Callus and Cell Suspension Cultures from Dormant Stems of Peach

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Explants of peach [*Prunus persica* (L.) Batsch.] have been derived from buds, stems, leaves, or anthers collected during the growing season (Heuser, 1972; Stiles, 1974; Tukey, 1937; Bradley and Dahmen, 1971; Hammerschlag, 1983). Although Ochatt and Caso (1983) produced shoots from dormant buds, it appears no research has been reported on, callus production directly from dormant stem tissue. Ability to derive callus from dormant tissue would be advantageous, since it would allow callus to be obtained during the entire year.

Current season's growth from several genotypes of peach (Table 1) was collected at the Appalachian Fruit Research Station, Kearneysville, W.Va., on 6 or 30 Nov., and 2 or 6 Dec. 1988. Buds were removed and stems cut into ≈ 10 -cm lengths. This material was soaked in 95% ethanol for 5 min and transferred to a solution of 15% Clorox : 0.01% Triton X-100 (v/v) for 4 min. Stems were then rinsed three times in sterile distilled water and cut into 1-cm-long segments. These were debarked and either quartered longitudinally or kept whole. Xylem and bark strips were cultured separately on either a Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) containing 3% sucrose, 0.5 μ M IBA, and 5, 10, or 50 μ M 2,4-D, or Woody Plant Medium (WPM) (Lloyd and McCown, 1981) containing sucrose, 0.5 μ M IBA, and 5 μ M 2,4-D (Sigma, St. Louis). Both media contained 1.2% agar (w/v) and the pH of each was adjusted to 5.8 before autoclaving. Cultures were maintained in the dark at 21C for 6 to 8 weeks.

No callus grew from bark strips. In contrast, substantial growth was observed on both whole and quartered stems within 2 weeks (Fig. 1 A and B). Explants taken in November were sometimes contaminated by *Cytospora* spp., whereas all tissue collected in December was not. All genotypes produced callus on each medium, although the greatest amount occurred at 1 or 5 μ M 2,4-D (data not shown).

To induce suspension cells, 1-cm³ blocks

of callus were trimmed from larger pieces and individually immersed in a single well of a six-well culture dish containing 3 ml of liquid MS medium (without IBA) per well. Either the 2,4-D or the BAP concentration was varied. Two replicate wells/treatment for each genotype were used. This procedure maintained a high cell : volume ratio.

Plates were incubated in the dark at 21C for 24 days on a rotary shaker at 100 rpm. Subsequently, 0.1-ml samples were drawn from the wells and the number of single cells and clumped cells recorded. The most successful hormone treatments are presented in Table 1. The greatest success in obtaining suspension cells was achieved in 'Sunhigh', which produced a soft, paste-like callus on both solidified MS and WPM. The second-most prolific suspension cell producer was KV862478; [(Empress dwarf OP) OP] $\times$ PI 442380. Callus obtained from this selection was hard and appeared resistant to sloughing cells. Therefore, physical appearance of callus was not associated with the ability to produce suspension cells. In all genotypes, suspension cells appeared as an even mixture of single cells, clusters of two to five cells, and larger clusters up to an estimated 100 cells/cluster. Morphologically, cells were evenly distributed between elongated, highly vacuolated cells and smaller, isodiametric cells with dense cytoplasm (Fig. 1C). After 4

Table 1. Optimal concentrations of growth regulators for production of suspension cells in five genotypes of peach.

Genotype	Growth regulator concn (μ M)		Cells/ml ^a
	2,4-D	BAP	
Sunhigh	1.0	5.0	289,900 $\pm$ 7,150
KV862478 ^b	1.0	5.0	46,310 $\pm$ 2,530
Bailey	5.0	0.5	7,410 $\pm$ 240
Boone County	0.5	1.0	1,530 $\pm$ 57
Reliance	0.5	0.5	160 $\pm$ 15

^aMean of three counts/well $\pm$ 1 SD.

^bSee test for parentage.

months in culture, stationary-phase cultures maintained cell densities similar to those given in Table 1.

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Fig. 1. Callus and suspension cell cultures derived from dormant stem tissue of peach. (A) Effect of 50 μ M and (B) 5 μ M 2,4-D in MS medium on callus production in 'Bailey'. Bar = 0.5 cm. (C) Suspension cells derived from callus of 'Sunhigh'. Bar = 500 μ m.

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