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Short communication

Clonal propagation of papaya in vitro

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Abstract. A procedure for the rapid tissue culture propagation of papaya is being developed. Tissue culture methods using apices of nursery and orchard trees of *Carica papaya* cv. "Sunrise Solo" were evaluated. The explants were established in a modified Murashige and Tucker (1969) basal medium with half-strength inorganic salts, $0.5\,mg\,l^{-1}$ 6-benzylaminopurine (BA) and $0.2\,mg\,l^{-1}$ naphthaleneacetic acid (NAA). Established explants were transferred to a proliferation medium consisting of Murashige and Tucker (1969) basal medium, $0.5\,mg\,l^{-1}$ BA and $0.1\,mg\,l^{-1}$ NAA, which caused extensive multiplication of shoots. Rooting was induced at a higher frequency by subculturing plantlets onto media with indole-3-butyric acid (IBA) than with NAA.

Introduction

Papaya is conventionally propagated by seed and therefore cultivation is hindered by problems due to the inherent heterozygosity and dioecious nature of this crop [7]. Although conventional techniques of asexual propagation such as grafting and rooted cuttings [1] exist, they are often tedious and impractical when carried out on a large scale. Tissue culture propagation could offer a valuable alternative, and a reliable procedure for tissue culture propagation of papaya by using shoot tips of field trees, has been reported [3]. However, bacterial contamination limited success [2, 3, 4].

In this study attempts were made to develop a procedure for the rapid tissue culture propagation of papaya by using the apices of small *lateral branches* on trees, rather than shoot tips of mature trees. This method of propagation could be used to supply homogeneous plants for both commercial and research purposes. As the sex of the explants is known, the required proportion of male to female plants could be planted directly in the field. This method could also facilitate the rapid propagation of selected genotypes.

Materials and methods

Shoot tips from the small side shoots that develop on the main stem of field grown trees; and shoot tips from young nursery trees of *Carica papaya* cv. "Sunrise Solo", were collected. The larger leaves and flower primordia were trimmed off and the remaining apical tissue was agitated in distilled water, containing a few drops of detergent, for 10 to 15 minutes, to minimize the flow of latex. The tissue was then rinsed in 70% ethanol for 1 minute and was surface-sterilized in 1% sodium hypochlorite for 15 to 20 minutes, followed by 3 rinses in sterile distilled water. The exposed surfaces of the tissue were then trimmed, leaving only the *apex* (2 × 2 mm) which was placed into culture. Two growth media were evaluated for the in vitro establishment of the cultures. Basal medium 1 (BM1) consisted of Murashige and Skoog [5] salts and organic mixture. It was supplemented with 10.76 mg l^{-1} kinetin, 1.86 mg l^{-1} NAA and 30 g l^{-1} sucrose [2]. Basal medium 2 (BM2) consisted of Murashige and Tucker [6] basal medium, which was modified by reducing the inorganic salts to half-strength with the normal organic mixture and 30 g l^{-1} sucrose. BM2 was supplemented with 0.5 mg l^{-1} BA and 0.2 mg l^{-1} NAA. Cultures were established in 23 mm × 100 mm culture tubes containing 14 ml of medium and closed with polypropylene caps.

After 90 days on growth medium (BM2) the enlarged apices were transferred to a proliferation medium (BPM) containing MT salts and the organic mixture plus 0.5 mg l^{-1} BA, 0.1 mg l^{-1} NAA and 30 g l^{-1} sucrose. Gibberellic acid (GA_3) was incorporated in the BPM for the fourth and fifth subcultures. The proliferating explants were cultured in 100 ml Erlenmeyer flasks, containing 25 ml of medium. The flasks were capped with aluminium foil.

The multiple shoots were separated during each of the subcultures and placed into 25 mm × 150 mm culture tubes containing 20 ml of culture medium, for root induction. NAA was only used on shoots from the second and fourth subcultures, while IBA was used on shoots of the second, third, fifth, sixth and eighth subcultures.

The pH of the media was adjusted to 5.7 prior to sterilization and all media were solidified with 8 g l^{-1} agar. All the growth regulators were added before autoclaving for 15 minutes at a temperature of 121 °C and a pressure of 1.2 kg cm^{-2}. Cultures were maintained in a growth chamber at a temperature of 26 °C and a light intensity of 16 W m^{-2} in a 16 h light/8 h dark cycle.

The rooted plants were removed from the agar and dipped in a Benlate solution (1 g l^{-1}) for 1 to 5 minutes. They were then transferred to 70 mm plastic pots containing a vermiculite:perlite mix (2:1 by volume), with a glass dome inverted over it to maintain the humidity.

Results

Establishment of shoot apices in culture

Bacterial contamination of explants was not experienced to any great degree during the course of this investigation. Only 4% of the cultures were contaminated when explants were isolated during the hot summer months. During late summer and autumn 29% of the cultures were contaminated.

No stimulation of growth occurred on BM1 and explant survival was very poor. After 30 days in culture, 89% of the explants on BM1 were chlorotic and eventually died. None of the explants established on this medium were transferred to BPM.

Growth was stimulated by BM2 and cultures were established from 80 to 90% of the pistillate and bisexual explants. Contrary to the results of previous authors [4], who found that the establishment period was less for shoot tips from staminate plants, only 13% of the staminate explants in this study survived the initial establishment period. Although this small number of staminate explants could be established, the response was in no case equal to that of pistillate or bisexual explants and none were transferred to the BPM. The reason for this unusual response remains unclear.

New growth of pistillate and bisexual explants was visible after 2 weeks in culture, and small discrete plants developed within 60 to 90 days. A limited amount of callus formed at the base of these plants. When explants reached this stage they were subcultured onto BPM.

Multiplication of shoots from apices established in culture

Individual shoots were excised from the proliferating cultures and subcultured onto fresh BPM at 30 to 40-day intervals. The average multiplication was 7-fold per subculture (Table 1). The cultures have now been maintained for more than one year (10 subcultures) without decrease in the multiplication factor per subculture.

Although extensive multiplication occurred on the BPM, the shoots were very compact and with shortened internodes and small leaves. The inclusion of GA_3 in the proliferation medium resulted in elongated shoots (7 to 15 mm in length), having larger leaves.

Multiplication factor of the control treatment was significantly greater than that of the GA_3 treatments (Table 2). The multiplication factor of the cultures decreased on all the levels of GA_3, in comparison with the multiplication factor of the control treatment without GA_3. During the second culture period with GA_3 the multiplication factor of the cultures on BPM

Table 1. Mean multiplication factor/subculture of *C. papaya* shoots at different subcultures when grown on proliferation medium with 0.5 $mg\,l^{-1}$ BA and 0.1 $mg\,l^{-1}$ NAA.

No. days in culture	No. cultures	No. developing plants	Multiplication factor (mean)
90 (establishment)			
134 (1st subculture)	10	35	3.50 ×
160	15	93	6.20
190	15	125	8.33
210	25	144	5.76
250	25	183	7.32
280	25	278	11.12
320	25	197	7.88
350	25	174	6.96
400 (50 day interval)	25	218	8.72
440 (10th subculture)	20*	187	9.35
Mean multiplication factor/subculture			7.51

* Subculturing of some explants was discontinued due to abnormal growth.

Table 2. Effect of gibberellic acid on stem elongation and multiplication of *C. papaya* shoot cultures grown on the BPM with GA_3 for two different subcultures.

GA_3 level ($mg\,l^{-1}$)	Days in culture	Multiplication	Cultures with elongated shoots
Control (BPM)	210	8.00 × *	0
0	240	7.57*	2
0.2	210[a]	5.53	12*
	240[b]	3.53	11*
0.5	210	6.07	11*
	240	3.07	10*
1.0	210	6.73	11*
	240	4.80	11*
2.0	210	4.00	6
	240	3.93	9
5.0	210	5.80	5
	240	4.07	10

Fifteen cultures per treatment were evaluated.
a. First culture with GA_3.
b. Second culture with GA_3.
*Treatments of which the means differ significantly at a 5% level, according to the Tukey test.

with GA_3, decreased even more. In cultures grown in the presence of 0.2, 0.5 and 1.0 $mg\,l^{-1}$ GA_3 the shoots were significantly longer than in the other treatments.

Rooting

IAA did not induce rooting (data not shown), while IBA produced roots on about one third to half of shoots when tested at the fifth, sixth and eighth subcultures of the proliferation stage. NAA, when tested at the second and fourth subcultures, and IBA at the third subculture led to rooting of less than one tenth of the shoots (Table 3). Although the results were variable and the rooting response was sporadic, it seemed if the shoots were more prone to rooting with increasing subcultures.

Rooted plants have been transferred to soil, but none of these plants survived due to wilting.

Table 3. Effect of various auxins on root induction on shoots of *C. papaya* grown in vitro. The basal medium consisted of Murashige and Tucker (1969) salts and organic mixture plus 30 $g\,l^{-1}$ sucrose.

Treatment	Level ($mg\,l^{-1}$)	No. of replicates	No. of subculture	Rooting percentage
NAA*	0.5	25	Second	11%
	1.0	20		9%
	5.0	25		4%
NAA	1.0	25	Fourth	4%
IBA**	1.0	65	Third	7%
IBA	2.0	20	Fifth	38%
IBA	1.0	20	Sixth	35%
	2.0	20		31%
	4.0	20		35%
IBA	2.0	25	Eighth	39%
	4.0	25		57%

*NAA was only used on 2nd and 4th subcultures.
**IBA was used on 2nd, 3rd, 5th, 6th and 8th subcultures.

Discussion

During the cooler months 29% of the primary explants were contaminated. This figure was considered satisfactory, since contamination rates of 80 and 95% have been reported [2, 3].

Cultures from pistillate and bisexual plants responded equally well and exhibited swelling of the apical dome and new growth of outer leaves after 2 to 3 weeks in culture. The established cultures were easily stimulated to extensive multiplication. This occurred by the stimulation of axillary buds and small shoots were formed in the axil of each petiole.

Although the tissue is still proliferating after ten subcultures, some cul-

tures exhibited abnormal growth, showing symptoms of vitrification. Subculturing of these was discontinued.

It was also noted that cultures from different clones of cultivar "Sunrise Solo", but the same sex, varied greatly in response to the BPM; while cultures from the same clone, but different sex – pistillate or bisexual – showed no significant difference in multiplication rate (data not shown).

Since the growth of the proliferating cultures was very compact, the stem elongation property of GA_3 was evaluated in an effort to alter this habit. The inclusion of GA_3 in the proliferation medium resulted in "branched" cultures with elongated shoots, but the multiplication rate of the cultures decreased.

Although this antagonism between the multiplication and extention of the shoots exists, it may be worthwhile to transfer compact cultures to a proliferation medium with GA_3, for a few subcultures. At a level of $1\ mg\,l^{-1}$ the multiplication rate was still fairly satisfactory, while plants with elongated shoots were obtained These plants could be handled more easily than cultures not grown on GA_3.

Rooting of shoots could be induced on NAA as well as IBA, but the rooting frequency on NAA was very low. It was noted that the ability of the shoots to form roots improved with increasing subcultures. This was also true for the subsequent shoot growth concomittant with rooting. However, this response was sporadic and seemed to be related to specific clones of the same cultivar only. Media trials are continuing with the aim of improved root development and subsequent shoot growth.

The poor survival of the plants when transferred to soil is still limiting success. Trials concerning potting media, maintenance of humidity, and incorporation of vesicular-arbuscular mycorrhizal fungi in the potting medium to improve survival, are continuing.

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