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Partial Organogenesis in Tissue Cultures of *Averrhoa carambola*[1]

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Abstract. **Callus was initiated from seedling leaf explants and from leaves of recent vegetative flushes of mature *Averrhoa carambola* L. trees on solid Murashige and Skoog medium with added growth regulators. Seedling-derived callus demonstrated a strong dependence on the presence of exogenous cytokinin, 1-5 mg/liter 6(γ, γ-dimethylallylamino) purine (2iP) with 0.2 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D), for induction and growth. Shoot differentiation was stimulated from seedling callus by increasing the concentration of available cytokinin. Callus induction and growth from young leaves of mature trees was auxin-dependent (2-5 mg/liter 2,4-D with 0.5 mg/liter 2iP. Organogenesis from callus cultures of mature leaf origin was not obtained.**

The carambola or star fruit *Averrhoa carambola* L. can be grown in the continental U.S. only in South Florida. Trees have normally been vegetatively propagated by veneer grafting but tissue culture was investigated as an alternative propagation method.

Young seedlings and established mature trees of 'Golden Star' carambola, a recent cultivar with low oxalic acid content, were removed from seedlings and were cut into 5×5 mm squares. In spring and early summer, young leaves were removed from vegetative flushes of mature trees. Leaflets that were in transition from the characteristic copper-red of young leaves to green were most suitable as explant sources, and were cut into 5×5 mm squares.

Explants were surface-sterilized after a brief immersion in absolute alcohol by continuous agitation in 1% sodium hypochlorite for 10 min. Excess sterilant was removed by transferring explants through 3 changes of sterile distilled water. Leaf pieces were transferred aseptically to test tubes containing basal medium (1) with 8 g/liter Difco Bacto agar, 30 g/liter sucrose and 0.5 mg/liter cysteine. Growth regulators were present in different combinations of 0.5-10.0 mg/liter 2iP and 0.2-5.0 mg/liter 2,4-D. The pH was adjusted to 5.7 with IN KOH and the media were sterilized by autoclaving at 1 kg/cm^2 and 120°C for 15 min.

Cultures were grown in an environmentally controlled room. Temperature was 25-28°C and light was provided by Agro Lite at 3 klx during a 16 hr photoperiod.

Vigorously growing callus was induced from seedling leaf explants within 5-6 weeks after the date of culturing with several growth regulator concentrations (Table 1). Callus growth was rapid on most media, and formed a hard, creamy-white plate-like mass. High cytokinin and low auxin ratios caused the callus to turn bright green. High concentrations of 2,4-D resulted in slightly more friable callus. The optimum growth regulator formulation for callus growth was 1-2 mg/liter 2iP together with 0.2 mg/liter 2,4-D. Higher levels of cytokinin inhibited callus growth, but were essential for plant regeneration.

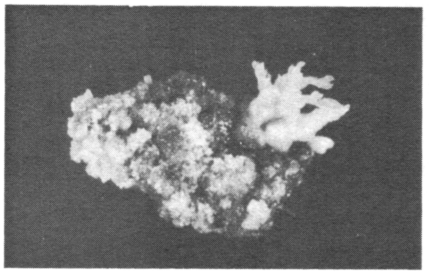

Fig. 1. Regeneration of *Averrhoa carambola* from callus of seedling leaf origin on Murashige and Skoog medium with 2 mg/liter 2iP and 0.2 mg/liter 2,4-D.

Differentiation of carambola shoots was observed only on media that contained high cytokinin concentrations (2-10 mg/liter 2iP and 0.2 mg/liter 2,4-D) and most frequently in senescing cultures. Without regular subculturing onto fresh medium, the callus would begin to turn brown after 2-3 months. Multiple shoot differentiation was observed to occur from small white or green areas of callus adjacent to necrosing tissue (Fig. 1). Shoots were dissected from the callus and were transferred to fresh media containing high levels of cytokinin (10 mg/liter 2iP and 0.2 mg/liter 2,4-D). Stimulation of lateral bud growth did not occur. Attempts to induce root development by transfer of shoots to media containing auxin alone (1-5 mg/liter NAA or IBA) and to basal media with half strength major elements were unsuccessful. After 2½ years in culture, carambola callus has retained its shoot regeneration potential.

Relatively slowly growing callus was obtained from leaf explants from mature carambola trees. In contrast to seedling leaves, mature leaf explants were insensitive to callus induction by 2iP (Table 1). Callus induction and growth were clearly dependent on auxin, 2,4-D. Callus growth was slow and cultures were white and slightly friable. Altering the cytokinin: auxin ratio did not alter the appearance of the callus. Increased cytokinin concentrations did not cause the callus to turn green; organogenesis was never observed.

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Table 1. *In vitro* effect of 2iP and 2,4-D on seedling and mature leaf explants of carambola.

Growth regulator (mg/liter)		Avg increase of callus (g fresh wt)[z]	Callus appearance	Shoot differentiation
2,4-D	2iP			
Seedling				
10.0	0.5	6	white, thin, friable	–
5.0	0.5	5	" " "	–
2.0	0.5	11	" ", hard	–
1.0	0.5	14	" " "	–
0.5	1.0	15	green, thick, "	–
0.5	2.0	16	" " "	+
0.5	5.0	12	" " "	+
0.5	10.0	5	" " "	+
Mature				
5.0	0.5	4	white, thin, friable	–
2.0	0.5	5	" " "	–
1.0	0.5	2	" " "	–
0.2	1.0	2	" ", hard	–
0.2	2.0	2	" " "	–
0.2	5.0	–	–	–
0.2	10.0	–	–	–

[z]Mean of 3 replicated experiments. g (fresh weight) increase measured after 60 days. Inoculum = 1 g.

Literature Cited

1. Murashige and Skoog medium with 2 mg/liter 2iP and 0.2 mg/liter 2,4-D. bioassays with tobacco tissue cultures. *Physiol. Plant.* 15:473-497.