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Somatic Embryogenesis in Cell Cultures of *Carica stipulata*[1]

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Abstract. **Somatic embryogenesis was induced in cell suspension cultures of callus derived from peduncle explants of *Carica stipulata* Badillo. The presence of activated charcoal stimulated embryogenesis particularly when BA and NAA were both in the growth medium. Plantlets derived from embryos have been successfully transferred to potting mixture.**

Carica stipulata, although having no commercial importance, is related to the papaya (*C. papaya* L.). *C. stipulata* is resistant to infection by papaya ringspot virus (PRV) which causes a serious disease of papayas throughout the New World tropics (1). Virus resistance is conferred by a single dominant gene (5). The two *Carica* species are sexually incompatible, and efforts to achieve hybridization through the use of mediator pollen and embryo culture have not been successful.

Previous studies have indicated that *Carica* species have a reasonably high morphogenetic potential. *In vitro* propagation of papaya is feasible not only from shoot tip cultures (7) but also by stimulation of adventitious shoot formation from callus (11). Haploid plants have been obtained from cultured anthers of *C. papaya, C. cauliflora* Jacq., and *C. stipulata* (8). Litz and Conover (9) have proposed an *in vitro* scheme for effecting interspecific hybridization between papaya and PRV-resistant *C. stipulata* by inducing protoplast fusion between the 2 species.

The exploitation of protoplast fusion in plant improvement depends on the ability to regenerate plants from cells efficiently. In this report we present a procedure for large-scale production of somatic embryos from cell cultures using *C. stipulata* as a model.

Peduncles were removed from mature, container-grown plants of *C. stipulata* and 1-cm pieces were disinfested by rinsing briefly in absolute alcohol followed by immersion in 1% (wt/vol) sodium hypochlorite for 10-12 min with continuous agitation. After transfer through 3 rinses in sterile, distilled water (10 min each), tissue was placed in culture on sterile plant growth medium composed of Murashige and Skoog medium (10) with 30 g/liter sucrose, 2 μM 6-benzylamino purine (BA), 1 μM naphthaleneacetic acid (NAA) and 8 g/liter Difco Bacto agar. The pH was adjusted to 5.8 with 1N KOH before sterilization at 120°C and 1 kg/cm^2 for 15 min. Cultures were maintained in a growth room at 25-27°C with a photoperiod of 16 hr light at 1.5 klux supplied by Agro Lite fluorescent tubes and 8 hr darkness.

Equal quantities of friable peduncle callus were transferred from solid medium into 125 ml Erlenmeyer flasks containing 30 ml Murashige and Skoog medium at pH 5.8. The growth regulator components were altered accordingly: 1) 2 μM BA and 1 μM NAA (6); 2) 2 μM BA; 3) no growth regulators; 4) 1 μM NAA. Activated charcoal (1% wt/vol) was added to half of the flasks in each treatment. There were 6 flasks in each treatment and the experiment was repeated once. The pH was adjusted to 5.8 prior to autoclaving. Cultures were agitated at 150 rpm on a rotary shaker for 1 month to facilitate the break up of cell clumps. Environmental conditions were 1.5 klux illumination with a 16 hr photoperiod at 25-27°C. The flasks then remained stationary for another 8 weeks before the cultures were decanted and examined.

Rapidly growing, friable callus was obtained on solid medium. After transfer of callus inoculum to liquid media, cell cultures continued to increase rapidly in flasks with NAA alone and in the control treatment

Table 1. Effect of growth regulators and activated charcoal on somatic embryogenesis of *Carica stipulata*.

Treatment	1% activated charcoal	BA (μM)	NAA (μM)	Avg no. embryos/ flask
1	–	2	1	45
	+	2	1	1560
2	–	2	0	0
	+	2	0	10
3	–	0	0	0
	+	0	0	13
4	–	0	1	0
	+	0	1	10

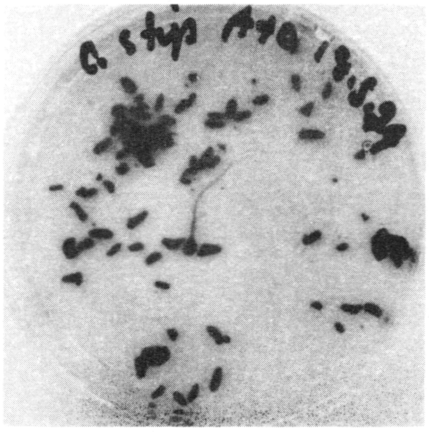

Fig. 1. Torpedo-shaped somatic embryos of *C. stipulata* prior to transfer to solid medium.

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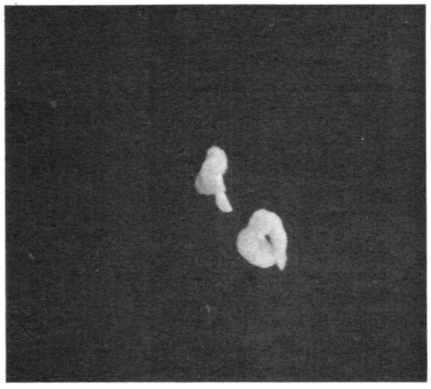

Fig. 2. *C. stipulata* plantlets derived from somatic embryos and grown on solid medium.

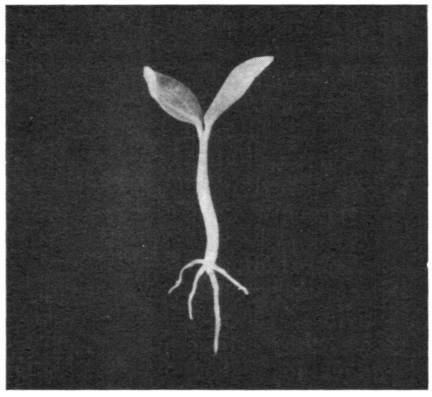

Fig. 3. *C. stipulata* plant derived from somatic embryo.

containing no growth regulators. Embryogenesis did not occur in any treatment during or immediately after the initial 1 month culture period when the flasks were being agitated. Adventitious embryo formation became apparent in some of the stationary flasks about 6-8 weeks after agitation ceased.

Treatments containing either BA or NAA alone as well as the control treatment in which no growth regulators were present were ineffective in stimulating somatic embryogenesis. The addition of activated charcoal was associated with limited somatic embryo production in these treatments (Table 1). In the treatments that contained both NAA and BA, a greater number of somatic embryos was observed; however, activated charcoal in combination with both growth regulators stimulated the formation of a large number of somatic embryos in each flask (Fig. 1).

Initially globular and then heart and torpedo shaped embryos were observed. Embryo development proceded more rapidly in media containing activated charcoal. Embryos at the torpedo stage have been transferred to medium without growth regulators and plantlets have been recovered (Fig. 2, 3). Many embryos failed to develop normally and formed roots or shoots only.

The induction of somatic embryogenesis in cell cultures is generally dependent on the presence of a particular component in the growth medium (6). Auxins have been considered to be essential for induction of competency and for the subsequent stages of embryo development (4). Somatic embryogenesis in *C. stipulata* cell lines was obtained only when NAA was present in embryo induction media that also contained activated charcoal. Although cytokinins generally inhibit embryogenesis, embryos were recovered from culture media containing BA; however the presence of activated charcoal was also necessary. Fridborg et al. (3) and Drew (2) have demonstrated that activated charcoal can positively influence embryogenesis in cell culture of some plant species. There are indications that certain phenolic compounds that are produced by tissue cultures can be selectively absorbed by activated charcoal (3). Phenolic inhibitors of plant growth regulator action could have been absorbed by activated charcoal and thereby stimulated somatic embryogenesis in *C. stipulata* cell cultures. It is clear that both NAA and BA are essential for induction of somatic embryogenesis in *C. stipulata* cell cultures, but only when activated charcoal is also present.

Regeneration of large numbers of adventitious embryos from cell cultures of *C. stipulata* should facilitate the recovery of a high proportion of hybrid plants following the fusion of protoplast of different *Carica* species. Somatic embryogenesis of *Carica* species can also be adapted as a rapid propagation technique.

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