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TISSUE CULTURE PROPAGATION OF PAPAYA[1]

RICHARD E. LITZ AND ROBERT A. CONOVER
IFAS, Agricultural Research and Education Center, University of Florida, 18905 SW 280 St., Homestead, FL 33031

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Abstract. **A procedure for the rapid tissue culture propagation of papaya** (Carica papaya **L.) has been developed as part of a project to obtain virus tolerance in this crop. The success of this technique is dependent upon 1) time of year, 2) the nature of the primary explant, and 3) the hormone component of the growth medium. This propagation method should enable one to multiply disease-tolerant varieties useful to the industry of South Florida.**

The papaya disease caused by distortion ringspot virus has effectively limited the production of this crop in South Florida for several years (1). A breeding program at Homestead under the direction of Prof. R. A. Conover has been in existence since 1975 (2) and efforts have been directed toward introducing tolerance to the virus from plant accessions from many tropical countries. At the same time, attention has been focused on obtaining high yielding papaya lines that bear fruit of good quality.

In order to preserve these desirable characteristics, and still retain tolerance to distortion ringspot, a reliable method for the clonal propagation of papaya is required. However, papayas have traditionally been propagated by seed, and considerable variability occurs as a result of open pollination. In some parts of the world, multibranched papayas are favored, and excised branches are routinely rooted. The papaya that is grown in Florida is generally non-branching. Even after the decapitation of the plant to induce a branching habit, this papaya does not lend itself to clonal propagation. Nor has grafting been very successful.

Tissue culture has been developed as an alternative to conventional propagation methods, and has been successfully adapted to many tropical plants. Therefore, a tissue culture unit was established at Homestead during the summer of 1976 in order to formulate *in vitro* systems for the preservation of breeding lines and for the rapid clonal propagation of papaya plants.

There have been 2 attempts to define a tissue culture propagation system for papaya. One of these (3) obtained proliferating plants from tissues isolated from 5-6 cm seedlings on a simple medium with kinetin. However, this formulation has been ineffective for tissues from mature papaya plants whose characteristics are known. The other system (4) was formulated for use with explants from mature papaya tissues, and caused rapid callus induction from petiole segments. Embryo and shoot formation was induced from this callus by a more complex medium. This system is not ideal for the plant breeder or propagator because papaya callus is heterogeneous in origin, and the cells have different ploidy levels.

Materials and Methods

Papaya plants (*Carica papaya* L.) of any state of development have been used. However, after some trial and error only the apical regions of the plants were retained. The larger petioles were discarded, leaving only the apex and a few small (1-2 mm diam) petioles. The small petioles including the axillary buds at their bases were removed and with the apex were rinsed in 95% ethanol, and sterilized for 15-20 min in continuously agitated 20% (v/v) Clorox. Following 3 rinses in sterile, distilled water, the explants were placed into culture.

The basal salts and organic mixture of Murashige and Skoog (5) was used together with different concentrations of plant growth substances in a latin square design: 5 μM-50 μM kinetin with 0.5 μM-10 μM α naphthalene acetic acid (NAA) and 3 μM-10 μM β indole acetic acid (IAA) with 0.2 μM-9 μM 6-benzyl amino purine (BA). The mixtures were solidified with 8 g/l Difco Bacto agar and pH was adjusted to 5.7 before sterilization at 15 psi and 120° C. Cultures were maintained at 28° C with 16 hr light (3500 lux) and 8 hr darkness.

Results

Initially, axillary buds were used from various positions on the plants; however, except for those buds that originated near the apex, these explants were difficult to surface-sterilize, and were usually flower buds. Callus could be induced from these tissues, but axillary shoot formation without callus induction was not possible. Efforts to improvise new media formulations were restricted by the adverse weather conditions during last winter, when frost damage and microbial contamination severely limited the number of cultures that could be established successfully. Bacterial contamination of the primary explants during the winter months appeared to be related to the slower rate of growth of the plants at this time of year. Contamination has continued to be a hindrance to rapid progress, and in excess of 80% of all tissues placed into sterile culture ultimately must be discarded because of it.

Various formulations of growth substances were attempted (Table 1) including those that had appeared in the literature. The most satisfactory results were obtained either when low levels of BA and NAA (2.0 μM BA + 0.5 μM NAA) were present in the medium or when high concentrations of kinetin together with relatively high concentrations of NAA (50 μM kinetin + 10 μM NAA) were used. The establishment period for the explant on these formulations was about 3 months, by which time the first indication of proliferation was evident. The medium that contained kinetin and NAA produced plantlets with thick, expanded, dark green leaves, but no callus formation occurred. Pro-

Table 1. The effect of hormone levels on papaya explants (Murashige and Skoog basal medium).

Auxin conc		Cytokinin concentration: Kinetin, Low conc	Kinetin, High conc	BA, Low conc	BA, High conc
NAA	0	—[z]	deformed growth	—	—
	low	—	—	shoot formation	—
	high	callus	shoot formation	callus	callus
IAA	low	—	callus	callus	—
	high	callus	callus	callus	—

[z]Medium formulation according to (3).

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liferation, however, was very slow. Small, discrete plantlets with typical young papaya leaves were formed on the medium containing BA and NAA. There was a limited amount of superficial callus induction at the cut edges of the tissue. The medium with BA and NAA was preferable as a proliferation formulation (Table 2). Explants that had been transferred from media that contained kinetin and NAA onto media with BA and NAA began to grow and proliferate very rapidly. This formulation has since been used as an establishment medium.

Table 2. Optimum formulation for establishment and proliferation of papaya (Murashige and Skoog basal medium).

Hormone components	Plantlet formation	Proliferation
2.0 μM BA + 0.5 μM NAA	Small, discrete leaves compact growth	very rapid
50 μM Kinetin + 10 μM NAA	Large, hard leaves spreading habit	good establishment slow proliferation

Proliferation of the explants occurred by the stimulation of axillary buds whereby a small plantlet was formed in the axil of each petiole. The axillary growth resulted in an extremely bushy appearance of the cultures (Fig. 1). Division was effected by excision of the nodes and by sub-culturing them on fresh proliferation media. The rate of increase of the cultures was extremely rapid, with a six-fold increase in the number of plants occurring during any 3 week period (*Table* 3). Thus, from a single explant, theoretically several hundred million identical plants could be obtained within twelve months.

Fig. 1. A rapidly proliferating papaya culture two weeks after subculturing on Murashige and Skoog basal medium with 2.0 μM BA + 0.5 μM NAA.

Table 3. Theoretical proliferation data[z] for papaya on Murashige and Skoog Basal medium supplemented with 2.0 μM BA and 0.5 μM NAA.

Date	No. culture
Day 1	1
Day 90[y]	2
110	12
130	72
150	432
170	2,592
Day 250	3,359,232
Day 270	20,155,392
etc.[x]	

[z]Theoretical proliferation data have been based on 4 months observation of proliferating cultures.
[y]The establishment period for the primary explant was approximately 90 days.
[x]This rate of increase would yield several hundred million plants/annum.

Young plants that have been transferred from proliferation media onto Murashige and Skoog basal medium (without hormones) reached an easily handled size in 3 weeks. Rooting was induced by the inclusion of 0.5 μM NAA in the medium.

Discussion

An extremely rapid *in vitro* clonal propagation method for papaya has been developed. If this propagation system should prove to be economically viable, it will be possible to release for the first time, named varieties of high yielding papayas with tolerance to distortion ringspot virus. Furthermore, the parent plants of these new varieties could be maintained indefinitely as part of an on-going crop improvement program.

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