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Micropropagation of *Morus alba* CVS$_1$ from Shoot Apices of Mature Trees

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Abstract. Shoot apices of mature tree of *Morus alba* cv S_1 were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations and combinations of cytokinins and auxins. Multiple shoots were proliferated only in cytokinin supplemented media. Maximum number of shoots per explant was obtained from cultures grown on medium supplemented with 1 mg/l 6-benzylaminopurine and 1 mg/l kinetin. Adventitious rooting occurred well after transferring excised shoots on half-strength Ms medium containing 0.5 mg/l each of naphthaleneacetic acid and 3-indolebutyric acid. Plantlets were acclimated and successfully established in soil under natural conditions.

Introduction

Mulberry is of great economic importance for sericulture industries because its foliage is used as food for silkworms. Some species of mulberry are cultivated for their sweet edible fruits and useful timber [1]. In many countries delicious jellies are prepared from mulberry fruits. Conventionally mulberry is propagated by seed and cutting but some species with high nutritive value are difficult to root. There have been increasing interest in using tissue culture as a means of clonal propagation of woody plants. This true in those plants, in which cuttings are difficult to root, particularly when they are taken from mature trees [2]. In mulberry bud culture has given successful results to provide multiple shoots and afterwards plantlets as well [2-11]. In the present paper the morphogenic potentials of shoot tip explants of mature trees of *M. alba* cv S_1 are demonstrated.

Materials and Methods

Actively growing shoot tips were collected from a 12-year-old tree of *Morus alba* cv S_1 and washed several times with distilled water. Surface disinfection was done with $HgCl_2$ (0.1%w/v) for two minutes after a rinse of 70% ethanol. The material was then washed with sterilized double distilled water for 10 minutes giving 6-7 changes. The explains were cultured on NS medium [12] supplemented with cytokinins : 6-benzylaminopurine (BAP) and kinetin and the auxins: naphthaleneacetic acid (NAA), 3-indolebutyric acid (IBA) and to 2,4-dichlorophenoxy-acetic acid (2-4-D). Hormones were used either individually or in some combinations (Table 1) and added in the medium before autoclaving. In vitro raised shoots were rooted in half-strength MS medium and sucrose (1,5%) with IBA or NAA at a concentration of 0.5 mg/1 either individually or in conjunction. The pH of the medium was adjusted 5.8 before autoclaving at 121° C for 20 minutes and solidified with 0.7% (w/v) Deface Batch-agar. The cultures were grown at 26± 2°C with 16 h photoperiod under alight intensity of 60 $\mu E\ m^{-2}\ s^{-1}$ provided by warm white fluorescent tubes.

Table 1. Effect of growth regulators on morphogenetic response of shoot tip explants of *M.alba* on MS medium after 5 weeks of culture. Each value is an average of 3 replication consisted of 10 culture tubes

Hormone concentration (mg/1)			% Explants with shoots	Number of shoots per explants	% Explants with roots	% Explants with callus	Number of response
BAP	0.5		57.27± 0.31	2.27± 1.28	-	-	LS
	1.0		66.77± 2.06	4.09± 2.02	-	-	LS
	2.0		65.28± 1.16	6.26± 3.10	-	-	LS
	3.0		54.66± 2.11	2.02± 0.09	-	-	LS
Kn	0.5		47.33± 1.24	2.33± 0.15	-	-	LS
	1.0		46.11± 0.32	2.49± 1.23	-	-	LS
	2.0		43.00± 2.06	3.10± 1.06	-	-	LS
	3.0		48.98± 0.61	3.00± 2.11	-	-	LS
BAP	+	Kn			-	-	
0.5	+	0.5	67.96± 2.03	7.62± 1.76	-	-	HS
1.0	+	1.0	77.66± 1.13	8.35± 2.35	-	-	HS
BAP	+	IBA					
0.5	+	0.5	-	-	18.22± 1.61	-	PR
1.0	+	1.0	-	-	22.56± 1.23		PR

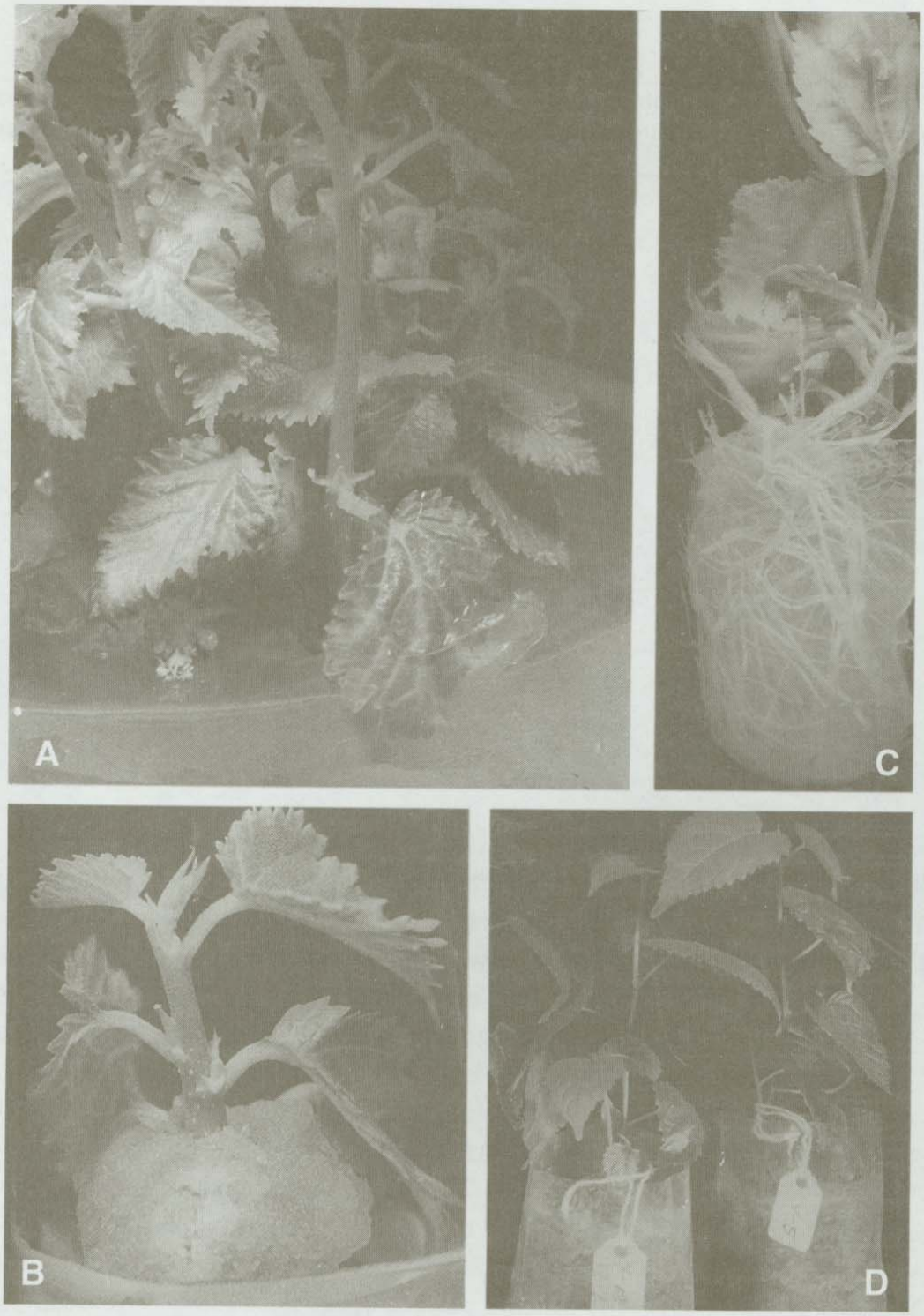
Table 1. (Contd.)

Hormone concentration (mg/l)			% Explants with shoots.	Number of shoots per explants	% Explants with roots	% Explants with callus	Number of response
BAP	+						
0.5	+	0.5	-	-	70.32± 2.03	-	PR
					81.20± 1.76	-	PR
BAP	+	2,4-					
0.5	+	0.5	20.71± 1.21	1.01± 0.13	-	86.89± 1.27	WFC
1.0	+	1.0	-	-	-	85.17± 1.23	WFC

- = No response,
LS = Long shoots,
HS = Healthy shoots,
PR = Profuse roots,
WFC = White friable callus.

Results

Shoot tip explants cultured on different combinations showed their first response by unfurling of leaves and axially bud enlargement within 1-2 weeks of culture (Table 1). Media supplemented with BAP and kinetin alone or in combination produced multiple shoots in 43-77% cultures. Less shooting was obtained with kinetin at the concentrations tested compared to BAP supplemented medium of corresponding concentrations. The mean number of shoots per explant was 3.10 recorded on the medium containing 2.0 mg/1 kinetin but the number was double (6.26) at the same concentration of BAP. However, maximum frequency of multiple shoot proliferation (77.66%) and the highest number of shoot (8.35) per explant were achieved when BAP and kinetin were added together at a concentration of 1 mg/1 each (Fig. A) followed by a same combination at 0.5 mg/1 each. It has been observed that media containing auxin (2,4-D) supplemented with BAP led to the development of one single shoot and stimulated callus formation at the base. Growth of this shoot became stunted (Fig. B, Table 1). In addition shoot formation was inhibited when BAP was added in IBA or NAA containing media and root initiation was stimulated.



Figs. A-D. Micropropagation of *Morus alba* from shoot apices. A multiple shoot proliferation in MS medium supplemented with BAP and Kn at a concentration of 1 mg/1 each after 4 weeks of culture. B. stunted single shoot with callus at the base in MS medium fortified with BAP and 2,4-D at a concentration of 0.5 mg/1 each after 4 weeks of culture. C. Induction of adventitious roots on half MS with IBA and NAA at a concentration of 0.5 mg/1 each after 4 weeks of culture. D. Two transferred plantlets on soil after 3 weeks of transfer.

From proliferated shoot clumps individual shoots (approximately 3 cm in length) were excised and transferred to rooting media. Shoots stimulated to root with auxin were induced in medium containing 1.0 mg/1 each of BAP and kinetin. Root initiation started within 7-9 days of culture. Both IBA and NAA at 0.5 mg/1 were equally effective for root induction but some callus formation was observed at NAA supplemented medium (Table 2). IBA and NAA in combination on the medium on the other hand, promoted rooting frequency, root number and length, and inhibited basal callusing, In this medium the roots also produced profuse lateral roots (Fig. C) which helped the plantlets to establish in the soil quickly.

Table 2. Induction of roots on *in vitro* raised shoots of *Morus alba* L. after five weeks in half-strength MS Medium supplemented with auxin, Each value is an average of 3 replications and each replication consisted of 10 culture tubes..

Auxin (mg/1)	% of shoots rooted	Root number per shoot	Root length(cm)
IBA 0.5	70.33±2.33	12.53±1.22	6.3±0.98
NAA 0.5	67.87±4.21	13.28±2.04	5.8±1.01
IBA 0.5+ NAA 0.5	85.08±4.68	18.41±2.14	8.2±1.93

For maximum shoot production the stock cultures were transferred to fresh medium after each batch of microcutting harvest and in this way they were kept for continuous shoot production for several months. It has been observed that the number of microcuttings produced per subculture, length and quality of microcutting and quality increased gradually up to 7th subculture and then declined.

For transfer to the soil, plantlets were taken out of culture tubes, washed thoroughly to remove any remains of medium and planted in polybag with 1:1 non-sterile garden soil and compost. During the first week of transfer the plantlets were covered with polybags to maintain humidity. Within 3 weeks after transfer to soil the plants began to form new leaves and resumed growth (Fig.D) and later on transferred to the field. Plantlets taken from double auxin supplemented medium surviced in the soil much better than those taken from medium supplemented with single auxin, 80% versus 60%, respectively.

Discussion

The result of the present experiment clearly show that rapid clonal propagation of *Morus alba* is possible through *in vitro* culture of shoot tip explants at a much faster rate than any conventional methods of propagation. Such a multiple shoot proliferation from shoot tip explants of mulberry has been reported [13], with shoot proliferation in cytokinin enriched media and rhizogenesis in an auxin supplemented media. In present

paper, we report better result of shoot proliferation in double cytokinin supplements and stout root with laterals in double auxin supplements. Similar results were reported in *Artocarpus heterophyllus* for shoot and root induction [14] and in guava for root induction [15]. An equimolar concentration of BAP and kinetin also allowed better results for multiple shoot in other species o fruits [16] and ornamental plants [17]. This might be due to the "synergism" between the two growth hormones. In eucalyptus highest number of shoots were proliferated by the synergistic action of NAA (0.1mg/1)and BA(1.0mg/1) in modified MS media (18) which correlates with our findings. Further it has been reported that, the addition of kinetin (4.0mg/1) along with IAA (1.0mg/1) stimulated the growth of pea buds (19), whereas, the nutrient solution having kinetin alone had little effect which also proves that, action of more than one hormone sometimes enhanced frequency of in vitro shoot proliferation. Even in intact plants it has been established that, cytokinins universally promote the out growth of shoots, including that of side shoots (20). Using the method described above, rapid and mass propagation of mulberry plants can be achieved by in vitro shoot tip culture.

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الإكثار الدقيق لأشجار المورس ألبا من القمم النامية للمجموع الخضري للأشجار البالغة

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ملخص البحث . تمت تنمية القمم النامية لأشجار (المورس ألبا) البالغة على بيئة موراشيج وسكوج مضاف إليـــها تراكيز مختلفة من السيتوكينينات والأكسينات. تم إكثار الأنسجة في البيئة المضاف إليها الستوكينين فقط. وكـــان أكبر عدد من المجموع الخضري تم الحصول عليه لكل نسيج مزروع من المزارع النامية على بيئة نمو مضاف إليها ١ مليجرام لكل ١٦ بيترايل أمينو مليجرام و ١ مليجرام كينتين. نمت الجذور الليفية بصورة جيدة عند نقل المجمـــوع الخضري على بيئة أم.أس (نصف قوه)، تحتوي على ٥ر٠ مليجرام لكل لتر لكل من حامض نفتالين اسيتك وحامض اندول بيوتيرك. كما تمت أقلمة النباتات الصغيرة ومن ثم زراعتها بنجاح في التربة تحت الظروف الطبيعيـــــة.