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MICRO-PROPAGATION OF RAMIE {*BOEHMERIA NIVEA* (L) GAUD} THROUGH SHOOT-TIP AND NODAL SEGMENT CULTURE

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ABSTRACT

Multiple-shoots were obtained from shoot-tip and nodal segments derived from seven week's old *in vitro* grown seedlings when cultured on modified medium supplemented with 1.5 mg/l BAP. Root development from *in vitro* regenerated individual shoots of ramie was obtained when cultured onto modified MS medium supplemented with 0.5 mg/l BAP and 2.0 mg/l IBA. About 60% of these in vitro developed plantlets of ramie were successfully established in the soil. The in vitro propagation of ramie would enable faster rate of multiplication, conservation of germplasm and prevent deterioration of crops.

INTRODUCTION

The strongest fibre, ramie is an excellent source of industrial fibre for textile industries. The most usual method of propagation of this crop is through rhizome. But the rhizomes are bulky in nature, can be stored for a maximum period of 10 days and cause problems during transportation. However, these problems can be overcome by regenerating plantlets with the help of micro-propagation techniques. The *in vitro* developed plantlets can be easily maintained, stored and transported. Since the crop is vegetatively propagated, there is enough scope for fixing heterosis once the heterotic combinations are obtained. Thus, the crop can be propagated and maintained for an indefinite period of time after fixation of heterosis and hybrid vigour. Once the heterotic combinations are identified, the regenerated hybrid plants can be subjected to rapid multiplication using shoot-tip and nodal segments through micro-propagation techniques.

The present investigation deals with micro-propagation of ramie.

MATERIAL AND METHODS

Shoot tips (1-1.5 cm) and nodal segments (1-2 cm) of *B. nivea* were collected from 7 weeks old seedlings grown under *in vitro* condition (in the tissue-culture laboratory of the Dept. of Plant-Breeding and Genetics of Assam Agricultural University, Jorhat). The explants were surface sterilised with 0.1% mercuric chloride solution for 2 minutes followed by 2-3 times washing in sterilised distilled water. Shoot-tips and nodal segments were excised under aseptic condition and inoculated into culture tubes (25 x 150 mm) containing 20 ml modified MS media supplemented with various combinations and concentrations of growth regulators, *viz.*, MS_1 (0.5 mg/l BAP), MS_2 (1.0 mg/l BAP), MS_3 (1.5 mg/l BAP), MS_4 (2.0 mg/l BAP), MR_1 (0.5 mg/l BAP + 0.5 mg/l IBA), MR_2 (0.5 mg/l BAP + 1.0 mg/l IBA), MR_3 (0.5 mg/l BAP + 1.5 mg/l IBA) and MR_4 (0.5 mg/l BAP + 2.0 mg/l IBA). The pH of the medium in each case is adjusted at 5.8 before autoclaving. The cultures were maintained at 25 ± 2^0C and 12 hr light (2500 lux, Phillips TL 40 W/4 fluorescent tubes)/12 hr dark cycles. The results were presented in Table 1, 2 and 3. The data were tested by analysis of variance and least significance difference.

RESULTS AND DISCUSSION

The supplementation of BAP (2.0 mg/l) to the modified MS (Murashige and Skoog, 1962) medium (MS_4) gave the highest percentages (77.50% and 65.00%) of shoot proliferation of ramie from shoot-tip and nodal

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Table 1. Shoot-tip culture of *B. nivea* on modified MS media

Media	No. of shoot tips inoculated	Percentage of cultures responded	Days to shoot elongation	After 7 weeks of culture		
				No. of shoots developed/ shoot tip	Length of shoots (cm)	No. of leaves/ shoot
MS_1	40	62.50_c	20.50_c	1.60_c	2.18_c	5.60_c
MS_2	40	65.00_c	21.40_c	3.80_b	4.78_b	7.40_b
MS_3	40	72.50_b	24.60_b	6.00_a	6.36_a	9.80_a
MS_4	40	77.50_a	26.20_a	3.40_b	4.94_b	8.20_b
S.Ed.±		1.58	0.45	0.47	0.41	0.50
CD (0.05)		3.35	0.98	1.01	0.87	1.05

Means within columns separated by Duncan's Multiple Range Test (P=0.05).

Table 2. Nodal segment culture of *B. nivea* on modified MS media

Media	No. of nodal segments inoculated	Percentage of cultures responded	Days to shoot initiation	After 7 weeks of culture		
				No. of shoots developed/ shoot tip	Length of shoots (cm)	No. of leaves/ shoot
MS_1	40	47.50_d	23.60_c	1.40_c	0.95_c	5.20_d
MS_2	40	52.50_c	24.80_b	1.80_{bc}	2.80_{ab}	6.40_c
MS_3	40	57.50_b	27.00_a	3.20_a	3.58_a	9.60_a
MS_4	40	65.00_a	26.80_a	2.60_{ab}	2.16_b	7.80_b
S.Ed.±		1.11	0.46	0.38	0.39	0.50
CD (0.05)		2.37	0.99	0.82	0.82	1.05

Means within columns separated by Duncan's Multiple Range Test (P=0.05).

Table 3. Rooting of *B. nivea* on various media

Media	No. of shoots inoculated	No. of cultures responded	Days to root initiation	After 7 weeks of culture	
				No. of roots developed/ shoot	Length of roots (cm)
MR_1	40	78.00_d	21.20_a	10.40_c	6.52_d
MR_2	40	83.50_c	16.20_c	11.20_c	7.84_c
MR_3	40	87.50_b	14.40_d	13.80_b	12.42_b
MR_4	40	90.50_a	18.00_b	17.60_a	14.92_a
S.Ed.±		0.87	0.41	0.44	0.40
CD (0.05)		1.83	0.87	0.94	0.86

Means within columns separated by Duncan's Multiple Range Test (P=0.05).

segments respectively after 4 weeks of culture (Table 1 and 2). After 7 weeks of cultures, the number of multiple shoots, average length of shoots and number of leaves developed per shoot were significantly higher in MS_3 medium as compared to the other media tested. Cointry *et al.* (1993) reported multiple shoot regeneration from shoot-tip of linseed in

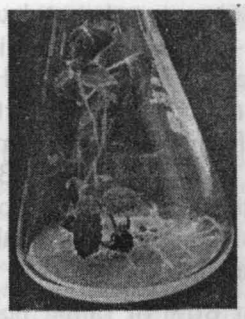

Fig. 1. Explant for micropropagation

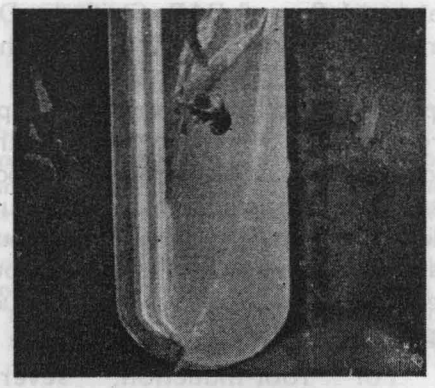

Fig. 2. Multiple shoot production from shoot-tip on MS medium supplemented with 1.5 mg/l BAP

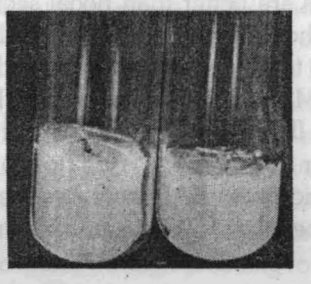

Fig. 3. Multiple shoot production from nodal segments on MS medium supplemented with 1.5 mg/l BAP

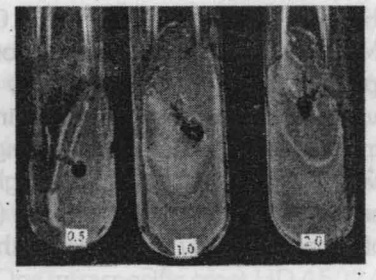

Fig. 4. Multiple shoot production from nodal segments on MS medium supplemented with BAP (0.5, 1.0 and 2.0 mg/l)

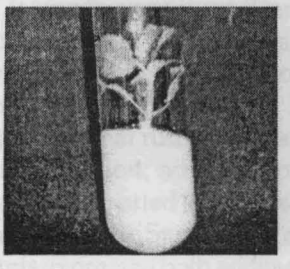

Fig. 5. Rooting of *in vitro* developed shoots on MS media supplemented with BAP (0.5 mg/l) and IBA (2.0 mg/l)

Fig. 6. *In vitro* developed seedlings on soilrite-perlrite (10:1) mixture

Murashige and Skoog (1962) medium containing 1.0 mg/l BAP. Similarly, Del Rosario and Corcolon (1991) reported that NAA and Kinetin caused the development of multiple shoots in cultures of ramie shoot-tips. George *et al.* (1989) also reported the development of multiple shoots from shoot-tip explant of sesame in MS medium supplemented with only 8 mg/l BAP. Thus, the supplementation of BAP at various concentrations seems to be essential to induce multiple shoots in ramie.

For root-induction, several combinations and concentrations of BAP and IBA were tried. The results are presented in Table 3. Roots were initiated and developed when *in vitro* regenerated single shoots were excised and transferred to all the tested media. A combination of 0.5 mg/l BAP and 2.0 mg/l IBA (MR_4) was found to be the best for root development. Root-initiation generally takes place within 18-20 days of inoculation in MR_4 medium. The average number and length of roots were found to be significantly higher in MR_4 medium after 7 weeks of culture (Table 3). Root-initiation usually occurred near the cut-surface of the shoot-explants' basal end. Bajaj and Gill (1986) reported root-development from shoot-tips of cotton in auxin supplemented MS medium. However, Dumanois *et al.* (1986) reported root-development from shoot-tip of ramie on MS medium with both Kinetin and IBA. In the present investigation, a combination of BAP and IBA was found to be essential for root-development in ramie.

About 60% of ramie plants were successfully hardened and successfully established in the soil.

In conclusion the MS medium supplemented with 1.5 mg/l BAP is found to be the best for the explants (shoot-tip and nodal segment) for multiple shoot production. The shoot tips are better than nodal segment for multiple shoot production because of the higher cytokinin to auxin ratio present in the shoot tip. The MS medium with 0.5 mg/l BAP and 2.0 mg/l IBA is found to be the best medium for better root system. Addition of IBA in combination with BAP increases the number of roots per shoot and the length of roots.

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