

Cotton plants transformed with a bacterial degradation gene are protected from accidental spray drift damage by the herbicide 2,4-dichlorophenoxyacetic acid

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The agronomic performance of broad leaved crop plants such as cotton would be greatly improved if genetically-engineered resistance to broadleaf herbicides could both protect the plants from accidental spray drift damage and allow the suppression of problem broadleaf weeds by chemical means. Following *in vitro* modification and the addition of plant expression signals, the gene for 2,4-*D* monooxygenase, a bacterial enzyme that degrades the broadleaf herbicide 2,4-dichlorophenoxyacetic acid (2,4-*D*), was introduced into cotton plants by *Agrobacterium*-mediated transformation. First generation homozygous progeny of regenerated transgenic cotton plants carrying this gene exhibited up to a 50-100 fold increase in tolerance to 2,4-*D* compared with untransformed controls, and glasshouse trials suggest that the genetically-engineered plants would be completely protected from spray drift of 2,4-*D*, at least up to the normal field application rates commonly used on neighbouring cereal crops.

Keywords: transgenic cotton plants; 2,4-*D* herbicide tolerance; herbicide detoxification

Introduction

There are many situations in modern agriculture where herbicides cannot be efficiently employed because of the lack of suitable chemicals that can differentiate between crop plants and weeds. Within a dicotyledonous (broadleaf) crop such as cotton, for example, broadleaf weeds such as Noogoora burr (*Xanthium occidentale*), which have a similar herbicide sensitivity spectrum to cotton, must be removed by mechanical or hand cultivation. Difficulties have consistently arisen in multi-cropping systems where monocotyledonous crops such as wheat or sorghum, which can tolerate a range of herbicides directed at broadleaf weeds, are grown in rotation with sensitive dicotyledonous crops. Thus in Australia, cotton plants can be severely damaged by the inadvertent drift of the volatile herbicide 2,4-dichlorophenoxyacetic acid (2,4-*D*) initially applied to adjacent cereal crops or by trace contaminants of 2,4-*D* left in the tanks of aerial spray rigs.

One possible solution to these problems in areas where multi-cropping is practised, lies in the development by genetic engineering of crops which are tolerant to herbicides that cause spray drift damage. Numerous genes for herbicide tolerance have been developed over the past five years (reviewed by Schulz *et al.*, 1990) and used to produce transgenic plants tolerant to herbicides such as glyphosate, sulfonylureas, glufosinate (De Block *et al.*, 1987) and bromoxynil (Stalker *et al.*, 1988). The latter two conferred resistance through the genetic engineering of a herbicide detoxification pathway in the plant cells. A major advantage of this detoxification strategy is that it removes the need to first identify the site(s) of action of the herbicide in the plant, since the aim is to intercept the chemical before it reaches its target(s). Also, if the herbicide has multiple sites of action each of the susceptible targets can be protected simultaneously with a single detoxification mechanism. This strategy is particularly appropriate to developing plants tolerant to the synthetic plant auxin 2,4-*D* and we recently validated this strategy in transgenic tobacco as described below.

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Transgenic tobacco plants with increased tolerance to 2,4-D (Lyon *et al.*, 1989; Streber and Willmitzer, 1989) have been produced by employing an appropriately modified gene for a 2,4-D monooxygenase from the bacterium *Alcaligenes eutrophus*. This enzyme catalyzes the first step in the bacterial degradation of 2,4-D and cleaves the acetate side chain of 2,4-D to give glyoxylate and 2,4-dichlorophenol (Don *et al.*, 1985). The latter compound is between 50 and 100 times less toxic to tobacco plants than 2,4-D, as measured by leaf disc regeneration and seed germination assays (Lyon *et al.*, 1989). In this paper we report the successful adoption of the 2,4-D detoxification strategy to a commercially important broadleaf crop with the production of transgenic cotton plants that should be completely protected from any accidental damage by this herbicide.

Materials and methods

Construction of the binary vector

In vitro manipulation of the 2,4-D monooxygenase gene (*tfdA*) from *A. eutrophus* strain JMP134 and the construction of plant expression fusions using the Cauliflower Mosaic Virus (CaMV) 35S promoter, Alfalfa Mosaic virus coat protein gene leader region and the nopaline synthase (*nos*) 3' polyadenylation signal was described in Lyon *et al.* (1989). Although we could assay the expression of the *tfdA* gene phenotypically in regenerated transgenic plants by their resistance to 2,4-D, we previously had difficulty in assaying 2,4-D monooxygenase activity enzymatically in plant tissues using an *in vitro* assay that performed well with extracts of bacteria expressing the enzyme. Since the transformation of cotton is a slow and laborious process taking up to a year, an easily assayable marker gene linked to the 35S-*tfdA* gene was considered valuable for use in screening calli at early stages in the transformation process. A binary transformation vector carrying both a plant-selectable kanamycin resistance gene (*Npt II*) marker and a screenable histochemical marker was therefore constructed from pGA470 (An *et al.*, 1985) by blunt end ligation of the filled *Hin dIII*-*Eco* RI35S-GUS (β -glucuronidase encoded by the *GusA* gene from *Escherichia coli* (Jefferson *et al.*, 1987)) gene of p35SGN100 (Bogusz *et al.*, 1990) into the filled *Eco* RI site of pGA470. The orientation containing the 35S-GUS gene transcribed in the opposite direction to the selectable marker gene was arbitrarily selected. The 35S-*tfdA*-*nos* construct was then cleaved with *Hin dIII* and introduced into the binary vector at its unique *Hin dIII* site such that the *tfdA* gene would be transcribed in the same direction as the *npt II* gene and opposite to that of the GUS gene. A schematic representation of the final binary vector plasmid is presented in Fig. 1. Triparental mating was employed to transfer the binary vector construct to the super-virulent disarmed

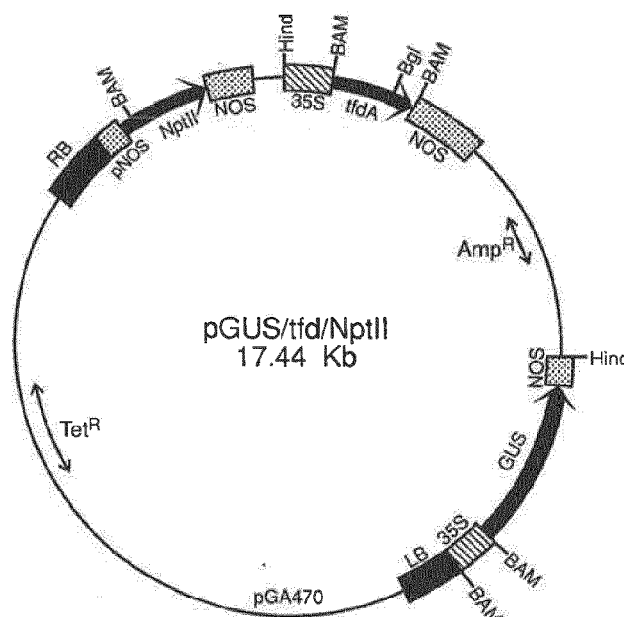


Fig. 1. Schematic representation of the pGUS-*tfdA*-*Npt II* binary vector plasmid used in this study. The GUS and *tfdA* genes were cloned between cauliflower mosaic virus 35S promoters and nopaline synthase (*Nos*) 3' polyadenylation signals and inserted into the binary vector plasmid pGA470 as described in the Materials and methods section. Restriction endonuclease sites are *Bam*, *Bam* HI; *Bgl*, *Bgl* II; *Hin* d, *Hin* dIII. The arrows indicate the directions of gene transcription. *Npt II* designates the neomycin phosphotransferase gene which mediates kanamycin resistance in transformed plant, p*NOS*, the promoter of the nopaline synthase gene; *Amp*^R and *Tet*^R designate the bacterial genes for ampicillin and tetracycline resistance, respectively. LB and RB designate the left and right borders of the T-DNA, respectively.

Agrobacterium tumefaciens strain AGL1 (Lazo *et al.*, 1991) and constructions were verified by restriction and DNA blotting analysis.

Transformation and regeneration of cotton plants

Cotton seedling explants were transformed by *A. tumefaciens* infection and transgenic plantlets regenerated by somatic embryogenesis essentially as described by Firoozabady *et al.* (1987). Cotyledons from 12-day seedlings of *Gossypium hirsutum* cv Coker 315 were transformed by co-cultivation with *Agrobacterium* AGL1 containing the GUS-*tfdA*-*npt II* binary vector construct. Transgenic kanamycin-resistant callus was selected on 35 mg l⁻¹ kanamycin for six weeks, then numbered and tested for GUS activity after 2–3 months. Selected GUS positive callus was cultured on embryogenesis medium until globular embryos developed and germinated (4–6 months). Plantlets were developed from these embryos

over the next 2–3 months using the procedure reported by Cousins *et al.* (1991) and were hardened off in a growth cabinet before being placed in soil pots and transferred to a biosafety containment glasshouse for seed propagation.

Analysis of plant tissue for the presence of introduced genes

Colonies of cotton callus growing on antibiotic selection media and small leaf samples of regenerated plants were assayed for GUS activity using 4-methyl-umbelliferyl glucuronide as the enzyme substrate (Jefferson *et al.*, 1987) and were scored relative to the level of UV fluorescence of control non-transformed tissues. Globular embryos were analysed for GUS activity using X-glucuronide as the enzyme substrate and were scored on the basis of the development of a blue colour. Selected callus and leaf material was assayed for neomycin phosphotransferase activity according to the method of Reiss *et al.* (1984). DNA from floral buds and young leaves was purified by a modification (K. Jacobsen, personal communication) of a previously published technique for the extraction of nucleic acids from cotton (Hughes and Galau, 1988) or using the CTAB method of Taylor and Powell (1982). The DNA samples were employed as templates in polymerase chain reactions (PCR) using primers selected from each end of the *tfdA* coding sequence and a kit supplied by Perkin-Elmer or cleaved with restriction endonucleases and analysed by Southern blotting as described in Mitchell *et al.* (1989).

2,4-D toxicity tests

F₁ seeds generated by self-pollination of wildtype and transgenic plants were germinated in soil in a biosafety containment glasshouse. At four to eight weeks post germination, young plants were sprayed to run-off using a pump-action garden sprayer with a range of 2,4-D (iso-

propyl ester in 50% acetone and 0.1% Tween 80) concentrations (0, 10, 30, 100, 300, 600, 900 and 1200 ppm). Plants were observed for detrimental effects over a period of three weeks.

Results

Transformation, regeneration and propagation of Coker 315 cotton

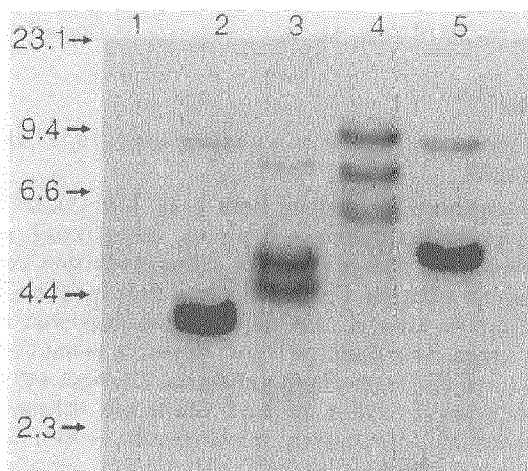
Fully expanded cotyledons from over 100 Coker 315 seedlings were cut into 4–6 pieces, co-cultivated with *Agrobacterium* and cultured for callus production under kanamycin selection. Cotyledon pieces from each seedling were kept separate in case individual seedlings were of a more regenerable genotype. Most cotyledon pieces developed some degree of callusing along the cut edges and foci of more prolific callus, each of which was believed to represent an independent transformation event, were subcultured onto selective media. After further subculture, the darker green, less friable callus material was assayed for GUS activity. From a total of 267 such samples, 83 were found to give strong GUS reactions while most of the remainder were clearly not expressing GUS enzyme and were discarded. This result was unexpected as all the tissues would presumably have to be transformed with the *npt II* gene (and therefore also the GUS gene) in order to proliferate on the kanamycin-containing medium over a number of subcultures.

Callus samples (49) were eventually selected for transfer onto embryogenesis medium and incubated for extended periods with limited subculture. During this time, 24 of them developed a cream-coloured granular appearance and, in 11 cases, subsequently produced globular embryos which were seen to germinate and grow cotyledon- and root-like structures. Embryos were trans-

Table 1. Regenerated cotton plants transformed with the GUS-*tfdA*-*npt II* gene construct

Transformation event	Plantlets in vitro	Plants in bud	Flower mutants	Dead	Infertile	Fertile
1	1	1	–	–	1	–
34	3	2	–	–	2	–
71	1	1	–	–	–	1
72	15	4	–	–	–	4
75	50	6	–	2	3	1
100	24	3	1	1	1	–
108	12	3	–	1	2	–
112	18	4	–	2	2	–
165	50	10	2	1	1	6
177	9	1	–	–	1	–
193	18	7	3	1	3	–
Total	201	42	6	8	16	12

A.



B.

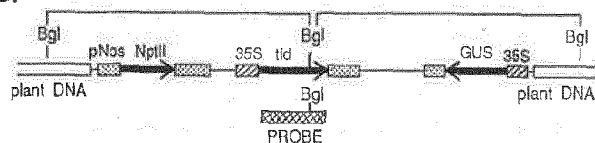


Fig. 2. (A) Genomic Southern blot analysis of 2,4-D tolerant cotton plants. Of the total genomic DNA, 10 µg from selected progeny plants of the four transgenic cotton lines were digested with *Bgl* II, separated on 0.7% agarose, transferred to Hybond N-membrane and probed with the *tfdA* coding region probe. Lane 1, Coker 315 control; lane 2, 71-1 progeny plant No. 5; lane 3, 72-11 progeny plant No. 4; lane 4, 75-24 progeny plant No. 16; lane 5, 165-5 progeny plant No. 29. Molecular markers in kilobase pairs are indicated at the side of lanes. (B) Schematic outline of the expected structure of the T-DNA inserted into the plant genome. The predicted fragments generated by *Bgl* II digestion and the position of the *tfdA* probe used in the Southern blot are indicated. (Not to scale.)

ferred onto germination medium and grown until true leaves and substantial root systems were present, at which time they were hardened-off and transplanted to soil in pots. Of 201 germinating embryos that were treated in this manner, 42 plants (derived from 11 different transformation events) survived long enough in the glasshouse to form floral bud or bud-like structures (Table 1). Twelve of these plants (from four different transformation events: 71, 72, 75 and 165) were found to be self-fertile, being able to set bolls and produce viable seed. Only one

member of each of these groups was analysed further as plants derived from the same embryogenic callus lines were assumed to be derived from a single transformation event. The remaining plants failed to develop seed as they were not self-fertile (16), possessed critical mutations in floral morphology (6), or died before setting bolls (8) (Table 1) and were discarded. GUS and NPT II *in situ* assays on young leaves indicated that all the fertile plants were expressing both the GUS and NPT II gene products at high levels (not shown).

Analysis for transgenic plants for the presence of *tfdA*

Evidence for the presence of *tfdA* in transgenic plants and their progeny was provided by Southern blotting, PCR analysis and (ultimately) phenotypic expression. In most primary transformants, there was, as expected, a strong correlation between the presence of the *tfdA* gene and the detection of GUS and NPT II enzyme activities, suggesting that the transformants had received an entire copy of the T-DNA and that this was stably inherited in the fertile plants. Southern blotting of DNA from progeny of the four fertile transformants (Fig. 2) revealed that the *tfdA* gene was inserted at different genomic positions in all of them, confirming that they were the result of independent transformation events. The T-DNA insert contains a single *Bgl* II site near the 3' end of the *tfdA* coding region. Southern blots of DNA digested with this enzyme should therefore contain two bands (whose size would be determined by the positions of flanking *Bgl* II sites in the plant DNA), one (containing over 90% of the *tfdA* coding region) hybridizing more strongly than the other. Multiple independent insertions should have more than one pair of these bands. Only one of the fertile transformants (71-1) contained a single T-DNA insertion (lane 2) while the other three transformants (72-11 and 75-24 and 165-5) appear to have received multiple (probably two) copies of the T-DNA (lanes 3-5). The faint upper band in Lane 2 is the result of partial digestion of the DNA and was not present in other blots of DNA digested with *Bgl* II or other enzymes that cut once within the T-DNA insert (not shown). These findings on T-DNA insert numbers are supported by seed segregation data for both GUS activity and 2,4-D tolerance (see below). Plant 165-5 is interesting since the Southern blot only shows a single pair of hybridizing bands, yet the GUS and 2,4-D resistance segregate as if there are two or more independent insertions. It is possible that the 5.0 kb band is a doublet and that the other end of the T-DNA is a large fragment that did not transfer efficiently to the membrane. While PCR analysis holds promise as a rapid means for detecting the presence of introduced genes in transgenic plants, we experienced difficulties in obtaining consistent results with cotton DNA using primers internal to the *tfdA* gene, and hence relied on the Southern blotting data to provide confirmation of transformation.



Spraying of young cotton plants with 2,4-D

Progeny seed produced by self-fertilisation of four of the original transformants (71-1, 72-11, 75-24 and 165-5), together with wildtype seed, was germinated in soil and grown for approximately four weeks before small leaf samples from each plant were assayed for GUS activity. As indicated above, segregation of the GUS gene within the selfed progeny suggested that this gene existed as a single site T-DNA insertion 71-1 (21 of the 28 plants tested expressed GUS activity), but as multiple independent insertions in 72-11, 75-24 and 165-5. Provided that the T-DNA was inherited as a stable entity in the transgenic plants, the *tfdA* gene would also segregate in this manner. GUS assays on progeny plants were therefore used to ensure that, where possible, subsequent spraying experiments included GUS negative progeny as internal controls at each of the herbicide concentration steps. This was not possible with 165-5 progeny as all 28 progeny expressed GUS.

Groups of 28 R_1 cotton plants (eight progeny from each of the R_0 transgenic parents 71-1, 72-11 and 165-5 and four wild type Coker 315 plants) were sprayed with one of four concentrations of 2,4-D (0 ppm, 30 ppm, 100 ppm and 300 ppm) and observed for detrimental effects over the next three weeks (eg. Fig. 3). Wild type plants and GUS negative transgenic progeny were severely affected even by the lowest level of herbicide (30 ppm) (Fig. 3B), where GUS positive transgenic progeny from all three original transformants showed only slight damage at the highest 2,4-D concentration (eg. Fig. 3D for 71-1 progeny plants). At 300 ppm, damage to the transgenic plants comprised browning and eventual loss of some new shoot tips and squares (flower buds), together with slight stem twisting, however, all plants were able to recover quickly, with normal shoots and squares evident after 2-3 weeks (Fig. 3D). There appeared to be no substantial differences in the tolerance at 300 ppm within the GUS positive R_1 progeny of any of the three groups of plants or between the three transformant groups, and all GUS positive plants proved to be tolerant to 2,4-D at 300 ppm 2,4-D.

In order to determine the effective increase in tolerance to 2,4-D accorded through possession of the *tfdA* gene, three GUS expressing R_1 transgenic progeny from 71-1 were sprayed with each of 600 ppm, 900 ppm and 1200 ppm solutions of the herbicide and compared with wild type cotton that had been sprayed with 10 ppm 2,4-D. Damage to the transgenic plants at 900 ppm was approximately equivalent to that observed for the wild type plants sprayed with 10 ppm, indicating that the transgenic plants were at least 90-fold more resistant to

the herbicide than wild type cotton (data not shown).

R_2 seed was collected from individual 2,4-D resistant R_1 plants. Twelve seeds from each were germinated and tested for GUS expression in young leaves using the histochemical stain. Progeny of 71-1-7 were all GUS positive while for plant 71-1-8 only 8 out of 12 were GUS positive. These results are consistent with 71-1-7 being homozygous for the single T-DNA insert and 71-1-8 being hemizygous, designations confirmed by Southern blot analysis (not shown). Progeny from 72-11, 75-24 and 165-5 R_1 plants segregated GUS in various ways, consistent with the multiple inserts present in the original R_0 plants. The R_2 progeny were sprayed at four weeks with 600 ppm 2,4-D, a dose that seemed to discriminate between different plants when used against the R_1 progeny. Plants were assessed after one, two and three weeks.

At one week there were distinct differences between the different groups of plants. The 71-1 R_2 progeny were the most tolerant to 600 ppm 2,4-D with the homozygous 71-1-7 progeny suffering very little damage, other than some mild burning of the young leaves present at the time of spraying. New leaves generated subsequent to spraying were undamaged. Segregating progeny from 71-1-8 showed three classes of damage. The GUS negative plants (4 out of 12) were badly burnt and twisted and all of the shoots, including axillary buds were dead. Among the GUS positive plants (8 out of 12) there was either very little damage (3 out of 12) similar to the homozygous 71-1-7 plants or moderate damage where the older leaves were burnt and the new leaves were slightly distorted but otherwise green and healthy. The other progeny plants of the independent transformants showed varying degrees of damage, but in all cases the segregating GUS negative plants were killed by 600 ppm 2,4-D. In general the 75-24 GUS positive R_2 progeny segregated three phenotypes, from very little damage in a few plants, to moderate damage in some and severe damage in others. The severely damaged plants still contained viable axillary buds and began to recover after two weeks. R_2 progeny of 72-11 and 165-5 plants had similar phenotypes except that there were more of the severely damaged plants at one week. By three weeks most of the GUS positive progeny from all four groups had recovered and were flowering and setting seed.

Discussion

The herbicide 2,4-D is active against most dicotyledonous weed species and, although its use has been actively suppressed in some European countries, in Australia and the USA, it is still widely used as a weed control agent on

Fig. 3. Spraying of young cotton plants with 2,4-D. Wild type (Coker 315) on the right of each panel and transgenic (71-1) R_1 progeny plants on the left of each panel were sprayed with (A) 0 ppm 2,4-D, (B) 30 ppm 2,4-D, (C) 100 ppm 2,4-D or (D) 300 ppm 2,4-D. (Photographs were taken three weeks after spraying.)

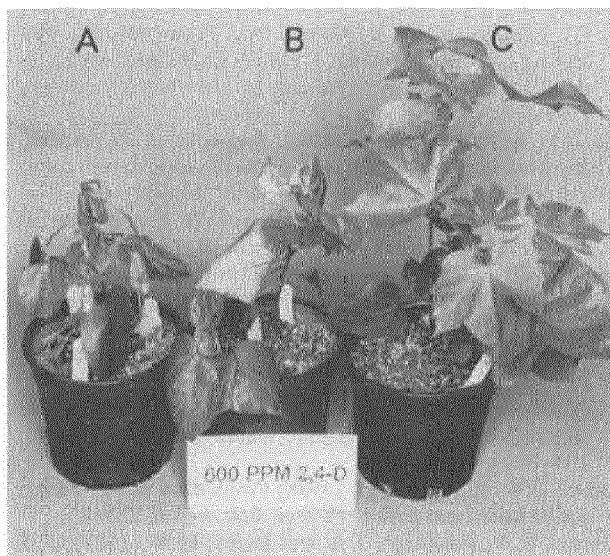


Fig. 4 Spraying of R_2 -segregants of plant 71-1-7 with a discriminating dose of 2,4-*D* (600 ppm). (A) *tfdA* null segregants, (B) segregants hemizygous for the *tfdA* gene and (C) segregants homozygous for the *tfdA* gene. (Plants were photographed two weeks after spraying).

monocot crops such as wheat and sorghum. Weed control is a serious issue in many agricultural situations where productivity can be severely limited if weeds are not suppressed by cultivation or treatment with chemical weedicides. In broad-acre crops cultivation can have deleterious effects on soil structure and result in soil loss or degradation, chemical weedicides provide an acceptable alternative provided that they have no long-term environmental or health impacts (Combella, 1989). 2,4-*D* might be considered an old-fashioned herbicide and has been scorned because of its use with the structurally related, but biologically different, herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) in Agent Orange in mass forest defoliation in Vietnam, but it does have many of the properties that could be considered desirable in an herbicide. Unlike 2,4,5-T, 2,4-*D* is biodegradable, surviving only five or six weeks in the soil (Noble and Hargreaves, 1992), due to the action of numerous species of bacteria, yeasts and fungi (e.g. Don and Pemberton, 1981). It is relatively safe for humans (Mullison, 1988) and does not contain the contaminating dioxins that caused the banning of 2,4,5-T when a link with specific human cancers was discovered. No such link has been clearly demonstrated for 2,4-*D* (Mullison, 1988 and references therein). 2,4-*D* has been carefully assessed by Australian authorities and registered for use as an herbicide. It will continue to remain a component of Australian cropping systems and problems with spray drift damage to dicot crops, such as cotton, will remain a problem in multi-

cropping systems, unless tolerant varieties can be developed.

We are interested in engineering 2,4-*D* resistant cotton that will be tolerant to drift damage caused by 2,4-*D* to make multi-cropping strategies more effective and allow greater flexibility in herbicide usage. In earlier work we demonstrated the potential of the gene (*tfdA*) for the first enzyme in the 2,4-*D* degradative pathway of the bacterium *A. eutrophus* (Streber *et al.*, 1987) to detoxify 2,4-*D* when expressed in transgenic tobacco. This result suggested that the introduction of the *tfdA* gene into a commercial species such as cotton should also allow 2,4-*D* detoxification and protect the plants from accidental spray drift damage.

A relatively efficient transformation frequency for cotton cotyledons was achieved by employing the super virulent *A. tumefaciens* strain AGL1 and screening putative transformed callus tissue for GUS activity at an early stage. GUS positive reactions were recorded from a high percentage of the kanamycin resistant callus recovered after *Agrobacterium* infection, giving a transformation frequency (transformants/seedling) of 60%. We have assumed that T-DNA transfer proceeds from the right border to the left border and that the presence of the GUS gene indicates the successful transfer of the entire T-DNA. We have not yet determined why many of the remaining callus cultures failed to produce a GUS positive reaction, despite being capable of surviving on kanamycin-containing media, but presume it was because of incomplete transfer of the T-DNA (e.g. van Lijsebettens *et al.*, 1986), rather than 'escapes' from selection on kanamycin. About 50% of the callus lines produced embryogenic callus when subcultured onto embryo development media and eleven of these (46%) produced germinating embryos that eventually developed into whole plants. Thus a quite workable regeneration rate of approximately 22% was achieved for the transformed callus lines using our screening strategy for a co-transferred screenable marker gene (GUS). Once in the glasshouse, however, only plants from four of the callus lines were able to produce fully fertile flowers, set fruit and produce acceptable yields of viable seed. This indicates that cells from the majority of callus lines may have undergone deleterious mutations, or other forms of somaclonal variation whilst in culture, that prevented the regeneration of normal fertile plants. Such a result is not unexpected given the length of time (over twelve months in some instances) that the cells spent in culture. We have observed similar abnormalities in plants regenerated using auxins other than 2,4-*D* in the culture medium, so cannot directly attribute these effects to toxicity by this specific compound. More rapid turnaround in tissue culture through more intensive subculture regimes and/or induction of embryogenesis in liquid culture has subsequently reduced the frequency of abnormal plants by significantly reducing the time needed for plant regeneration. Although some of our transgenic

plants may have had partial fertility (e.g. Cousins *et al.*, 1991), we did not attempt to recover seed from any male sterile transgenic plants by cross-pollination with pollen from fertile non-transgenic plants.

The level of herbicide resistance obtained in our transgenic cotton is promising, with engineered plants able to survive spraying with 2,4-D at concentrations more than 90-fold higher than that tolerated by wild type cotton. Following calibration trials with the commercial 2,4-D herbicide, Nufarm Estericide 800, on wild type tobacco in the field and transgenic tobacco (Lyon *et al.*, 1989) in the glasshouse, we estimate that the minimum effective field application rate of 280 grams 2,4-D per hectare would be approximately equivalent to the 300 ppm rate used in this and our previous work in tobacco (data not shown). This would mean that the transgenic cotton would suffer no damage if it were accidentally exposed in a spray drift situation to the minimum weed control rate of 2,4-D. 2,4-D can, however be used at much higher rates for some problem weeds and unintentional exposure to these higher doses of the herbicide could cause significant damage and loss in crop productivity. The 2,4-D tolerance gene is currently being transferred into all of our existing commercial cotton varieties from our best transformant (71-1) by traditional breeding techniques with recurrent back-crosses and this material should be ready for commercial release as 2,4-D drift tolerant cultivars within the next five years.

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