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Mulberry *(Morus alba* L.) micropropagation

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Abstract : Shoot buds of shoot tip and nodal stem cutting of *Morus alba* were cultured on MS medium to produce multiple plantlets. Higher rate of shoot induction, multiplication and plantlet regeneration were observed on MS medium with exogenous addition of growth hormones *viz.* BAP (1.5 mg l^{-1}) and IBA (0.5 mg l^{-1}). Plantlets on transfer to rooting medium of ½ MS with IBA (1.0 mg l^{-1}) produced vigorous rooting. From single shoot bud of mulberry plant about 225 plantlets within 70-75 days were obtained. Successful transfer and survival of 90-95 percent of regenerants to soil have been accomplished. Therefore, with high multiplication rate, present study provides the scope for rapid clonal multiplication of a superior clone of *M. alba.*

Key words: Mulberry, shoot bud, apical bud, axillary bud, exogenous growth hormones, shoot induction, root induction, multiplication

Introduction

Mulberry *(Morus alba* L.) is an economically important woody shrub or small tree. It has been exploited for commercial production of silk mainly as feed crop for the silkworm *(Bombyx mori* L.). It is a chief crop in countries where sericulture industry is undertaken. (Ishikawa and Hirao, 1963; 1965). The silkworm is eventually monophagous. It can survive and grow for a period or even produce poor cocoons on leaves of some other plants than mulberry, but only mulberry leaves can support its normal cocooning and reproduction. Why the silkworm prefers mulberry leaves is not fully understood. It is likely that the silkworm is attracted to some fragrance and is equipped with special organs which respond to the taste of leaves. The silk proteins, which are composed of fibroin and sericin, are totally derived from the mulberry leaves. There is evidence that the content of sugars in the leaves is largely related to that taste (Ishiksawa and Hirao, 1963). Thus the high contents of proteins and sugars characterize this crop as a good diet for the silkworm. Mulberry grows in temperate to tropical regions. However, it has been commercially cultivated mainly in China, Japan, Korea, India and Russia, where most of the cocoons in the world have been produced.

Conventional methods of vegetative propagation of mulberry are grafting and cutting. Root stocks are usually prepared from 1 year old seedlings and scions are taken from branches before buds break. The grafts were grown in the field next year. However, the draw back in the soft wood cutting is, the successful rooting depends on so many factors regarding physiological states of the cutting and environmental conditions to have enough rooting ability. Micropropagation by tissue culture is an ideal method of propagation for plants with less rootable varieties or which are difficult to produce through seeds. There are only a few reports of regeneration of mulberry plants in tissue culture (Mhatre

Table 1. Response of axillary / apical buds of mulberry to various growth regulators.

MS+growth regulators (mg l^{-1})	Explants	Shoot induction (%)		Shoot multiplication (%)		Plantlet regeneration (%)		Nature of response on 35th day after inoculation
		15th day	35th day	15th day	35th day	15th day	35th day	
BAP+IAA	T_1	25.32	42.13	22.19	26.06	18.68	21.72	Green shoots with small leaves
1.0 + 0.5	T_2	23.16	38.16	20.26	25.17	16.17	19.23	Green long shoots
BAP+ IAA	T_1	30.34	48.72	27.18	36.665	23.32	27.16	Multiple shoots
1.5 + 0.5	T_2	28.54	41.08	24.49	34.12	21.03	25.00	Clustered shoots with minute leaves
BAP + IBA	T_1	33.78	54.16	32.16	38.91	28.67	23.18	Healthy long multiple shoots
1.0 + 0.5	T_2	30.30	45.15	28.79	34.16	24.28	29.81	Vigorous shoots
BAP+IBA	T_1	40.47	62.69	38.58	44.56	35.18	39.59	Vigorous multiple shoots
1.5 + 0.5	T_2	36.13	52.67	34.61	40.78	30.65	35.73	Healthy multple shoots
BAP + NAA	T_1	26.64	46.33	22.46	27.64	18.32	21.67	Healthy long branched multiple shoots.
1.0 + 0.5	T_2	25.32	41.53	21.23	25.75	16.56	19.72	Healthy long multiple shoots
BAP+NAA	T_1	32.28	50.50	30.14	36.22	25.71	29.16	Vigorous branched shoots
1.5 + 0.5	T_2	31.17	45.36	28.65	34.15	23.93	27.38	Branched shoots
K+IAA	T_1	22.14	25.13	18.87	23.93	13.85	16.73	Long shoots pale green leaves
1.0 + 0.2	T_2	21.06	23.77	17.98	21.67	11.16	14.33	Moderate growth
K+IBA	T_1	22.08	27.71	17.98	21.67	11.16	14.33	Moderate growth
1.0 + 0.2	T_2	20.25	25.18	16.01	20.13	10.79	13.98	Multiple shoots
K + NAA	T_1	21.00	24.56	15.81	18.32	9.32	12.25	Multiple branched shoots
1.0 + 0.2	T_2	18.68	20.19	14.23	16.43	8.71	10.18	Moderate shoots
CD (5%)		**0.86**	**0.98**	**0.68**	**0.72**	**0.59**	**0.64**	

1. Each treatment repeated thrice with 20 tubes and experimented twice.
2. Only effective hormones of suitable concentrations have been tabulated.
3. T_1 : axillary buds T_2 : apical buds

et al., 1985; Oka and Ohyama, 1986). Sharmila *et al.* (1990) had presented a suitable method for quick *in vitro* multiplication of axillary buds from mature tree of *M. alba* and its suitable sericulture variety *M. alba* var. Mandalaya for commercial utilization.

Materials and Methods

Plant material

Shoot tips and lateral buds were taken from non -woody shoots while they were growing vigorously in the University green house at Sericulture Department, Tamil Nadu Agricultural University, Coimbatore. After surface sterilization with 0.1% (W/V) mercuric chloride solution for 5 min. terminal buds were excised from young leaves under a dissecting microscope, giving rise to small explants of 5 mm in length. Lateral buds were cut into 1 node segments and were taken at a short distance from the top of the shoot. Explants were rinsed 4-5 times by sterile distilled water under aseptic condition inside a laminar air flow chamber.

Culture medium

Explants were cultured on MS medium (Murashige and Skoog, 1962) supplemented with various concentrations and combinations of BAP (6-benzyl amino purine), K (Kinetin), IAA (Indole acetic acid), IBA (Indole butaric acid) and NAA (1-naphthalene acetic acid) (Table 1).

Culture condition

The pH of the medium was adjusted to 5.7 before solidifying the medium with 0.8% (W/V) Difco Bacto agar and dispensed at 20 ml per test tube. All media were autoclaved at 103 K Pa for 25 minutes and cultures were maintained in a culture room at 25±2°C and a relative humidity of 60% with 16:8 hr Light: Dark photoperiod under white fluorescent light (3000 lux). For each treatment 20 tubes were inoculated for one replication and replicated thrice. Each experiment was repeated twice.

Subculturing was done after 20-22 days of culturing. Full grown shoots (2.5 cm and above) with atleast a pair of leaves were cultured for rooting on MS medium consisting of half of the concentrations of inorganic salts, 2% (W/V) sucrose and auxins at a range of concentrations. Finally, plantlets with well developed roots were gradually acclimatized and transferred to the soil under high humidity in the green house.

Results and Discussion

Bud meristems are good materials for producing multiple shoots (Oka and Ohyama, 1986). Pre-existing meristems are easy to develop into shoots and are likely to be genetically stable. In this experiment the response of axillary and apical buds significantly vary to a great extend depending on the growth regulators used. Axillary buds when cultured on MS medium containing both auxins and cytokinins showed the best response in shoot induction, multiplication and plantlet regeneration. Infact, different morphogenetic expressions and behaviours of the explant were observed in different auxin and cytokinin combinations within 15 days of inoculation (Table 1). The levels of inorganic salts in MS medium were satisfactory in culturing of auxiliary and apical bud explants. Carbon sources are essential as energy source and important as osmoticum as well. In most plant tissue cultures, sucrose is the best carbohydrate (Gamborg, 1981). In the presence of sucrose, the bud developed a few outer most leaves within 10 days and maintained viability of the meristem for more than 30 days.

Table 2. Response of *in vitro* formed shoots for rooting in 1/2 MS medium containing different auxins.

Auxins (mg l^{-1})		Root induction (%)		Average No.of roots		Average root length(cm)		Nature of response on 35th day after culturing
		15th day	35th day	15th day	35th day	15th day	35th day	
IAA	0.2	11.35	18.42	2.3	3.2	1.2	2.3	Very poor rooting
	0.5	20.48	28.06	4.7	5.0	1.5	2.6	Very poor rooting
	1.0	25.31	34.17	5.2	5.8	1.3	2.5	Roots necrotic
IBA	0.2	14.56	33.81	2.5	3.8	1.8	3.0	Vigorous rooting
	0.5	65.72	80.14	5.1	6.2	2.1	4.2	Root length and girth are good
	1.0	**80.09**	**92.28**	**7.3**	**8.6**	**3.5**	**7.6**	Vigorous rooting, more length and girth
NAA	0.2	12.68	30.73	2.8	3.5	1.5	2.7	Moderate rooting
	0.5	53.35	75.14	5.0	5.6	2.0	2.8	Moderate rooting
	1.0	72.25	86.32	5.6	6.2	3.5	4.7	Rooting with branches
2,4-D	0.2	12.00	26.32	2.4	3.3	1.4	2.5	Moderate rooting
	0.5	26.32	31.17	4.8	5.4	1.9	2.7	Roots slender
	1.0	28.14	39.36	5.3	6.0	3.2	3.8	Moderate rooting
CD (5%)		**0.65**	**1.08**	**0.38**	**0.52**	**0.08**	**0.13**	

As is common in bud cultures of other plants, mulberry buds absolutely require a cytokinin for their growth. Kinetin was less effective than BAP over a wide range of concentrations. The optimum concentration of BAP was between 0.5 and 2.0 mg 1^{-1} (Shrivastava and Chawla, 2001.). Bud explants were swollen slightly after 4-5 days of culturing. Development of multiple shoots were observed after 10-15 days of culture period. Most distinguishable multiple shoots were noted on MS medium containing BAP (1.5 mg l^{-1}) + IBA (0.5 mg 1^{-1}') and most of the shoots were able to produce complete plantlets. The shoot induction percentage was the highest (62.69%) on the medium with 44.56 per cent shoot multiplication rate and 39.59 per cent plantlet regeneration rate on 35th day after inoculation. The multiple shoots were healthy and vigorous (Sharmila *el al.,* 1990).

The appropriate concentration of agar was 0.8 per cent (W/V), because the medium should be hard enough to hold the explants in an upright position. In 35 days, 6-15 shoots proliferated in the medium, which could be used as explants in the next subculture or transferred to rooting medium. Thus, stock cultures of shoots could be maintained indefinitely by sub culturing at an interval of 30 days.

When the cultured bud developed 5-6 leaves and 5-7 roots on the rooting medium, it was taken out of the culture vessel with care not to damage the roots and transferred to netted mini nursery pots with vermiculate or sand after thoroughly removing the debris of agar. The plant was acclimatized in the mist chamber for about 15 days, supplied with 5 ml of 1/10 MS medium nutrients every 7 days and sufficient amount of water everyday. Then, it was transferred to shade house, followed by gradual exposure to the environmental air and light with ordinary intensity. After 30 days the pot was finally placed in the normal size nursery pot filled with garden soil mixture.

Since the control of adventitious shoot development or somatic embryogenesis is very difficult in mulberry tissues, meristem culture is an accessible tool for micro propagation *in vitro*. Standard method for the culture of shoot buds has been described. Maximum number of shoots were obtained after 35 days of culture. However, more new shoots could be obtained with further sub-culturing. Perhaps exogenous supply of growth hormones (BAP + IBA) might have influence on induction of such shoots (Madhusudan *et al.,* 1998).

Thus, the results provided a practical method for quick *in vitro* production of the economically important plant, mulberry at a faster rate than those conventional method of propagation. It has been found that a single shoot bud of *M. alba* may produce 225 plants within 70-75 days of culture period and successful transfer and survival of 90-95 per cent of regenerants to the soil. Therefore, with high multiplication rate, present study provides the scope of rapid clonal multiplication of a superior clone of *M. alba* as well as a suitable sericultural variety for commercial utilization of mulberry.

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