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The effects of promoter on transient expression in conifer cell lines *

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Summary. Protoplasts from suspension cultures of somatic embryos of white spruce (*Picea glauca* Moench Voss) were electroporated with plasmids containing the chimeric genes for chloramphenicol acetyl transferase (CAT) or β-glucuronidase (GUS), under control of one of three promoters. Transient CAT gene expression of approximately equal magnitude resulted when the CAT gene was fused to either the cauliflower mosaic virus (CaMV) 35S promoter or the nopaline synthase (NOS) promoter. When the CAT gene was fused to a tandem repeat CaMV 35S promoter (pPBI-363), CAT enzyme activity compared to NOS or 35S promoters increased up to eightfold (cell line WS-34), and were up to 100-fold greater than control (electroporated without plasmid). Comparatively, protoplasts of black spruce (*Picea mariana* Mill) and jack pine (*Pinus banksiana* Lamb.), electroporated with pPBI-363, produced increases in CAT activity compared to control of 90-fold and 70-fold, respectively. White spruce (WS-34) protoplasts were subsequently electroporated with the GUS gene fused to the tandem repeat CaMV 35S promoter. Comparatively, GUS enzyme activity increased up to tenfold compared to GUS fused to a CaMV 35S promoter. The results indicated that transient expression of the CAT and GUS genes was influenced by the type of promoter and cell line used, as well as by electroporation conditions.

Key words: Conifer – Electroporation – Promoter – CAT – GUS

Introduction

The traditional practices of silviculture and tree improvement are highly significant in modern forestry programs. However, such methods may not have the capacity to address all the needed requirements in a timely manner. In such instances, the alternative approaches offered by in vitro methods offer potential.

One of several such methods with application to genetic transformation and, thereby, to genetic improvement is electroporation (Shigekawa and Dower 1988). This is especially attractive when used in conjunction with protoplasts that are capable of regeneration to somatic embryos (Attree et al. 1987, 1989). Because electroporation avoids the host-range limitations of *Agrobacterium*-mediated transfer methods, it has the further advantage of being useful for the rapid evaluation of the functionality of plasmid constructions (Werr and Lörz 1986), for assessing transient gene expression (Fromm et al. 1985; Bates et al. 1988) and stable transformation (Langridge et al. 1985; Shillito et al. 1985; Fromm et al. 1986).

Recently, transient chloramphenicol acetyl transferase (CAT) gene expression in conifer protoplasts was obtained in white spruce, using electroporation (Bekkaoui et al. 1988) and polyethylene glycol (PEG)-mediated DNA uptake (Wilson et al. 1989). Both reports described the use of 35S cauliflower mosaic virus (CaMV) promoter-driven CAT, and compared the effects on transient expression of linear versus circular plasmid. In each study single, but different, cell lines of white spruce were used. However, a comparison of the variation in transient CAT activity among genotypically distinct cell lines of both black spruce and of jack pine showed that substantial differences in transient gene expression could occur (Tautorus et al. 1989).

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The purposes of this study, therefore, were three-fold: (1) to determine the effects of promoter strength on transient gene expression in white spruce (*Picea glauca* Moench Voss), black spruce (*Picea mariana* Mill), and jack pine (*Pinus banksiana* Lamb.), (2) to determine the variability in transient CAT activity among genotypically distinct white spruce cell lines and, thereby (3) to determine if our previous results with transient GUS expression in white spruce (Bekkaoui et al. 1988) could be attributable to the cell line and promoter that had been used.

Materials and methods

Plant material

Embryogenic callus cultures of white spruce [*Picea glauca* (Moench) Voss] (WS-3, WS-33, WS-34) were initiated from immature zygotic embryos by the methods of Hakman and Fowke (1987). After callus induction on agar-solidified LP medium (von Arnold and Eriksson 1981) with 1% sucrose, 2 mg l^{-1} 2,4-dichlorophenoxyacetic acid (2,4-D), and 1 mg l^{-1} N^6-benzylaminopurine (BA), the cultures were transferred to liquid conditions, where they were maintained as suspensions by weekly subculture in the above medium. The white spruce cultures were maintained for at least 2 years. The cell line from black spruce [*Picea mariana* (Mill.)] (BS-F) and that from jack pine [*Pinus banksiana* (Lamb.)] (JP-2) were produced by Tautorus et al. (1989). BS-F is an embryogenic cell suspension culture similar in appearance to the equivalent white spruce cultures, but derived from mature zygotic embryos; JP-2 is a non-embryogenic cell line also derived from mature zygotic embryos. BS-F and JP-2 were cultured in ½ LM medium (Litvay et al. 1981) modified after Tautorus et al. (1989), with the same phytohormonal composition as used with white spruce. BS-F and JP-2 were maintained for at least 3 months.

Protoplast isolation and culture

Protoplasts from 5- to 7-day-old suspension cultures were isolated following the methods of Bekkaoui et al. (1987). Cells from actively growing suspension cultures were collected on Miracloth (Chicopee Mill/NY) and transferred to petri dishes (100 × 25 mm; Nunc, Inc./IL) containing a cell-wall-digesting solution (10 ml per 3.0 g fresh wt of cell suspension). This consisted of 0.5% (w/v) Cellulase (Onozuka R10, Yakult Honsha, Tokyo), 0.25% each of Driselase (Sigma Chemical, USA) and Pectinase (Sigma), 5 m*M* $CaCl_2 \cdot 2H_2O$, and 0.5 *M* mannitol (pH 5.8).

Cells were incubated in enzyme solution for 4–7 h on a gyratory shaker (50 rpm) at room temperature in the dark. After purification on a sucrose-mannitol gradient, the protoplasts were resuspended in their respective culture media supplemented with 0.37 *M* glucose. Yields of protoplasts for the three species were between 0.2 and 2.4×10^6 per gram fresh weight, as determined by haemocytometer counts. Protoplast viability was measured immediately after purification and at the time of CAT or GUS assay, by scoring 500 protoplasts for the exclusion of phenosafranine (Widholm 1972). Protoplast viability after electroporation was checked using samples suspended in a known volume, as detailed in Bekkaoui and Dunstan (1989). Viabilities immediately after purification were 75%–85% for all species. The data presented are expressed both as a function of the estimated number of surviving protoplasts at the time of assay and as a function of the total activity per cuvette. The results are based on two to six independent experiments, involving separate protoplast isolations and subsequent assays. Data are expressed either as mean values ± standard deviation or mean values with variability.

Electroporated and non-electroporated protoplasts of each species were plated at a density of 5×10^4 protoplasts ml^{-1} (20 ml per petri dish, 100 × 25 mm) in their appropriate liquid media, supplemented with 0.37 *M* glucose and 5 m*M* glutamine. Cultures were placed for 20–22 h in high humidity under diffuse light (7–10 μE m^{-2} s^{-1}) at 25° ± 1 °C, before being collected for enzymatic assays.

Electroporation

A 0.8-ml aliquot, containing 1×10^6 protoplasts in culture medium supplemented with 0.37 *M* glucose, was placed in a Bio-Rad (Bio-Rad, Richmond/CA) 1-ml electroporation cuvette with a 0.4-cm space between the electrodes. Sonicated calf thymus DNA was added as carrier DNA at a concentration of 50 μg ml^{-1}. After addition of plasmid DNA at various concentrations (0–100 μg ml^{-1}), cuvettes were kept on ice for 10 min before being placed in a Bio-Rad Gene Pulser apparatus for electroporation at various voltages (capacitance was standard at 960 μF). This generated an exponential decay pulse of time constant varying between 70 and 170 ms for all species, depending on the voltage (300–700 V cm^{-1}). After electroporation, the protoplast samples were kept on ice for 10 min before dilution and culture.

Plasmids

NOS, CaMV 35S, and tandem CaMV 35S promoter-driven CAT. The pNeoΔ 7 plasmid (Bevan 1984), carrying the NOS promoter and NOS terminator, was used to construct three CAT vectors differing in their promoter. These were NOS-CAT, CaMV 35S-CAT, and tandem CaMV 35S-CAT. This was achieved by removal of the NOS promoter from the pNeoΔ 7 as a HindIII-BamHI fragment, and insertion of the respective fragment carrying either the CaMV 35S promoter (from pCaMVCN, Pharmacia, USA) or the tandem CaMV 35S promoter [from the pCa2 plasmid (Kay et al. 1987)]. The CAT gene cassette was then isolated from the pJS133 plasmid (Shiou and Smith 1988) as a BamHI fragment, and was inserted in the correct orientation into BamHI-digested pNeoΔ 7 and both its derivatives, as described above. The resulting promoter-CAT constructs were designated pPBI-361 (NOS-CAT), pPBI-362 (CaMV 35S-CAT), and pPBI-363 (tandem CaMV 35S-CAT) (Fig. 1). In some experiments with white spruce cell lines the plasmid pCaMVCN (Pharmacia) was used.

Tandem CaMV 35S promoter-driven GUS. The plasmid pBI221, incorporating the CaMV 35S promoter and the GUS gene from *E. coli*, was obtained from Clontech (USA). The CaMV 35S promoter from pBI221 was substituted by the HindIII-BamHI fragment of the tandem CaMV 35S promoter isolated from the pCa2 plasmid (Kay et al. 1987). The derivative plasmid was designated pPBI-364 (Fig. 1).

All plasmids were maintained in *E. coli* strain DH5-α and prepared by CsCl gradient centrifugation. Plasmids were sterilized by ethanol precipitation and dissolved in the appropriate culture medium at 1 mg ml^{-1}.

CAT assay

The method of Gorman et al. (1982) modified by Fromm et al. (1985) was used to determine CAT activity, with [^{14}C]-chloramphenicol (CM) used at 0.2 μCi (60 mCi $mmol^{-1}$, NEN, Du-

pont). Samples were collected 20–22 h after electroporation. As a control, 0.25 units of commercial CAT was used (Pharmacia, USA). Chloramphenicol and its acetylated forms were resolved by TLC and Visualized by autoradiography on X-OMAT Kodak film for 3–4 days, and the radioactive spots were quantified by liquid scintillation counting. The percentage of acetylated CM (=% CM conversion) was calculated as cpm acetylated CM/cpm total CM. Commercial CAT control produced 92.2% ± 5.6% acetylated chloramphenicol (AcCM).

GUS assay

Protoplasts were assayed following the methods of Jefferson et al. (1987). Briefly, this involved collection by centrifugation, and homogenization by grinding in lysis buffer [50 m*M* NaH_2PO_4 (pH 7.0), 10 m*M* β-mercaptoethanol, 10 m*M* EDTA, 0.1% (v/v) Triton X-100, and 0.1% (w/v) Sarkosyl buffer]. Aliquots of 100 µl were assayed in lysis buffer containing 1 m*M* MUG (4-methylumbelliferyl glucuronide) substrate (Sigma) made up to a final volume of 500 µl. The reaction was allowed to proceed for 0.5, 1, 2, and 15 h at 37 °C, and was terminated by adding 4.5 ml of 0.2 *M* Na_2CO_3. Fluorescence emission was measured using a fluorescence spectrophotometer (Turner, Model 111, Palo Alto/CA), with an exitation wavelength of 365 nm and an emission filter of 455 nm. The fluorometer was calibrated by making standard curves using methylumbelliferone (MU) at known concentrations. The GUS activity is reported in pmol MU min^{-1}.

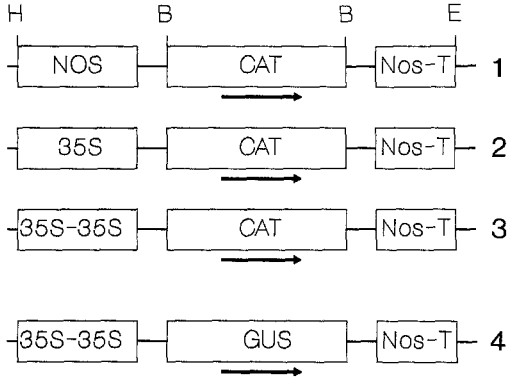

Fig. 1. Plasmid constructs used in transformation experiments using the CAT reporter gene and the GUS reporter gene. The restriction enzyme sites in these constructs are: H = HindIII; B = BamHI; E = EcoRI. The promotors are, 1: NOS (pPBI-361), 2: CaMV 35S (=35S) (pPBI-362), 3 or 4: the tandem repeat CaMV 35S (=35S–35S) (pPBI-363 with CAT, pPBI-364 with GUS). Nos-T is the NOS terminator

Results

Effect of cell line on transient CAT expression

The effect of cell line (genotype) on transient CAT expression in white spruce was seen when protoplasts from each of the three cell lines of white spruce were electroporated at 300 V cm^{-1}, in the presence or the absence of 100 µg ml^{-1} of pCaMVCN (Table 1). Less than 0.05% CM conversion per 10^4 surviving protoplasts was observed when electroporation occurred in the absence of DNA. Protoplasts of WS-34 and WS-33 showed the highest CAT activities with 0.14% and 0.16% AcCM per 10^4 surviving protoplasts, respectively. With pCaMVCN, the difference in CAT activity between these lines and WS-3 was about twofold. However, better survival of WS-34 protoplasts after electroporation was reflected in a greater value for total activity (7.1% AcCM) compared with WS-33 (3.5% AcCM). It was found that detectable levels of AcCM were also obtainable with WS-34 protoplasts after electroporation with only 50 µg ml^{-1} plasmid (data not presented).

Comparison of three promoter-CAT constructs

Two white spruce cell lines, WS-3 and WS-34, were electroporated with the promoter constructs pPBI-361 (NOS), pPBI-362 (35S), and pPBI-363 (tandem repeat 35S). For WS-3 cell line, CAT transient activity was similar for pPBI-361 (NOS) or pPBI-362, whereas for WS-34 a marginal twofold increase was obtained with

Table 1. Effect of white spruce cell line on transient CAT activity, using pCaMVCN

Cell line	Repeat[a]	Plasmid[b] (µg ml^{-1})	Protoplasts[c] ($\times 10^4$)	AcCM[d] per 10^4 protoplasts	AcCM[d] total
WS-3	4	0	30.1 ± 10.1	0.023 ± 0.012	0.7 ± 0.3
	6	100	21.7 ± 9.4	0.08 ± 0.05	1.7 ± 0.6
WS-33	2	0	28	0.018	0.5
	2	100	22	0.16	3.5
WS-34	4	0	66.3 ± 9	0.009 ± 0.005	0.6 ± 0.4
	6	100	52.3 ± 11.6	0.14 ± 0.05	7.1 ± 3.6

[a] No. of repeat experiments. Data are means ± standard deviation; where not so indicated, variability from mean did not exceed 50%
[b] Carrier DNA was added at 50 µg ml^{-1}. Electroporation at 300 V cm^{-1}
[c] No. of surviving protoplasts. Viabilities of non-electroporated controls were: WS-3 = 69.3% ± 14.8%; WS-33 = 48.5% ± 14.9%; WS-34 = 76% ± 10.9%
[d] AcCM is the cpm CM acetylated/cpm total CM × 100. CAT enzyme control gave 92.2% ± 5.6% AcCM. Background in non-electroporated controls was: WS-3 = 0.006%; WS-33 = 0.006%; WS-34 = 0.002%, per 10^4 surviving protoplasts

Table 2. Relative transient CAT activity in protoplasts of white spruce lines WS-3 and WS-34, electroporated at 300 V cm^{-1} in the presence or absence of various promoter-CAT constructs

WS-3					WS-34			
Plasmid[a]	Repeat[b]	Protoplasts[c] ($\times 10^4$)	AcCM[d] per 10^4 protoplasts	AcCM[d] total	Repeat[b]	Protoplasts[c] ($\times 10^4$)	AcCM[d] per 10^4 protoplasts	AcCM[d] total
0	4	30.1 ± 10.1	0.023 ± 0.012	0.65 ± 0.3	4	66.3 ± 9	0.009 ± 0.005	0.6 ± 0.4
pPBI-361	3	27 ± 9.2	0.04 ± 0.03	1.2 ± 0.7	2	62	0.06	3.6
pPBI-362	3	23 ± 9.6	0.06 ± 0.04	1.4 ± 0.7	4	54.8 ± 12.5	0.12 ± 0.06	6.7 ± 4.4
pPBI-363	3	22.3 ± 10.9	0.11 ± 0.03	2.5 ± 0.9	2	49	0.92	45.0

[a] 100 μg ml^{-1}. Carrier DNA was added at 50 μg ml^{-1}
[b] No. of repeat experiments. Data are means ± standard deviation; where not so indicated, variability from mean did not exceed 45%
[c] No. of surviving protoplasts. Viabilities of non-electroporated controls were: WS-3 = 69.3% ± 14.8%; WS-34 = 76% ± 10.9%
[d] AcCM is the cpm CM acetylated/cpm total CM × 100. CAT enzyme control gave 92.2% ± 5.6% AcCM. Background in non-electroporated controls was: WS-3 = 0.006%; WS-34 = 0.002%, per 10^4 surviving protoplasts

Table 3. Transient CAT activity in jack pine and black spruce protoplasts electroporated with pPBI-363

Cell line[a]	Repeat[b]	Plasmid[a] (μg ml^{-1})	AcCM[c] per 10^4 protoplasts	AcCM[c] total
JP-2	4	0	0.013	0.5
	2	100	0.90	35.0
BS-F	2	0	0.021	0.8
	2	50	0.74	29.0
	2	100	1.92	75.0

[a] Carrier DNA was added at 50 μg ml^{-1}. Mean protoplast viabilities – JP-2 electroporated at 300 V cm^{-1}: 39.0% ± 1%; BS-F electroporated at 250 V cm^{-1}: 39.0% ± 1.5%. Viabilities of non-electroporated controls – JP-2 = 64% ± 1.4%; BS-F = 64% ± 3.5%
[b] No. of repeat experiments. Data are mean values, variability from the mean did not exceed 50%
[c] AcCM is the cpm CM acetylated/cpm total CM × 100. CAT enzyme control gave 92.2% ± 5.6% AcCM. Background in non-electroporated controls was: JP-2 = 0.002%; BS-F = 0.003%, per 10^4 surviving protoplasts

the latter construct (Table 2). The level of CAT activity in each cell line differed most noticeably with pPBI-363. With the tandem repeat 35S promoter, there was a twofold increase in CAT activity for WS-3 compared to the NOS or 35S promoter. For WS-34, however, the comparative increase was approximately eightfold over the 35S promoter. The resulting increase in magnitude of CAT activity over controls (electroporated without DNA) was tenfold for WS-3 and 100-fold for WS-34.

Transient gene expression with pPBI-361 and pPBI-362 was also assessed with jack pine and black spruce. With these two species, either promoter gave comparable CAT activity (data not presented), a result in agreement with that of Tautorus et al. (1989) using pNCN (NOS promoter) and pCaMVCN (35S promoter) from Pharmacia. With pPBI-363 in jack pine there was an approximate 70-fold increase of CAT activity over control with no plasmid (Table 3). In black spruce, a 35-fold increase in CAT activity over control was obtained after electroporation with 50 μg ml^{-1} plasmid DNA; at 100 μg ml^{-1} an approximately twofold further increase was observed (Table 3), indicative that the response was no longer linear.

Effects of voltage and plasmid concentration

In an attempt to maximize transient CAT expression, protoplasts of WS-34 were electroporated with pCaMVCN at various voltages. Data (not presented) showed that the highest CAT activity per 10^4 surviving protoplasts was obtained at 500 V cm^{-1} (mean of 7.6% AcCM). However, this voltage was deleterious to protoplast viability (mean viability of 5×10^4). Comparatively, for 400 V cm^{-1}, the mean protoplast viability was 19×10^4 (2.6% AcCM per 10^4 surviving protoplasts). Consequently, this voltage was selected in the subsequent comparison of the effect of pPBI-363 DNA concentration, which was varied between 0 and 100 μg ml^{-1}, to determine the minimum concentration adequate for CAT detection. Increases in enzyme activities (as expressed by CM conversion) were of similar proportions to the increases in DNA concentration up to 50 μg ml^{-1} (Table 4). Data for 100 μg ml^{-1} suggests that CM concentrations may no longer have been saturating, indicative that the optimal concentration for maximal conversion lay between 50 and 100 μg ml^{-1}. CAT activity was detectable as low as 1 μg ml^{-1} (0.06% CM conversion), and reached experimentally convenient concentrations between 5 μg ml^{-1} and 50 μg ml^{-1}.

Transient expression with the GUS reporter gene

It was previously found that a high level of background resulted from electroporation of WS-3 with pBI221 (Bek-

Table 4. Effect of pPBI-363 concentration on transient CAT activity in white spruce WS-34 electroporated at 400 V cm^{-1}

Plasmid[a] (μg ml^{-1})	Repeat[b]	AcCM[c] per 10^4 protoplasts	AcCM[c] total
0	2	0.021	0.1
1	2	0.06	1.5
5	2	0.36	8.6
10	2	0.52	12.5
20	2	1.33	32
50	2	2.75	66
100	2	3.92	94

[a] Carrier DNA was added at 50 μg ml^{-1}. Mean viability of non-electroporated protoplasts was 76% ± 10.9%. Mean viability of protoplasts electroporated in the absence of DNA was 45% ± 3.7%. Mean viability of protoplasts electroporated in the presence of DNA was 24% ± 6.3%

[b] No. of repeat experiments. Data are mean values, variability from the mean did not exceed 35%

[c] AcCM is the cpm CM acetylated/cpm total CM × 100. CAT enzyme control gave 92.2% ± 5.6% CM. Background in non-electroporated controls was 0.002% per 10^4 surviving protoplasts

Table 5. Comparative transient GUS activities in white spruce cell lines WS-3 and WS-34, electroporated at 300 V cm^{-1} with either pBI221 or PBI-364

Cell line/ plasmid[a]	Repeat[b]	Protoplasts[c] (× 10^4)	Activity[d] per 10^4 protoplasts	Activity[d] total
WS-3				
0	2	27	0.01	0.31
pBI221	2	13	0.03	0.31
pPBI-364	2	17	0.07	1.10
WS-34				
0	2	48	0.01	0.52
pBI221	2	9	0.11	1.04
pPBI-364	2	21	1.29	27.01

[a] 100 μg ml^{-1}. Carrier DNA was added at 50 μg ml^{-1}

[b] No. of repeat experiments. Data are mean values, variability from the mean did not exceed 50%

[c] No. of surviving protoplasts. Viabilities of non-electroporated controls were: WS-3 = 69.3% ± 14.8%; WS-34 = 76% ± 10.9%

[d] Activity is expressed as pmol MU min^{-1}

kaoui et al. 1988); this reduced the significance of any transient GUS activity that might have been present. The results with CAT assays suggested a comparative weakness of the CaMV 35S promoter (in pBI221) when used with the poorly expressing WS-3 cell line. To test this hypothesis, protoplasts from WS-3 and WS-34 were electroporated in the presence of either pBI221 or pPBI-364 (tandem repeat CaMV 35S-GUS) at 100 μg ml^{-1}. Compared to the control, WS-3 electroporated with pBI221 showed a slight increase in GUS activity per 10^4 surviving protoplasts, which was offset by a reduction in protoplast viability (Table 5). When electroporated with pPBI-364, there was a further doubling in detectable GUS activity, resulting in a total threefold increase in activity over control. Comparatively, WS-34 electroporated with pBI221 produced a tenfold increase in GUS activity per 10^4 protoplasts compared to the control, although a reduction in protoplast viability resulted only in a twofold increase in total activity. With pPBI-364, the comparable levels of GUS transient expression were 125 times (per 10^4 surviving protoplasts) and 50 times (total activity) control (Table 5).

Discussion

This experimentation has shown the importance of promoter selection in obtaining transient CAT and GUS expression in conifer protoplasts. The CaMV 35S and NOS promoters have previously been shown to be active in conifers (Bekkaoui et al. 1988; Dandekar et al. 1987; Gupta et al. 1988; Sederoff et al. 1986), and were found to promote similar levels of transient CAT expression in jack pine and black spruce (Tautorus et al. 1989). Results with transient CAT activity in protoplasts of the poorly expressing cell line WS-3 are in agreement with the latter observation. However, results obtained with the better expressing WS-34 suggest a marginal increase in expression with the CaMV 35S promoter. For comparison, in results with angiosperm species, the use of the CaMV 35S promoter has usually given higher levels of gene expression than the NOS promoter (Bevan et al. 1985; Fromm et al. 1985; Junker et al. 1987). The tandem repeat CaMV 35S gave noticeably higher levels of gene expression than the CaMV 35S and NOS promoters in white spruce line WS-34, and substantially increased CAT activity compared to control in each of the three species, a result similar to that obtained for expression of the *NPT-II* gene in tobacco (Kay et al. 1987). The increased levels of expression with the tandem CaMV 35S promoter will be significant in the detection of transient activity in weakly expressing cell lines, and in the use of the GUS assay, for which background levels can be problematic (Bekkaoui et al. 1988).

A further consequence of obtaining higher levels of expression is that it is now possible to use less plasmid DNA during transfection. The lowest concentration of the CaMV 35S-CAT (pCaMVCN) that had been previously used in transient expression systems in white spruce was 100 μg ml^{-1} (Bekkaoui et al. 1987; Wilson et al. 1989). With PEG-mediated transformation of this species, a linear increase in transient CAT activity with pCaMVCN was observed up to 150 μg ml^{-1}, with 75% CM conversion (Wilson et al. 1989). Using the tandem CaMV 35S promoter in pPBI-363 there was a linear increase in enzyme activity to approximately 50 μg ml^{-1}

with an optimal value for maximal conversion probably between 50 $\mu g\ ml^{-1}$ and 100 $\mu g\ ml^{-1}$. However, it was also possible to obtain CM conversions between 5 $\mu g\ ml^{-1}$ and 50 $\mu g\ ml^{-1}$ adequate to afford experimentation with AMV RNA-4 non-translated leader sequences (R.S.S. Datla, J. Hammerlindl, G. Pilate, F. Bekkaoui, D.I. Dunstan, W.L. Crosby, unpublished results) and antisense constructs.

Transient CAT activity in white spruce protoplasts was influenced by cell line, an observation in agreement with the findings of Tautorus et al. (1989) for jack pine and black spruce. Because each cell line was originated from genotypically distinct zygotic embryos, such variation in transient gene expression may ultimately be a reflection of genotypic differences. This suggests a need to use more than one cell line (genotype) when using transient assays. The variation between the poorly expressing WS-3 and the better expressing WS-34 cell lines was accentuated using the GUS reporter gene. WS-3 protoplasts electroporated with pBI221 were previously found to produce a high background GUS-like reaction in the absence of the GUS-containing plasmid (Bekkaoui et al. 1988). This background masked any transient GUS activity there may have been. Although a background reaction also occurred with WS-34, when electroporated with pBI221, there was a twofold increase in total activity over background. This is of similar magnitude to the level of total transient GUS activity obtained by PEG treatment of white spruce protoplasts (Wilson et al. 1989). By comparison, electroporation of WS-34 with pPBI-364 gave a total activity of 50-fold over background, indicating the value of selecting suitable cell lines and promoters on transient expression assays.

The experimentation described was designed as a means to assess the functionality of plasmid constructions, with a view to obtaining stable transformation. It should also be possible to use such a system for comparative studies of gene function in gymnosperms and angiosperms, e.g., by comparing the ability of conifer cells to regulate angiosperm promoters using chimeric reporter constructs.

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