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AGROBACTERIUM-MEDIATED TRANSFORMATION OF PEACH (*PRUNUS PERSICA* L. BATSCH) LEAF SEGMENTS, IMMATURE EMBRYOS, AND LONG-TERM EMBRYOGENIC CALLUS

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SUMMARY

Peach leaf segments, immature embryos, and long-term embryogenic calli have been transformed *in vitro* with the engineered *Agrobacterium tumefaciens* strain A281 containing pGA472. All three tissue sources proliferated callus which grew on a medium containing 100–200 mg/l kanamycin or 10–20 mg/l G-418 as selective agents. These calli were shown to produce neomycin phosphotransferase. The results of Southern analyses were consistent with the incorporation of foreign DNA into the genome of leaf, embryo and embryogenic peach callus.

Key words: Southern analysis; tissue culture; G-418; kanamycin; embryogenesis; genetic improvement.

INTRODUCTION

Agrobacterium-mediated transformation offers the opportunity to genetically manipulate plants by insertion of functional non-plant genes (4,5,6,15), by transferring genes from one plant species to another (2), or by affecting the regulation of native genes (7,19). Such genetic manipulations may be particularly useful in perennial tree crops where long generation cycles and space requirements for large segregating populations make genetic improvement through hybridization and selection especially time-consuming and expensive. The manipulation of one or a few economically important characteristics of an established cultivar would be a useful complementary approach to hybridization for tree fruit improvement, since for many fruit species relatively few major cultivars are favored by growers and consumers and these make up the bulk of production.

Few woody perennials have been successfully transformed and regenerated with the notable exceptions of poplar, apple and walnut (5,10,13). Preliminary reports from our lab using *Agrobacterium tumefaciens* strain A281 containing pGA472 indicated that this vector produced galls on peach stems inoculated *in vitro*. The pGA472 plasmid carries the neomycin phosphotransferase gene that confers kanamycin resistance to transformed plant cells (1). When explanted on selective medium with 200 mg/l kanamycin these galls proliferated, suggesting transformation (17). Transformation of peach stem tissue *in vitro* with other engineered *Agrobacterium* strains has been reported (9). The purpose of this study was to develop procedures for *in vitro* *Agrobacterium*-mediated transformation of peach leaf tissue, sexual embryos, and immature embryo-derived somatic embryos.

MATERIALS AND METHODS

Tissue culture media and growth conditions. The basal medium for all tissue culture described in this report consisted of full strength or diluted Murashige and Skoog (MS) salts (14) with the addition of vitamins, (in mg/l); myo-inositol, 100; nicotinic acid, 0.5; pyridoxine HCl, 0.5; thiamine HCl, 0.4; p-aminobenzoic acid, 0.1, and sucrose, 30 000. Co-cultivation medium was 1/4 strength MS salts in the basal medium with 0.5 g/l casein hydrolysate, 0.6 μM benzyladenine (BA) and 8 μM napthaleneacetic acid (NAA). Acetosyringone (Aldrich, Milwaukee, WI) (10 μM final concentration) was filter sterilized and added after autoclaving. Agar ("TC Agar"; Hazleton Biologics, Lenexa, KS) was incorporated into solidified media at 7 g/l. Media were adjusted to pH 5.7–5.8 with 1 N KOH or 1 N HCl and autoclaved at 1.4 kg cm^{-2} for 20 min. Media were poured into 100 × 15 mm petri plates (25 ml/plate). Explants were cultured under 45–50 $\mu E\ m^{-2}s^{-1}$ of mixed warm white (General Electric) and Vita-lite full spectrum fluorescent (Duro-Test Corp., North Bergen, NJ) lamps at 24 ± 1° C with a 16 h photoperiod.

Long-term embryogenic callus. Embryogenic callus was derived from immature, 40–50 day post-bloom embryos isolated from fruit of open-pollinated seedlings of a red leaf peach breeding selection 14DR60 following the general methods of Hammerschlag, et al. (8). Briefly, embryos were placed on callus initiation medium (CIM) (4.5 μM 2,4-D + 0.44 μM BA) for 9 weeks with transfers to fresh medium every 3 weeks. Calli were then placed on nodular callus-inducing medium (NCM) (2.2 μM BA + 0.27 μM NAA) for 9 weeks with transfers to fresh medium at 3-week intervals. Nodular calli were then transferred to

shoot-inducing medium (SIM), (4.4 μM BA + .05 μM NAA). Nodular calli that did not form shoots on SIM were transferred back to NCM. Calli were maintained on NCM for approximately 4 years, with transfers to fresh NCM every 3-5 weeks. These long-term embryogenic calli became growth regulator-independent, produced embryo and shoot-like structures, but formed few shoots (18). They were used as the source of embryogenic callus for transformation studies.

Tissue sensitivity to antibiotics. The sensitivity of peach tissue to kanamycin and G-418 ("Geneticin", Sigma, St. Louis, MO) was determined by plating leaf pieces and embryogenic calli on half strength MS salts basal medium with kanamycin at 0-300 mg/l or G-418 at 0-30 mg/l. The leaf culture medium also contained 10 μM kinetin (K) and 20 μM NAA to stimulate cell proliferation. Tissue sensitivity was evaluated on the basis of the number of explants producing callus or callus fresh weight.

In order to further define levels of antibiotics appropriate for selection and maintainence of transformed cells, the sensitivity of transformed leaf callus (LE1; *see* Fig. 1) and untransformed leaf callus was compared on selective media containing kanamycin at 20-160 mg/l and G-418 at 10-80 mg/l. The source of untransformed leaf callus was a culture which had been growing for 4-5 months (3-4 weeks between transfers to fresh medium) on basal medium with 1 μM K and 10 μM NAA. Calli (transformed and non-transformed) were divided into 9-11 mg fresh weight pieces and explanted on to selective media.

Culture of bacterial strains and plasmid isolation. Agrobacterium tumefaciens strain A281 containing the engineered plasmid pGA472 (1) was used in this study. The engineered plasmid consists of a wide host-range replicon, a chimeric *nos-npt* gene, left and right T-DNA border sequences and a multiple cloning site. It also contains the *cos* site of bacteriophage lambda and the ColE1 origin of replication between the T-DNA borders.

A. tumefaciens cultures were maintained frozen at −80° C in LB medium containing 7.5% dimethyl sulfoxide and selective antibiotics. Bacteria were streaked on agar-solidified LB medium supplemented with kanamycin (50 mg/l) and tetracycline (10 mg/l) several days before use and single colonies were picked as needed. Cultures for transformation experiments were grown overnight with vigorous agitation at 28° C in liquid LB medium containing the selective antibiotics. Overnight cultures were diluted 1:10 with the same medium and grown to A_{600} = 0.5. These cultures were centrifuged at 1000 $\times g$ for 15 min and the pellet resuspended in basal plant culture medium containing 10 μM acetosyringone immediately prior to tissue inoculation.

DNA used as a control to test for the presence of unincorporated plasmid DNA in putative transformants was extracted from *A. tumefaciens* A281(pGA472). One liter of *Agrobacterium* was grown up and plasmid extracted using the lysis by SDS protocol for large plasmids (11). The DNA was separated from RNA and the majority of chromosomal DNA on a CsCl gradient.

The plasmid pGA472 used for probe isolation was grown in *E. coli* strain MC1000, isolated and purified by standard methods (11).

Transformation of excised leaf segments. Young expanding leaves were collected from greenhouse seedlings of peach rootstock cultivar Tennessee Natural and rinsed under running deionized water for 5-10 minutes. They were surface sterilized in a solution of 10% bleach (0.525% NaOCl) + 0.005% Tween 20 for 10 min then rinsed 3 times with sterile deionized water. Leaf margins and the tip and base of each leaf were removed. The remainder of the leaf was cut into sections (approx. 1 cm × 2 cm) with each segment bisected by the leaf midrib. Leaf sections were immersed for 5-10 seconds in the *Agrobacterium* suspension (A_{600} = 0.5), blotted on sterile filter paper and incubated for 2 days on co-cultivation medium. After 2 days, leaf sections were rinsed in sterile water, blotted dry and placed on half strength MS salts basal medium with 50 mg/l casein hydrolysate, 0.6 μM BA, 8 μM NAA, carbenicillin (300-500 mg/l), cefotaxime (100 mg/l), and kanamycin (200 mg/l) or G-418 (20 mg/l). Sixty-six leaf segments were plated on kanamycin and 58 were plated on G-418. Leaf segments were transferred at 3 week intervals to fresh medium with antibiotics. The number of calli per leaf segment was recorded after 10 weeks of culture. Each callus was scored as a putative transformation event.

Transformation of immature embryos. Immature seed (approximately 55 days post bloom) were excised from fruit of an open-pollinated doubled haploid peach selection (PER 2D) obtained from T. Toyama (Washington State University). The seed were surface sterilized in a solution of 10% bleach + 0.005% Tween 20 for 10 min and rinsed 3 times with sterile deionized water. Immature embryos were dissected away from endosperm and immersed for four hours in co-cultivation medium containing *A. tumefaciens* at a concentration of 20 μl of an overnight culture (A_{600} = 0.5) per 60 ml of medium. Embryos were then blotted dry on sterile filter paper and cultured on agar-solidified CIM in the dark at 22° C for 3 days. They were then removed from CIM, washed with 100 ml of liquid CIM containing 500 mg/l carbenicillin and cultured on agar-solidified CIM with 500 mg/l carbenicillin for 12 days. Following this initial culture period embryos were transferred to CIM with 100 mg/l kanamycin, 250 mg/l carbenicillin and 100 mg/l cefotaxime. Embryos were transferred to fresh CIM with antibiotics every three weeks.

Transformation of embryogenic callus. Long term, growth regulator-independent embryogenic callus was suspended in liquid medium consisting of half strength MS salts basal medium (8 g callus/25 ml medium) and macerated in a blender at high speed for 3 seconds. Macerated tissue was filtered through a 60 mesh (230 u opening size) stainless steel screen and the tissue remaining on the screen was preincubated for 24 hours on co-cultivation medium. Tissue was then removed from the co-cultivation medium, immersed for 15-20 seconds in the *Agrobacterium* culture (A_{600} = 0.5), at 3 g tissue/10 ml *Agrobacterium* culture and refiltered through a 60

mesh screen. The cells remaining on the screen were then placed back on co-cultivation medium. Following a 2-day co-cultivation, the tissue was washed 4 times with 50 ml each time of half strength MS salts basal medium and placed on basal medium with 300 mg/l carbenicillin, 100 mg/l cefotaxime, and 200 mg/l kanamycin.

NPT II assay. After at least 3 months of growth on selective medium, proliferating calli from leaf segments, embryos and embryogenic callus cultures were selected as putative transformants for further study. These calli were assayed for the presence of neomycin phosphotransferase (NPT II) activity using a simplified dot blot procedure (12). An average of 15 μg total protein, as measured by the Bradford protein assay (3), was used for each sample. The results of the assays were quantitated by liquid scintillation. *E. coli* containing pKC7 (16) was used as a positive control source of NPT II activity.

DNA extractions, restriction digests and Southern analyses. Callus and leaf material were frozen in liquid nitrogen and stored at −80° C. DNA was extracted using the solutions from a commercially available DNA extraction kit (Oncor, Inc., Gaithersburg, MD) with modifications of the Oncor procedure. Two grams of frozen tissue were ground to a fine powder in liquid nitrogen with a mortar and pestle and transferred into 10 ml of 0° C Oncor Wash Buffer (phosphate buffered saline). One ml of Oncor Lysis Buffer I (surfactant) was added and the solution was left at 0° C for 15 min. The extract was filtered through two layers of miracloth (Cal Biochem, San Diego, CA) and the filtrate was centrifuged at 1000 ×*g* at room temperature for 5 min. The supernatant was poured off and the pellet was resuspended in 0.3 ml of Suspension Buffer I (tris buffered saline). An equal volume of Oncor Lysis Buffer II (buffered urea, detergent) and 15 μl of Oncor Protein Digesting Enzyme (proteinase K solution) were added. After incubation at 60° C for 50 min, boiled RNase A (Sigma) was added to the solution to a concentration of 100 mg/l and the incubation was continued at 60° C for an additional 10 min. The solution was extracted with equal volumes of phenol twice, phenol/chloroform/isoamyl alcohol (25:24:1, v:v:v) twice, and chloroform/isoamyl alcohol (24:1, v:v) twice. One half volume of Oncor DNA Conditioner I (ammonium acetate) and three volumes of cold 100% ethanol were added. After incubation at −70° C for 20 min the DNA was collected by centrifugation at 17 000 ×*g* for 20 min. The pellet was washed with 70% ethanol and dried. The pellet was resuspended in water and the nucleic acids were quantitated by the absorbance at 260 nm. Typically 25 to 200 μg of nucleic acid were recovered per gram of starting material.

Restriction digests were performed as suggested by the suppliers of the restriction enzymes (Promega, Madison, WI and BRL, Gaithersburg, MD).

Southern analyses were done using an Oncor Probe Tech I apparatus and Oncor reagents (Oncor) according to the instructions supplied by the manufacturer. Prehybridizations, hybridizations and washes were done using a Sure Blot kit (Oncor). The hybridization was done in 50% formamide and 6 × SSC at 45° C and the final wash was at 0.1 × SSC, 52° C for one hour.

Probe construction. The 2.4 kb BamHI fragment from the T-DNA region of the plasmid DNA was separated by electrophoresis. isolated from low melting point agarose (BioRad, Richmond, CA), and radioactively labeled using random primers according to the instructions with the BioRad Random Primer DNA Labeling Kit.

RESULTS

Antibiotic sensitivity. Excised leaf segments which had not been exposed to *Agrobacterium* produced callus on the control medium without antibiotics and at concentrations of 10–40 mg/l kanamycin or 5 mg/l G-418. Explants necrosed and died on media with higher concentrations of kanamycin or G-418 (Table 1). Total necrosis of explants progressed more slowly on kanamycin than on G-418 (approximately 3 versus 2 weeks). Small calli (1 mm diameter or less) developed on explants at the higher levels of kanamycin (80 and 160 mg/l) and 10 mg/l G-418 but these calli did not continue to grow and died. All leaf segments were completely necrotic on 20 mg/l G-418.

Growth of embryogenic calli on selective medium was variable, particularly using kanamycin as the selective agent. This variability may be related to the larger mass of embryogenic callus tissue exposed to selection when compared with leaf segments. Little or no growth occurred with G-418 in the medium (Table 2) and necrosis was generally more rapid than with kanamycin (2 vs 3 weeks).

Selection of transformants. Leaves co-cultivated with *A. tumefaciens* formed calli on selective medium containing 200 mg/l kanamycin or 20 mg/l G-418. At least one callus formed on 64% of the leaf pieces on kanamycin and 38% of the leaves produced 1 callus on G-418. On kanamycin 26% of the leaf pieces had 5 or more calli and

TABLE 1

SENSITIVITY OF PEACH LEAF PIECES TO KANAMYCIN AND G-418 BASED UPON CALLUS DEVELOPMENT AFTER 4 WEEKS OF CULTURE ON HALF STRENGTH MS SALTS BASAL MEDIUM WITH 10 μ*M* KINETIN AND 20 μ*M* NAA

Kanamycin Concentration (mg/l)	% Explants callusing
0	100[a]
10	100
20	100
40	88
80	38[b]
160	38[b]
G-418 (mg/l)	
0	100
5	38
10	13[b]
20	0
40	0
80	0

[a]N = 8 leaf pieces/treatment.
[b]Small calli (1 mm or less) which subsequently died.

TABLE 2

EFFECTS OF KANAMYCIN AND G-418 ON GROWTH OF EMBRYOGENIC PEACH CALLUS[a]

Concentration mg/l	Mean Individual Callus Fresh Weight (mg)[b]
No antibiotic	65.6 ± 11.7[c]
G-418	
5	8.8 ± 0.9
10	11.7 ± 1.1
20	10.0 ± 0.8
30	8.1 ± 1.0
Kanamycin	
25	35.0 ± 6.3
50	31.7 ± 5.6
75	17.7 ± 3.6
100	22.2 ± 2.3
150	25.7 ± 2.9
200	19.7 ± 1.7
250	13.2 ± 1.1
300	14.8 ± 1.6

[a]Cultured on half strength MS salts basal medium. Data collected after 10 weeks of culture.

[b]32 Calli per treatment. 64 Control calli. Initial weights of calli were 10–15 mg.

[c]Standard errors.

on G-418 5% had 5 or more calli (Table 3). Continual growth of calli after at least 3 transfers to fresh selective medium suggested that the putative transformants were stable. Continued growth after transfer to half strength MS salts basal medium with antibiotics but without growth regulators indicated that calli were growth regulator-independent. Two of these, LE1 and LE2, were selected for further analyses.

From approximately 200 immature embryos, ten produced calli that survived at least 4 subcultures onto selective medium (3 weeks between each subculture). Continued growth of calli upon transfer to half strength MS salts basal medium with antibiotics but without growth regulators indicated that these calli were growth regulator-independent. Two lines were selected for further analyses (DHE1 and DHE2).

A total of 15 g fresh weight of macerated embryogenic calli were exposed to *Agrobacterium* and transferred to selective medium in 2 separate tests. In the first trial one vigorously-growing callus (EM1) was isolated. This putative transformed callus line did not produce somatic embryos or shoot-like structures typical of untransformed long-term embryogenic callus. In the second transformation experiment 16 calli were selected for growth on antibiotic-containing media. All of these calli produced somatic embryos and shoot-like structures. Seven were selected for further analyses (EM2-8).

NPT II assay. Calli growing vigorously on selective medium from leaf pieces, immature embryos and from embryogenic callus, were tested two separate times for NPT II activity. All gave positive signals on the autoradiograms. A positive control (*E. coli* MC 1061 with pKC7) also gave a positive signal. Embryogenic peach callus and leaves not exposed to *Agrobacterium* gave no detectable signal. Liquid scintillation counts for NPTII positive dot blots ranged from 200–630 cpm/10 μg protein for embryos and embryogenic calli which was 2-6× over controls. LE1 was 106 × over the leaf control. LE2 was not included in this first test. In the second test, counts for embryogenic calli and embryos ranged from 25–124 cpm/10 μg protein which was 4–25 × over controls. Counts/min/10 μg protein for leaf-derived calli were 519 (LE1) and 144 (LE2) which were 13 × and 3.5 × respectively over leaf controls.

Southern analysis. To determine if integration of T-DNA from the pGA472 plasmid into the plant genome had occurred, DNA from twelve putative transformants, 2 from leaves (LE 1-2), 2 from embryos of the doubled haploid (DHE 1-2), and 8 from long-term embryogenic callus (EM 1-8) was examined by Southern analysis (Fig. 1). DNA was also extracted from peach embryogenic callus not exposed to *Agrobacterium* (EM CONT) and from greenhouse-collected leaves (LE CONT). The plant DNA was digested with BamHI to assay for the 2.4 kb internal BamHI T-DNA fragment and digested with HindIII to assay for a novel HindIII-HindIII plant-T-DNA hybrid fragment. Both would be present if the complete T-DNA had been integrated into the plant genome. Any integration event fragment based on where it integrated into the plant genome. If free plasmid were present, the 2.4 kb BamHI fragment and a 15.56 kb HindIII fragment representing the intact, linear plasmid would be detected. Uncut DNA was also resolved on the gel to demonstrate lack of free plasmid which would migrate faster than uncut genomic DNA.

DNA from the transforming *A. tumefaciens* strain yielded the expected 2.4 kb BamHI fragment and the 15.56 kb HindIII fragment when hybridized with the 2.4 kb BamHI fragment (Fig. 1). The leaf control and the embryogenic callus control had no detectable fragments hybridize indicating no random homology with the probe sequence. Eleven of the twelve putative transformants yielded the expected 2.4 kb BamHI fragment, the 2.4 kb fragment for EM4 is detectable on a longer exposure. LE 1 yielded a slightly smaller fragment. Southern analyses with HindIII-EcoRI digestions probed with the HindIII-EcoRI fragment of pGA472 indicated that a deletion of part of the T-DNA had taken place in LE 1 (data not shown). Two additional fragments are detected in DHE 1

TABLE 3

TRANSFORMATION FREQUENCY OF PEACH LEAF SEGMENTS EXPOSED TO *A. TUMEFACIENS* A281(pGA472) BASED UPON CALLUS PRODUCTION ON SELECTIVE MEDIUM CONTAINING KANAMYCIN OR G-418

	% Leaves Producing the Indicated Number of Calli on[a]	
No. Calli/Leaf	Kanamycin (200 mg/l)	G-418 (20 mg/l)
0	36	62
1	10	22
2	14	9
3	14	0
4	0	2
5	6	2
5+	20	3

[a]Total number of leaves in kanamycin treatment 66, in G-418 treatment 58.

and one other additional fragment is detected in EM5 which may also indicate that other events have taken place with a deletion of some of the T-DNA. All twelve putative transformants have a unique HindIII-sized fragment that is not the same size as that of the whole pGA472 plasmid. All but two of the HindIII digestions yielded more than one unique fragment (EM 4 and EM 7 yielded 1 fragment). We believe that these multiple fragments represent more than one integration event. The uncut DNA of all twelve putative transformants yielded one fragment that co-migrated with uncut genomic DNA (data not shown). The Southern analyses support the conclusion that these three sources of peach tissue have been transformed with T-DNA and it has been integrated into the peach genome.

Growth of transformed callus on selective media. After 28 days of culture on selective media, growth of transformed leaf-derived callus (LE1) on kanamycin at 20–160 mg/l was similar if not slightly higher than growth on non-selective medium (Table 4). Growth was reduced at all levels of G-418, particularly at 80 mg/l. After 73 days of culture on selective medium transformed leaf callus grew as well on 20-160 mg/l kanamycin and 10–20 mg/l G-418 as on non-selective medium. Growth was reduced at 40–80 mg/l G-418. Untransformed callus grew more slowly on the basal non-selective medium than the transformed callus because the transformed callus was growth regulator-independent and required no exogenous growth regulators. The levels of BA (0.1 μM) and NAA (1.0 μM) used in this test were too low to permit optimum growth of the untransformed callus which has an absolute requirement for exogenously-supplied growth regulators. Nevertheless, on non-selective medium it more than doubled its fresh weight between 28 and 73 days of culture. Untransformed callus was able to survive and grew slowly for at least 28 days on kanamycin at 20–160 mg/l and G-418 at 10–20 mg/l. It did not survive this period on G-418 at 40–80 mg/l nor did it survive for 73 days on any selective medium.

Discussion

This study indicates that peach leaf tissue, embryos, and embryogenic callus are amenable to *Agrobacterium*-mediated transformation. We have demonstrated growth of callus on selective medium (using kanamycin or G-418), positive NPT II assays, and the detection of unique insertion events through Southern analyses. All indicate transformation. This report presents, to our knowledge, the first demonstration of T-DNA integration into the peach genome by Southern analysis. These analyses present additional evidence of transformation in peach to what has been previously reported through octopine assays, growth on cytokinin-free medium and DNA slot blot assays (9) and support the suggestion that peach tissues are amenable to *Agrobacterium*-mediated transformation.

The transformation of embryogenic peach callus is significant because plant regeneration from this tissue

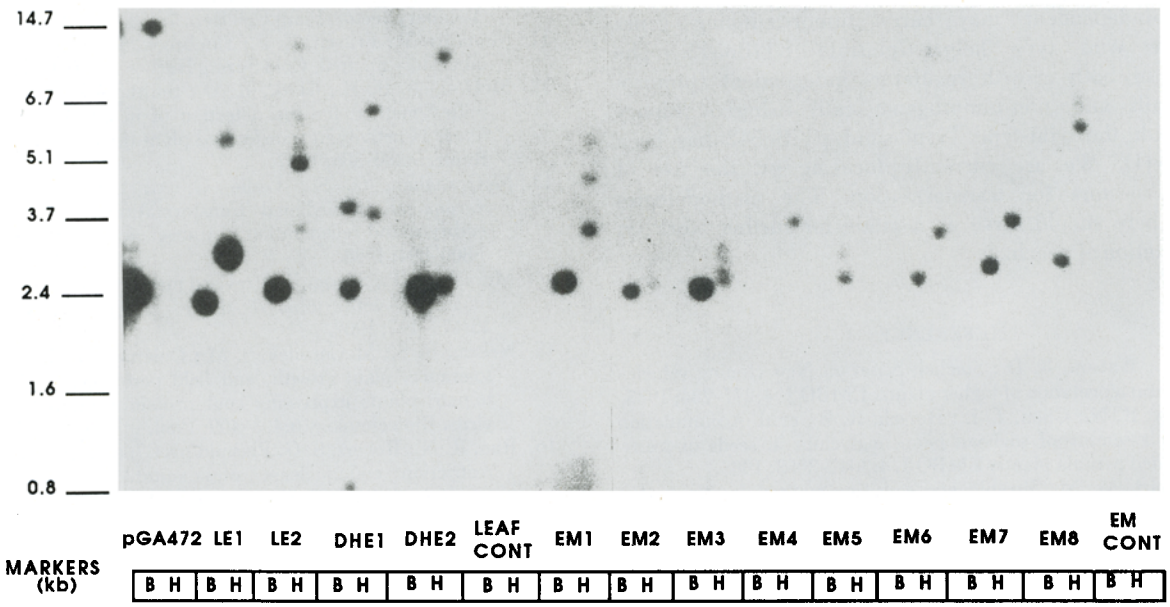

FIG. 1. Southern analysis of the twelve putative transformed calli. DNA was extracted from callus of two leaf samples (LE 1-2) two embryos (DHE 1-2) and from eight embryogenic calli (EM 1-8), that had been selected for growth on selective media following exposure to *A. tumefaciens* A281(pGA472). pGA472 DNA (pGA472) was also extracted from the *A. tumefaciens* strain used in the transformations. DNA was extracted from two control tissues that had not been exposed to *A. tumefaciens* nor selected on kanamycin or G-418. The DNA was digested with BamHI (B) and a second sample with HindIII (H) and resolved on a 0.7% agarose gel. Lambda DNA digested with Pst I and lambda DNA digested with EcoRV were also resolved and a subset of the fragment sizes are indicated on the left of the figure in kilobase pairs. All of the putative transformants exhibit an internal fragment of 2.4 kb in the BamHI digest (except LE 1 — *see* text for explanation) and exhibit a unique fragment (different from that of pGA472) in the HindIII digest. This is consistent with integration of the T-DNA into the genomes of these callus lines. The two controls have no detectable fragments.

TABLE 4

FRESH WEIGHT (mg) OF TRANSFORMED AND NON-TRANSFORMED LEAF DERIVED CALLI CULTURED AT 16 H PHOTOPERIOD ON MS MEDIUM PLUS VITAMINS, 3% SUCROSE, 0.1 μM BA AND 1.0 μM NAA, WITH AND WITHOUT SELECTIVE ANTIBIOTICS

	Transformed		Untransformed	
	28 Days	73 Days	28 Days	73 Days
No Selection	44.8 ± 2.2[a]	174.6 ± 21.1	14.2 ± 0.7	30.5 ± 1.4
Kanamycin				
20	58.5 ± 2.8	194.0 ± 9.1	14.3 ± 0.3	dead
40	54.3 ± 5.6	179.2 ± 20.1	13.2 ± 0.6	dead
80	62.7 ± 2.7	167.0 ± 24.6	14.7 ± 0.6	dead
160	48.0 ± 4.1	171.8 ± 20.0	12.7 ± 0.6	dead
G-418				
10	34.3 ± 0.9	166.8 ± 12.3	11.7 ± 0.4	dead
20	27.5 ± 1.3	179.3 ± 9.7	10.5 ± 0.4	dead
40	24.0 ± 1.2	115.3 ± 9.8	dead	dead
80	17.2 ± 1.0	69.7 ± 7.3	dead	dead

[a]Standard errors; 6 calli per treatment.

has been reported (8). We did not obtain transformed plants from the transformed embryogenic calli developed in this study because typically long term embryogenic peach cultures produced few if any normal shoots (18). Seven of the eight transformed embryogenic callus lines (EM2 through 8) produced somatic embryos and shoot-like structures typical of untransformed long-term embryogenic peach callus. One transformed line (EM1) produced a friable callus that did not form somatic embryos. This callus line, along with leaf-derived transformed calli (LE1 and LE2) which were growth regulator-independent may carry tumor-inducing genes from the wild type helper plasmid pTiBo542. The isolation of both growth regulator- independent (tumorogenic) calli and non-tumorogenic calli using a binary vector with the wild-type Ti plasmid pTiBo542 has been reported (1). We are currently focusing on the use of disarmed vectors for transformation. The methodologies reported here should serve as a guide for further work on transformation of peach.

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