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Lateral Bud Culture of Papaya (*Carica papaya*) for Clonal Propagation

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Abstract: *In vitro* culture of lateral buds of field grown mature plants were tested in MS medium supplemented with different concentrations of BAP and NAA. Seasonal endophytic contamination was suppressed by shaking propagules for 2 h in 300 mg L^{-1} rifampicin before surface sterilization. Maximum survival rate was found in MS medium supplement with 1.0 mg L^{-1} BAP plus 0.20 mg L^{-1} NAA. The highest number of shoots was produced in MS medium containing 0.50 mg L^{-1} BAP and 0.20 mg L^{-1} NAA. Both the highest multiplication rate and the longest shoot were found in MS medium supplemented with 0.50 mg L^{-1} BAP plus 0.20 mg L^{-1} NAA. On the same treatment an increasing trend was observed in multiplication rate of shoot upto 5th subculture which decreased thereafter. First subculture produced the highest shoot length which decreased with the increase in number of subculture. Half strength MS medium supplemented with 1.0 mg L^{-1} IBA was found as the best treatment to produce root in the culture media. Rooted plantlets were transplanted successfully.

Key words: *In vitro*, *Carica papaya*, lateral bud, clonal propagation

INTRODUCTION

Papaya (*Carica papaya* L.), an economically important crop of the tropics and sub tropics, is commercially propagated by seed. Since papaya is a polygamous species, many forms of inflorescences have been reported by many authors (Reuveni *et al*., 1991). Papaya is conventionally propagated by seed and therefore cultivation is hindered by problems due to the sex reversal, inherent heterozygosity and dioeciously nature of the crop (Veerannale, 1984; Rajeevan and Pandey, 1986). To avoid these problems tissue culture propagation could offer a valuable and a reliable procedure for propagation of papaya. Litz and Conover (1978 and 1981), Drew (1988), Winner (1988) and Rahman *et al.* (1992) reported a procedure based on shoot tips of field grown mature trees. The unbranched nature of papaya limits supply of shoot tips for initial culture. Moreover, excision of shoot tips from adult plant is hazardous and often results in death. This problem can be overcome by using lateral bud as explants for *in vitro* propagation. In our country, protocol has been established from papaya seedling but there is no recognized protocol for *in vitro* regeneration of papaya from mature plants and in this study, attempts have been made for rapid tissue culture of papaya by using lateral buds from mature field grown plants to establish suitable protocol for rapid plant regeneration *in vitro* from mature plants.

MATERIALS AND METHODS

The experiment was conducted in the Tissue Culture Laboratory of Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur during June 2004 to March 2005. Lateral buds consisting of the top young unexpended leaves collected from field grown mature plants were used as explants in this study. The buds were shaken in an antibiotic, rifampicin (RIF) at 300 mg L^{-1} to suppress the bacterial contamination. The buds were then cleaned with soap water and treated for 7 min with mercuric chloride (0.1%) containing a few drops of Tween-20. This was followed by 3-4 washings with sterile distilled water. The explants

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were agitated in sterile distilled water for 2-3 h in horizontal shaker at 160 rpm before inoculation to minimize the flow of latex into medium. The MS (Murashige and Skoog, 1962) was used as basal medium with different concentration of BAP (0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 3.0 and 4.0 mg L^{-1}) with different concentration of NAA (0.10, 0.20 and 0.30 mg L^{-1}). Individual elongated shoots were excised carefully and transferred to half strength MS media with Indole-3-Butyric Acid (IBA) at different concentrations (0.10-0.5 mg L^{-1}). The pH of the media was adjusted at 5.8 prior to autoclave and 8 g L^{-1} agar was added to solidify the media. The cultures were kept under approximately 2000 lux fluorescent light at 25±1°C with 16 h photoperiod.

RESULTS AND DISCUSSION

Establishment: A high rate of contamination was appeared as the cloud like growth at the base of the explants at the establishment stage of the explants. Shaking the lateral buds with RIF (300 mg L^{-1}) was found to be most efficient in reducing contamination. Lateral buds shaken in rifampicin increased the uncontaminated cultures to 50% (Fig. 1). Reuveni *et al.* (1990) first applied FIR in suppressing bacterial contamination I culture of papaya and contamination rate reduced to 20%. RIF has also been reported in suppressing bacterial contamination in culture of other plant species (Bastiaens *et al.*, 1983; Phillips *et al.*, 1981; Young *at al.*, 1984). Buds survived and grew better at 0.50 and 1.0 mg L^{-1} BAP with all level of NAA (Table 1). Maximum number of surviving buds (53.33%) was recorded at 1.0 mg L^{-1} BAP supplemented with 0.20 mg L^{-1} NAA. Maximum number of explants produced shoot (31.67%) in MS medium supplemented with 0.50 mg L^{-1} BAP plus 0.20 mg L^{-1} NAA. New growth was visible after 3-4 weeks of culture bud. The buds were considered to be established only when new growth spread approximately 1-2 cm diameter within 30-40 days. Bud cultured appeared very compact with highly shortened internodes and reduced leaf lamina (Fig. 2). The buds were transferred with a basal cut to fresh establishment. The buds were transferred with a basal cut to fresh establishment medium for accelerating growth.

Multiplication of shoots from buds established in culture: Individual shoots were excised from the proliferating culture and subcultured into fresh MS medium supplemented with different concentration of BAP (0.50-2.0 mg L^{-1}) along with different concentration of NAA (0.10-0.3 mg L^{-1}). The first subculture was made after 90 days of cultures and the subsequent culture at an interval of 20 days. Total six subcultures were tested and

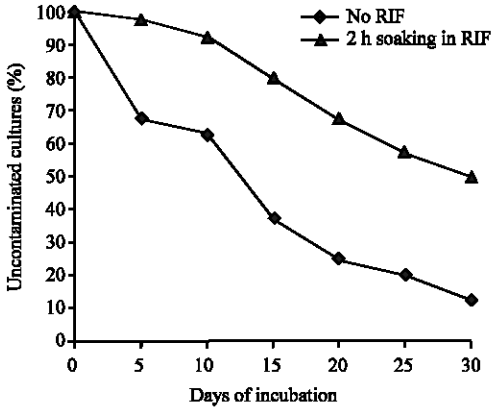

Fig. 1: Percentage of uncontaminated cultures of papaya explants following 2 h soaking treatment with rifampicin (RIF 300 mg L^{-1})

Table 1: Effect of growth regulators on the establishment of lateral bud explants

Growth regulators (mg L^{-1})			
BAP	NAA	% explants survived*	% explants produced shoots
0.00	-	11.67h	-
0.10	-	16.67g	4.167i
0.20	-	18.33fg	4.167i
0.30	-	17.50fg	6.667hi
0.40	-	20.83f	6.167hi
0.50	-	34.17d	18.33de
1.00	-	41.67c	21.67cd
2.00	-	32.50d	22.50cd
3.00	-	28.33e	14.17ef
4.00	-	27.50e	9.167gh
0.50	0.10	45.83b	28.33ab
0.50	0.20	49.17b	31.67a
0.50	0.30	49.17b	29.17ab
1.00	0.10	52.67a	30.83a
1.00	0.20	53.33a	25.83bc
1.00	0.30	48.33b	22.50cd
2.00	0.10	34.17d	20.00d
2.00	0.20	33.33d	20.00d
2.00	0.30	27.50e	12.50fg
SE(±)		0.84	0.70
Mean		33.86	18.21

*Data after 30 days of inoculation. Within a column means followed by same letter(s) did not differ significantly at 1% level

the treatment MS medium supplemented with 0.50 and 0.20 mg L^{-1} was found to be the best for shoot multiplication and shoot length (Table 2 and 3). An increasing trend in shoot multiplication rate was observed with the increase in subculture number upto 5th subculture which decreased there after. In the present study, average 12.73 fold shoot multiplication rate and 2.83 cm shoot length was observed (Table 4). The highest shoot length was found from 1st subculture (Fig. 3) which slightly decreased in the subsequent cultures. With addition of BAP and NAA in the MS medium Litz and Conover (1978), Hossain *et al.* (1993), Winner (1988) and Bhuyain and Akond (2000) obtained almost similar result.

Table 2: Effect of growth regulators on multiplication rate at different in papaya

Growth regulators (mL)		Subculture					
BAP	NAA	1st subculture	2nd subculture	3rd subculture	4th subculture	5th subculture	6th subculture
0.50	0.10	4.40cd	8.60cde	7.60c	6.80c	5.60c	3.80cd
0.50	0.20	7.90a	12.60a	13.60a	14.40a	16.20a	11.80a
0.50	0.30	6.20b	9.40cd	8.00c	7.00c	6.40c	4.60c
1.00	0.10	6.40b	9.20cd	8.00c	6.60cd	5.40cd	3.80cd
1.00	0.20	7.80a	11.20b	11.60b	11.0b	9.80b	7.80b
1.00	0.30	5.80b	9.8c	5.80d	5.40de	4.20de	3.40cd
2.00	0.10	5.20bc	8.20de	5.00de	4.60e	3.60e	2.60de
2.00	0.20	4.00cd	7.40e	4.00ef	3.00f	2.20 f	1.60e
2.00	0.30	3.40 d	5.60f	3.20f	2.60f	1.80f	1.40e
SE(±)		0.25	0.31	0.50	0.55	0.64	0.48
Mean		5.67	9.11	7.42	6.82	6.13	4.53

Within a column means followed by same letter(s) did not differ significantly at 1% level

Table 3: Effect of growth regulators on shoot length at different subculture in papaya

Growth regulators (mL)		Shoot length (cm)					
BAP	NAA	1st subculture	2nd subculture	3rd subculture	4th subculture	5th subculture	6th subculture
0.50	0.10	2.10b	2.06b	1.88b	1.70c	1.50c	1.24c
0.50	0.20	3.24a	3.12a	3.00a	2.80a	2.56a	2.26a
0.50	0.30	2.20b	2.14b	1.70c	1.46cd	1.28cd	1.04cd
1.00	0.10	1.74c	1.64c	1.40c	1.24de	1.00d	0.90de
1.00	0.20	3.12a	3.02a	2.82a	2.52b	2.02b	1.98b
1.00	0.30	1.94bc	1.56c	1.36c	1.18e	0.96de	0.72ef
2.00	0.10	1.24d	1.14d	0.96d	0.84f	0.66ef	0.54fg
2.00	0.20	1.02d	1.02d	0.90de	0.74fg	0.62ef	0.46gh
2.00	0.30	0.98e	0.76e	0.70e	0.56g	0.44f	0.26h
SE(±)		0.13	0.12	0.12	0.11	0.10	0.10
Mean		1.98	1.84	1.64	1.45	1.24	1.04

Within a column means followed by same letter(s) did not differ significantly at 1% level

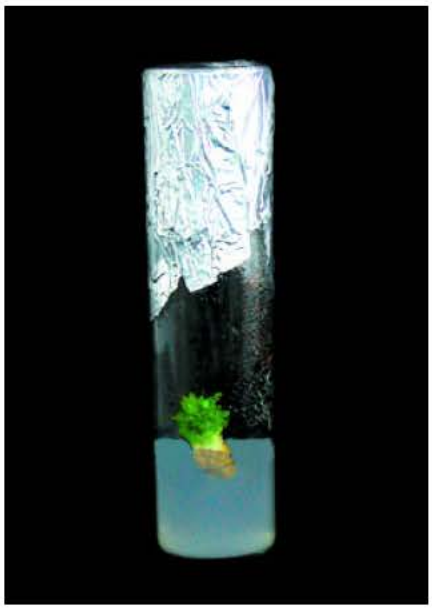

Fig. 2: Growth of lateral bud in establishment medium (0.50 mg L^{-1} BAP + 0.20 mg L^{-1} NAA) after 40 days of incubation

Fig. 3: Multiple shoot in 1st subculture in MS medium containing 0.50 mg L^{-1} BAP+0.20 mg L^{-1} NAA

Rooting: In this study for rooting different concentrations IBA (0.10, 0.20, 0.30, 0.40, 0.05, 1.0, 1.5 and 2.0 mg L^{-1}) in half strength MS medium were tested for rooting but 0.10, 0.20, 0.30, 0.40 and 2.0 mg L^{-1} IBA produced no root. Half strength MS supplemented with 1.0 mg L^{-1} IBA gave the best result for all the characters studied (Table 5). The

Fig. 4: Rooted plantlet in half MS supplemented with 1.0 mg L^{-1} IBA

Fig. 5: A papaya seedling produced from *in vitro* culture of lateral bud

treatment 1.5 mg L^{-1} IBA produced very few numbers of roots with very short length. Large callus at the base of the shoot was found and no plantlet survived from this concentration. Root of elongated shoots was obtained at relatively high frequency when IBA at 1.0 mg L^{-1} was incorporated in the medium (Fig. 4). Litz and Conover (1978) recommended rooting to be carried out on half strength MS supplemented with NAA (0.10-1.5 mg L^{-1}) or IBA (0.10-1.0 mg L^{-1}), but IBA induced the best result.

Table 4: Multiplication rate of shoots at different subcultures in MS medium supplemented with 0.50 mL^{-1} BAP and 0.20 mL^{-1}

No. of subculture	Age of culture (days)	Multiplication rate*	Shoot length (cm)*
1st	90	7.90±0.45	3.24±0.21
2nd	110	12.60±0.89	3.12±0.08
3rd	130	13.60±0.55	3.01±0.15
4th	150	14.40±0.56	2.80±0.16
5th	170	16.20±0.84	2.56±0.17
6th	190	11.8±0.83	2.26±0.11
Average		12.73	2.83

*Mean± standard deviation

Table 5: Effect of IBA on rooting of papaya shoots

Growth regulators (mg L^{-1})	Days to rooting	% shoot produced root	No. of roots	Root length (cm)
0.10				
0.20	-	-	-	-
0.30			-	-
0.40	-	-	-	-
0.50	22.20b	24b	2.00b	1.08b
1.0	11.80c	50a	5.00a	2.18a
1.5	25.20a	28b	2.80b	0.66c
2.0	-	-	-	-
SE(±)	1.53	2.16	0.72	0.51
Mean	19.73	34.00	3.27	1.31

Within a column means followed by same letter(s) did not differ significantly at 1% level

Rooted plantlets were transplanted to plastic pot containing mixture of compost and soil and covered with polythene to protect from dehydration. After hardening they were transferred to net house (Fig. 5) until planted in the soil.

CONCLUSIONS

In our laboratory, this experiment became helpful to develop a protocol for *in vitro* regeneration of papaya plant from mature plant using lateral bud. Although the survival rate of the plantlets were low, its improvement is possible for commercial purposes and the present study indicated the possibility of producing papaya plants from mature plants through later bud culture. In the culture media, formation of calli is a great problem to develop root and further study will help to remove the problem.

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