

# *Agrobacterium tumefaciens*-mediated transformation of *Arabidopsis thaliana* root explants by using kanamycin selection

(herbicide resistance/neomycin phosphotransferase II/plant regeneration/tumor-inducing Ti plasmid/transgenic plants)

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**ABSTRACT** Culture conditions were developed that induce *Arabidopsis thaliana* (L.) Heynh. root cuttings to regenerate shoots rapidly and at 100% efficiency. The shoots produce viable seeds *in vitro* or after rooting in soil. A transformation procedure for *Arabidopsis* root explants based on kanamycin selection was established. By using this regeneration procedure and an *Agrobacterium* tumor-inducing Ti plasmid carrying a chimeric neomycin phosphotransferase II gene (*neo*), transformed seed-producing plants were obtained with an efficiency between 20% and 80% within 3 months after gene transfer. F<sub>1</sub> seedlings of these transformants showed Mendelian segregation of the kanamycin-resistance trait. The transformation method could be applied to three different *Arabidopsis* ecotypes. In addition to the *neo* gene, a chimeric *bar* gene conferring resistance to the herbicide Basta was introduced into *Arabidopsis*. The expression of the *bar* gene was shown by enzymatic assay.

*Arabidopsis thaliana* (L.) Heynh. has many advantages as a model system for plant molecular biology (1, 2). The small plant has a generation time of only 4–6 weeks, and a single plant can be grown to maturity on as little as 1 cm<sup>2</sup>. The haploid genome size of *Arabidopsis* is only 70,000 kilobases (kb), which is roughly the size of the *Drosophila melanogaster* and *Caenorhabditis elegans* genomes (3–5). Like these model animal systems, *Arabidopsis* has excellent genetics. Many developmental and physiological mutations are characterized and mapped (6), although most of these gene loci have not been cloned. Because of its small genome size, *Arabidopsis* should be ideally suited for cloning such genes by insertional mutagenesis and/or complementation experiments using shotgun transformation of cosmid clones (7). One obstacle in the development of these gene-cloning strategies was the lack of a rapid and efficient *Arabidopsis*-transformation procedure. Several *Arabidopsis*-transformation procedures using leaf material infected by *Agrobacterium tumefaciens* have been published (8–10). In our hands the regeneration of plants by these protocols was generally not efficient and took 4–5 months or more. Therefore, we examined the regenerative response of *Arabidopsis* roots.

We found that *Arabidopsis* root explants have a high potential for rapid shoot regeneration. Fertile plants can be regenerated reproducibly within 2–3 months. We incorporated this regeneration method with *A. tumefaciens* infection to develop an efficient and rapid transformation procedure using kanamycin (Km) selection.

## MATERIALS AND METHODS

**Arabidopsis Strains.** *A. thaliana* seeds collection number C24 were provided by M. Jacobs (Vrije Universiteit Brussels); the *Arabidopsis* ecotype Landsberg *erecta*, by M. Koornneef

(Landbouwwuniversiteit Wageningen); and *Arabidopsis* ecotype Columbia, by G. Rédei (Columbia University).

**Agrobacterium Strains.** *A. tumefaciens* C58C1Rif<sup>R</sup> containing the nononcogenic tumor-inducing Ti plasmid pGSFR1161 was obtained from J. Botterman (Plant Genetic Systems, Ghent, Belgium). pGSFR1161 is a cointegrate vector between pGV2260 (11) and pGSFR161 (Fig. 1). Between the borders of the transferred DNA (T-DNA; portion of the Ti plasmid that is transferred to plant cells), the construct contains a chimeric *bar* (13) and *neo* gene (from transposon Tn5) under control of the T-DNA T<sub>R</sub> promoter (the dual promoter from the T<sub>R</sub>-DNA of an octopine Ti plasmid) (14); the *neo* gene encodes neomycin phosphotransferase II (NeoP-Tase II), and the *bar* gene confers resistance to the herbicide Basta. The binary vector pCLH88 (C. Bowler, personal communication) contains a *neo* gene under the control of the T-DNA nopaline synthase gene (*nos*) promoter and was used together with pGV2260 in an *A. tumefaciens* C58C1Rif<sup>R</sup> background. *Agrobacterium* containing the nononcogenic Ti plasmid pGV2260 alone was used as a control strain in transformation experiments. Bacteria were grown overnight in Luria broth at 28°C with swirling (200 rpm).

**Tissue Culture Conditions.** *Arabidopsis* seeds were vernalized for 7 days at 4°C before germination. Seeds were surface-sterilized for 2 min in 70% EtOH, transferred to 5% NaOCl/0.5% NaDodSO<sub>4</sub> for 15 min, rinsed five times with sterile distilled water, and placed on 150 × 25 mm Petri dishes containing germination medium (GM) (Table 1) to germinate. Petri dishes were sealed with gas-permeable medical tape (Urgopore, Chenove France). Plants were grown at 22°C in a 16-hr light/8-hr dark cycle. The same growth-room conditions were used for tissue culture procedures.

All plant media were buffered with 2-(*N*-morpholino)ethanesulfonic acid at 0.5 g/liter (pH 5.7; adjusted with 1 M KOH), solidified with 0.8% Difco Bacto agar, and autoclaved at 121°C for 15 min. Hormones and antibiotics were dissolved in dimethyl sulfoxide and water, respectively, and were added to the medium after autoclaving and cooling to 65°C.

**Transformation of Arabidopsis Root Explants.** Intact roots were incubated for 3 days on solidified 0.5/0.05 medium (Table 1). Roots were then cut into small pieces of about 0.5 cm (herein referred to as “root explants”) and transferred to 10 ml of liquid 0.5/0.05 medium; 0.5–1.0 ml of an overnight *Agrobacterium* culture was added. The root explants and bacteria were mixed by gentle shaking for about 2 min.

Abbreviations: PAcTase, phosphinothricin acetyltransferase; NeoP-Tase II, neomycin phosphotransferase II; Km, kanamycin; Km<sup>S</sup> and Km<sup>R</sup>, Km sensitive and resistant; 2,4-D, 2,4-dichlorophenoxyacetic acid; T-DNA, portion of the Ti plasmid that is transferred to plant cells; T<sub>R</sub> promoter, dual promoter from the T<sub>R</sub>-DNA of an octopine Ti plasmid; GM, germination medium; CIM, callus-inducing medium; SIM, shoot-inducing medium.

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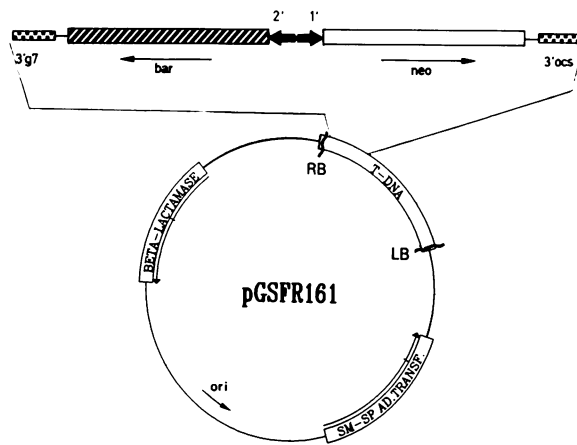


FIG. 1. Schematic representation of pGSFR161. The chimeric *bar* and *neo* genes are under the control of the 2' and 1'  $T_R$  promoter, respectively, and are inserted at opposite orientation between the T-DNA border repeats of pGV1500 (12). The respective genes have termination and polyadenylation signals of the T-DNA *ocs* (3' *ocs*) and the T-DNA gene 7 (3' *g7*). RB, right border; LB, left border.

Subsequently, the root explants were blotted on sterile filter paper to remove most of the liquid medium and cocultivated for 48 hr on 0.5/0.05 agar. The explants were then rinsed in liquid 0.5/0.05 medium containing 1000 mg of vancomycin (Sigma) per liter. The pieces were blotted and then incubated on 0.15/5 agar (Table 1) supplemented with 750 mg of vancomycin and 50 mg of Km per liter. Three weeks after infection with agrobacteria containing a chimeric *neo* gene, green Km-resistant ( $Km^R$ ) calli were formed in a background of yellowish root explants. At this point the root explants were transferred to fresh 0.15/5 agar containing only 500 mg of vancomycin and 50 mg of Km per liter. Three weeks later most green calli had formed shoots. Transformed shoots were transferred to 150 × 25 mm Petri dishes containing GM to form roots or seeds or both. In these Petri dishes, many regenerants formed seeds without rooting. Rooted plants could also be transferred to soil to set seed.

**Enzymatic Assays.** Assays for NeoPTase II were performed as described by Reiss *et al.* (21); the assay for phosphinothricin acetyltransferase (PACTase) has been described by De Block *et al.* (13). For both assays, transformed shoots free of agrobacteria were used.

**DNA Methods.** Total *Arabidopsis* DNA was isolated as described by Dellaporta *et al.* (22). Genomic hybridizations were performed as described by Simoens *et al.* (7).

## RESULTS

**Capacity of Root Explants to Form Shoots.** To test their regenerative capacity, we incubated *Arabidopsis* C24 root explants from 4-wk-old, sterile-grown plantlets for 7 or 14 days on PG1 or R3 CIM (Table 1). They were then transferred to 0.15/5 or 0.05/7 (auxin/cytokinin ratio) SIM (Table 1). Roots that were callus-induced for 7 days were completely covered with shoots after 4 wk of incubation on SIM. By contrast, roots that were transferred to SIM after 14 days of callus induction produced no shoots but formed brown degenerating callus instead.

Omitting the callus induction step and culturing roots immediately onto SIM resulted in shoot formation at the proximal end of the explant only. Fig. 2 summarizes the different morphogenetic pathways that can be induced from *Arabidopsis* root explants by manipulating culture conditions. Clearly, the combination of callus induction followed by shoot induction was critical for efficient formation of shoots.

The ontological pathway leading to rapid shoot formation from *Arabidopsis* roots is illustrated in Fig. 3 a–e. Induction

Table 1. Plant media

	CIM				SIM	
	GM	R3*	PG1*	0.5/0.05	0.05/7*	0.15/5*
Salts + vitamins	MS	MS	B5	B5	MS	B5
Sucrose, g/L	10	30	—	—	30	—
Glucose, g/L	—	—	20	20	—	20
IAA, mg/L	—	5	—	—	0.05	0.15
2,4-D, mg/L	—	0.5	2	0.5	—	—
2ipAde, mg/L	—	—	—	—	7	5
Kin, mg/L	—	0.3	0.05	0.05	—	—

L, liter; IAA, indole-3-acetic acid; Kin, kinetin; 2ipAde,  $N^6$ -(2-isopentenyl)adenine; CIM, callus-inducing medium; SIM, shoot-inducing medium; MS, Murashige & Skoog medium (19); B5, Gamborg B5 medium (20).

\*Data for R3, PG1, 0.05/7 and 0.15/5, were from refs. 15–18, respectively.

with 2,4-dichlorophenoxyacetic acid (2,4-D) resulted in a slight thickening of the root explants (Fig. 3a). When this tissue was transferred to high cytokinin/auxin-containing medium, root hairs covered the entire surface within 5–10 days (Fig. 3b). A few days later, new areas of growth, green and devoid of root hairs, became visible on the root surface. After 2–3 wk on SIM, the first shoots developed from these green areas (Fig. 3c). Finally, after only 3–4 wk of tissue culture, the original root was completely converted in a dense growth of shoots (Fig. 3d). These shoots were transferred to 150 × 25 mm Petri dishes containing GM. About 50% of the shoots grew roots in 2–4 wk. Rooted shoots could then be transferred to soil to flower and to produce seed. However, almost all shoots, regardless of whether they had roots or not, would form siliques and viable seeds in the 150 × 25 mm Petri dishes (Fig. 3e). In these aseptic conditions, typically 30–100 seeds per regenerant were produced.

**Influence of 2,4-D and Callus-Induction Period on Morphogenesis.** Since our initial experiments suggested that the concentration of 2,4-D in CIM and the length of the callus-induction period were determinative for homogenous shoot formation, we further tested the influence of these parameters on regeneration from root cuttings. We incubated root explants on CIM containing 0.2, 0.5, 1.0, 2.0, and 5.0 mg of 2,4-D and 0.05 mg of kinetin per liter. Explants were transferred to 0.15/5 SIM after 0, 4, 7, 10, and 14 days of incubation on each 2,4-D-containing CIM. Optimal shoot regeneration was achieved when we incubated the root explants for 4 days on a medium containing 0.5 mg of 2,4-D per liter prior to shoot induction (Fig. 4). Significantly more aberrant shoots were obtained with 2,4-D at 0.2 mg/liter. Increasing concentrations of 2,4-D and/or longer periods of callus induction resulted in a severe decrease in the regeneration efficiency. For example, 10 days of callus induction on a medium containing 2 mg of 2,4-D per liter and subsequent transfer to SIM resulted in thickening of the explants and formation of root hairs. However, further differentiation,

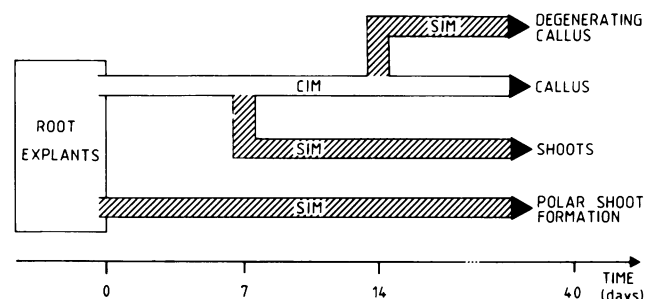


FIG. 2. Summary of different possible morphogenetic pathways with *Arabidopsis* C24 root explants combining CIM and SIM.

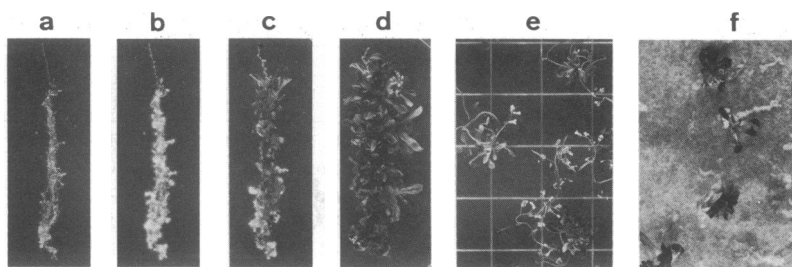


FIG. 3. Regeneration sequence of *Arabidopsis* C24 root explants. A root system was incubated for 4 days on the 0.5/0.05 CIM (Table 1) and subsequently transferred to the 0.15/5 SIM (Table 1). Differentiation on SIM is shown after 1 day (a), 4 days (b), 17 days (c), and 25 days (d). The first siliques are formed 2 wk after transfer of shoots to hormone-free GM—that, is within 6 wk of tissue culture (e). *Arabidopsis*-transformed shoots are induced on root explants after  $\pm$  5 wk of selection on 0.15/5 SIM containing 50 mg of Km per liter (f).

such as green callus and shoot formation, was completely blocked. Higher concentrations of 2,4-D for longer periods resulted in total loss of morphogenetic potential even before root hairs were formed (Fig. 4).

**Influence of Antibiotics on Regeneration from Roots.** To develop a rapid and efficient *Agrobacterium*-mediated transformation method for *Arabidopsis*, we assayed the action of a number of antibiotics that are currently used as selectable markers in plant transformation procedures. We found that 50 mg of Km, 10 mg of G418, or 25 mg of hygromycin per liter completely blocked regeneration from untransformed roots and, therefore, could be used to select for transformed cells.

Preliminary transformation experiments indicated that cefotaxime, an antibiotic commonly used to kill *Agrobacterium* after cocultivation with plant material, severely inhib-

ited regeneration from *Arabidopsis* root explants. Therefore, in addition to cefotaxime, three other *Agrobacterium*-inactivating antibiotics were tested for root explant toxicity at the effective concentrations of 0.5 and 1.0 g/liter (D.V., unpublished data). Except for slight vitrification, vancomycin and triacillin showed no inhibitory effects on regeneration from root explants. By contrast, carbenicillin was slightly inhibitory and cefotaxime inhibited almost totally the root regeneration. Because the pGV2260 (11) and pGSFR1161 Ti plasmids we used contained a  $\beta$ -lactamase (*bla*) gene capable of inactivating both carbenicillin and triacillin, vancomycin (750 mg/liter) was used in subsequent transformation experiments to stop *Agrobacterium* growth after cocultivation.

**Transformation of Root Explants.** After 3 days of callus induction on 0.5/0.05 medium, roots were cut into small pieces (0.5 cm) and cocultured on the same medium for 48 hr with an *A. tumefaciens* strain conferring Km<sup>R</sup> phenotype to plant cells after transfer of the T-DNA. Three to 4 wk after transfer to the 0.15/5 SIM containing 50 mg of Km per liter, green transformed calli (1–2 mm) in a background of yellowish root explants were formed. More than 80% of these green calli formed shoots after transfer to fresh SIM (Fig. 3f). Transformed shoots were transferred to GM for seed set. Using this method based on Km selection, we could reproducibly achieve transformation efficiencies between 20% and 80% (root explants forming seed-producing transformants per total number of infected root explants) (Table 2). Root explants infected with *Agrobacterium* harboring the T-DNA-less pGV2260 Ti plasmid produced no green calli or shoots on Km-containing medium (Table 2).

DNA from four randomly chosen Km<sup>R</sup> regenerants was analyzed by DNA gel-blot hybridization to determine the T-DNA copy number. The data in Fig. 5b, lanes 1–3, suggest that each of three transformants have one T-DNA insert hybridizing to both right- and left-border probes. However, one transformant (Fig. 5b, lane 4) probably contains four inserts. One DNA fragment of this transformant (marked by the arrowhead) hybridized to both right- and left-border probes. This hybridization pattern and the length of the fragment suggest that two of the four inserts might be arranged in a directly repeated tandem structure. In addition, both NeoPase II and PacPase activities were found in all transformants tested (Fig. 5c and d).

**neo Gene Segregation in the F<sub>1</sub> Progeny of Transformants Confirms the Low-Copy-Number T-DNA Insertion.** Seeds from 10 randomly chosen transformants were germinated on GM containing 50 mg of Km per liter. Two weeks after germination, Km<sup>S</sup> seedlings had completely etiolated cotyledons, and development of roots and leaves was completely inhibited. By contrast, Km<sup>R</sup> plants had produced two to three pairs of leaves and a normal, elongating root (Fig. 6). All 10 seed lines tested showed both Km<sup>R</sup> and Km<sup>S</sup> plants.  $\chi^2$  analysis confirms that they all segregate for one or two functional T-DNA copies (Table 3).

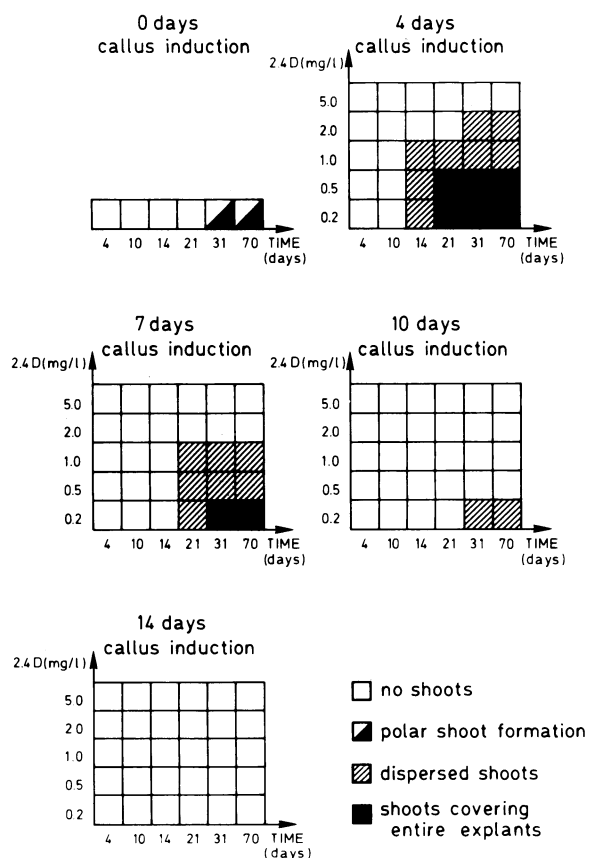


FIG. 4. Effects of the 2,4-D concentration in CIM and of the length of the induction period on regeneration of plants from root explants. Roots of *Arabidopsis* C24 were incubated for 0, 4, 7, 10, and 14 days on CIM containing different 2,4-D concentrations (ordinate) and 0.05 mg of kinetin per liter before transfer to 0.15/5 SIM. Each different combination of parameters was evaluated after 4, 10, 14, 21, 31, and 70 days of tissue culture (abscissa).

Table 2. Transformation efficiency of root explants

Agrobacterium strain	Chimeric selectable marker gene and promoter (P)	Arabidopsis ecotype	Root explants, no.		Transformation efficiency*
			Inoculated	With green calli on medium with Km (50 mg/liter)	
pGSFR1161	<i>PT<sub>R</sub>-neo</i>	C24	251	81	32.0
pGV2260		C24	119	0	0
pGSFR1161	<i>PT<sub>R</sub>-neo</i>	C24	443	131	29.6
pGV2260		C24	51	0	0
pCLH88*	<i>Pnos-neo</i>	C24	171	170	99.4 <sup>†</sup>
pGV2260		C24	28	0	0
pGSFR1161	<i>PT<sub>R</sub>-neo</i>	Landsberg <i>erecta</i>	118	104	88.1 <sup>†</sup>
pGV2260		Landsberg <i>erecta</i>	29	0	0

Root explants ( $\pm 0.5$  cm of a plant root system) were transformed as described. After 4 wk of selection on medium containing Km at 50 mg/liter, the number of green calli was determined.

\*% of root explants forming green calli.

<sup>†</sup>Root explants form one to three green calli.

**Genotype Effects on Root Regeneration and Root Transformation.** All experiments described above were performed with *A. thaliana* collection number C24. We also tested the root regeneration and transformation of *Arabidopsis* ecotypes Landsberg *erecta* and Columbia. *Arabidopsis* Landsberg *erecta* roots were both regenerated (D. V., unpublished data) and transformed equally efficiently as C24 roots (Table 2). Root explants of *Arabidopsis* ecotype Columbia could also be transformed efficiently. Every infected Columbia root explant developed one to three independent green calli. However, when compared with C24, these calli were both slower and less efficient in shoot formation. Additional experiments are required to circumvent these regeneration problems in *Arabidopsis* ecotype Columbia.

## DISCUSSION

A short preculture on 2,4-D-containing CIM and subsequent incubation on SIM with a high cytokinin concentration transforms *Arabidopsis* roots into a dense growth of shoots in only a few weeks time (Fig. 3). Similar conditions have been applied (17, 18) to induce shoot formation from *Convolvulus* and *Arabidopsis* ecotype Wassilewskija (WS) leaf material. The short preculture on the 2,4-D medium was an essential step in the regeneration procedure, as immediate incubation of roots on shoot-inducing medium led to shoot formation at the proximal end of the root only (Figs. 2 and 4). This polar shoot formation has been reported for roots of several other plant species and is thought to result from the endogenous auxin gradient that exists in roots (23, 24). The high incidence of shoot formation and the histological simplicity of *Arabidopsis* roots make this an elegant system to study initiation and development of shoots. By sectioning root explants at several stages during regeneration, the tissues giving rise to shoot primordia can be identified.

In contrast to previous reports (9, 10), we successfully used Km selection to develop a reliable *A. tumefaciens*-mediated root transformation procedure (Fig. 3f).

Infected root explants reproducibly yielded fertile transformants with an efficiency between 20% and 80%, which is considerably higher than in previous reports (8, 9, 10, 25). Seeds obtained from transformants germinated on Km-containing medium, resulting in both etiolated (Km<sup>S</sup>) seedlings and phenotypically normal (Km<sup>R</sup>) plants, further evidenced the inheritance and Mendelian segregation of the *neo* gene (Fig. 6). Segregation analysis and genomic-blot analysis revealed that most transformants contained only one T-DNA insert (Table 3; Fig. 5 a and b). Km also can be used efficiently as a selectable marker in *Arabidopsis* leaf disc transformation (M.V.L., unpublished data).

We also introduced a chimeric *bar* gene into *Arabidopsis*. The *bar* gene encodes PACtase, which inactivates phosphi-

nothricin, the active compound of the commercial herbicide Basta (Hoechst, Frankfurt). Unlike other marker genes that

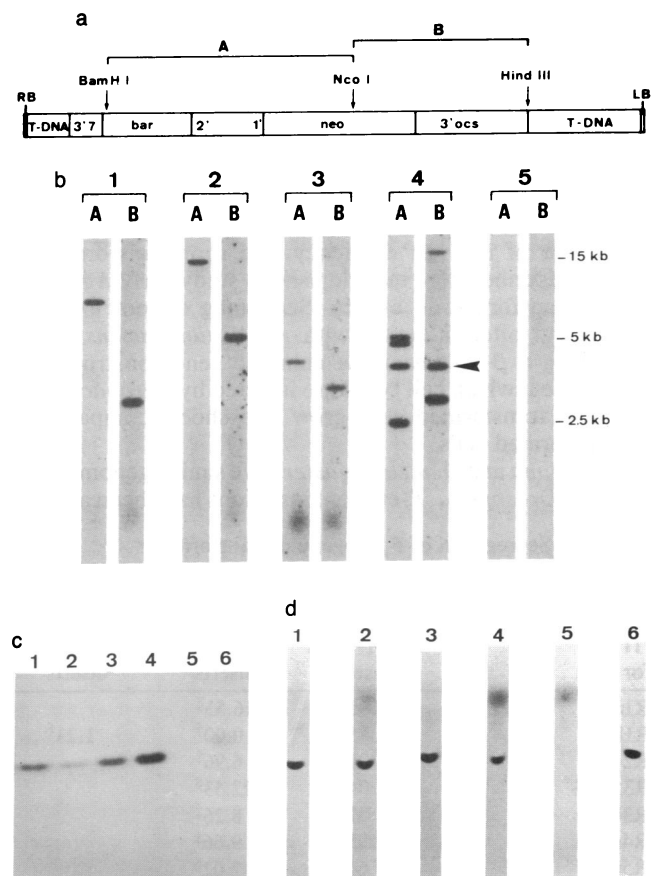


FIG. 5. Molecular analysis of *Arabidopsis* root-derived transformants. (a) T-DNA region of the pGSFR1161 vector. A is the 1.6-kb *Bam*HI-*Nco*I fragment; B is the 1.3-kb *Nco*I-*Hind*III fragment. Both were used as probes in genomic hybridizations. Notation is as in Fig. 2. (b) Genomic hybridizations. DNA from four transformants (lanes 1-4) and from an untransformed regenerant (lane 5) was digested with *Nco*I, separated on agarose gels, and blotted onto nylon membranes. Lanes: A, hybridization to the *Bam*HI-*Nco*I probe; B, hybridization to the *Nco*I-*Hind*III probe. Except for the band marked by the arrowhead, which is probably the internal fragment of a direct T-DNA repeat, all bands hybridizing to both probes are composite fragments between T-DNA and plant DNA. (c) NeoPTase II assays. Lanes: 1-4, transformants; 5, negative control (C24 plant); 6, positive control (purified NeoPTase II enzyme). (d) PACtase assays. Lanes: 1-4, transformants; 5, negative control (untransformed regenerant); 6, positive control (purified PACtase).

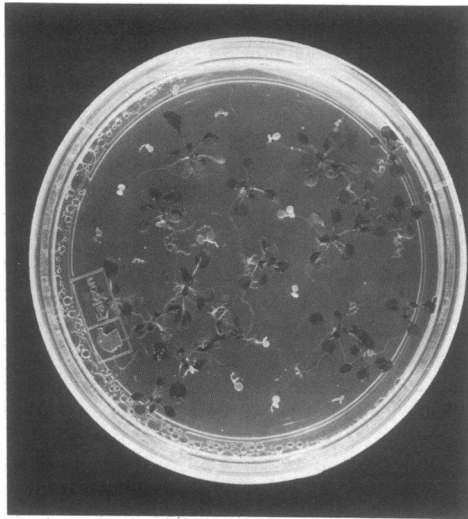


FIG. 6. Segregation of  $F_1$  progeny of transformant R72.  $F_1$  seeds were germinated on GM containing Km at 50 mg/liter. Two weeks after germination,  $Km^S$  seedlings are etiolated while  $Km^R$  seedlings grow phenotypically normal.

can be used in *Arabidopsis*, the *bar* gene is easily selected for at the adult plant level, which facilitates gene mapping. We demonstrated PACtase activity in transformed shoots (Fig. 5d). Moreover, herbicide spray tests showed cosegregation of Basta resistance with T-DNA in  $F_1$  progeny of *Arabidopsis* transformants (M.V.L., unpublished data).

Because of its high efficiency, the root transformation system described here may be used to study early *Agrobacterium* transformation events. Sectioning of root explants during and after infection with an *A. tumefaciens* strain containing a  $\beta$ -glucuronidase chimeric gene construct (26) could reveal which cell types are infected by *Agrobacterium* and how transformed cells grow into shoots compared to untransformed cells.

*A. thaliana* and *D. melanogaster* have similar genome sizes (3, 4). In *Drosophila*, *P*-element insertions have been a useful

Table 3. Segregation of  $F_1$  progeny of transformants

Trans- formant	Number of $F_1$ plants		$\chi^2$ *		
	$Km^R$	$Km^S$	One T-DNA insert	Two T-DNA inserts	Three T-DNA inserts
R16	49	12	0.66 <sup>†</sup>	16.53 <sup>§</sup>	
R18	23	2	3.85 <sup>‡</sup>	0.00 <sup>†</sup>	1.23 <sup>†</sup>
R24	18	5	0.02 <sup>†</sup>	6.96 <sup>‡</sup>	
R35	23	9	0.04 <sup>†</sup>	22.53 <sup>§</sup>	
R38	16	5	0.02 <sup>†</sup>	8.26 <sup>‡</sup>	
R44	34	8	0.25 <sup>†</sup>	9.66 <sup>‡</sup>	
R50	42	10	0.64 <sup>†</sup>	12.02 <sup>§</sup>	
R72	18	17	9.15 <sup>‡</sup>	99.89 <sup>§</sup>	
R85	20	3	1.17 <sup>†</sup>	0.79 <sup>†</sup>	12.95 <sup>§</sup>
R102	39	17	0.60 <sup>†</sup>	51.51 <sup>§</sup>	

$F_1$  seeds were germinated on GM (Table 1) containing Km at 50 mg/liter. Two weeks after germination, the number of  $Km^R$  and  $Km^S$  seedlings was determined.

\*Because of the small numbers of seeds the Yates correction term was used in the  $\chi^2$  calculations.

<sup>†</sup> $P > 0.05$ .

<sup>‡</sup> $0.05 > P > 0.001$ .

<sup>§</sup> $P < 0.001$ .

tool for the molecular cloning of developmentally important regulatory genes with a frequency of *P*-linked mutations of roughly 10% (27). In *Arabidopsis*, it is not yet known what fraction of the T-DNA insertions produces detectable mutations. We screened the progeny of 10 independent *Arabidopsis* root transformants and of about 50 independent leaf disc transformants. So far, this strategy did not yield any mutant clearly linked to the T-DNA (M.V.L., unpublished data). However, it is too early to conclude whether the T-DNA will be an effective mutagen in *Arabidopsis*.

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