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Improved efficiency for T-DNA-mediated transformation and plasmid rescue in *Arabidopsis thaliana*

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Abstract. A vector was constructed for the isolation of gene fusions to the *lacZ* reporter gene following T-DNA integration into the genome of *Arabidopsis thaliana*. To facilitate the generation of tagged *A. thaliana* plants, we established a modified method for high-frequency transformation of *A. thaliana* by *Agrobacterium tumefaciens*. The main modification required was to inhibit the methylation of T-DNA in the transformed calli. Apparently, cytosine residues of the *nos-nptII* gene used as a selectable marker were methylated, and the expression of this gene was suppressed. Treatment of the calli with the cytosine methylation inhibitor 5-azacytidine led to a dramatic increase (from 3% to 96%) in the regeneration of transformed (kanamycin-resistant) shoots. A total of 150 transgenic plants were isolated, and in 17 of these expression of the *lacZ* reporter was detected by *in situ* staining. The T-DNA insert together with flanking plant DNA sequences was cloned into *Escherichia coli* by plasmid rescue from some of the T_3 transformants that harbored one copy of the integrated T-DNA. Comparison of the rescued DNA with the corresponding DNA of the transgenic plant showed that most of the rescued plasmids had undergone rearrangements. These rearrangements could be totally avoided if an *mcrAB* (*m*odified *c*ytosine *r*estriction) mutant of *E. coli* was used as the recipient in plasmid rescue.

Key words: T-DNA mutagenesis – Gene tagging – DNA methylation – Methylation restriction – Plasmid rescue

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Introduction

Insertional tagging of genes either with naturally occurring transposons or by integration of foreign DNA (e.g., T-DNA) into the plant genome has opened up new possibilities for the identification and cloning of plant genes. The use of T-DNA of *Agrobacterium tumefaciens* as an insertion element provides an efficient way to generate both insertion mutants (Feldmann et al. 1989; Yanofsky et al. 1990; Feldmann 1991; Van Lijsebettens et al. 1991) and gene fusions (Andre et al. 1986; Teeri et al. 1986; Koncz et al. 1989; Goldsborough and Bevan 1991; Herman et al. 1990; Koncz et al. 1990; Fobert et al. 1991; Kertbundit et al. 1991; Topping et al. 1991). As we (Teeri et al. 1986) and others (Andre et al. 1986; Koncz et al. 1989) have shown, the integration of T-DNA into the plant genome can indeed be employed to create fusions of reporter genes to plant regulatory sequences and to identify plant promoters. T-DNA-generated gene fusions appear to occur at high frequency independently of the genome size, suggesting that the T-DNA integrates preferentially to actively transcribed regions of the plant genome (Koncz et al. 1989; Kertbundit et al. 1991).

To facilitate the use of random T-DNA-mediated fusion mutagenesis as a general method for tagging plant genes a genetically well-defined model system is an advantage. Such a system is provided by *Arabidopsis thaliana*, which is becoming widely used as the model plant in molecular biology research (Meyerowitz 1989). The generation of a large number of T-DNA-tagged *A. thaliana* plants and the identification and cloning of new promoter or enhancer elements requires optimization of both the transformation procedures as well as the development of efficient means for cloning of the tagged genes. Although *A. thaliana*, like other *Cruciferae*, originally proved to be rather recalcitrant in *Agrobacterium*-

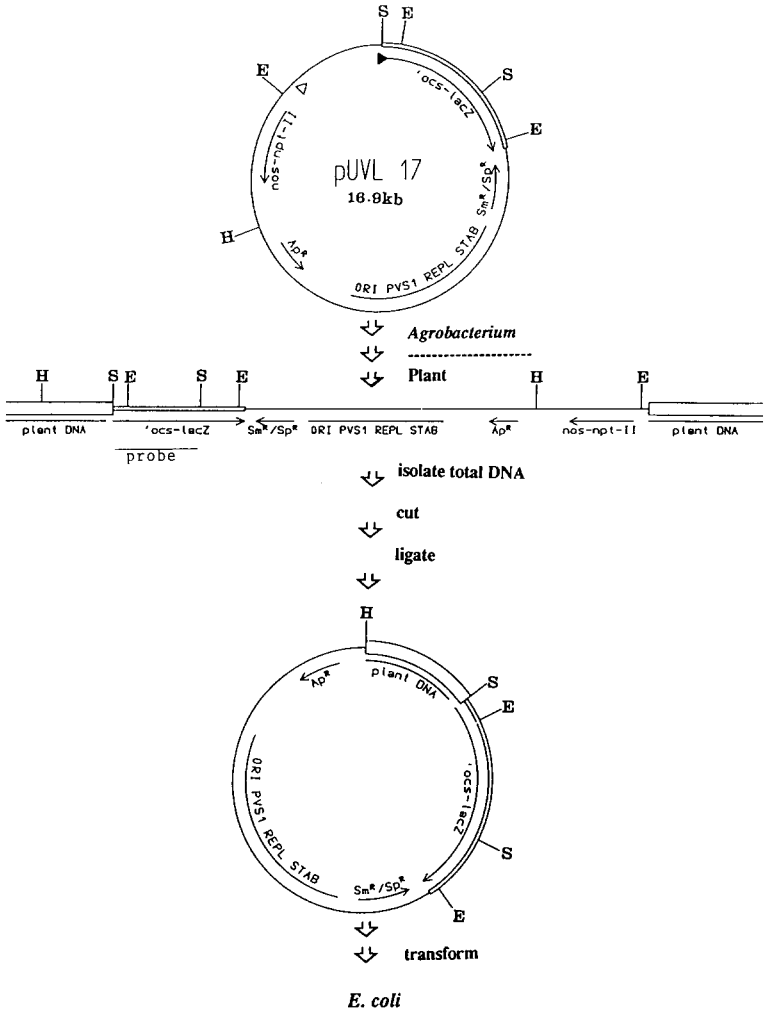

Fig. 1. Schematic representation of the gene tagging vector pUVL17 and cloning of the disrupted plant DNA by plasmid rescue. The vector harbors a plasmid rescue cassette with antibiotic resistance marker (Sm^r/Sp^r, Ap^r) and a broad host range origin of replication (ORI PVS1 STAB), all functional in bacteria; *nos-nptII* for selection (Km^r) of transformed plants and *'ocs-lacZ* for generation of plant gene-reporter gene fusions upon integration. *Open triangle*, T-DNA left border repeat; *closed triangle*, T-DNA right border repeat; *H*, *E* and *S* indicate *Hin*dIII, *Eco*RI and *Sst*I restriction sites, respectively. The indicated location of the *Hin*dIII site in the plant DNA is arbitrary

mediated transformation, several recent studies indicate that a fairly efficient transformation rate can be achieved (Schmidt and Willmitzer 1988; Valvekens et al. 1988; Koncz et al. 1990; Marton and Browse 1991; Clarke et al. 1992).

Several methods can be employed for the cloning of the T-DNA-tagged plant sequences: (1) the screening of genomic libraries derived from the tagged individuals using inserted T-DNA sequences as hybridization probes (Kertbundit et al. 1991); (2) inverse polymerase chain reaction (PCR) (Triglia et al. 1988) using T-DNA-derived sequences as primers (Vankateswarlu and Nazar 1991); and (3) direct plasmid rescue provided that the integrated T-DNA contains a replication origin and antibiotic resistance markers functional in *E. coli* (Koncz and Schell 1986; Koncz et al. 1989; Mayerhofer et al. 1991; Behringer and Medford 1992). Although the plasmid rescue technique should provide a simple way for the cloning of plant DNA sequences disrupted by the T-DNA insertion, problems have been encountered with the stability of the plant sequences in the rescued plasmids. A high frequency of rearrangements was observed in plant sequences that were cloned into *E. coli* (Ott and Chua 1990; Behringer and Medford 1992).

The aim of our work is to develop a large-scale T-DNA fusion tagging system for *Arabidopsis thaliana*. In this paper we report the conditions for obtaining a very high frequency of transformation (about 96%) of *A. thaliana* by *Agrobacterium tumefaciens*. Furthermore, we show that cloning of the integrated T-DNA together with flanking plant DNA sequences can be effectively accomplished by a single-step plasmid rescue procedure and that rearrangements in the rescued plant or T-DNA sequences can be avoided using an *E. coli* host containing mutations in the modified cytosine restriction system (*mcrAB*).

Materials and methods

Construction of the T-DNA tagging vector

The binary vector pUVL17 was constructed using standard recombinant DNA techniques as described by Sambrook et al.

(1989). A schematic map of this construct is outlined in Fig. 1. A 7.8-kb *Hin*dIII-*Kpn*I fragment containing the ampicillin resistance (Ap^r) and streptomycin-/spectinomycin resistance (Sm^r/Sp^r) bacterial selectable markers and the broad host range replication origin of the plasmid pGSC1702 (kindly provided by Plant Genetic Systems NV, Gent, Belgium) was cloned into *Hin*dIII-*Bam*HI deleted (3.3 kb) plasmid pHTT27 (Teeri et al. 1989) harboring the two T-DNA borders, *nos-nptII* for selection of transformed plants and *ocs-lacZ* as a reporter gene. The *ocs* enhancer sequences (Leisner and Gelvin 1988) were deleted from −371 bp to −116 bp, leaving a truncated *'ocs* promoter. In order to detect plant enhancer elements upon insertion the truncated *'ocs-lacZ* reporter cassette was placed next to the right border of the T-DNA. The unique *Hin*dIII restriction site in pUVL17 is used to facilitate the rescue of tagged plant sequences disrupted by integrated T-DNA. This vector (pUVL17) was then introduced into *Agrobacterium tumefaciens* (C58C1 rif^r) containing the non-oncogenic Ti plasmid pGV2260 (Deblaere et al. 1985) by triparental mating (Fraley et al. 1983).

Plant transformation and tissue culture

Roots from axenically grown (24 °C under a 16 h day, 100 μE m^{-2} s^{-1}) 5-week-old seedlings of *Arabidopsis thaliana*, ecotype C24 were isolated for *Agrobacterium*-mediated transformation. The transformation procedure and tissue culture conditions were essentially the same as those described by Valvekens et al. (1988) but with the following modifications: isolated roots were preincubated on solidified callus-inducing medium (CIM) for 4 days, cut into small segments (1–2 mm) and transferred to 20 ml liquid CIM. 3′,5′-Dimethoxy-4′ hydroxyacetophenone was added (0.2 mg/l) to the mixture prior to infection with *Agrobacterium*. The bacteria used for infection were propagated overnight in YEB medium (Vervliet et al. 1975) containing appropriate antibiotics at 28 °C and collected by centrifugation. The bacterial pellet was then resuspended in 10 m*M* $MgSO_4$, added to the root explants and mixed gently for about 15 min. The excess liquid was poured off, and the roots were blotted on sterile filter paper. After 2 days of cocultivation on solid CIM, the roots were rinsed 3–4 times in liquid CIM to wash off the bacteria, blotted on sterile filter paper and transferred to selective shoot induction medium (SIM: 50 mg/l kanamycin and 1000 mg/l vancomycin). The 2-isopentenyladenine (2IPAde) used by Valvekens et al. (1988) in SIM was replaced by 2.0 mg/l benzylaminopurine (BAP). After 7 days of incubation, explants with differentiated morphogenic sectors were transferred to fresh selective SIM supplemented with 3.0 mg/l of either 5-azacytidine or 5-azathymine. The calli were incubated on this medium for only 3 days and then subcultured immediately to fresh selective SIM for shoot regeneration. Vancomycin and kanamycin selections were omitted when the regenerated shoots were isolated for seed production. Growth chamber conditions were maintained at 22 °C under a 16-h day (90 μE m^{-2} s^{-1}) and 90% relative humidity.

NptII assay, Southern analysis and in situ detection of LacZ activity

The neomycin phosphotransferase (NptII) activity in plant tissues was analyzed as decribed by McDonnell et al. (1987). Plant DNA was isolated essentially as in Dellaporta et al. (1983), and hybridization was performed as in Southern (1975). The number of T-DNA copies in transgenic plants was estimated by the segregation pattern of their T_2 progeny on selective medium (50 mg/l kanamycin, Km), and some of these estimations were verified by Southern analysis. The DNA isolated from the pUVL17-tagged plants was digested with *Hin*dIII and *Eco*RI in order to obtain (a) three defined internal plasmid fragments (the 3.0-kb *'ocs-lacZ* fragment, the 8.4-kb fragment containing the plasmid replication origin as well as bacterial selectable marker genes and the 2.8-kb *nos-nptII* fragment) and (b) two variable junction fragments (Fig. 1). The *Sst*I (2.4-kb) fragment containing the right border of the T-DNA and *'ocs-lacZ* was isolated from pUVL17 and used as a hybridization probe. To screen for fusions of the *'ocs-lacZ* reporter gene to plant regulatory elements 146 primary transformants (Km^r and $NptII^+$) were isolated. Leaf explants of about 2 × 2 mm in size were fixed in 2% glutaraldehyde and stained with X-Gal as reported previously (Teeri et al. 1989). β-Galactosidase positive phenotype were distinguished based on the blue color in these tissues.

Plasmid rescue

DNA was isolated from T_3 progeny of some of the self-pollinated transgenic plants (lines 4, 11, 82 and 87) that contained a single copy of the integrated T-DNA. After digestion with *Hin*dIII (10/μg DNA), phenol extraction and ethanol precipitation, the DNA fragments were separated by electrophoresis in 0.8% Sea Plaque agarose (FMC BioProducts, USA). Fragments in the 11–25 kb size range were recovered by melting and freezing (Sambrook et al. 1989) and purified by extracting with phenol, chloroform and ether. After ethanol precipitation the DNA was resuspended in a 10 m*M* Tris-Cl, 1 m*M* EDTA (pH 8.0) buffer. From each sample 5 μg DNA was allowed to self-ligate in a volume of 1.5 ml at 16 °C for 24 h in the presence of 50 U T4-ligase. Self-ligated fragments were then transformed into competent cells of two *E. coli* strains, DH5α and DH5α *mcrAB* (Raleigh and Wilson 1986; Raleigh et al. 1988; Raleigh 1992), by the standard $CaCl_2$ transformation procedure (Sambrook et al. 1989). As a control, both intact and *Hin*dIII-digested plasmid DNA as well as *Hin*dIII-digested and self-ligated DNA from nontransformed control plants were similarly transformed into these *E. coli* cells.

Results

Transformation and regeneration of Arabidopsis thaliana

One of the prerequisites for efficient T-DNA-mediated gene tagging in any plant species is the generation of a large population of transgenic individuals, and for this a high transformation frequency is essential. Transformation of *A. thaliana* C24 root explants with the enhancer probe vector pUVL17 by *A. tumefaciens* resulted in the efficient development of calli, but problems were encountered in regenerating shoots from these calli under selective conditions. In contrast, prolonged subculture of the calli on kanamycin-containing media led to a gradual decay of the callus tissue. The omission of kanamycin selection at this stage resulted in the efficient (55/55) regeneration of shoots (Table 1), but further analysis revealed that only a minority of these (14/55) had been transformed. It has been shown that the phenotypic instability and inactivation of the transgenes present on the integrated T-DNA can be caused by DNA methylation (Gelvin et al. 1983; Hepburn et al. 1983; Amasino et al. 1984; Matzke et al. 1989). To test whether methylation and subsequent inactivation of the *nos-nptII* marker gene was the reason for the problems in shoot regeneration, the calli were treated with the methylation inhibitor 5-azacytidine. The results of these experiments (Table 1)

Table 1. Effect of methylation inhibitors on the regeneration of shoots from transformed calli of *A. thaliana*

	Treatment[a]			
	Azacytidine	Azathymine	Control 1[b]	Control 2[bc]
Number of calli tested	128	76	244	55
Number of calli producing shoots	123	3	8	55
Number of calli producing NptII$^+$ shoots	123	3	8	14
Transformation frequency (%)	96.1	3.9	3.3	25.4

[a] After 1 week of incubation on selective shoot induction medium (SIM) root explants with differentiated morphogenic sectors were transferred to fresh SIM supplemented with 3.0 mg/l of either 5-azacytidine or 5-azathymine. The calli were incubated for 3 days on this medium and then transferred back to SIM supplemented with kanamycin for shoot production
[b] Regeneration of shoots without methylation inhibitor
[c] Regeneration of shoots without kanamycin selection

Table 2. Analysis of transgenic plants

Type of analysis		Number of transgenic lines tested
Segregation pattern[a] (Km^r:Km^s ratio)	3:1	48
	15:1	41
	Others	59
Southern analysis[b] (number of T-DNA copies)	1	9
	2	8
	3 or more	11
Expression of the *lacZ* gene fusion[c]	*lacZ*$^+$	17
	lacZ$^-$	129

[a] The ratio of kanamycin-resistant (Km^r) to kanamycin-sensitive (Km^s) T_2 seedlings was determined 15 days after germination on selective medium (50 mg/l kanamycin)
[b] DNA from T_2 plants was digested with *Hin*dIII and *Eco*RI and characterized by Southern hybridization using the 2.4 kb *Sst*I fragment containing *'ocs-lacZ* from pUVL17 as the hybridization probe
[c] Leaf explants from primary transformants were stained in situ for β-galactosidase activity

demonstrate that most of the 5-azacytidine-treated calli (123/128) regenerated NptII-positive shoots and could produce transformed seeds. In contrast to the results with the C-methylation inhibitor, 5-azacytidine, azathymine pretreatment had no effect on shoot regeneration; this frequency was not significantly different from that of untreated control (Table 1). With 5-azacytidine treatment shoot regeneration started usually 18 days after culture initiation and continued for a further 10–15 days. The number of induced shoots per callus varied from one to seven, but only one shoot/callus was selected for seed production and further analysis.

Analysis of the transgenic plants

About 150 transgenic plants were generated with pUVL17 and subjected to progeny analysis (Table 2). The number of T-DNA copies in transgenic plants was estimated by determining the segregation pattern of the kanamycin marker in the T_2 progeny, and for some of the plants the results were verified by Southern blot hybridization. Of the 148 lines tested, 48, 41 and 59 were shown to have 1, 2, and 3 or more copies of the integrated T-DNA, respectively (Table 2). To verify these results 28 transgenic lines were tested by Southern analysis (Table 2). The data obtained from Southern analysis corresponded to those obtained from the segregation experiments. In all of the lines tested a positive hybridization was observed to the internal 3.0-kb *Eco*RI *'ocs-lacZ* fragment of pUVL17 (Fig. 1). In addition, a variable hybridizing fragment probably representing the plant DNA-T-DNA junction was detectable in the different transformants tested. This observed variation in the size of the junction fragment is in agreement with random integration of T-DNA into the plant genome.

To analyze for the fusion of the truncated *'ocs-lacZ* reporter gene to plant regulatory elements, we stained leaf tissues from 146 primary transformants in situ for detection of β-galactosidase activity. Of all the transformants tested 17 (11.6%) exhibited a LacZ-positive phenotype (Table 2). The intensity of LacZ staining varied greatly among the different transformed lines: in some the stain was barely detectable; others stained deep blue (data not shown).

Plasmid rescue

The vector pUVL17 harbors a plasmid rescue cassette (Fig. 1) to facilitate cloning of the tagged plant sequences. To test the efficacy of the rescue procedure we selected 4 of the transgenic plant lines (nos. 4, 11, 82 and 87) that contained a single copy of the integrated T-DNA. DNA from these plants was isolated, cleaved with *Hin*dIII, ligated and transformed to the *E. coli* strain DH5α. On average about 30 ampicillin-resistant (Ap^r) colonies per 5 μg of plant DNA were obtained. The structure of 5 random plasmid clones rescued from each plant line was characterized by restriction analysis and Southern hybridization (Fig. 2, Table 3). Restriction analysis indicated that while most of the rescued clones had undergone rearrangements, it appeared that the rearrangements were mainly in the rescued plant DNA-T-DNA junction fragments as all of the rescued plasmids contained the two vector fragments of defined size (the 8.4-kb *Hin*dIII-*Eco*RI fragment harboring the replication origin, Sm^r/Sp^r, Ap^r marker genes and the 3.0-kp *Eco*RI fragment

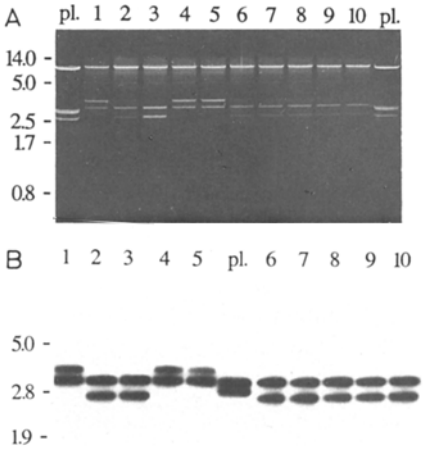

Fig. 2 A, B. Characterization of plasmids rescued into *E. coli* strains DH5α and DH5α *mcrAB* from a transgenic plant (line 87) by restriction analysis and Southern blot hybridization. All plasmids were digested with *Hin*dIII and *Eco*RI. *Lanes 1–5*, Plasmids rescued in DH5α; *lanes 6–10*, plasmids rescued in DH5α *mcrAB*; *pl.*, pUVL17. Size markers are indicated in kb on the *left*. **A** Restriction pattern characterized by agarose gel electrophoresis and visualized by staining with ethidium bromide. **B** Southern hybridization of the gel shown in **A** using the 2.4-kb *Sst*I fragment containing *'ocs-lacZ* from pUVL17 as the hybridization probe

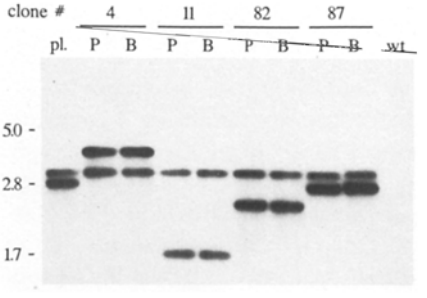

Fig. 3. Comparison of plasmids rescued into DH5α *mcrAB* with the corresponding genomic DNA of respective transformed plant lines by Southern hybridization. The DNA isolated from both rescued plasmids (*B*) and transformed plants (*P*) was digested with *Hin*dIII and *Eco*RI. The 2.4-kb *Sst*I fragment containing *'ocs-lacZ* from pUVL17 was used as the hybridization probe. *pl.*, pUVL17; *4*, *11*, *82* and *87*, independent lines of transgenic plants; *wt*, nontransformed wild-type *A. thaliana* DNA as a control. Size markers in kb are indicated on the *left*

harboring the *'ocs-lacZ* reporter gene) and these corresponded exactly to those of pUVL17. This was verified by Southern analysis (Fig. 2B, Table 3), which showed that the rearrangements had indeed occurred in the junction fragment. Within this fragment alterations were seen not only in the rescued plant sequences (Fig. 2B, lanes 1–5) but sometimes also in the T-DNA (Table 3, transgenic plant line no. 11 in which 3 of the 5 rescued plasmids failed to hybridize to the T-DNA junction probe).

Table 3. Characterization of the rescued plant DNA-T-DNA junction fragments by restriction analysis and Southern blot hybridization

Transgenic plant line	Recipient *E. coli* strain[a]	Size (kb) of junction fragment	Number of *Eco*RI sites[b]	Hybridization[c]
4	DH5α 1	5.0	0	nd
	DH5α 2	5.0	0	nd
	DH5α 3	6.7	1	nd
	DH5α 4	6.7	1	nd
	DH5α 5	6.7	1	nd
	DH5α *mcrAB*	6.7	1	+
11	DH5α 1	4.7	0	+
	DH5α 2	1.9	0	–
	DH5α 3	6.0	0	–
	DH5α 4	8.4	3	–
	DH5α 5	4.7	2	+
	DH5α *mcrAB*	4.7	2	+
82	DH5α 1	2.7	0	nd
	DH5α 2	2.7	0	nd
	DH5α 3	2.7	1	nd
	DH5α 4	2.7	0	nd
	DH5α 5	2.7	0	nd
	DH5α *mcrAB*	2.7	1	+
87	DH5α 1	3.6	0	+
	DH5α 2	3.6	0	+
	DH5α 3	5.1	1	+
	DH5α 4	3.6	1	+
	DH5α 5	3.6	0	+
	DH5α *mcrAB*	3.6	1	+

[a] All five randomly analyzed plasmid clones rescued in DH5α *mcrAB* from an individual transgenic plant line were equal in size and identical in both restriction and hybridization pattern; from DH5α 5 random plasmid clones (1–5) were characterized

[b] Number of *Eco*RI sites in the rescued plant sequences

[c] Hybridization to the junction fragment. All plasmids were digested with *Eco*RI and *Hin*dIII and characterized by Southern analysis using the 2.4-kb *Sst*I fragment containing *'osc-lacZ* from pUVL17 as the hybridization probe. nd, Not determined; +hybridized; –not hybridized

Many *E. coli* strains including DH5α are capable of restricting methylated DNA (Raleigh and Wilson 1986; Heitman and Model 1987; Ross et al. 1989; Waite-Rees et al. 1991). The *mcr* loci are responsible for the detection of methylated cytosines (Noyer-Weidner et al. 1986; Raleigh and Wilson 1986; Heitman and Model 1987; Raleigh et al. 1989; Raleigh 1992), the main type of methylation in plant DNA (Gruenbaum et al. 1981; Woodcock et al. 1989). Thus it was a distinct possibility that the rearrangements observed in the rescued plasmids were caused by the *mcr* system. In order to test this we employed an *E. coli mcrAB* mutant strain for the plasmid rescue experiments; this strain (DH5α *mcrAB*) should not restrict C-methylated foreign DNA. All of the 5 randomly tested plasmid clones rescued in DH5α *mcrAB* from a

particular transgenic plant line were identical in size and in both their restriction (Fig. 2A, lanes 6–10) and hybridization patterns (Fig. 2B, lanes 6–10). Comparison of the DNA rescued in DH5α *mcrAB* with the corresponding DNA of the transgenic plants by Southern analysis showed that the hybridization patterns were identical (Fig. 3), suggesting that no rearrangements had taken place in the rescued plant DNA or T-DNA sequences.

Discussion

This paper is a part of our effort for developing a large-scale T-DNA- mediated gene tagging system in *Arabidopsis thaliana*. We are constructing a T-DNA-tagged library of several thousand plants that should help us to identify and isolate essentially any plant gene of interest. One of the most crucial steps in constructing such a library is to obtain a very high frequency transformation of *A. thaliana* by *A. tumefaciens*. In the initial stages of our work we could not obtain efficient transformation of *A. thaliana* by following published protocols (Schmidt and Wilmitzer 1988; Valvekens et al. 1988; Marton and Browse 1991). Problems were encountered in shoot regeneration (Table 1) when most of the calli failed to regenerate transformed shoots under selective conditions. However, improved regeneration of shoots could be obtained by omitting kanamycin selection at this stage, which suggested that the selectable marker gene *nos-nptII* is not properly expressed. Several reports indicate that the T-DNA sequences upon integration into the plant genome may undergo methylation and become inactivated (Gelvin et al. 1983; Hepburn et al. 1983; Amasino et al. 1984; Matzke et al. 1989). To test whether this was the explanation in our case we treated the calli with the C-methylation inhibitor 5-azacytidine and found that indeed, the block in regeneration was removed. These results indicate that the problems we encountered were caused by inhibition of the expression of the *nos-nptII* gene in the integrated T-DNA rather than T-DNA loss or mutation. The most evident interpretation of the data is that cytosine residues in the *nos-nptII* gene became methylated in the transformed calli and that the resulting invactivation of this *nptII* marker gene could be prevented by 5-azacytidine treatment.

Southern blot analysis suggested a random integration of the T-DNA into the genome of *A. thaliana* since the plant DNA-T-DNA junction fragments appeared to vary in size in the different transformed plant lines tested. In order to determine the number of plant lines with fusions of the *'ocs-lacZ* reporter gene to plant regulatory elements we screened leaf tissues from primary transformants for β-galactosidase activity. Of the 146 plants tested 17 (11.6%) appeared to be LacZ-positive (Table 2). This frequency is well within that (from 5% to 73%) observed for T-DNA-mediated gene fusions in several plant species, e.g. tobacco (Koncz et al. 1989; Fobert et al. 1991; Topping et al. 1991), potato (Goldsborough and Bevan 1991) and *A. thaliana* (Koncz et al. 1989; Kertbundit et al. 1991).

Plasmid rescue can provide an efficient technique for recovery of the tagged plant sequences (Koncz and Schell 1986; Koncz et al. 1989; Mayerhofer et al. 1991; Behringer and Medford 1992). However, cloning by rescue has been hampered by rearrangements in the rescued plant DNA sequences (Ott and Chua 1990; Behringer and Medford 1992). Further, Ott and Chua (1990) reported that the *E. coli* strain DH5α is capable of rearranging not only plant DNA but also sequences within the T-DNA itself (deletion derivatives accounted for 26% of the rescued plasmid clones tested). Our results indicate that the observed rearrangements are caused by *E. coli's* ability to restrict C-methylated DNA (Noyer-Weidner et al. 1986; Raleigh and Wilson 1986; Heitman and Model 1987; Raleigh et al. 1989; Raleigh 1992). This *m*odified *c*ytosine *r*estriction (*mcr*) system is present in several of the commonly used *E. coli* strains including DH5α. By employing an *mcrAB* mutant of *E. coli* DH5α for plasmid rescue we were able to avoid the rearrangements completely. All plasmids rescued from a particular transformed plant line were identical to each other and their structure corresponded exactly to that of the respective tagged genomic DNA in the plant.

In conclusion, we have optimized the conditions for *A. tumefaciens*-mediated transformation of *A. thaliana*, thus allowing large-scale gene tagging in this species. Furthermore, we demonstrate that the T-DNA-tagged plant DNA sequences can be efficiently cloned by plasmid rescue, provided that DNA rearrangements due to modified cytosine restriction are avoided by using a proper *E. coli* recipient strain.

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