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Peach Regeneration from Callus Derived from Embryos of Selected Cultivars

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Abstract

Thirteen peach cultivars were used in experiments to produce somaclonal variants from embryo culture. In the first and second years of the project, a total of 5,172 immature peach embryos were excised and used in the protocol. Initially, 45% of the immature embryos formed friable callus in the first three weeks of culture. One hundred plantlets (1987) 85 plantlets (1988) formed in culture, however a large number failed to develop further due to their inability to form roots and some rooted plantlets were lost during acclimation to greenhouse conditions. The two year study produced 54 plantlets which formed root systems. The difficulty in obtaining large numbers of plants from adapted cultivars limits this in vitro technique for use in screening programs for pathogen resistance in peach in Ontario.

Introduction

In vitro culture of *Prunus persica* (L.) Batsch, has been carried out by a number of investigators. One of the most common and successful techniques for this recalcitrant species utilizes excised meristems and shoot tips which are grown and multiplied on a number of complex media (2, 3, 8, 11, 13, 14). Other researchers have developed regeneration systems for *Prunus* spp. with embryo cultures (9), embryo-derived callus cultures (4, 7, 10), and root callus cultures (1). Hammerschlag (4) provided an extensive and detailed list summarizing all types of tissue culture techniques used for in vitro culture of *P. persica* and related species. Toxin-selected (5) and non-selected (6) embryo callus cultures of peach have produced plantlets with increased levels of resistance to the bacterial spot pathogen *Xanthomonas campestris* pv. *pruni*.

For the peach breeder interested in breeding for disease resistance, the ideal procedure would provide large numbers of rooted somaclonal variants which are readily acclimatized to greenhouse conditions. The peach canker fungi, *Leucostoma persoonii* and *L. cincta* are the major biotic factors limiting peach production southern Ontario. Since peach cultivars that are immune or highly resistant to the peach canker fungi have not been identified, the long term goal of the project was to obtain by somaclonal selection a source of novel genetic material with increased resistance to the peach canker fungi. The objective of this study was to determine if peach cultivars important to the industry in southern Ontario could be adapted to an in vitro regeneration regime.

Materials and Methods

Plant Material

Plant material was obtained from two distinct sources. Embryos from the cultivars 'Redhaven,' 'Brighton,' 'Sentry,' 'Vivid,' 'Garnet Beauty' and 'Harbrite' were obtained from 3-year-old, peach trees grown in a protected environment structure (PES) located at the Horticultural Research Institute of Ontario (HRIO) in Vineland Station. Embryos from the cultivars 'Redhaven,' 'Babygold 5,' 'Harson,' ' Harbrite,' 'Harrow Diamond,' 'Veeglo,' 'Veecling,' 'Redskin' and 'Candor' were obtained from 4-year-old, field-grown peach trees at the Victoria Farm. Trees from which seeds were to be collected were enclosed in muslin tents during the

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bloom period to ensure a high proportion of self-pollination. Average date of full bloom was recorded as March 28 in the PES and May 11 for the field-grown peaches.

Tissue Culture

Peach seeds were excised from the green fruit, sterilized in a solution of 0.5% sodium hypochlorite (5-10 min), and washed three times in sterile distilled water. To obtain callus with the highest totipotency, only embryos with a percent fill (PF_1) of 2.5 to 3.5 were used. The percent fill (PF_1) value for each embryo was determined at approximately 45-55 days following full bloom with a dissecting microscope by calculating embryo length/seed length X 100 (9). In 1987, only embryos with ratios 2.5-3.5 were utilized, however, in 1988, embryos with PF_1 values of 2.0 to 4.0 were included in the study. Embryos were removed from the seeds, wounded and placed onto the friable callus-inducing medium (a basal medium composed of Murashige and Skoog salts (12) supplemented with 555.1 μM myoinositol, 4.06 μM nicotinic acid, 2.43 μM pyridoxine HCl, 1.18 μM thiamine HCl, 87.6 μM sucrose, and 0.6% agar, plus 4.5 μM 2,4-dichlorophenoxyacetic acid and 0.44 μM benzyladenine (BA) as described by Hammerschlag (7). The cultures were incubated in the dark at 22-24°C. The smooth white friable callus which developed from some embryos was subcultured every 3 weeks for 3 months. Only the white to cream-colored friable callus was transferred to nodular callus-inducing medium (NC) (7), which consisted of basal medium supplemented with 0.27 μM α-naphthylacetic acid (NAA) and 2.2 μM benzyladenine. The cultures were incubated at 26°C, 16 h photoperiod of 35 μmol $m^{-2}s^{-1}$ with cool white fluorescent lights.

Once the white to cream-colored nodules became visible, they were transferred to the shoot inducing medium. This medium consisted of basal medium supplemented with 0.05 μM NAA and 4.4 μM BA. The photoperiod, temperature and light conditions remained the same as for the NC culture. Once peach shoots reached a height of 1 to 5 cm, the clumps of plants were separated and placed on hormone-free ½ strength basal medium. Each shoot was placed aseptically into the ½ basal medium and stored in the dark at 3 to 5°C for 6 weeks. At the end of the acclimatization period the shoots were transferred to a root inducing medium (RM). The RM contained the basal medium supplemented with 7.3 μM para-aminobenzoic acid (PABA), 2.9 μM NAA, 16 h photoperiod at 80 μmol $m^{-2}s^{-1}$ and 14 days incubation. Plantlets which formed roots were transferred to an elongation medium consisting of 1/2 strength MS salts, 0.05 μM NAA and 11.0 μM BA. Once the roots reached 5 to 10 mm they were transferred to the greenhouse conditions. The plantlets which failed to form roots were placed onto a lower concentration of PABA and the procedure was repeated. The majority of the plantlets rooted at the original concentration. Since root size was critical to further survival of the plantlets, only plantlets with roots between 5 to 10 mm in length were moved onto the next stage.

Plantlets with roots were transferred to a 6 cm peat pot (2:1:1 foam:peat:soil) and acclimated in a mist chamber maintained at 23 to 28°C with a 16 hr photoperiod. Plantlets were exposed to mist for 6 sec/64 min for 2 days, 4 sec/64 min for 5 days, 4 sec 2 to 3 times/day for 7 days, and finally no mist for 7 days. Acclimated plants were then transferred to a greenhouse bench with supplemental lighting, water and fertilizer (1:1:1 N:P:K 50 ppm for 3 wk then 100 ppm for 2 wk) as needed. The 15 to 30 cm tall plants were then forced into dormancy under ambient light conditions and 5 to 10°C temperature for 30 days and stored at 1°C in a refrigerated storage for 90

days. On April 1, the plants were returned to the greenhouse (15 to 20°C and ambient light and photoperiod), transferred to 12 cm pots after 2 wk, fertilized after 4 wk (50 ppm 1:1:1) and then acclimated to field conditions for 7 to 14 days in an exterior site protected from winds. At the time of planting, regenerated plants were 45 to 75 cm in height. Data on the relationship between PF_1 value and days after full bloom on percent survival of primary callus in 1987 were analyzed with simple linear regression (to determine those cultivars with slopes significantly different from 0) and the Kruskal-Wallis procedure (to determine significant differences among cultivars) (SAS Institute, Cary, NC).

Results and Discussion

In 1987 and 1988, a total of 5,172 immature peach embryos were excised and cultured. The survival of the callus was affected greatly by the developmental stage of the excised immature peach embryo. Hammerschlag et al. (7) used PF_1 values and the days after bloom period to determine the optimum physiological stage for immature embryos to form highly regenerative friable callus.

In 1987, the highest survival of primary callus from immature embryos occurred when the PF_1 values were approximately 2.5 to 5.5 (Fig. 1). No differences among cultivars could be determined with the Kruskal-Wallis procedure. The cultivars 'Harrow Diamond,' 'Harbrite,' and 'Babygold 5' exhibited significant negative regressions, suggesting the percent survival of primary callus declined as PF_1 value increased. None of the other cultivars exhibited regression line slopes significantly different from 0. The highest survival of the callus was obtained from 49 to 51 days following bloom (Fig. 2). Again, the Kruskal-Wallis procedure showed no significant differences among cultivars in percent callus survival. The culitvars 'Harrow Diamond,' 'Harbrite,' and 'Babygold 5' exhibited significant negative regressions, suggesting that percent survival of primary callus declined with days after full bloom. All of the other cultivars exhibited regression slopes of 0. Both days after bloom and PF_1 values for determining the 'readiness' of the immature peach embryos for peach culture were unreliable. The determination of the PF_1 values became more difficult under adverse weather conditions. In 1988, high temperatures during bloom followed by low rainfall in the post bloom period made this estimation technique virtually ineffective because there was no uniformity in the PF_1 ratios within cultivars or even within individual trees.

The numbers of immature embryos cultured and the survival of the friable callus to the first subculture for each cultivar and each experiment is shown in Table 1. The last column represents the percentage recovery of rooted plantlets in relation to the total number of embryos excised. The low success rate (about 1%) is somewhat discouraging when one examines the large number of immature embryos which were initially plated. In both years, the technique yielded only 100 (1987) and 85 (1988) plantlets prior to the rooting stage. The technique provided by Hammerschlag (personal communication) did not yield a significant number of rooted plantlets in our studies and the protocol that we developed was only marginally better. Only 54 plantlets (about 1% of the total number of embryos plated) were rooted successfully and planted in the field over the course of these experiments. These plants currently are being evaluated for horticultural characteristics and pathogen resistance at the HRIO in Vineland Station. Preliminary observations indicate the presence of significant somaclonal variation in harvest date of fruit in individuals derived from 'Harrow Diamond' and 'Harbrite' (N. W. Miles, unpublished data).

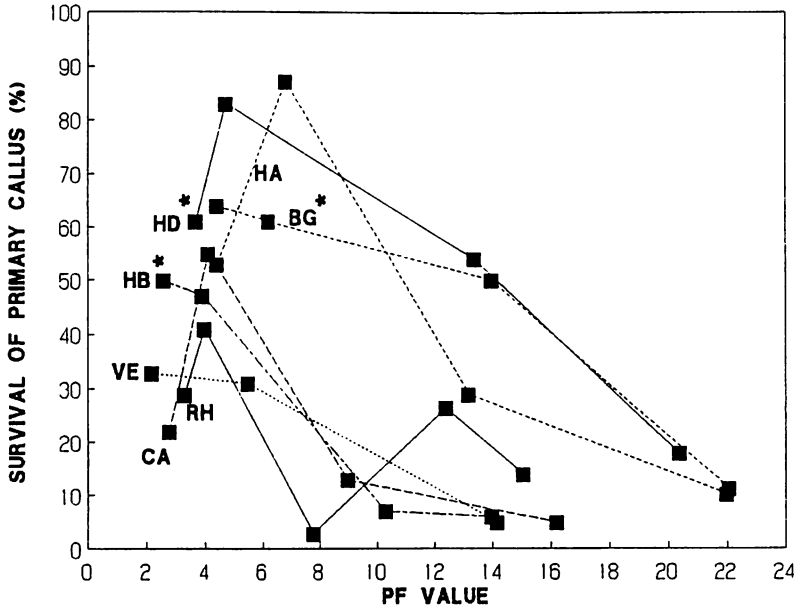

Figure 1. The effect of PF_1 value on the percent survival of the primary peach callus in the 1987 season. The abbreviations HD, HB, HA, BG, VE, RH, and CA refer to the cultivars 'Harrow Diamond,' 'Harbrite,' 'Harson,' 'Babygold 5,' 'Veeglo,' 'Redhaven,' and 'Candor,' respectively. Asterisk indicates cultivars with regression slopes different from 0.

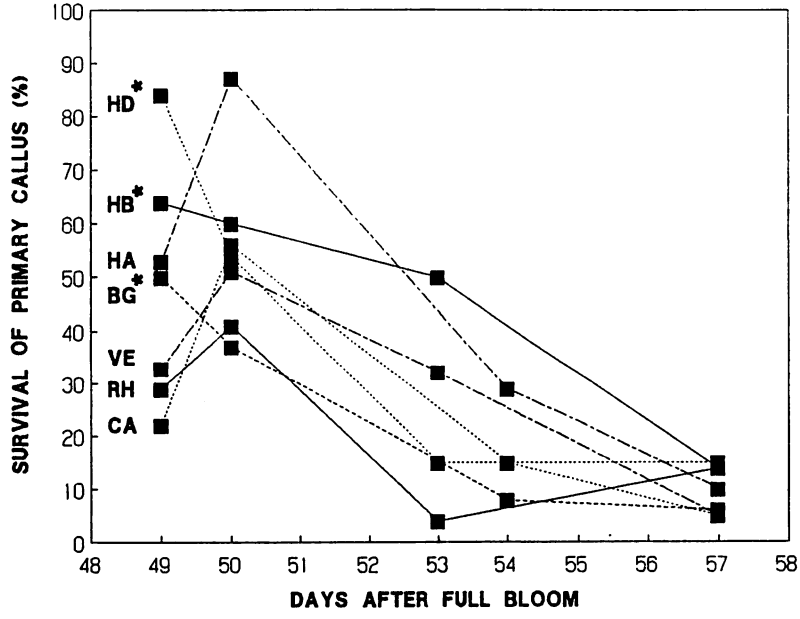

Figure 2. The effect of days after bloom on the survival of the friable peach callus in the 1987 seson. The abbreviations HD, HB, HA, BG, VE, RH, and CA refer to the cultivars 'Harrow Diamond,' 'Harbrite,' 'Harson,' 'Babygold 5,' 'Veeglo,' 'Redhaven,' and 'Candor,' respectively. Asterisk indicates cultivars with regression slopes different from 0.

Table 1. Total number of immature peach embryos excised and number that survived to first subculture and produced plantlets in the 1987 and 1988 seasons.

Cultivar	# Embryos excised	# Surviving to first culture	# Rooted plantlets	Percent recovery
Field cultivars 1987				
Harrow Diamond	268	108	3	1.1
Candor	84	34	-	0
Harbrite	355	126	6	1.7
Harson	166	59	-	0
Redhaven	193	74	-	0
Babygold 5	179	29	-	0
Veeglo	132	23	-	0
TOTAL	1,377	453	9	0.7
PES cultivars 1988				
Redhaven	251	102	1	0.4
Brighton	264	180	-	0
Vivid	173	107	6	3.5
Garnet Beauty	270	81	-	0
Sentry	56	18	1	1.8
Harbrite	184	144	4	2.2
TOTAL	1,198	632	12	1.0
Field cultivars 1988				
Harrow Diamond	491	336	-	0
Harbrite	404	159	-	0
Harson	426	303	-	0
Veeglo	306	126	1	0.3
Babygold 5	274	209	-	0
Veecling	102	84	-	0
Redhaven	171	123	31	18.1
Redskin	423	400	1	0.2
TOTAL	2,597	1,740	33	1.3

The difficulty in obtaining large numbers of peach plants via this in vitro technique limits its use in the screening for resistance to pathogens. However, Hammerschlag (6) has been able to obtain bacterial spot resistant plants after screening small numbers of peach regenerants. In contrast to whole plant screening, screening cells against pathotoxins with the goal of obtaining individuals with increased tolerance to certain diseases is an alternative approach which does utilize large populations. The inability of the cultivars to differentiate and produce large numbers of regenerants may be attributed to the technique as well as intrinsic differences among peach cultivars. Cultivar differences were observed in an earlier study (7), although the cultivars in the earlier study generally yielded greater numbers of rooted plantlets. The second possible dificiency in the technique was the inability to accurately determine when the majority of the immature peach embryos were at the optimum condition for in vitro culture. The collection and culture of embryos in sub-optimum condition was observed in 1988 when the sampled fruitlets varied considerably in the size of the immature embryos. A degree-day model for embryo development, with the model initialized for date of full bloom, may facilitate the collection of fruit with embryos at the optimum stage for in vitro culture (A. R. Biggs, unpublished data). However, it is likely that individual cultivars would require separate models due to the cultivar variation in embryo size optimal for survival and callus production, as well as the intrinsic differences among cultivars in bloom date, ripening date, and, presumably, rate of embryo development.

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Bud Distribution and Yield Potential in Peach

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Abstract

One year old shoots of 50 cultivars of peach (*Prunus persica* (L.) Batsch) differing in genetic and geographic origin were studied. Bud density and distribution showed marked differences among genotypic groups. Cultivars originating in climates with less risk of low winter temperatures generally had lower bud densities based on a high proportion of blind nodes or nodes with only one bud. Higher bud densities were associated with a higher proportion of nodes with 3 buds which generally results in higher flower and fruit densities that would require more labor for thinning.

Fruit yield is a complex trait that is influenced by such factors as climate and management practices (1, 4, 7) as well as by genetic differences among genotypes (3, 10) and by the genotype-environment interaction (5, 9). Some phenotypic factors influencing yield of perennial crops have been reported. For blueberries, Siefker and Hancock (11) observed that the most important factors associated with yield were the number of canes per bush, followed by the number of berries per cane. The main yield components for sour cherries were the number of 4 year old limbs per tree and fruit set (4) whereas in 3 pear cultivars studied by Kappel (6), yield was associated with fruit set and fruit growth.

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