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Somatic embryogenesis and plant regeneration in *Carica papaya* L. tissue culture derived from root explants

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ABSTRACT

The regeneration potential of shoot tip, stem, leaf, cotyledon and root explants of two papaya cultivars (Carica papaya cv. 'Solo' and cv. 'Sunrise') were studied. Callus induction of these two cultivars of papaya showed that the shoot tips and stems are most suitable for forming callus, while leaves, cotyledons and roots are comparatively difficult to induce callus. Callus induction also varied with the varities. Somatic embryogenesis was obtained from 3-month-old root cultures. A medium containing half strength of MS inorganic salts, 160 mg/l adenine sulfate, 1.0 mg/l NAA, 0.5 mg/l kinetin and 1.0 mg/l GA_3 was optimal for embryogenesis. The callus maintained high regenerative capacity after two years of culture on this medium. Plants derived from somatic embryos were obtained under greenhouse conditions.

ABBREVIATIONS

MS: Murashige and Skoog's (1962) medium, NAA: Naphthaleneacetic acid, IAA: Indole-3-acetic acid, GA: Gibberellic acid, PRV: Papaya ring spot virus.

INTRODUCTION

Papaya (Carica papaya L.) plants in Taiwan have been almost completely infected by ring spot virus. This disease has become the single most serious problem of the Taiwan papaya industry. Therefore, it is of great importance to achieve a method of papaya regeneration in order to breed ring spot virus resistant strains by means of tissue culture. Recent achievements in papaya tissue culture have been reviewed by Litz (1984).

Embryogenesis in papaya callus has been obtained from seedling petiole segments (De-Bruijne et al., 1974) and stem segments (Yie and Liaw, 1977). These two reports describe a three-step and a two-step culture procedure, respectively, to obtain somatic embryos. In these methods, it is necessary to transfer the callus to another medium for embryogenesis. Although various explants have been utilized for callus induction and regeneration, (Litz and Conover, 1983; Arora and Singh, 1978a), a comparative study on the suitability of various explants has not been made. In this study, the callus induction and regeneration potential of various sources of explants from two papaya cultivars were compared. We also describe in vitro induction of somatic embryos in cultured root of Carica papaya L., the long-term maintenance of strong embryogenesis capacity, and plantlet formation from somatic embryos.

MATERIAL AND METHODS

Plant Materials. Seeds of two papaya varities, Carica papaya L. 'Solo' and 'Sunrise', were supplied by the Feng-Shen Tropical Horticultural Experiment Station of Taiwan Agricultural Research Institute. The seeds, after being washed for 1 h in tap water, were immersed in 75% ethanol for 1 min, and then in 0.1% sodium hypochlorite for 10 min. After several rinses with sterilized water, they were sown on MS agar medium in 2.5x13 cm test tubes. Shoot tip, stem, leaf, cotyledon and root (with root-tip removed) were excised from 4 to 6-week-old seedlings with three leaves (5 to 8 cm in height). They were cut into 3-5 mm segments and were cultured.

Culture Media and Environment. A modified Murashige and Skoog (1962) medium was used as basal medium. This medium contained half strength inorganic salts, 0.5 mg/l thiamine-HCl, 1.0 mg/l pyridoxin-HCl, 5.0 mg/l nicotinic acid, 2.0 mg/l glycine, 1.0 mg/l casein hydrolysate, 100 mg/l myo-inositol, 160 mg/l adenine sulfate, 30 g/l sucrose, and 8 g/l agar. Different combinations of 1.0 mg/l NAA with kinetin (0, 0.5 mg/l) and gibberellic acid (GA_3) (0, 1.0 mg/l) were used as supplements. The pH was adjusted to pH 5.8 with 1N KOH and 1N HCl before adding agar. Sterilization was by autoclaving at 1.1 kg/cm^2 (112°C) for 10 min. GA_3 was filter-sterilized.

Embryogenic callus was subcultured on medium containing NAA, kinetin, and GA_3. Germinated somatic embryos were isolated and cultured on medium containing NAA only.

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Cultures were maintained at 25 ± 2°C in a growth room with 16 h light (2000 lux) and 8 h darkness.

Re-establishment of Plants in Soil. The *in vitro* regenerated plantlets were removed from culture tubes, washed free of agar medium and potted in moist mixture of sand : soil (1/1 : v/v), and covered with a beaker to maintain high humidity. One percent IAA was sprayed on the plantlets for two weeks. The plantlets were then transplanted to soil and cultured under greenhouse conditions.

RESULTS

Callus was induced from papaya shoot tip, stem, leaf, cotyledon and root cultures (Table 1). Most of the explants formed callus at the cut ends after 10 days. Considerable variation in the development of callus among single explants was observed. The result of callus formation after 3 weeks, and the regeneration of somatic embryos after 3 months are shown in Table 1. Optimal callus formation took place on 1/2 MS medium containing 1.0 mg/l NAA and 0.5 mg/l kinetin. Shoot tips and stems are most suitable explants for forming callus, while leaves, cotyledons and roots are comparatively recalcitrant to callus induction. Callus was initiated mainly from the base of the shoot tip, and at the cut end of stem segments. Those explants without callus proliferation were usually swollen presumably due to expansion growth of tissue. Roots were occasionally from the vein region of swollen leaf explants. Callus induction also varied with different cultivars; the explants from 'solo' produced more callus than the explants from 'Sunrise' on medium containing 1.0 mg/l NAA.

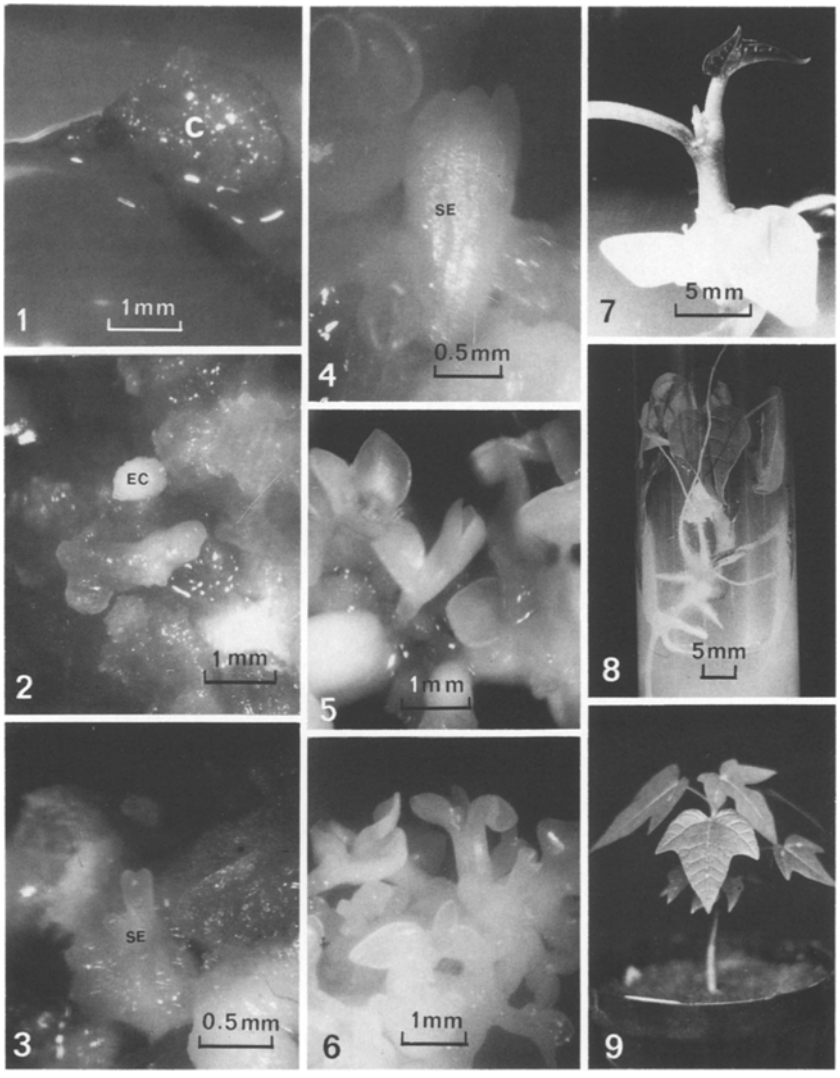
Embryogenesis occurred only on calli from root culture. Stem and shoot tip explants showed abundant callus proliferation, however, no embryogenesis was observed (Table 1). Although a small quantity of callus was induced from root explants after one month; some of the browning explants produced callus later on (Fig. 1). Somatic embryo formation from root callus occurred (Fig. 2) after three months when compact and hard structures were seen on the surface of the callus. Different stages of developing embryos were observed on the surface of yellow callus (Fig. 3-6). The embryogenic callus regenerated more than one hundred somatic embryos on each explant when subcultured on the medium. For both cultivars of papaya the optimal medium for embryogenesis was 1/2 MS modified medium with 1.0 mg/l NAA, 0.5 mg/l kinetin, and 1.0 mg/l GA_3, in which 30% of the root cultures of 'Sunrise' and 20% of the root cultures of 'Solo' regenerated embryos. Callus and somatic embryo formation was sustained for more than two years by subculturing portions of callus with several somatic embryos.

During the subculture of embryogenic callus and somatic embryos, new callus and embryoids were formed, a few somatic embryos developed hypocotyl, tri-lobed leaves (Fig. 7) and roots; roots were generally formed directly from the callus and not from somatic embryos. The shoots produced roots after they were isolated and transfered into NAA containing medium for one month (Fig. 8). More than forty rooted plantlets were successfully transferred to pots and cultured under greenhouse conditions (Fig. 9).

Table 1. Effects of growth regulators on callusing[(a)] and somatic embryogenesis (E) in tissue culture of two papaya cultivars (Solo and Sunrise). The 1/2 MS medium (1962) was used as the basal medium. Data were collected after 1 month culturing in 16 h light (2000 lux) and 8 h darkness at 25 ± 2°C.

	NAA 1.0 mg/l	NAA 1.0 Kinetin 0.5	NAA 1.0 GA_3 1.0	NAA 1.0 Kinetin 0.5 GA_3 1.0
Explants	Solo/Sunrise	Solo/Sunrise	Solo/Sunrise	Solo/Sunrise
Shoot tip	++/+	+++/+++	++/++	/++
Stem	++/+	+++/+++	0/+	+/++
Leaf	++/0	+/++	0/0	0/0
Cotyledon	0/+	0/++	0/0	0/0
Root	++(E)/+	+/+	0(E)/0(E)	0(E)/+(E)

(a) degree of callusing: 0, not visible; +, limited, usually at the cut surface; ++, localized; +++, abundant.



Figs. 1-9. Callus induction, somatic embryogenesis and plant regeneration from root explants of papaya.

1. Callus (C) induced from the browning root explant after 1 month of culture.
2. Embryogenic callus (EC) appeared on root callus after 3 month of culture.
3. Somatic embryos (SE) on the surface of root callus.
4. Bipolar torpedo-shaped somatic embryo (SE).
5. Mature somatic embryos with fully formed cotyledons.
6. Numerous somatic embryos on root callus.
7. A shoot with tri-lobed leaf and hypocotyl.
8. A plant with well developed roots.
9. A plant grown in soil.

DISCUSSION

Embryogenesis and organogenesis have been obtained from calli derived from papaya seedling petiole (Arora, 1978a) and stem segments (Yie and Liaw, 1977). However, no embryogenesis was observed on seedling cotyledon culture (Litz and Conover, 1983). Our results show that it is easier to induce callus on shoot tips and stem segments in the same culture medium and for the root segments it is easier to induce embryogenesis on GA_3 containing medium. This shows that the success of callus induction and embryogenesis of papaya is dependent on sources of the explant and the medium components.

Optimal papaya callus formation from the various explants was obtained on a medium containing both NAA and kinetin. The results of Arora and Singh (1978a, b) and Litz and Conover (1983) indicate that addition of cytokinin to the NAA medium increases the growth of callus. In the reports of Yie and Liaw (1977) and Arora and Singh (1978b), callus derived from papaya seedling stem segments can give rise to adventitious shoots or embryos. But in our study, no regeneration was observed on any of the stem cultures. This may be due to the medium containing high auxin (1.0 mg/l), and the lack of transferring the callus to medium with higher concentrations of cytokinin (1.0 - 2.0 mg/l kinetin) and lower concentrations of auxin (0 - 0.05 mg/l IAA) (Yie and Liaw, 1977), which is critical for regeneration.

DeBruijne et al. (1974) suggested that embryogenesis seemed to be dependent on a relatively low sucrose concentration. Litz and Conover (1982) have demonstrated that a medium containing coconut water is necessary for embryogenesis in ovule callus. The results of the present study show that embryogenesis of papaya root explant can be obtained on a defined medium with the usual concentration (3%) of sucrose and without the addition of coconut milk. The papaya root cultures produced somatic embryos on the medium supplemented with NAA only. We consider that auxin is essential for induction of embryogenic callus in papaya as in wild carrot (Halperin and Wetherell, 1964). Kinetin is probably not critical for induction of embryogenic callus, but the presence of GA_3 in the medium increases embryogenesis. The result is similar to that obtained in alfalfa (Kao and Michayluk, 1981) which shows that gibberellic acid, together with auxin, plays an essential role in the determination of embryogeny.

Embryogenesis provides a simple and quick method to obtain a large number of plants. Papaya root culture can be used for rapid propagation for rare and special plants. Because there is more than one root in a plant, the seedling can still live after some roots have been excised.

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