strong apical dominance even in the presence of BAP (Lakshmanan *et al.* 1997).

A significant effect of explanting season on shoot regeneration was observed in this study. In all cultivars, nodal explants collected during April to September showed better shoot forming response than the explants taken during October to March. Similar results have been obtained in *M. australis* (Pattnaik *et al.* 1996), *M. cathayana, M. ihou* and *M. serrata* (Pattnaik and Chand 1997), and *M. nigra* (Yadav *et al.* 1990). Better shoot forming ability of nodal explants collected during April to September may be attributed to the most active growth phase of plant material.

Nodal explants excised from shoots grown on a medium containing high BAP concentration (4 mg dm^{-3}) required transfer to a low BAP concentrations (1 - 1.5 mg dm^{-3}) for further growth and shoot proliferation in cvs. Chinese White and Goshoerami. Similar observations were also reported in *Picea abies* (Ewald and Suss 1993) and *Anacardium occidentale* (D'Silva and D'Souza 1992). Continued exposure of explants to high BAP concentrations during induction and proliferation stages seem to cause high accumulation of cytokinins which inhibits further shoot development. The nodal explants of Kokuso-27, Ichinose and Rokokuyaso excised from shoots induced exhibited normal growth and shoot proliferation upon subculturing on the same medium.

Ohyama and Oka (1987) reported that carbon source for micropropagation varies with different cultivars of mulberry. In contrast, our study showed that sucrose was most favorable carbon source for shoot formation in all cultivars. Sucrose has also been reported to be favorable

for micropropagation of shoots in *M. laevigata* (Islam *et al.* 1993). Oka and Ohyama (1982) have shown that fructose is superior over sucrose for multiplication of shoots in *M. alba*.

Multiple shoots produced *in vitro* were proliferated further as nodal explants by repeated subculturing at 4-week intervals. The number of shoots per explant in all cultivars increased upto 5 subcultures and thereafter shoots multiplication rate declined. A similar effect of subculturing on multiplication of shoots has been reported in *M. australis* (Pattnaik *et al.* 1996), *M. cathayana* and *M. ihou* (Pattnaik and Chand 1997).

Rooting of nodal explants excised from *in vitro* raised shoots was achieved in the presence of an auxin. NAA proved to be most favourable auxin for root formation in Chinese White, Kokuso-27 and Rokokuyaso, whereas IBA was very effective for root formation in Ichinose and Goshoerami. NAA and IBA have also been reported to be potent auxins for rooting in other mulberry species. (Jain *et al.* 1990, Pattnaik *et al.* 1996, Pattnaik and Chand 1997).

The number of plants produced per explant after 12 weeks of culture was found to vary with the cultivars. Rokokuyaso gave the best response whereas Ichinose gave the least response. A genotypic difference with respect to total number of plants formed per explant has been previously reported in various *Morus* species (Pattnaik and Chand 1997, Tewary *et al.* 1995).

References

Bhau, B.S.: Tissue culture studies of some difficult-to-root temperate varieties of *Morus alba* (L.) and *Morus multicaulis* (PERR.). - Ph.D. Thesis, University of Jammu, Jammu 1999.

Bhau, B.S., Wakhlu, A.K.: Effect of genotype, explant source and growth regulators on organogenesis in *Morus alba* L. - Plant Cell Tissue Organ Cult. **66**: 25-29, 2001.

Chattopadhyay, S., Datta, S.K.: A rapid clonal propagation of mulberry tree *(Morus alba)* through tissue culture. - In: Proceedings of the 76th Ind. Sci. Cong. Part III (Ab). Pp. 208-209. 1990.

D'Silva, I., D'Souza, L.: *In vitro* propagation of *Anacardium occidentale* L. - Plant Cell Tissue Organ Cult. **29**: 1-6, 1992.

Duncan, D.B.: Multiple range and multiple F-tests. - Biometrics **11**: 1-42, 1955.

Enomoto, S.: Preservation of genetic resources of mulberry by means of tissue culture. - JARQ **21**: 205-210, 1987.

Ewald, D., Suss, R.: A system for repeatable formation of elongating adventitious buds in Norway spruce tissue cultures. - Silvae Genet. **42**: 169-175, 1993.

Fotadar, R.K., Ahsan, M.Q., Dhar, K.L., Dhar, A.: Screening of mulberry varieties for rooting and induction of rooting by the use of growth regulators. - Sericologia **30**: 347-361, 1990.

Gamborg, O.L., Miller, R.A., Ojima, K.: Nutrient requirements for suspension cultures of soybean root cells. - Exp. Cell Res. **50**: 151-158, 1968.

Hammatt, A., Ridout, M.S.: Micropropagation of common ash (*Fraxinus excelsior*). - Plant Cell Tissue Organ Cult. **13**: 67-74, 1992.

Hossain, M., Rahman, S.M., Zaman, A., Joarder, O.I., Islam, R.: Micropropagation of *Morus laevigata* Wall. from mature tree. - Plant Cell Rep. **11**: 522-524, 1992.

Islam, R., Zaman, A., Joarder, O.I., Barman, A.C.: *In vitro* propagation as an aid for cloning of *Morus laevigata* Wall. - Plant Cell Tissue Organ Cult. **33**: 339-341, 1993.

Jain, A.K., Dandin, S.B., Sengupta, K.: *In vitro* propagation through axillary bud multiplication in different mulberry genotypes. - Plant Cell Rep. **8**: 737-740, 1990.

Lakshmanan, P., Lee, C.L., Goh, C.J.: An efficient *in vitro* method for mass propagation of a woody ornamental *Ixora coccinea* L. - Plant Cell Rep. **16**: 572-577, 1997.

Lloyd, G., McCown, B.: Commercially feasible micropropagation of mountain laurel, *Kalmia latifolia,* by use of shoot tip culture. - Int. Plant Prop. Soc. Proc. **30**: 421-427, 1980.

Marks, T.R., Simpson, S.E.: Factors affecting shoot development in apically dominant *Acer* cultivars *in vitro*. - J. hort. Sci. **69**: 543-551, 1994.

Mhatre, M., Bapat, V.A., Rao, P.S.: Regeneration of plants from the culture of leaves and axillary buds in mulberry (*Morus indica* L.). - Plant Cell Rep. **4**: 78-80, 1985.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassays with tobacco tissue cultures. - Physiol. Plant. **15**: 473-497, 1962.

Ohyama, K., Oka, S.: Mulberry. - In: Bonga, J.M., Durzan, D.J. (ed.): Cell and Tissue Culture in Forestry. Pp. 272-284. Martinus Nijhoff Publishers, Dordrecht 1987.

Oka, S., Ohyama, K.: Sugar utilization in mulberry (*Morus alba* L.) bud culture. - In: Proceedings of the 5th Int. Cong. Plant Tissue Cell Cult. Pp. 67-68. 1982.

Oka, S., Ohyama, K.: Mulberry (*Morus alba* L.) - In: Bajaj, Y.P.S. (ed.): Biotechnology in Agriculture and Forestry. Vol. I - Trees. Pp. 384-392, Springer-Verlag, Berlin - Heidelberg - New York 1986.

Patel, G.K., Bapat, V.A., Rao, P.S.: *In vitro* culture of organ explants of *Morus indica:* Plant regeneration and fruit formation in axillary bud culture. - Z. Pflanzenphysiol. **111**: 465-468, 1983.

Pattnaik, S.K., Chand, P.K.: Rapid clonal propagation of three mulberries, *Morus cathayana* Hemsl., *M. ihou* Koiz. and *M. serrata* Roxb., through *in vitro* culture of apical shoot buds and nodal explants from mature trees. - Plant Cell Rep. **16**: 503-508, 1997.

Pattnaik, S.K., Sahoo, Y., Chand, P.K.: Micropropagation of a fruit tree, *Morus australis* Poir. Syn. *M. acidosa* Griff. - Plant Cell Rep. **15**: 841-845, 1996.

Tewary, P.K., Sarkar, A., Kumar, V., Chakraborti, S.: Rapid *in vitro* multiplication of high yielding mulberry (*Morus* sp.) genotypes V_1 and S_{34}. - Indian J. Sericol. **34**: 133-136, 1995.

Vieitez, A.M., San-Jose, M.C., Vieitez, E.: *In vitro* plantlet regeneration from juvenile and mature *Quercus robur* L. - J. hort. Sci. **60**: 99-106, 1985.

Vijaya Chitra, D.S., Padmaja, G.: Clonal propagation of mulberry (*Morus indica* L. cultivar M-5) through *in vitro*

culture of nodal explants. - Scientia Hort. **80**: 289-298, 1999.

Wakhlu, A.K., Bhau, B.S.: A review of tissue culture studies in mulberry (*Morus*). - Sericologia **40**: 1-20, 2000.

Yadav, U., Lal, M., Jaiswal, V.S.: Micropropagation of *Morus nigra* L. from shoot tip and nodal explants of mature trees. - Scientia Hort. **44**: 61-67, 1990.

Yao, D.Y., Batchelor, C.A., Koehler, M.J., Harris, P.J.C.: *In vitro* regeneration of *Prosopis* species (*P. chilensis, P. cineraria* and *P. juliflora*) from nodal explants. - Chinese J. Bot. **1**: 89-97, 1989.

Zaerr, J.B., Mapes, M.O.: Action of growth regulators. - In: Bonga, J.M., Durzan, D.J. (ed.): Tissue Culture in Forestry. Pp. 231-255. Martinus Nijhoff / Dr. W. Junk Publishers, The Hague - Boston - London 1982.