FIELD TESTING OF POTATO LINES GENETICALLY MODIFIED FOR CHLORSULFURON RESISTANCE

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Summary. A field experiment was conducted to evaluate lines of the potato cultivar Iwa that had been genetically modified for chlorsulfuron resistance. Transformed lines with one to three copies of a gene encoding a chlorsulfuron-insensitive form of acetolactate synthase were compared with a non-transformed line.

Crop response was assessed to one and two applications of 20 g/ha chlorsulfuron. In the absence of chlorsulfuron, the non transformed line yielded 80.1 t/ha, which was reduced to 30.6 t/ha by two chlorsulfuron applications. In comparison, the transformed lines showed minimal or no damage in response to the herbicide treatments.

In the absence of chlorsulfuron, the transformed lines had reduced yields in comparison to the untransformed line. This was attributed to somaclonal variation arising during the cell culture and regeneration phase of transformation.

INTRODUCTION

The development of crop plants with resistance to a specific herbicide has been achieved by conventional plant breeding and more recently by the application of plant molecular biology techniques (1, 8). The prerequisites for engineering herbicide resistance in a crop plant are the availability of a suitable cloned gene that confers herbicide resistance and the ability to transform foreign genes into the crop species of interest. The rapid development of plant genetic engineering in recent years has already resulted in over 135 regulatory approved small scale field trials on herbicide-resistant transgenic plants in a wide range of crops (2).

There have been relatively few reports of the performance of genetically modified, herbicide resistant crops under large scale field conditions. The present paper details such an experiment using genetically modified potatoes (*Solanum tuberosum* L.) transformed for resistance to the broad spectrum herbicide chlorsulfuron. Crop & Food Research (formerly Crop Research Division DSIR) developed a series of transformed chlorsulfuron-resistant potato lines of the New Zealand cultivar Iwa (3). These lines resulted from *Agrobacterium* - mediated transformation using the binary vector pKIWI110 (7), with all lines expressing 3 foreign genes: a chimeric selectable marker gene conferring kanamycin resistance (NOS-NPTII-NOS), a chimeric reporter gene conferring ß-glucuronidase activity (355-GUS-OCS), and a gene encoding a chlorsulfuron-insensitive form of the enzyme acetolactate synthase cloned from a chlorsulfuron-resistant mutant of *Arabidopsis thaliana* L. (5).

METHODS

Three transformed lines were selected for this study, since they showed high levels of resistance to chlorsulfuron and were indistinguishable from the parent cultivar in phenotypic appearance and yield during initial small scale field trials (Conner et al, unpublished results). The 3 lines, SCI1, SCI2, and SCI4 had two, three and one copy of the chlorsulfuron-resistant gene respectively.

The experiment, conducted at the Lincoln Research Farm (Crop & Food Research) was a randomised block, split plot design. Potato genotype was the main plot, consisting of the three chlorsulfuron-resistant lines and a non-transformed line of the cultivar Iwa. Split plot factors consisted of the weed control treatments; hand weeding, a single or a double application of chlorsulfuron and an untreated control. Main plots were 16x6 m and sub plots 8x3 m which were randomly assigned in four replicate blocks. The total experimental area was 1920 m^2.

Regulatory approval for the field trials was received from the Minister for the Environment on 27 October 1989, following submissions to the Interim Assessment Group for the Field Testing and Release of Genetically Modified Organisms.

Seed potatoes were planted on 11 November 1989 in 750 mm rows and grown at optimum soil fertility and with irrigation to maintain soil moisture. Chlorsulfuron was applied as Glean at 20 g/ha in 200 L water as an overall spray on 18 December 1989, when the crop was 150 mm above the top of the mould and again on 20 January 1990, at which stage the crop canopy had achieved almost full cover.

Potatoes were harvested during the third week of May 1990 using a Faun 1600 single row potato harvester. Only tubers from the middle two rows of each plot were used for further analysis. The tubers were graded to establish the yield of table-grade potatoes. Orthogonal contrasts were used to assess significance of difference between treatments and lines.

RESULTS AND DISCUSSION

Weed populations in all treatments were not significant and unlikely to have a major effect on the yield of potatoes. Thus there were limited differences in potato yield between unsprayed and hand weeded plots (Tables 1 and 2). The low incidence of weeds allowed for realistic estimates of the impact of genetic transformation for chlorsulfuron resistance on the yield of potatoes and for determination of the effect of chlorsulfuron on untransformed and transformed lines.

Total yield of potatoes in transformed lines was reduced, compared to the untransformed line in those treatments except for SCI 4 hand weeded not receiving chlorsulfuron (Table 1). In particular the line SCI 1 showed a significant yield penalty. Addition of a single or double application of chlorsulfuron severely reduced yield in the untransformed line (data analysis not presented), but not in the transformed lines. There were significant differences in the yield of table potatoes between all transformed lines and the untransformed line, except between hand weeded, untransformed (62.8 t/ha) and SCI 4 (57.5 t/ha) (Table 2). The unsprayed, untransformed line produced a significantly greater yield of table potatoes than any of the transformed lines, by at least 17.4 t/ha. A single application of chlorsulfuron severely reduced the yield of the untransformed line, without any major yield penalty to the transformed lines. Similarly the double application of chlorsulfuron severely reduced the yield of the untransformed line but allowed a yield of >39.0 t/ha in transformed lines.

The combined results in Tables 1 and 2 indicated that transformed lines produced smaller tubers and a reduced proportion of total yield as table potatoes.

There was no significant crop damage in transformed lines receiving chlorsulfuron treatments. Any yield reduction associated with genetic transformation was not linked to obvious foliar damage.

The total yield of the unsprayed, untransformed line of cultivar Iwa of 80.1 t/ha represents a highly productive potato crop for Canterbury conditions. This was almost 20% greater than the total yield for the corresponding transformed lines SCI 2 and SCI 4, while SCI 1 had an even lower yield (Table 1). This reduced yield of the transformed lines could be attributable to:

1. a yield penalty associated with the possession and expression of the inserted genes,
2. a mutational event resulting from the insertion of the transferred genes into the potato genome,
3. somaclonal variation occurring during the cell culture and regeneration phase of transformation.

Table 1. Total potato tuber yield (t/ha). Contrast between untransformed and transformed lines

	Unsprayed	Hand weeded	Chlorsulfuron applications	
			1	2
Untransformed	80.1	74.4	54.1	30.6
SCI 1	48.7	52.4	61.0^{ns}	57.0
SCI 2	64.7	61.4*	62.0^{ns}	64.4
SCI 4	64.4	70.2^{ns}	63.2^{ns}	66.0

Significant contrasts between untransformed and SCI lines. All contrasts significant at $P<0.01$, except *($p<0.05$) and ns (non-significant).

The first two possibilities are regarded as being highly unlikely or rare events associated with plant transformation (4, 6), whereas somaclonal variation is considered to be of common occurrence and likely to account for the majority of phenotypic variation between transformed lines (4).

The possession of genes for chlorsulfuron resistance conferred a yield advantage for transformed lines under a herbicide regime of two applications (Tables 1 and 2). The absence of visible damage in transformed lines following chlorsulfuron applications, irrespective of the number of gene copies, indicated that the introduced genes were being expressed under field conditions. Although the transformed lines did not retain the yield performance of the parental cultivar, this study clearly established that resistance to chlorsulfuron was sufficient to protect the potato crop from the recommended field application rate of chlorsulfuron.

Table 2. Total table potato tuber yield (t/ha). Contrast between untransformed and transformed lines.

	Unsprayed	Hand weeded	Chlorsulfuron applications	
			1	2
Untransformed	68.1	62.8	30.1	4.8
SCI 1	32.5	33.9	45.5	39.0
SCI 2	43.5	38.0	40.5*	51.1
SCI 4	50.7	57.5^{ns}	45.9	40.9

Significant contrasts between untransformed and SCI lines. All contrasts significant at $p<0.01$, except *($p<0.05$) and ns (non significant).

The scale of the field evaluation of genetically modified potato lines was significant and allowed for an effective analysis of the performance of transformed plants in an appropriate agronomic situation. This is in contrast with the small scale of previous field evaluation studies with crop species that have been genetically modified for herbicide resistance (2). Although the transformed lines field tested in this study were indistinguishable from the parent cultivar in small scale field trials, these lines did not retain yield performance when trialled under the more rigorous experimental plot design and statistical analysis possible with larger scale field trials. This emphasises the need to produce a large number of independently selected transformed lines in the laboratory in order to recover lines with appropriate expression of the inserted genes, while maintaining the phenotype and yield performance of the parental cultivars.

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