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Culture of Papaya Explant in Solid - Liquid Media Sequence as a Rapid Method for Producing Multiple Shoots

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ABSTRAK

Tunas aksilari betik yang diperolehi daripada pokok matang dikultur di dalam medium pepejal MS + 0.1 mg/l BA + 500 mg/l kasein hidrolisat + 0.38 mg/l riboflavin menghasilkan kurang daripada 2 pucuk/eksplan dalam tempoh 2 hingga 18 minggu. Jika eksplan dikultur di dalam medium pepejal selama 10 minggu diikuti dengan 10 minggu lagi di dalam medium cecair penghasilan pucuk didapati meningkat 82 kali ganda.

ABSTRACT

Culturing papaya axillary buds obtained from mature field-grown trees on solid MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin produced less than 2 shoots per explant over a period of 2 to 18 weeks. However, 82 times more shoots were produced when the explants were cultured on solid medium for 10 weeks followed by another 10 weeks in liquid medium.

INTRODUCTION

The papaya is a popular dessert fruit. The plant is generally grown from seeds. This practice naturally gives rise to a marked variation in the yield, fruit type and quality. Such variation is undesirable if papaya is to be commercially grown on a large scale where consistency in quality and size of the fruits are important.

Propagation of papaya by tissue culture is one answer to the problem. Methods have been described to obtain plantlets from callus, apical and axillary bud explants from seedling and field-grown tissues (Arora and Singh 1978; Medhi and Hogan 1976; Yie and Liaw 1977; Rajeevan and Pandey 1983; Litz and Conover 1978; Drew 1988).

A practical method of clonal propagation is to induce *in vitro* direct formation of multiple shoots from explants derived from selected field-grown trees. These shoots can then be rooted and grown in the field. In this paper we report a method of producing multiple shoots.This method differs from those reported earlier (Litz and Conover 1978; Drew 1988; Rajeevan and Pandey 1986).

MATERIALS AND METHODS

Explant Sterilization

Explants for the experiment were obtained from mature field-grown trees of the popular local papaya variety known as Taiping. The axillary buds were surface sterilized for 20 m in 20% Clorox(R) solution to which a few drops of detergent were added. The explants were then rinsed with sterile distilled water three times and then soaked for 24 h in antibiotic solution containing 100 mg/l chloramphenicol and 100 mg/l streptomycin. After the antibiotic treatment, the explants were again rinsed in sterile distilled water and then incubated for 48 h on filter paper in a petri dish to which was added 4% sterile sucrose solution. The explants were again sterilized with 5% Clorox(R) solution for 5 m and were finally rinsed with sterile distilled water prior to culture.

Experiment 1

The explants were initially cultured on three different solid (8 g/l agar) Murashige and Skoog (MS) (1962) media containing various additives. K medium was made up of MS + 0.1 mg/l NAA

+ 0.5 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin. L medium was made up of MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin, and P medium was made up of MS + 1.0 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin + 80 mg/l adenine sulphate.

The three media used were those found to be the most effective for the production of multiple shoots from among the 64 media formulations evaluated earlier.

After 10 weeks on the solid media, the explants were then transferred into liquid media of similar formulations. The cultures were placed on an orbital shaker operating continuously at 120 rpm. Continuous light was provided at an intensity of about 2000 lux. The culture room temperature was set at 25 ± 2°C.

Twenty axillary buds were used for each treatment and the experiment was replicated 3 times. The number of shoots produced per explant was noted every two weeks before subculturing to fresh media. The experiment was terminated after 16 weeks in liquid media. The results were analyzed using Analysis of Variance.

Experiment 2

The explants used were obtained from the same source and sterilized in the same manner as described in the earlier experiment.

The axillary buds were initially cultured on a solid L medium (MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin) for 2, 4, 6, 8, 10, 12, 14, 16, and 18 weeks; they were then transferred to a liquid medium of the same formulation. The cultures in the liquid medium were subcultured every two weeks for 10 weeks. During each subculture the number of shoots produced was noted. Twenty explants were used for each treatment and the experiment was replicated 3 times. The culture conditions were similar to those in the previous experiment. The results were analyzed using Analysis of Variance.

To observe explant development axillary buds cultured on the solid medium at 4, 8, and 12 weeks were used for the preparation of histological sections following the standard paraffin method (Johansen, 1940).

RESULTS AND DISCUSSION

Explants cultured in the liquid L medium yielded the most number of shoots in comparison with those in the K and P media. After 10 weeks an explant in the L medium produced 118 shoots while only 65 and 36 shoots were produced per plant in the K and P media respectively (*Fig. 1*).

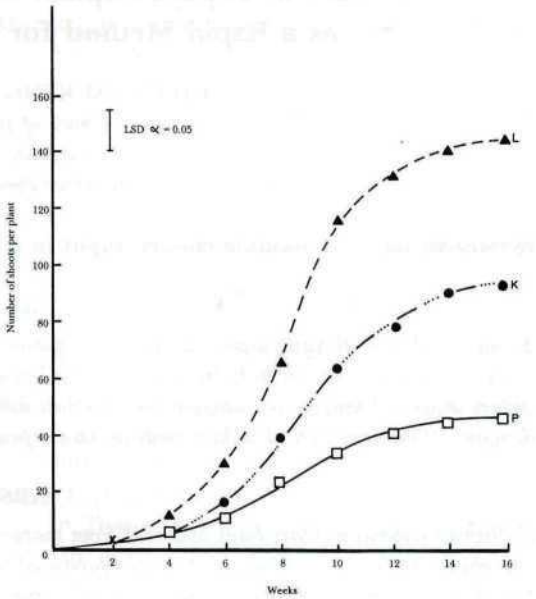

Fig. 1: Production of shoots from the axillary bud of Taiping *variety papaya over 16 weeks in liquid media. Shoots produced after the 10th week were abnormal. K= MS + 0.1 mg/l NAA + 0.5 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin. L = MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin. P = MS + 1.0 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin + 80 mg/l adenine sulphate*

The pattern of explant growth in the three liquid media differed markedly. In the L medium the explant grew into an elongated stem with shoots along its axis. In the K medium the stem was short and the shoots produced were in clusters. In the P medium, the stem was much shorter than that produced in the K medium and the shoots were clustered on the stem giving the appearance of a "rosette".

Prolonged culture of the explant in liquid media caused the shoots to become abnormal. The abnormal shoots produced leaves that were brittle, long, narrow and curled. Shoot abnormality is another problem encountered in *in vitro* culture of papaya. Earlier work by Litz and Conover (1978) reported that after 8 successive subcultures the regeneration ability of cultures was lost.

TABLE 1
Percentage of abnormal shoots produced from axillary bud explants after culturing in liquid media for varying duration

Weeks in liquid medium	% abnormal shoots produced		
	Media		
	K	L	P
2	0	0	0
4	0	0	0
6	0	0	0
8	0	0	27.3
10	33.0	16.7	66.7
12	83.3	92.0	100
14	100	100	100
16	100	100	100

K = MS + 0.1 mg/l NAA + 0.5 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin. L = MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin. P = MS + 1.0 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin + 80 mg/l adenine sulphate

TABLE 2
Production of multiple shoots when axillary bud explants were cultured initially on solid medium followed by culture in liquid medium

Initial culture Weeks in solid medium L	Number of multiple shoot produced in liquid medium L				
	Weeks				
	2	4	6	8	10
2	1.0	1.0	1.0	1.0	1.0
4	1.0	1.0	1.0	1.0	1.2
6	1.0	1.0	2.0	6.4	12.8
8	1.2	1.4	12.0	18.8	48.0
10	1.4	11.8	38.2	79.8	115.8
12	1.8	11.2	36.0	76.4	120.4
14	2.2	12.8	31.8	61.2	113.6
16	1.8	8.8	22.8	63.0	104.0
18	1.4	7.8	21.2	45.6	73.6

Medium L = MS + 0.1 mg/l BA + 500 mg/l casein hydrolysate + 0.38 mg/l riboflavin.

Explants cultured in the liquid P medium started to become abnormal after 8 weeks. At 10 weeks all the explants cultured in the K, L and P media became abnormal. By the 14th week all the shoots in all the media were abnormal. Of the three media, the P medium showed the greatest tendency to cause abnormality (Table 1).

The implication of this result is that while it is possible to produce many shoots rapidly in a liquid medium, culturing in liquid media should not extend more than 10 weeks.

If the explants had been initially cultured on a solid medium from 6 to 10 weeks before they were transferred to a liquid medium, the number of shoots produced per explant in the liquid medium increased (Table 2). However, when the explants were cultured on a solid medium for 18 weeks and then transferred to a liquid medium for another 2 weeks, the number of shoots produced were 2 shoots or less per explant. The implication of the result is that for a total culture period of 20 weeks (18 of which were on solid medium) only 1.4 shoots per explant were produced. On the other hand, by first culturing the explant on a solid medium for 10 weeks and then in a liquid medium for another 10 weeks, the number of shoots produced increased significantly, i.e.115.8 shoots per explant. This represented an increase of shoots by 82 times. The results indicate that the best procedure would be to culture the explants for 10 weeks on solid medium followed by 10 weeks in the liquid medium (Table 2).

The ability of the explants cultured on solid medium for 10 to 18 weeks to produce more shoots can be related to the growth and development of the growing points on the explant. Initially the axillary bud had only one apical shoot. After 4 weeks on solid medium anatomical studies showed that 3 shoot apexes were formed; after 8 weeks 5 shoot apexes were initiated, and after 12 weeks an axillary bud which initially had only one apical shoot now had 8 apical shoots (*Fig. 2*). The subsequent culture of the explant in liquid medium enhanced the growth and development of the shoots. The shoots elongated and produced many axillary buds. These buds developed rapidly and formed more shoots.

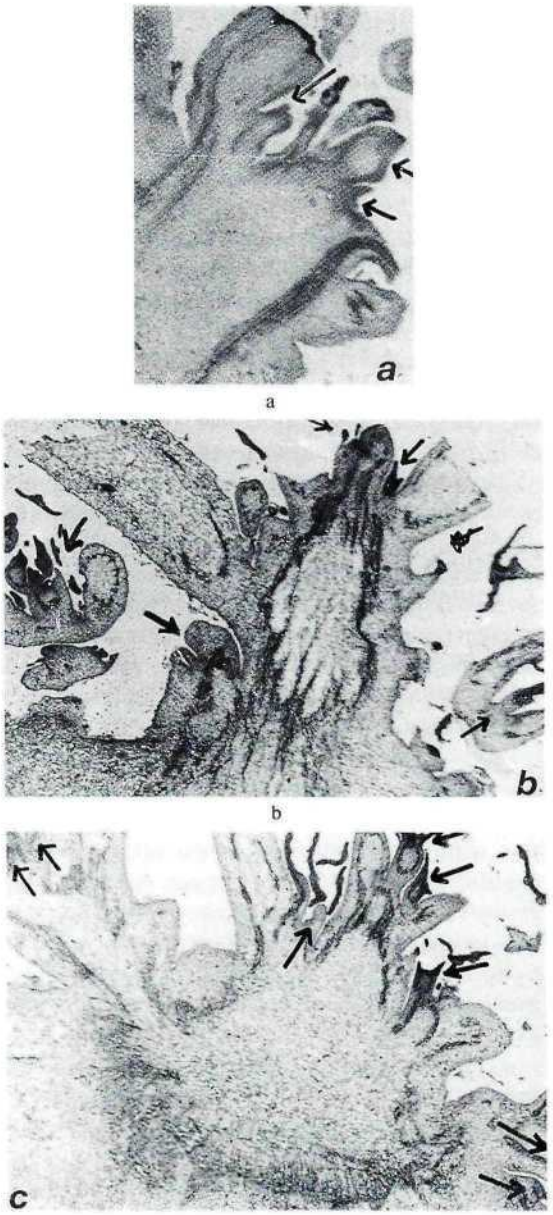

Fig. 2: Histological sections of axillary bud explant of papaya on solid medium at (a) 4 (b) 8 and (c) 12 weeks howing developing shoots as indicated by the arrows

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