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IN VITRO CLONAL PROPAGATION OF PAPAYA (*CARICA PAPAYA* L.)

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Abstract

A procedure for the rapid clonal propagation of (*Carica papaya* L.) was developed by culturing apices of mature field grown plants. The explants were successfully established on MS medium supplemented with 10 mg/l kinetin and 2 mg/l NAA. The growing explants upon transfer to a proliferation medium containing reduced levels of BA (0.5 mg/l) and NAA (0.1 mg/l) produced maximum number of shoots per explant. Shoots rooted well in IBA supplemented MS medium and plantlets developed were transferred to soil.

Introduction

Papaya (*Carica papaya* L.) is a rapidly growing, perennial herbaceous plant that can attain a maximum height of 10 m after 1-2 years. Laticifers occur in all organs and tissues. Plant can be dioecious or hermaphroditic, although many intermediate sex types also occur. Fruits range in shape from round to pyriform to elongate and individual fruit can weigh as much as 10 kg. Flesh may be yellow or red and can have a pleasant aroma with the flavor of a melon. Ripe papaya is a good source of pro-vitamin A and ascorbic acid. Papain, a proteolytic enzyme is extracted from its latex which is widely used in medicine for the treatment of bed sores and insect bites (Litz, 1986). Extracts from papaya plants have also been effective as a vermifuge and as a purgative. The occurrence of carpaine in papaya, an alkaloid has been used as amoebicide, diuretic and heart depressant (Chan & Tang, 1979).

Papaya is conventionally propagated by seed and therefore, cultivation is hindered by problems due to inherent heterozygosity and dioecious nature of the crop. Although conventional techniques of asexual reproduction such as grafting and rooted cuttings exist, they are often tedious and impractical when carried out on a large scale (Allan, 1964). Tissue culture propagation could offer a valuable alternative and a reliable procedure for large scale propagation of papaya. There were also attempts for propagation of papaya through tissue culture. However, many of them (Arora & Singh, 1978; De Bruijne *et al.*, 1974; Yie & Liew, 1977; Litz & Conover, 1981) involved callus step and dealt with seedlings only. Procedures of Litz & Conover (1978), Rajeevan & Pandey (1986) and Winnaar (1988) using lateral and apical buds is free from above drawback but considerable research is still required to make it economically feasible. In this paper, a mass propagation scheme for culture of apices of field-grown plants of Carica papaya 'Rajshahi-red' is described.

Materials and Methods

Shoot apices from 5 month old field grown plants of *Carica papaya* cv. 'Rajshahi-red' were removed and thoroughly washed under running tap water. The material was surface sterilized with $HgCl_2$ (0.1 w/v) for 5 min containing a few drops of Tween-20 followed by 3-4 rinses in sterile distilled water. The external surfaces of the tissue were then

trimmed, leaving only the apex which was placed on establishment medium consisting of MS medium (Murashige & Skoog, 1962) supplemented with different concentrations of benzyladenine (BA), kinetin or 2- ispentenyladenine (2ip) and naphthaleneacetic acid (NAA). Cultures were established in 25x150 mm culture tubes containing 15 ml of medium. Successfully established explants were transferred to a proliferation medium containing same basal medium with 0.1-1 mg/l BA or kinetin in combination with 0.1 mg/l NAA or indoleacetic acid (IAA). The proliferating explants were cultured in 250 ml flasks containing 50 ml of medium. Multiple shoots were separated and placed into 25x150 mm culture tubes with 15 ml of culture medium for adventitious root induction. Indolebutyric acid (IBA) or NAA was used on shoots of third subculture.

The pH of the media was adjusted to 5.7 prior to autoclaving and all media were solidified with 7 g/l Difco Bacto-agar. All the growth regulators were added before autoclaving for 15 min at a temperature of 121°C and a pressure 1.1 kg cm^2. Cultures were maintained in a growth chamber at a temperature of 26°C under 16 h photoperiod with a light intensity of 60-70 uE m^{-2} s^{-1} provided by warm-white fluorescent tubular lamps.

Results and Discussion

Preliminary observations revealed that phytohormones are essential for primary establishment and growth of shoot apices in papaya. No stimulation of growth occurred on MS basal medium only and the explants became chlorotic and eventually died. A series of attempts were made to establish shoot apices in culture using different cytokinins and auxin individually and mixed together. BA, kinetin and 2ip alone and in combination with NAA were capable of stimulation of shoot apices and kinetin-NAA combination was found to be most effective than any other combinations. Thus, only kinetin-NAA combination was used in the present study. Incubation in the dark and changes in sugar concentration did not appear to influence growth of explants. The

Table 1. Effect of kinetin and NAA in MS medium on the establishment of shoot apices of *Carica papaya*. Data recorded after 8 weeks of culture initiation. Twelve to 15 cultures per treatment were used.

Growth regulators (mg/l)		Survival	Growing
Kinetin	NAA	%	%
5	0.5	20	0
10	0.5	25	15
15	0.5	21	0
5	1	35	0
10	1	40	20
15	1	32	25
5	2	30	0
10	2	57	42
15	2	15	30

explants survived and grew better at 10 mg/l kinetin in combination with all levels of NAA and performances increased with the increasing level of NAA (Table 1). Maximum number of surviving explants (57%) and growing cultures (42%) were recorded at 10 mg/l kinetin and 2 mg/l NAA. New growth of shoot apices was visible within two weeks of culture initiation but the explants were considered to be established only when lamina of the leaves spreaded accompanied with elongation of petiole and internode within 7-8 weeks of culture. Further exposure to this medium caused more leaf expansion with no proliferation of shoots or roots. In some cases limited amount of callus was found to develop at the base of the explant.

After eight weeks of culture initiation, the explants were transferred to medium containing low levels of BA or kinetin in conjunction with NAA or IAA (Table 2). Within two weeks of transfer a number of axillary and adventitious buds oozed out from the nodal and internodal portions of the explants (Fig.1). Later, many of these buds developed as young shoots. However, capacity of these buds to develop shoots was different on different media preparation.

Both kinetin and BA at various concentrations were capable of inducing differentiation. Of the two cytokinins, BA was better than kinetin and of the auxins, NAA was more efficient than IAA. Thus, the best results for shoot formation were obtained using BA at 0.5 mg/l and NAA at 0.1 mg/l (Fig.2). This hormone balance is consistent with other reports (Drew, 1988; Winnaar, 1988). However, kinetin exerted favourable effects on shoot growth for easy and rapid handling during subculturing as reported by Rajeevan & Pandey (1986). Individual shoots from proliferating cultures were excised and placed in fresh medium at every four weeks interval. During the process of subculture a multiplication rate of 10-12 fold was achieved. Transfer of shoots on a nutrient medium without any growth regulators did not favour root development. Root formation occurred only when 0.1-1 mg/l IBA or NAA was added to the medium where IBA @ 1 mg/l

Table 2. Effect of BA or kinetin in presence of NAA or IAA in MS medium on prolif and growth of shoot apices of *Carica papaya*. Data recorded after 14 weeks of culture initiation (6 weeks of subculture). Ten cultures per treatment were used.

Growth regulators (mg/l)				No. of shoots per explant	Shoot length (cm)
BA	0.1	NAA	0.1	34.7±2.5	0.7±0.3
	0.5		0.1	48.4±3.0	0.8±0.2
	1.0		0.1	41.2±2.1	0.8±0.1
Kinetin	0.1	NAA	0.1	20.5±1.4	1.2±0.1
	0.5		0.1	28.6±2.2	1.4±0.3
	1.0		0.1	31.4±1.8	1.1±0.2
BA	0.1	IAA	0.1	30.2±2.6	0.7±0.1
	0.5		0.1	36.3±1.8	0.8±0.1
	1.0		0.1	33.7±1.1	0.9±0.3
Kinetin	0.1	IAA	0.1	15.5±0.9	1.4±0.2
	0.5		0.1	16.6±2.1	1.3±0.4
	1.0		0.1	13.2±1.7	1.3±0.3

Fig.1-3. Clonal propagation from shoot apices of papaya. 1. Development of shoot buds from nodal and internodal portions after 5 weeks of culture in MS medium supplemented with 10 mg/l kinetin and 2 mg/l NAA. 2. Development of young shoots after transfer onto MS medium with 0.5 mg/l BA and 0.1 mg/l NAA (4 weeks after transfer). 3. Induction of adventitious roots on MS medium containing 1 mg/l IBA, after 5 weeks of culture.

was found most effective. About 55% of shoots rooted in this medium and primary roots ranged from 5-12 in number (Fig.3). Shoots were more prone to rooting with increasing subcultures (data not shown). The subculture treatment might rejuvenate shoots with a high degree of regenerative competence. The rooted plants were transferred to soil and acclimated with natural environment. Transplantation success was 60%.

The study has demonstrated the feasibility of establishing proliferating differentiated cultures of papaya directly from field-grown plants apices without first inducing callus. Avoiding an intervening callus phase by inducing direct organogenesis, may on the other hand, produce a multitude of plants without risking clonal fidelity.

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