# Ethanol Vapor Is an Efficient Inducer of the *alc* Gene Expression System in Model and Crop Plant Species

**Justin P. Sweetman[1], Chengcai Chu, Nan Qu, Andrew J. Greenland*, Uwe Sonnewald, and Ian Jepson**

Crop Genetics Research, Syngenta, Jealott's Hill Research Station, Bracknell, Berkshire RG42 6EY, United Kingdom (J.P.S., A.J.G., I.J.); and Institut fur Pflanzengenetik und Kulturpflanzenforschung, Correnstrasse 3, 06466 Gatersleben, Germany (C.C., N.Q., U.S.)

We have demonstrated that low concentrations of ethanol vapor efficiently induce the *alc* gene expression system in tobacco (*Nicotiana tabacum* cv Samsun NN), potato (*Solanum tuberosum* cv Solara), and oilseed rape (*Brassica napus* cv Westar). For many situations, this may be the preferred method of induction because it avoids direct application of comparatively high concentrations of an ethanol solution. Although induction was seen with less than 0.4 $\mu$M ethanol vapor, maximal induction of the chloramphenicol acetyl transferase gene was achieved after 48 h in leaves of tobacco plants enclosed with 4.5 $\mu$M ethanol vapor. In the absence of ethanol, there is no detectable gene expression. Treatment of potato tubers with ethanol vapor results in uniform $\beta$-glucoronidase (GUS) expression. Vapor treatment of a single oilseed rape leaf resulted in induction of GUS in the treated leaf only and $^{14}$C-ethanol labeling in tobacco confirmed that the inducer was not translocated. In contrast, enclosure of the roots, aerial parts, or whole plant with ethanol vapor resulted in induction of GUS activity in leaves and roots. The data reported here broaden the utility of the *alc* system for research and crop biotechnology.

A number of gene expression systems that rely on chemical induction have been developed for plant use (for review, see Gatz, 1997; Gatz and Lenk, 1998; Jepson et al., 1998; Zuo and Chua, 2000). These systems allow gene activity to be induced in the plant at defined times during development. In this way, they avoid problems that may be associated with constitutive overexpression, and can uncover primary molecular events resulting from activation of a particular gene. For example, dexamethasone-dependent control of the flowering promoter CONSTANS and the homeotic protein heterodimer APETALA3/PISTILLATA has been used to identify genes that are the immediate targets of these transcription factors in Arabidopsis (Sablowski and Meyerowitz, 1998; Samach et al., 2000). An additional feature of chemical induction is that by varying dosage it may be possible to titrate the activity of a target gene (Salter et al., 1998).

Although extremely useful, these systems are not without problems. The tetracycline-inducible system works well in tobacco (*Nicotiana tabacum* cv Samsun NN), but it can have adverse effects in tomato (*Lycopersicon esculentum*), and it does not work at all in Arabidopsis (Gatz, 1997). Treatment of Arabidopsis with dexamethasone, a potent synthetic glucocorticoid, results in growth defects and pleiotrophic effects on gene expression (Kang et al., 1999). In many of these systems, the properties of the inducing chemical precludes widespread agricultural use.

The *alc* gene expression system, which is based on a regulon from *Aspergillus nidulans* (Pateman et al., 1983; Sealy-Lewis and Lockington, 1984; Felenbok et al., 1988), uses a comparatively benign inducer, ethanol, that may have uses in several crop situations. In plants, the *alcR* transcriptional regulator is expressed from the cauliflower mosaic virus (CaMV) 35S promoter such that in the presence of ethanol, ALCR induces expression of any gene fused to a modified *alcA* promoter (Caddick et al., 1998; Salter et al., 1998). The induction of gene expression using the *alc* system has been extensively studied after uptake of ethanol via the roots and after foliar sprays (Salter et al., 1998). Using these application methods, reporter gene expression in tobacco was induced after 2 h and reached a peak at 96 h after root drenching and 24 h after foliar sprays. More recently, ethanol vapor has been reported as an inducer in Arabidopsis (Roslan et al., 2001)

Here, we demonstrate in transgenic tobacco, potato (*Solanum tuberosum* cv Solara), and oilseed rape (*Brassica napus* cv Westar) that the *alc* gene switch is efficiently induced by low concentrations of ethanol vapor. Treatment with vapor avoids dosing plants with comparatively high concentrations of ethanol from foliar sprays or root drenches and in many cases may be the preferred method for gene induction. It may also be an effective way of activating genes in agriculture, particularly in postharvest crops.

## RESULTS

### Ethanol Vapor Induction in Tobacco

Transgenic tobacco containing the *alc* gene expression system treated with ethanol vapor showed a

[1] Present address: Genesis R&D, 1 Fox Street, Parnell, Auckland, New Zealand.

* Corresponding author; e-mail andy.greenland@syngenta.com; fax 44–1344–413638.

www.plantphysiol.org/cgi/doi/10.1104/pp.010937.

200-fold induction in chloramphenicol acetyl transferase (CAT) activity compared with uninduced controls, and 2- and 10-fold higher levels relative to plants treated by root drenching and leaf spraying, respectively (Fig. 1A). Sampling the headspace of the induction vessel and quantifying levels of ethanol demonstrated the sensitivity of the *alc* system to vapor. Equivalent levels of induction of the CAT gene were first detected with source solutions of 0.87 and 1.74 mM ethanol (undetectable and 0.4 $\mu$M ethanol in the headspace, respectively) and reached maximal levels between 1.48 and 197.4 $\mu$M ethanol in the headspace (Fig. 1B). Visual assessment of control and treated plants indicated no adverse effects arising from enclosure and the lack of induction in control leaves (Fig. 1B) showed that production of endogenous ethanol under these conditions was not a cause for concern. These results clearly demonstrate that ethanol vapor can provide efficient induction of the *alc* system in a dose-dependent manner in whole plants.

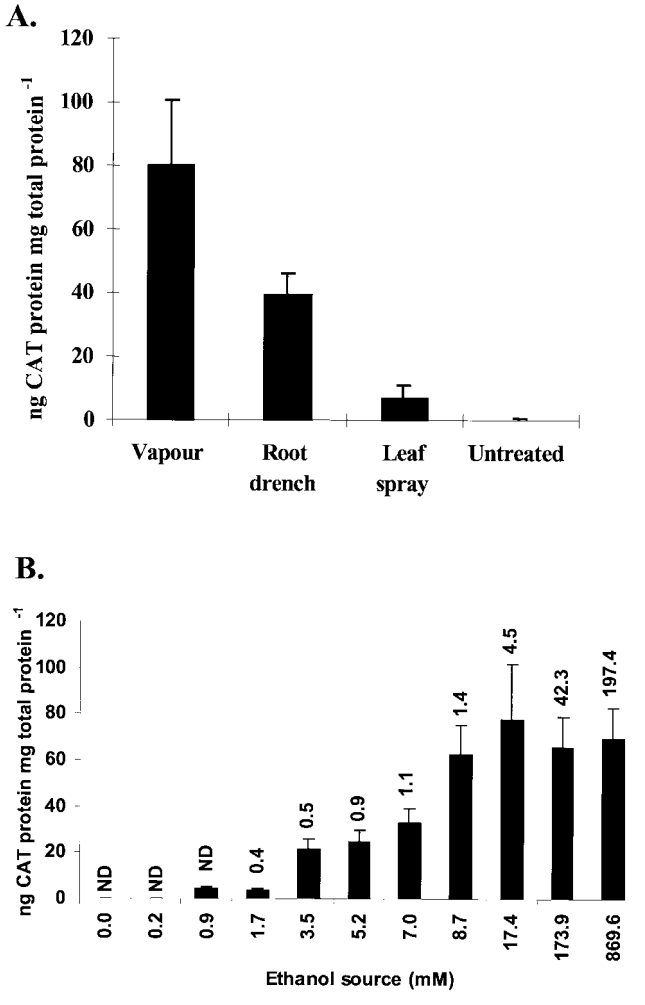


**Figure 1.** Ethanol vapor induction of reporter gene expression. CAT activity was determined by ELISA; the values are means of samples from three 56-d-old plants ± SD. A, Plants were left untreated in a separate growing area or induced with ethanol by vapor (enclosed with 40 mL of 0.7 M ethanol), root drench (40 mL of 0.7 M ethanol applied to the soil), and spraying (0.7 M ethanol until runoff). One leaf was taken from each replicate plant 48 h after treatment. B, Plants were enclosed with 40 mL of water or ethanol at the stated concentrations in a sealed glass vessel for 48 h. One leaf was taken from each replicate plant. Ethanol concentrations ($\mu$M) in the vessel headspace are shown above the corresponding induction columns and are the mean of 10 readings. ND, Not detected.

### Ethanol Vapor Induction in Potato Tubers

Tubers obtained from five *alc*-containing potato lines were treated with ethanol vapor and showed clear induction of $\beta$-glucoronidase (GUS) activity when compared with uninduced and non-transgenic controls (Fig. 2, A and B). A time course of induction in tubers from line 14 was undertaken by assessing GUS mRNA levels and enzyme activity at regular intervals over a 28-d period that included an initial 7-d treatment with ethanol vapor. GUS mRNA was first detected 6 and 24 h after exposure to vapor in the outer layer and core tuber samples, respectively, and was present through the end of the experiment at 28 d (Fig. 2C). GUS enzyme activity was first detected in the outer and core tuber samples at 24 h, reaching a maximum at 28 d after induction and apart from 7 d was always highest in the outer tuber samples (Fig. 2D).

### Spatial Induction in Tobacco and Oilseed Rape Plants

To investigate spatial induction by ethanol vapor in transgenic tobacco, a series of "bagging" experiments were performed and the amount and distribution of GUS activity in leaves, stems, and roots compared with that obtained after root drenching. Vapor treatment of whole plants or the aerial parts of plants resulted in higher levels of GUS activity in leaves than that obtained by root drenching (Fig. 3A). GUS levels were highest in roots when induced with ethanol vapor, although untreated roots showed higher background GUS (approximately 3% of the induced level) than was observed in other organs in this experiment (Fig. 3A). However, comparing the aerial effects of root drenching with root-only exposure to vapor, where expression in the leaves and stem was higher after the former treatment (Fig. 3A), suggests that induction in the aerial organs of the plant results from both transpired ethanol and vapor issuing from the soil after drenching. Induction in stems was very low in each of the treatments, suggesting that either uptake of vapor and transfer of transpired ethanol was comparatively poor or that the *alc* system was only weakly active in this part of the plant.

We examined transfer of ethanol between leaves in transgenic oilseed rape by enclosing a single leaf with a vapor source and staining for GUS in treated and non-treated leaves. Although a treated leaf was uniformly stained, no GUS activity was detected in the

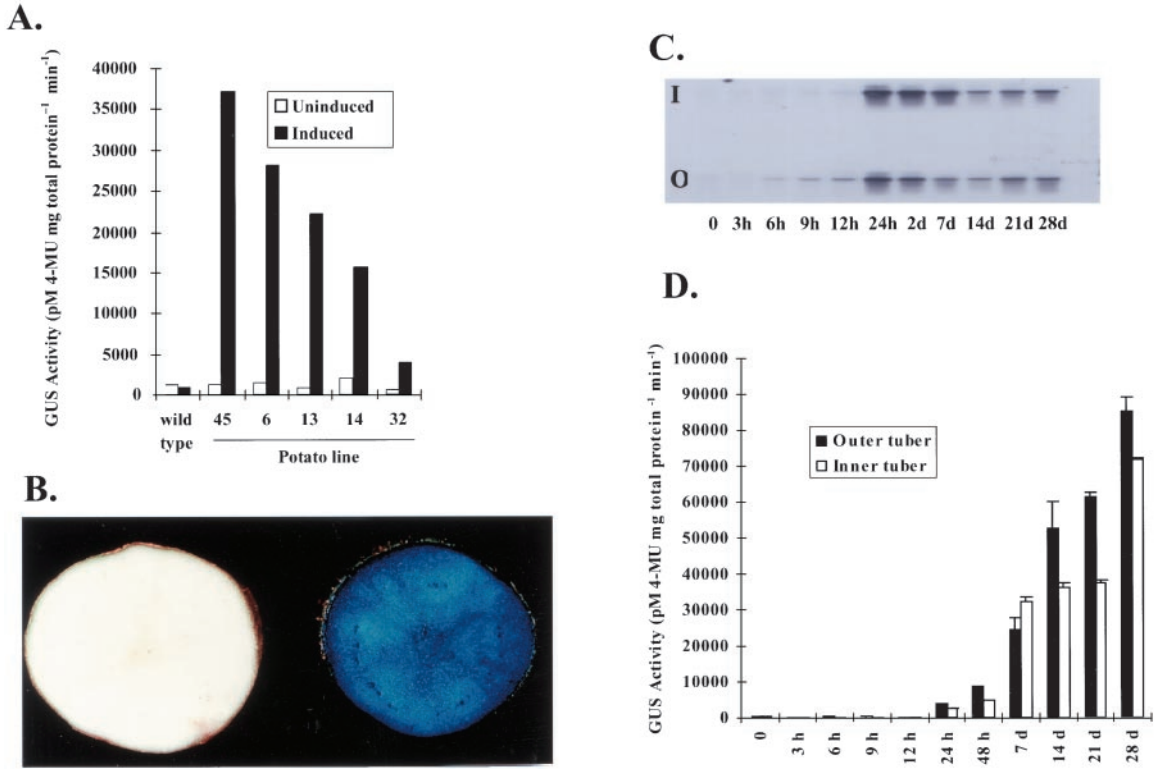


**Figure 2.** Induction in transgenic potato tubers. A, GUS activity in the mature tubers of 5 lines. Uninduced and induced tubers were enclosed with 100 mL of water or 0.7 M ethanol solution, respectively, for 7 d. B, Histochemical GUS staining in mature tubers from line 14. Induced tubers were enclosed with 40 mL of 0.7 M ethanol for 35 d. C, GUS mRNA levels. D, Enzyme activity in two regions of mature tubers from line 14 after ethanol vapor induction. "Outer tuber" (O) indicates the outer part of the tuber 1 to 3 mm below the epidermis and "inner tuber" (I) indicates the remainder of the tuber. Tubers were induced with 40 mL of 0.7 M ethanol for the first 7 d. During induction the ethanol concentration in the airspace was 9 $\mu$M. The fluorometric values are means of three replicate tubers ± SD.

adjacent non-treated leaf (Fig. 3B). By treating a single leaf on a tobacco plant with vapor containing $^{14}$C-ethanol, we confirmed that ethanol is not transported between leaves because the resulting phosphor image clearly shows localization of $^{14}$C to the treated leaf only (Fig. 3, C and D).

## DISCUSSION

These experiments demonstrate that the *alc* system is sensitive to low concentrations of ethanol vapor and, in all comparisons, the level of induction exceeds that seen with root drench and leaf spray applications. In accordance with earlier work (Caddick et al., 1998; Salter et al., 1998; Roslan et al., 2001), *alc* background activity in these studies is generally very low. We noted that root and foliar sprays must also partially result in induction by vapor. Care has to be taken not to unintentionally induce control plants when applying ethanol solutions in the same area (data not shown). Comparison of root application methods (Fig. 3A) suggests that the effect seen in foliage after root drenching is probably due to a combination of ethanol entering the transpiration stream and vapor issuing from the soil. Furthermore, although there were no visible signs of damage after any ethanol treatment, to explain the relative inefficiency of drenching and spray applications we cannot rule out that direct applications of 0.7 M ethanol (4% [v/v]) used in these experiments had a deleterious effect on the treated plants. We conclude that, where feasible, ethanol vapor is the preferred method for induction of the *alc* gene switch. We have used these vapor induction methods to induce gene expression in Arabidopsis (data not shown); others have taken similar approaches (Roslan et al., 2001).

In potato tubers, GUS mRNA and enzyme activity was present up to 21 d after removal of ethanol vapor. In contrast, invertase activity induced in tobacco leaves by root drenching with 0.18 M ethanol had returned to control levels by 9 d after treatment (Caddick et al., 1998). This may reflect relative mRNA and protein stabilities and/or the lower levels of ethanol metabolism in potato tubers compared with other plant organs (Cossins and Beevers, 1962).

By exposing different parts of a transgenic plant to ethanol vapor, it is possible to target expression of the *alc* system to particular organs. For example, vapor induction of a single oilseed rape leaf results in GUS expression in the treated leaf only. Labeling

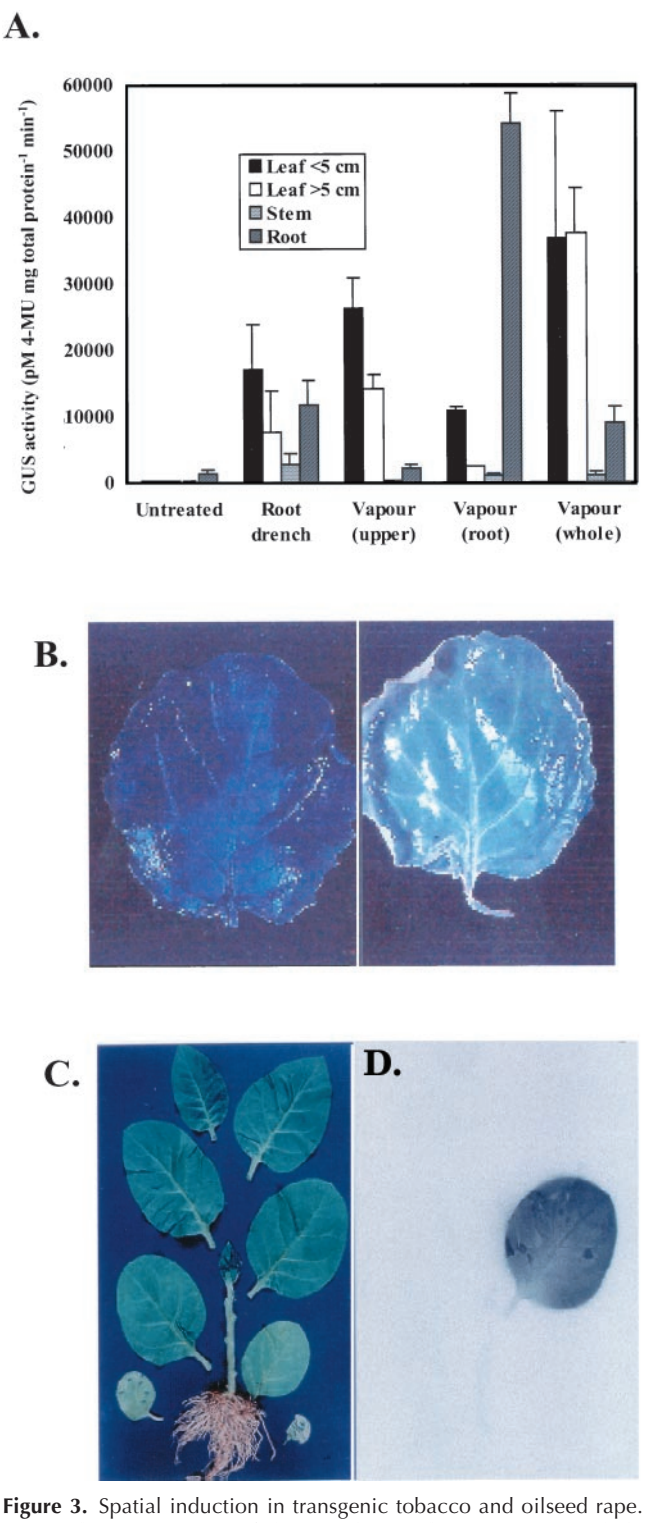


**Figure 3.** Spatial induction in transgenic tobacco and oilseed rape. GUS values are means of three replicate plants ± SD. A, Whole plants, roots, or aerial organs of 56-d-old tobacco were enclosed with 40 mL of 0.7 M ethanol for 48 h. Whole plants were root drenched with 40 mL of 0.7 M ethanol. B, Digitized image of a photograph of GUS staining of vapor-treated and non-treated adjacent leaves from oilseed rape. The induced leaf (left) was enclosed with 10 mL of 0.7 M ethanol for 48 h. The apparent blue tint visible in the non-treated control is an artifact arising from the background used during photography. C, Digitized image of a photograph of a 56-d-old tobacco plant after the third leaf was enclosed with 10 mL of 0.7 M ethanol containing $2 \times 10^4$ Bq mL$^{-1}$ $^{14}$C-ethanol for 48 h. D, Digitized image of the phosphor image of the radiolabeled plant.

experiments show that ethanol is not readily translocated within the plant, an observation supported by earlier studies in cottonwood (*Populus deltoides*; MacDonald and Kimmerer, 1993). Therefore, confined vapor treatment provides a powerful technique for inducing organ-specific gene expression. In earlier studies, Mett et al. (1996) took a different approach to demonstrate a similar effect. They used copper to induce gene expression in *Lotus corniculatus* nodules, where expression of the yeast (*Saccharomyces cerevisiae*) *ace1* gene, a metallo-regulatory transcription factor, was targeted with a nodule specific promoter. The same approach is possible with *alc* through use of a developmental or organ-specific plant promoter rather than the constitutive CaMV 35S promoter used in these studies to control expression of the transcriptional regulator gene, *alcR*. In this way, whole-plant induction by ethanol vapor would only result in *alc*-regulated gene expression in the tissue or organ expressing *alcR*. Experiments of this nature, using the *alc* system, are ongoing in our laboratories.

The *alc* system is a powerful technology for fundamental research and also has the potential for applied uses. We believe that these studies showing that ethanol vapor is a highly efficient inducer will only widen its applicability. Ethanol is inexpensive and biodegradable, and its vapor may be particularly useful in controlling gene expression in postharvest crops such as potato, cut flowers, or fruit. In potato, expression of a pyrophosphatase leads to nonsprouting tubers (Hajirezaei and Sonnewald, 1999), a phenotype that could be used to improve tuber storability. Coupling expression of an inhibitor of pyrophosphatase expression (e.g. its antisense gene) to the *alc* switch and treatment with ethanol vapor could be used to restore sprouting.

## MATERIALS AND METHODS

### Transgenic Plants, Growth, and Maintenance

In tobacco (*Nicotiana tabacum* cv Samsun NN) experiments with the CAT reporter gene, the existing homozygous line, AR10, containing the p35S.*alcR*, p*alcA*:CAT vector was used (Salter et al., 1998). For GUS reporter gene analysis, the *Escherichia coli uidA* gene fused to the CaMV 35S polyA addition sequence was introduced behind the *alcA*/minimal CaMV 35S chimeric promoter (Caddick et al., 1998) to produce a p35S:*alcR*, p*alcA*:GUS construct in a Bin19 plant transformation vector (Bevan, 1984). Transformation of tobacco, potato (*Solanum tuberosum* cv Solara), and oilseed rape (*Brassica napus* cv Westar) with this construct was as described previously (Bevan, 1984; Rocha-Sosa et al., 1989; Moloney et al., 1989, respectively).

Plants were grown in pots containing John Innes number 3 compost in a plant growth room at 60% relative humidity in a 16-h-light (25°C) and 8-h-dark (15°C) cycle (irradiance of 300 $\mu$M m$^{-2}$ s$^{-1}$). Of 20 kanamycin-resistant *alc*-GUS potato lines, five showed induced GUS activity in the leaves after a 0.7 M ethanol root drench; tubers from these lines were collected and used in subsequent experiments. Six *alc*-GUS oilseed rape transformants were similarly tested and from these, line 5 was self-pollinated and shown by segregation of kanamycin resistance (ratio 3:1; $P >$

0.7) to contain a single T-DNA locus. A homozygous line derived from the progeny of line 5 was used in all subsequent experiments.

## Ethanol Induction

In experiments with tobacco and oilseed rape, 56- and 35-d-old plants respectively were used throughout. For vapor induction, plants were placed in sealed 8-L glass containers with a beaker containing 40 mL of 0.7 M ethanol and transferred to a controlled environment room under the conditions described above. Root drenches were performed by application of 40 mL of 0.7 M ethanol solution to the soil in 7.5-cm pots. Normal watering of plants was resumed after application. Leaf sprays were done with a 0.7 M ethanol from a hand sprayer until run off. Samples were taken from the second leaf of all treated plants and their untreated controls at 48 h and assayed for reporter gene activity as described below. Controls were always placed in separate rooms to prevent induction by vapor emitting from root- and foliar-treated plants. For spatial induction, individual leaves, leaves and stem, or roots from *alc*-CAT tobacco and *alc*-GUS oilseed rape plants were enclosed in a 35.5- × 25.5-cm transparent plastic bag with 10 mL of a 0.7 M ethanol such that plants were not in direct contact with the liquid. The bag was sealed around the plant stem or leaf petiole and samples taken at 48 h and assayed for reporter gene activity. Potato tubers were induced in a sealed 40-L plastic container with 8 mL of 0.7 M ethanol for either 7 d or 35 d. Samples were taken and assayed for GUS activity at varying times after the start of the experiment.

## Quantification of Ethanol in the Airspace

Airspace samples were taken through the gas tap of sealed glass containers using a 1-mL Hamilton gas-tight syringe (Phenomenex, Macclesfield, Cheshire, UK) and injected into the manual port of a gas chromatograph (5890 Series II, Hewlett-Packard, Palo Alto, CA) fitted with a 30-m × 0.25-mm × 0.75-$\mu$m Heliflex film column (Alltech, Carnforth, Lancashire, UK) connected to a mass spectrometry detection quadruple. The injection port and oven temperatures were 280°C and 200°C, respectively. Helium carrier gas was used at 40 cm $s^{-1}$ in split injection mode. The chromatograph was controlled by a Hewlett-Packard vectra VL2 4/50 workstation. Ethanol peak area was integrated and quantified using a calibration curve prepared from standard ethanol solutions.

## $^{14}$C-Ethanol Uptake and Distribution

To investigate the distribution of ethanol during vapor induction, the second leaf of a 56-d-old tobacco plant was enclosed with 10 mL of 0.7 M ethanol containing 2 × $10^4$ Bq $^{14}$C-ethanol for 48 h. The whole plant was mounted on card, flash frozen with liquid nitrogen, and placed at −70°C under a 20- × 40-cm FujiBasIIIs phosphor imaging plate for 1 to 3 d. The plate was then imaged on a Fuji Bas-1500 phosphor imager using TINA software (Raytek Scientific, Sheffield, South Yorkshire, UK).

## Histochemical Staining

Oilseed rape leaves and hand-cut sections of potato tubers were stained for GUS activity for 2 to 15 h as described (Blume and Grierson, 1997) except that leaves were cleared by boiling in chloroform:water (1:1 [v/v]) for 5 min after staining.

## Enzyme Assays

CAT activity was assayed using a CAT ELISA kit (Boehringer Mannheim, Lewes, UK) and expressed as ng CAT protein mg total protein$^{-1}$. GUS activity was determined fluorometrically as described (Jefferson, 1987) with the addition of 20% (v/v) methanol to the assay buffer (Kosugi et al., 1990). 4-Methyl umbelliferone (4-MU) production was measured at three time points using a fluorimeter fitted with an LS-3B fluorescence spectrometer (Applied Biosystems, Warrington, Cheshire, UK). Measurements were performed in duplicate and GUS activity expressed as pM 4-MU mg total protein$^{-1}$ min$^{-1}$. A 4-MU standard was used for calibration. Total protein in cell-free extracts was determined by the method of Bradford (1976) using Bradford reagent (Bio-Rad Laboratories, Hemel Hempstead, Hertfordshire, UK) and bovine serum albumin as a standard.

## RNA Extraction and Northern Blotting

Total RNA was extracted from potato tubers as described (Logemann et al., 1987) and 20 $\mu$g per sample separated on a 1.5% (w/v) formaldehyde-agarose gel using conditions described by Sambrook et al. (1989). After electrophoresis, RNA was transferred to a nylon membrane (Hybond $N^+$, Amersham Pharmacia, Little Chalfont, Buckinghamshire, UK) and fixed by UV cross-linking (Stratalinker, Stratagene, La Jolla, CA). The filters were hybridized with a $^{32}$P-labeled GUS DNA probe at 65°C overnight in 0.5 M sodium phosphate, pH 7.2; 7% (w/v) SDS; 1% (w/v) bovine serum albumin; and 20 mM EDTA (Church and Gilbert, 1984). The filters were washed twice for 30 min in 2× SSC and 0.1% (w/v) SDS at 65°C and then exposed to photographic film overnight.

## ACKNOWLEDGMENTS

Mike Salter provided the *alc*-GUS construct. Helen Jones and Kath Howarth were responsible for transformation of tobacco and oilseed rape, and Andrea Knospe was responsible for the transformation of potato. Ian Hayhurst provided technical assistance with gas chromatography, and Sarah Vanderpump and Guy Henderson were responsible for cultivation of plant material. We acknowledge Jackie Paine and Nicola Robertson for useful technical discussions.

Received October 12, 2001; returned for revision January 4, 2002; accepted March 22, 2002.

## LITERATURE CITED

**Bevan M** (1984) Binary *Agrobacterium* vectors for plant transformation. Nucleic Acids Res **12:** 8711–8721

**Blume B, Grierson D** (1997) Expression of ACC oxidase promoter-GUS fusions in tomato and *Nicotiana plumbaginifolia* regulated by developmental and environmental stimuli. Plant J **12:** 731–746

**Bradford M** (1976) A rapid and sensitive method for quantitation of microgram quantities of protein using the principle of protein dye binding. Anal Biochem **72:** 248–254

**Caddick MX, Greenland AJ, Jepson I, Krause K-P, Qu N, Riddell KV, Salter MG, Schuch W, Sonnewald U, Tomsett AB** (1998) An ethanol inducible gene switch for plants: manipulation of carbon metabolism. Nature Biotechnol **16:** 177–180

**Church GM, Gilbert W** (1984) Genomic sequencing. Proc Natl Acad Sci USA **81:** 1991–1995

**Cossins EA, Beevers H** (1962) Ethanol metabolism in plant tissues. Plant Physiol 375–380

**Felenbok B, Sequeval D, Mathieu M, Sibley S, Gwynne DI, Davies RW** (1988) The ethanol regulon of *Aspergillus nidulans*: characterisation and sequence of the positive regulatory gene alcR. Gene **73:** 386–396

**Gatz C** (1997) Chemical control of gene expression. Annu Rev Plant Physiol Plant Mol Biol **48:** 89–108

**Gatz C, Lenk I** (1998) Promoters that respond to chemical inducers. Trends Plant Sci **3:** 352–358

**Hajirezaei M, Sonnewald U** (1999) Inhibition of potato tuber sprouting: Low levels of cytosolic pyrophosphate lead to non-sprouting tubers harvested from transgenic potato plants. Potato Res **42:** 353–372

**Jefferson RA** (1987) Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep **5:** 387–405

**Jepson I, Martinez A, Sweetman JP** (1998) Chemical inducible gene expression systems for plants. Pestic Sci **56:** 360–367

**Kang H-G, Fang Y, Singh B** (1999) A glucocorticoid-inducible transcription system causes severe growth defects in Arabidopsis and induces defense-related genes. Plant J **20:** 127–133

**Kosugi S, Ohashi Y, Nakajima K, Arai Y** (1990) An improved assay for $\beta$-glucuronidase in transformed cells: Methanol almost completely suppresses a putative endogenous $\beta$-glucuronidase activity. Plant Sci **7:** 133–140

**Logemann J, Schell J, Willmitzer L** (1987) Improved method for the preparation of RNA from plant tissues. Anal Biochem **163:** 16–20

**MacDonald RC, Kimmerer TW** (1993) Metabolism of transpired ethanol by Eastern Cottonwood (*Populus deltoides* Bartr.). Plant Physiol **102:** 173–179

**Mett VL, Podivinsky E, Tennant AM, Lochhead LP, Jones WT, Reynolds PHS** (1996) A system for tissue-specific copper-controllable gene expression in transgenic plants: nodule-specific antisense of aspartate aminotransferase-$P_2$. Trans Res **5:** 105–113

**Moloney MM, Walker JM, Sharma KK** (1989) High efficiency transformation of *Brassica napus* using *Agrobacterium* vectors. Plant Cell Rep **8:** 238–242

**Pateman JH, Doy CH, Olson JE, Norris U, Creaser EH, Hynes M** (1983) Regulation of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (AldDH) in *A. nidulans*. Proc Roy Soc Lond B **217:** 243–264

**Rocha-Sosa M, Sonnewald U, Frommer W, Stratmann M, Schell J, Wilmitzer L** (1989) Both developmental and metabolic signals activate the promoter of a class I patatin gene. EMBO J **8:** 23–29

**Roslan HA, Salter MG, Wood CD, White MD, Croft KP, Robson F, Coupland G, Doonan J, Laufs P, Tomsett AB et al.** (2001) Characterisation of the ethanol-inducible alc gene expression system in *Arabidopsis thaliana*. Plant J **28:** 225–235

**Sablowski RWM, Meyerowitz EM** (1998) A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes APETALA3/PISTILLATA. Cell **92:** 93–103

**Salter MG, Paine JA, Riddell KV, Jepson I, Greenland AJ, Caddick MX, Tomsett AB** (1998) Characterisation of the ethanol-inducible alc gene expression system for transgenic plants. Plant J **16:** 127–132

**Samach A, Onouchi H, Gold SE, Ditta GS, Schwarz-Sommer Z, Yanofsky MF, Coupland G** (2000) Distinct roles of CONSTANS target genes in reproductive development of Arabidopsis. Science **288:** 1613–1616

**Sambrook J, Fritsch EF, Maniatis T** (1989) Molecular Cloning: A Laboratory Manual, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

**Sealy-Lewis HM, Lockington RA** (1984) Regulation of two alcohol dehydrogenases in *Aspergillus nidulans*. Curr Genet **8:** 253–259

**Zuo J, Chua N-H** (2000) Chemical-inducible systems for regulated expression of plant genes. Curr Opin Biotechnol **11:** 146–151