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Silicon carbide fiber-mediated stable transformation of plant cells *

H. F. Kaeppler [1], D. A. Somers [1], H. W. Rines [2], and A. F. Cockburn [3]

[1] Department of Agronomy and Plant Genetics and Plant Molecular Genetics Institute, University of Minnesota, 411 Borlaug Hall, 1991 Upper Buford Circle, St. Paul, MN 55108, USA
[2] Plant Science Research Unit, US Department of Agriculture, Agricultural Research Service, St. Paul, MN 55108, USA
[3] Insects Affecting Man and Animals Research Laboratory, US Department of Agriculture, Agricultural Research Service, Gainesville, FL 32604, USA

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Summary. Maize (*Zea mays*, cv 'Black Mexican Sweet') (BMS) and tobacco (*Nicotiana tabacum*, cv 'Xanthi') tissue cultures were transformed using silicon carbide fibers to deliver DNA into suspension culture cells. DNA delivery was mediated by vortexing cells in the presence of silicon carbide fibers and plasmid DNA. Maize cells were treated with a plasmid carrying both the *BAR* gene, whose product confers resistance to the herbicide BASTA, and a gene encoding β-glucuronidase (*GUS*). Tobacco cells were treated with two plasmids to co-transfer genes encoding neomycin phosphotransferase (*NPTII*) and *GUS* from the respective plasmids. Thirty-four BASTA-resistant BMS colonies and 23 kanamycin-resistant tobacco colonies recovered following selection contained intact copies of the *BAR* gene and *NPTII* genes, respectively, as determined by Southern blot analysis. Sixty-five percent of the resistant BMS colonies and 50% of the resistant tobacco colonies also expressed *GUS* activity. Intact copies of the *GUS* gene were observed in Southern blots of all resistant BMS and tobacco colonies that expressed *GUS* activity. These results indicate that a simple, inexpensive DNA delivery procedure employing silicon carbide fibers can be used to reproducibly transform cells of both monocotyledonous and dicotyledonous plant species.

Key words: Genetic transformation – Silicon carbide fibers – *BAR* gene – Maize – Tobacco

Introduction

Plant genetic transformation strategies involve either removal of the cell wall followed by DNA uptake into protoplasts (Fromm et al. 1986; Rhodes et al. 1988; Shimamoto et al. 1989; Potrykus et al. 1987), the employment of *Agrobacterium* species as a natural vector for DNA delivery (Hohn et al. 1989; Klee et al. 1987), or physical penetration of the cell wall via methods such as microprojectile bombardment (Klein et al. 1987, 1989, 1990; McCabe et al. 1988; Gordon-Kamm et al. 1990; Fromm et al. 1990). In some agronomically important cereals, numerous difficulties have been encountered in attempts to adopt these transformation strategies for the establishment of routine transformation systems. Plant regeneration from protoplast cultures has been optimized in a few crop species, but requires substantial tissue culture development. The host range of *Agrobacterium* transformation appears to be limited mostly to dicotyledonous plant species (Hohn et al. 1989; Klee et al. 1987), although there are recent reports of monocot transformation using *Agrobacterium* (Gould et al. 1991; Raineri et al. 1990; Hess et al. 1990). Microprojectile bombardment has been the most successful method for transforming a wide range of plant species (Klein et al. 1988; McCabe et al. 1988; Gordon-Kamm et al. 1990; Fromm et al. 1990; Tomes et al. 1990; Finer and McMullen 1990; McCown et al. 1991), thus resulting in greater interest in the development of transformation procedures that involve DNA delivery into cells with intact cell walls.

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Correspondence to: D. A. Somers

An investigation into alternatives to microprojectile bombardment that also deliver DNA into plant cells by physical penetration may facilitate the development of inexpensive and simple plant transformation strategies. We have previously reported delivery of DNA into intact maize (*Zea mays*) and tobacco (*Nicotiana tabacum*) cells using minute, silicon carbide fibers (0.6 μm average diameter, 10–80 μm length) combined and vortexed with a suspension of tissue culture cells and plasmid DNA (Kaeppler et al. 1990). DNA delivery in this system is presumably due to cell-wall penetration by DNA-coated silicon carbide fibers. Cellular penetration is likely enhanced by frequent and forceful intercellular collisions encountered in the vortexed milieu (Kaeppler et al. 1990). DNA bound to the fiber surface is carried into the penetrated cell and may have the potential to become integrated into the nuclear genome and stably transform plant cells in a manner similar to that proposed for the transformation of animal cells with asbestos (Appel et al. 1988). In this report, we demonstrate that silicon carbide fiber-mediated DNA delivery effects the stable transformation of tissue cultures of both monocotyledonous (maize) and dicotyledonous (tobacco) plant species.

Materials and methods

Cell cultures

'Black Mexican Sweet' (BMS) maize nonregenerable cell suspension cultures were grown in liquid MS2D medium (Green 1977) containing MS salts (Murashige and Skoog 1962) and 150 mg/l asparagine, 0.5 mg/l thiamine-HCl, 20 g/l sucrose, and 0.5 mg/l 2,4-D, adjusted to pH 5.8 prior to autoclaving. Maize suspension cultures were subcultured by a 20-fold dilution into fresh MS2D medium every 7 days. Cells to be used as samples were collected 5–7 days following subculture.

Nonregenerable tobacco suspension culture Txd (*Nicotiana tabacum*, cv 'Xanthi') was kindly provided by the Monsanto Co., St. Louis, Mo. Tobacco suspension cultures were grown in liquid MS2D-T medium containing MS salts, B5 vitamins (Gamborg et al. 1968), 30 g/l sucrose, 5 μg/l kinetin, 200 mg/l inositol, 130 mg/l *L*-asparagine, and 4 mg/l parachlorophenoxyacetic acid, adjusted to pH 5.8. Cultures were subcultured by inoculating 9 ml of cell culture into 40 ml of fresh medium every 3–4 days. Cells to be used as samples were collected 2 days following subculturing.

Plasmids

Plasmids pNGI and pBARGUS were provided by Dr. M. Fromm, USDA Plant Gene Expression Center, Albany, Calif. pNGI (Klein et al. 1989) contains a β-glucuronidase (*GUS*) (Jefferson 1987) gene fused to the promoter and intron 1 sequences of maize alcohol dehydrogenase (*Adh1*), and a Tn5 transposon neomycin phosphotransferase II (*NPTII*) gene fused to the 35S cauliflower mosaic virus (CaMV) promoter (Fig. 1). Plasmid pBARGUS (Fromm et al. 1990) contains the *BAR* gene (Thompson et al. 1987) fused to the CaMV 35S promoter and intron 1 of maize *Adh1* and joined in opposite orientation to the *GUS* gene under the control of the *Adh1* promoter and intron 1 of maize *Adh1* (Klein et al. 1989) (Fig. 1). The *BAR* gene encodes phosphinothricin acetyl transferase, which confers plant cell resistance to phosphinothricin-containing herbicides such as

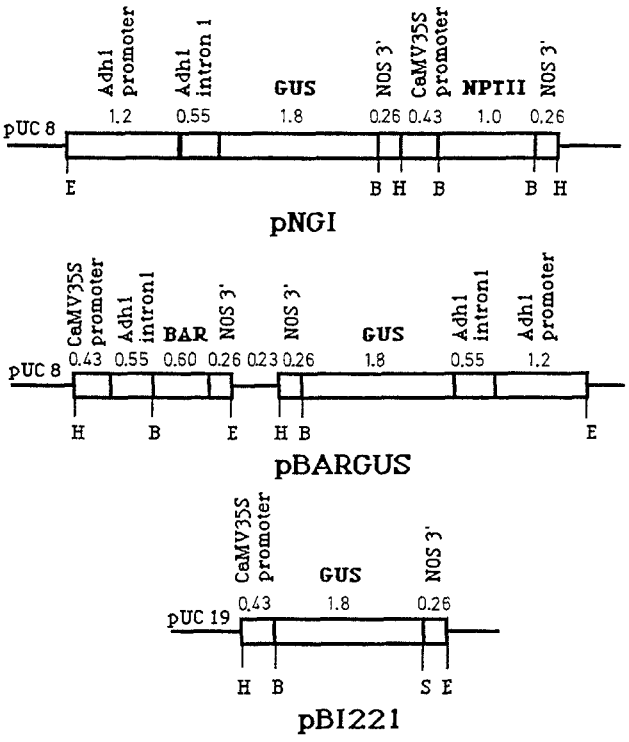

Fig. 1. Schematic representations of plasmids pNGI, pBARGUS, and pBI221 used in transformation experiments with silicon carbide fibers. Section lengths given in kilobases. *E*, *B*, *H*, and *S*=*Eco*RI, *Bam*HI, *Hin*dIII, and *Sst*I restriction sites, respectively

BASTA (Hoescht AG, Frankfurt/Main, Germany) (Thompson et al. 1987). Plasmid pBI221 (Fig. 1) was obtained from Clonetech Laboratories (Palo Alto, Calif.). pBI221 contains the CaMV 35S promoter-GUS-NOS poly-A signal cloned into pUC19 (Jefferson 1987).

Preparation of sterile suspensions of silicon carbide fibers

Silicon carbide fibers were handled with care to avoid inhalation that could result in possible lung damage. Dry fibers were handled only in a vented flow hood, and when possible, fibers were used in a liquid suspension. Sterile suspensions of fibers were made as follows. In a vented hood, approximately 50 mg of fibers were transferred into a preweighed 1.5-ml Eppendorf tube. The tube was capped and reweighed to determine the weight of the fibers. The top of the tube was then punctured with a needle and covered with two layers of aluminum foil. Tubes containing the fibers were autoclaved on a fast exhaust + drying cycle. After autoclaving, the appropriate volume of sterile, deionized H_2O was added to the tube to equal a 5% (w/v) suspension of fibers. The tubes were vortexed to suspend the fibers. Immediately before use, the tube was vortexed again, as fibers settle quickly. Fresh fiber suspensions have been observed to transfer DNA at much higher frequencies than older suspensions, therefore new fiber suspensions were made for each experiment. Material Safety Data Sheets on silicon carbide fibers were obtained from the Advanced Composite Materials Corp, 1525 S Buncombe Rd, Greer, SC 29651.

DNA delivery into cells

DNA was delivered into maize and tobacco suspension culture cells as follows. All preparations and treatments were performed

aseptically. Twenty-five microliters of plasmid DNA (1 μg/μl) was combined with 40 μl of a 5% (w/v) suspension of silicon carbide fibers (Silar SC-9 Whiskers, Advanced Composite Materials Corp., Greer, S. Car.) in a 1.5-ml Eppendorf centrifuge tube. The fibers averaged 0.6 μm in diameter and 10–80 μm in length. The suspension was mixed by vortexing for 5 s. Suspension culture cells were collected using vacuum filtration, rinsed with culture medium, and approximately 300 μl packed volume of cells added to each Eppendorf tube containing the plasmid DNA and suspended silicon carbide fibers. Culture medium (100 μl) was added to the mixture. The Eppendorf tube was capped, vortexed in an inverted position for 5–10 s, then returned upright and vortexed at top speed for 60 s using a desktop vortex unit (Vortex Genie 2, Scientific Industries, McGaw Park, I11).

Maize suspension culture cells were vortexed in the presence of silicon carbide fibers and the plasmid pBARGUS. Tobacco suspension culture cells were treated with silicon carbide fibers and plasmids pNGI and pBI221.

Selection for stable transformants

Samples of maize cells treated with pBARGUS and silicon carbide fibers, and control samples treated with fibers alone, were transferred into 60 × 20 mm disposable petri plates, and 2 ml of MS2D medum was added to each plate. Two days later, cells from a single treatment were divided into three equal portions, and each portion was evenly distributed in a thin layer over a 7-cm diameter filter support paper (Whatman #1) overlying 50 ml solidified selection medium (in 25 × 100-mm petri plates) consisting of MS2D medium lacking asparagine and containing 15 μl BASTA per liter (an equivalent of 3 mg/l of the active ingredient, phosphinothricin) solidified with 0.2% Gelrite (Scott Laboratories, West Warwich, R.I.). Support filter papers and overlying cells were transferred to fresh selection medium at 2-week intervals. Putatively transformed maize colonies growing in the presence of BASTA-containing medium were observed after 4–5 weeks of selection. When resistant colonies had grown to approximately 0.5 cm in diameter, they were placed directly onto the selection medium and subcultured every 2 weeks thereafter onto fresh selection medium.

Tobacco suspension culture cells treated with pNGI + pBI221 + silicon carbide fibers, and control samples treated with only fibers, were transferred to 25 × 100-mm disposable petri plates, and 10 ml medium was added to each plate. One week later, 10 ml of culture medium containing kanamycin was added to each petri plate, giving a final concentration of 150 μg/ml kanamycin. Following 1 week of selection in liquid medium, cells from a single treatment were divided into three equal portions, and each portion was evenly distributed in a thin layer over 7-cm diameter filter support papers (Whatman #1) and placed on Gelrite-solidified medium containing 100 μg/ml kanamycin. The cells were subcultured by transferring support papers to selection medium at 2-week intervals. Putative kanamycin-resistant, transformed tobacco colonies were observed after approximately 6 weeks of selection. Individual colonies were transferred directly onto the kanamycin selection medium and subcultured every 2 weeks thereafter.

DNA isolation and Southern blotting

Total nuclear DNA was isolated from BASTA-resistant maize colonies, kanamycin-resistant tobacco colonies, and nontreated controls according to Saghai-Maroof et al. (1984). DNA (8 μg) was digested with the enzymes listed below, resolved on a 0.8% agarose gel and transferred to Immobilon membranes (Millipore Corp, Bedford, Mass.). Maize DNA digested with *Bam*HI was probed with a ^{32}P-labelled 1.35-kb *BAR*-containing fragment obtained from *Bam*HI-digested pBARGUS. Maize DNA digested with *Bam*HI + *Eco*RI was probed with a ^{32}P-labelled 3.55-kb *GUS*-containing fragment obtained from *Bam*HI + *Eco*RI-digested pBARGUS.

Tobacco DNA digested with *Bam*HI was probed with a ^{32}P-labelled 1.0-kb *NPTII*-containing fragment obtained from *Bam*HI-digested pNGI. Tobacco DNA digested with *Bam*HI + *Eco*RI, which releases two *GUS*-containing fragments of sizes 3.55 kb (from pNGI) and 2.06 kb (from pBI221), was probed with a P^{32}-labelled 1.8-kb *GUS*-containing fragment obtained from *Bam*HI + *Sst*I-digested pB221.

Results

Maize cells treated with silicon carbide fibers and pBARGUS DNA exhibited transient expression of the *GUS* gene at a mean frequency of 40 GUS expression units (one unit = one blue cell or group of adjacent blue cells) per treatment. Untreated cells did not express *GUS* activity. An average of 3.4 BASTA-resistant maize colonies were recovered per vortexed sample of maize cells treated with pBARGUS + fibers following approximately 10 weeks of selection. BASTA-resistant colonies were not detected in the control plates of untreated cells, even after several weeks of selection.

A cotransformation experiment was conducted to transfer the selectable marker gene *NPTII* and the *GUS* gene into tobacco. In transient expression assays, tobacco cells treated with pNGI + fibers did not express *GUS* activity, presumably because the tobacco cells did not efficiently recognize and/or process the maize *Adh1* promoter-intron sequences. However, tobacco cells treated with pBI221 + fibers did express *GUS* activity. Tobacco cells treated with pBI221 + pNGI + fibers transiently expressed *GUS* activity at a mean frequency of 200 GUS expression units per sample. The growth of resistant colonies was not observed in any plate containing control samples. An average of 2.3 kanamycin-resistant tobacco colonies were recovered per sample treated with pNGI + pBI221 + fibers following approximately 10 weeks of selection on kanamycin.

Tests for coexpression of the linked *BAR* and *GUS* genes from pBARGUS in BASTA-resistant maize colonies and of the unlinked genes *NPTII* (from pNGI) and *GUS* (from pBI221) in kanamycin-resistant tobacco colonies were performed on subsamples of all resistant colonies following 12 weeks of selection. Approximately 65% of the BASTA-resistant maize colonies and 50% of the kanamycin-resistant tobacco colonies expressed GUS activity.

Stable integration of the exogenous plasmid DNA into the nuclear genome of the two recipient species was tested by Southern blot hybridization analysis (Southern 1975). DNA was isolated from nontransformed maize and tobacco tissue cultures and from BASTA-resistant maize colonies and kanamycin-resistant tobacco colonies

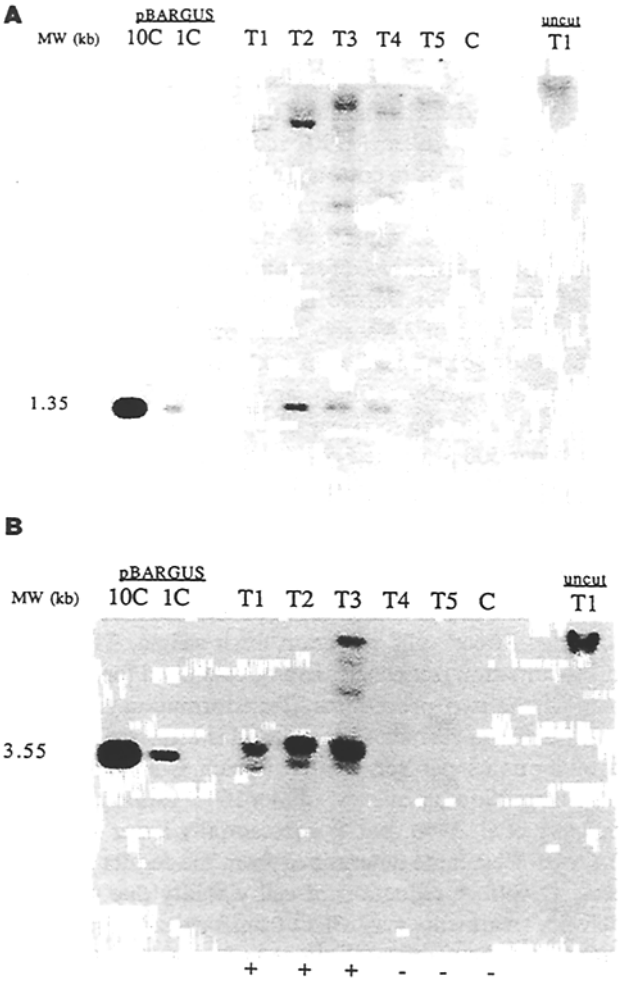

Fig. 2 A, B. Integration of the *BAR* and *GUS* genes transferred from pBARGUS into nuclear DNA isolated from BASTA-resistant maize tissue cultures. **A** Southern blot hybridization analysis of nucelar DNA from BASTA-resistant colonies (*T1–T5*) and a nontreated control (*C*) probed with the *BAR* gene. *Lanes 1* and *2* are ten copies and one copy, respectively, of a 1.35-kb *BAR* gene fragment obtained by *Bam*HI digestion of pBARGUS. *Lanes 3–7* are *Bam*HI-restricted nuclear DNA from tissue cultures *T1–T5*, and *lane 8* is a nontransformed control DNA (*C*). *Lane 9* is uncut genomic DNA from tissue culture *T1*. **B** Southern blot hybridization analyses of nuclear DNA isolated from BASTA-resistant colonies and a nontreated control (*C*) probed with the *GUS* gene. *Lanes 1* and *2* are ten copies and one copy of a 3.55-kb *GUS*-containing fragment obtained by *Bam*HI + *Eco*RI digestion of pBARGUS. *Lanes 3–7* are *Bam*HI + *Eco*RI-digested nuclear DNA from colonies *T1–T5*, and *lane 8* is a nontransformed control (*C*). *Lane 9* is uncut genomic DNA from colony *T1*. Results of assaying colonies *T1–T5* for *GUS* activity (+ = activity detected, − = no activity) are indicated at the *bottom* of gel lanes

after approximately 14 weeks of selection. Figure 2 displays the results from our analysis of transgenic maize tissue cultures. DNA isolated from nontreated control samples of maize did not hybridize with either the *BAR* or *GUS* probes (lanes marked C). Intact copies of the *BAR* gene that comigrated with the 1.35-kb *BAR* fragment from *Bam*HI-digested pBARGUS were present in nuclear genomic DNA of the five BASTA-resistant BMS colonies tested (Fig. 2 A). Restriction fragments of unpredicted sizes also hybridized to the *BAR* gene probe. Restriction fragment polymorphisms were not due to incomplete digestion of the DNA as determined by a subsequent Southern analysis of the same blots but reprobed with a maize single-copy probe (data not shown). Among the transformed BMS colonies shown, intact copies of the *GUS* gene were present only in BASTA-resistant colonies expressing the *GUS* gene, suggesting that the loss or rearrangement of the *GUS* gene during integration accounted for lack of *GUS* expression (Fig. 2 B). Unpredicted restriction fragment sizes of the *GUS* gene were also present in both *GUS*-expressing and nonexpressing colonies. Copies of the *BAR* and *GUS* genes were present only in the high-molecular-weight portion of uncut genomic DNA isolated from BASTA-resistant colonies (Fig. 2 A, B, and data not shown), indicating stable integration of the transgenes into the nuclear DNA. Maize colonies T3 and T4 were isolated from a single treated sample. The different DNA restriction fragment patterns of each colony indicated that T3 and T4 arose from different transformation events. Copy number estimates of the intact and rearranged *BAR* and *GUS* genes integrated into the genome of maize ranged from 1 to approximately 20.

In the tobacco cotransformation experiment, intact and rearranged copies of the *NPTII* gene were detected in nuclear genomic DNA isolated from all kanamycin-resistant tobacco colonies (Fig. 3 A). Intact copies of the *GUS* gene were detected as 2.06-kb and 3.55-kb fragments resulting from *Bam*HI + *Eco*RI digestion of pBI221 and pNGI, respectively, in blots of DNA isolated from kanamycin-resistant colonies (Fig. 3 B), indicating cointegration of *GUS*-encoding DNA from both pNGI and pBI221. Only colonies containing the 2.06-kb *GUS*-hybridizing fragment expressed the *GUS* gene when assayed for *GUS* activity. As in the transformed maize cells, copies of the *NPTII* and *GUS* genes were detected in the high-molecular-weight portion of uncut DNA isolated from kanamycin-resistant colonies, indicating stable integration into the nuclear genome. Colonies T2 and T3 were isolated from a single fiber + DNA treated sample, and colonies T4 and T5 were from another sample; however, the differing restriction fragment patterns of the integrated transforming DNAs from these colonies (Fig. 3) indicated that, as in maize, each of the tobacco transformants arose from unique events. Copy number estimates of intact and rearranged gene copies integrated into the tobacco genome also ranged from 1 to approximately 20.

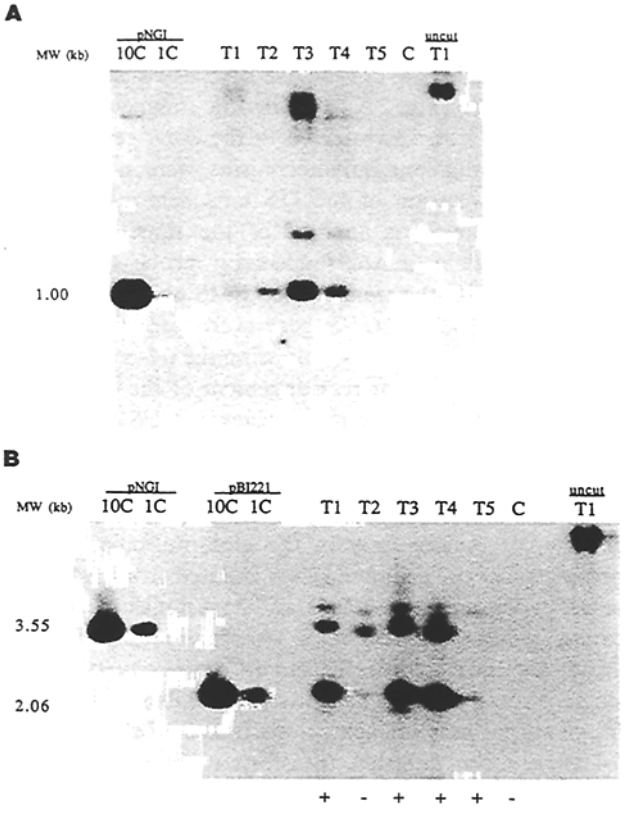

Fig. 3 A, B. Cointegration of the unlinked genes *NPTII* and *GUS* into the nuclear DNA isolated from kanamycin-resistant tobacco tissue culture. **A** Southern blot hybridization analysis of total nuclear DNA isolated from kanamycin-resistant colonies *T1–T5* and a nontransformed control (*C*) probed with the *NPTII* gene. *Lanes 1* and *2* are ten copies and one copy, respectively, of a 1.0 kb-*NPTII*-containing fragment. *Lanes 3–7* are *Bam*HI-restricted nuclear DNA from colonies *T1–T5*, and *lane 8* is a nontransformed control (*C*) probed with the *NPTII* gene. *Lane 9* is uncut nuclear DNA from colony *T1*. **B** Southern blot hybridization analysis of nuclear DNA isolated from kanamycin-resistant colonies *T1–T5* and a nontransformed control (*C*) probed with the *GUS* gene. *Lanes 1* and *2* are ten copies and one copy, respectively, of a 3.55-kb *GUS*-containing fragment obtained by *Bam*HI + *Eco*RI digestion of pNGI. *Lanes 3 and 4* are ten copies and one copy, respectively, of the 2.06-kb *GUS* fragment from pBI221. *Lanes 3–7* are *Bam*HI + *Eco*RI-restricted nuclear DNA from colonies *T1–T5*, and *lane 8* is digested DNA from a nontransformed control (*C*). *Lane 9* is uncut nuclear DNA from colony *T1*. Results of assaying colonies *T1–T5* for *GUS* activity (+ = activity detected, − = no activity) are indicated at the *bottom* of gel lanes

Discussion

Silicon carbide fiber-mediated DNA delivery effected the stable transformation of plant tissue cultures. Our evidence for stable transformation includes expression of both the selectable marker and reporter genes in both maize and tobacco tissue cultures, the detection of DNA fragments encoding intact copies of the *BAR* or *NPTII* genes, and the *GUS* gene, and integration of exogenous DNA into high-molecular-weight uncut nuclear DNA. Ratios of the number of stable transformants to the average number of transient expression events per treatment were 8.5% and 2.3% for maize and tobacco, respectively. Finer and McMullen (1990) reported a stable-to-transient ratio of 0.7% in cotton, Spencer et al. (1990) a ratio of 5.0% in maize, and Klein et al. (1988) a ratio of 1.9% in tobacco. Thus, while the ratios we observed were higher than that reported for cotton, they were similar to ratios reported for transformed maize and tobacco.

An average of 3.4 stably transformed maize cell cultures were selected per 300 µl packed cell volume treated with silicon carbide fibers and DNA. Spencer et al. (1990) reported the recovery of up to 20 stable transformants per 500 µl packed cell volume of 'Black Mexican Sweet' cells treated by microprojectile bombardment, whereas Klein et al. (1989) recovered an average of 56 transformed cultures per treatment of 2×10^5 'Black Mexican Sweet' suspension culture cells. Smith et al. (1984) previously estimated that there are 10^7 'Black Mexican Sweet' cells per gram fresh weight. Therefore, transformation frequencies can be estimated for comparison of the two procedures. Transformation frequency for the silicon carbide fiber method is estimated at 10^{-6} transformants per cell treated, which is similar to the transformation frequency of 4×10^{-6} estimated from Spencer et al. 1990, but is substantially lower than the 2.8×10^{-4} estimate determined from the results of Klein et al. (1989). A reduction of cell viability due to DNA delivery treatments may affect transformation frequency estimates. The viability of maize and tobacco suspension culture cells following the treatment of cells with silicon carbide fibers and vortexing were determined using Evans Blue (5 mg/ml) stain. Compared to the controls, maize cell viability was found to be reduced 29% and tobacco cell viability reduced 13% by treatment with silicon carbide fibers, which did not appreciably increase the estimated transformation frequency. It is likely that transformation frequency and cell viability can be increased with further modifications of the silicon carbide fiber DNA delivery procedure.

High frequencies of the coexpression of linked or unlinked genes may be desirable when the transforming gene of interest cannot be selected for in culture and cotransformation with a selectable marker must be performed. Coexpression frequencies observed in tissue cultures transformed using silicon carbide fibers compared favorably with frequencies reported for other methods of transformation. The frequency of the coexpression of linked genes (*BAR* and *GUS*) that we observed in maize (65%) was similar to the frequency reported in maize cells transformed by microprojectile bombardment (Spencer et al. 1990) and in soybean transformed by electroporation (Christou and Swain 1990). The frequency of the coexpression of unlinked genes (*NPTII* and *GUS*)

observed in the transformed tobacco tissue cultures from our study (50%) was similar to the coexpression frequencies of 18–27% reported in soybean cells transformed by electroporation (Christou and Swain 1990).

The copy numbers of integrated intact and rearranged DNA that we detected are similar to copy number estimates reported for tobacco and maize cells transformed by particle bombardment (Klein et al. 1988, 1989; Spencer et al. 1990). While methods of DNA delivery into plant cells vary considerably, copy numbers integrated into the genome by the different methods appear to be on the same order of magnitude (Klein et al. 1989; Fromm et al. 1986; Tomes et al. 1990). This may indicate that, although DNA may enter the cells by different means, there is a common mechanism of integration of the DNA once it enters a cell. Restriction fragments both larger and smaller than the predicted sizes were observed in our study, and have also been noted in other studies (Fromm et al. 1986; Klein et al. 1988, 1989; Spencer et al. 1990; Finer and McMullen 1990). Restriction fragment length polymorphisms observed in transgene sequences may originate from: (1) concatemerization of the transforming DNA by homologous recombination with concommitant loss and/or rearrangement of restriction sites prior to integration; (2) shearing or partial digestion of the DNA prior to integration; and (3) methylation of restriction sites within the integrated transgene. The formation of concatemers by homologous recombination of plasmids was first documented in transformed mammalian cells (Folger et al. 1982) and later in transformants of rice (Hayashimoto et al. 1990) and cotton (Finer and McMullen 1990). Such concatemers were shown to contain both head-to-head and head-to-tail arrangements of the linear plasmid molecules. These different sized concatemers could then integrate into several different loci in the plant genome (Hayashimoto et al. 1990). Shearing or partial digestion of the transforming DNA has been suggested as another cause of polymorphic fragments (Spencer et al. 1990; Finer and McMullen 1990). This mechanism may explain the appearance of fragments smaller than the size expected. Methylation of transforming DNA after integration into the plant genome has been observed by several researchers (Hobbs et al. 1990; Matzke and Matzke 1991; Linn et al. 1990). However, this would only be detected by methylation-sensitive restriction enzymes, and only if methylation occurs and blocks a restriction site of the diagnostic fragment resulting in a polymorphic fragment larger than expected. Despite the possible occurrence of rearrangements, shearing, and/or methylation of transgenes integrated into the plant genome, many transgenic plants and cell lines expressing high levels of various transgenes have been recovered (Finer and McMullen 1990; Gordon-Kamm et al. 1990; Linn et al. 1990; Fromm et al. 1990; McCown et al. 1991). Knowledge of the factors affecting transgene expression levels, such as gene copy number (Linn et al. 1990), position effects, and those factors discussed above, will likely be required for achieving optimum levels of transgene expression.

We also have investigated silicon carbide fiber-mediated DNA delivery into regenerable maize and oat tissue culture cells (data not shown). DNA delivery was achieved at a frequency similar to that achieved with the nonregenerable maize cultures reported in this study. DNA delivery into cells with plant regeneration capacity will enable the applications of silicon carbide fiber-mediated transformation to crop improvement. Advantages of the silicon carbide fiber-mediated transformation method over other procedures include the ability to transform walled cells (thus avoiding protoplast isolation), relative ease of the procedure, and very low equipment costs.

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