Genetic engineering for potato tuber moth resistance

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1 EXECUTIVE SUMMARY

Plants of potato cultivars, Ilam Hardy and Iwa, have been genetically engineered with vectors containing Bt genes known to code for insecticidal proteins that target potato tuber moth. The regeneration of transgenic lines was attempted following the selection of 1900 transformed cell colonies of Ilam Hardy and 120 transformed cell colonies of Iwa. Consistent with previous results, many of the regenerated lines had malformed plants or were very slow growing. From the transgenic plants produced, 10 independently developed lines of Ilam Hardy and 15 lines of Iwa were selected for resistance testing against potato tuber moth. All of these lines were transgenic for the *cry*IAc gene from *Bacillus thuringiensis*.

Testing *in vitro* plants from some of the transgenic Ilam Hardy lines established improved resistance to larvae of potato tuber moth. Subsequent challenges under greenhouse conditions established significantly reduced growth rates of potato tuber moth larvae when feeding on nine of the transgenic lines of Ilam Hardy and 12 lines of Iwa. Following assessment of plant appearance and tuber production under greenhouse conditions, nine Ilam Hardy lines and eight Iwa lines have been identified as worthy of field testing next summer.

2 INTRODUCTION

The use of genetic engineering to clone Bt genes from *Bacillus thuringiensis*, and to transfer and express these genes in plants, is well established to confer resistance to insects. This approach is currently being attempted in many crops throughout the world to develop resistant cultivars against specific insect pests. Research in North America and Europe has resulted in potato plants with resistance to Colorado beetle, tobacco hornworm, and aphids. In late 1994, transgenic Russet Burbank lines with Colorado beetle resistance received FDA approval for commercialisation in the USA. In early 1995, the USDA-APHIS granted these lines nonregulated status and considered them to have been proven safe for general use.

Potato tuber moth is the major insect pest of potato crops in New Zealand, and is especially prevalent in drier seasons. The resulting physical damage to potato tubers makes them unmarketable. Ilam Hardy is the most widely grown potato cultivar in New Zealand. It has a tendency to develop tubers close to the soil surface, which makes it one of the most susceptible cultivars to potato tuber moth damage. The use of Bt genes for resistance to potato tuber moth in this cultivar will offer an important component of an integrated pest management system for this pest. It will assist in the production of high quality tubers without insect damage, and in the marketing of tubers with minimal pesticide residues.

The objectives of this project was to complete plant regeneration from cell potato colonies transformed with *Agrobacterium*-based vectors containing Bt genes known to target potato tuber moth; to evaluate the transgenic lines for general phenotypic appearance *in vitro* and in a containment greenhouse; and to assess lines with normal appearance for resistance to potato tuber moth.

3 EXPERIMENTAL METHODS

Pieces of plant tissue (explants) from *in vitro* cultured potato plants were dipped in cultures of *Agrobacterium* strains containing binary vectors with Bt genes. The *Agrobacterium* was cultured with the plant tissue for two days to allow gene transfer to take place, then eliminated by incorporating specific antibiotics into the culture medium. The presence of a gene conferring kanamycin resistance to plant cells on the same vector as the Bt gene permits the transformed cells to be selected in tissue culture and regenerated into plants on culture media (the general methods are outlined in Conner et al. 1996, *Commercial Grower 51(8)*: 22-26).

The resulting plants were clonally multiplied via micropropagation and transferred to a containment greenhouse using our standard procedures (Conner et al. 1994, *New Zealand Journal of Crop and Horticultural Science* 22: 361-371) for observations on general plant appearance and tuber production.

Nine transgenic lines of Ilam Hardy were challenged *in vitro* with larvae of potato tuber moth. For these lines, five to six one-day-old potato tuber moth larvae were placed on single *in vitro* plants growing on medium supplemented with cefotaxime (200 mg/ℓ) and miconazole (20 mg/ℓ) to prevent contamination arising from the larvae. The plant weight and number of leaves from four replicate plants were determined after eight days.

The growth of potato tuber moth larvae feeding on the foliage of all the transgenic lines was determined using greenhouse-grown plants growing in 5 cm^3 plastic pots. Five recently hatched larvae were placed on each of four replicate plants for all transgenic lines and control plants (20 larvae per line). The weight of the larvae was measured at day 9 (normal time to pupation). A growth index (GI) of the larvae was calculated as:

$$GI = \ln(\text{final weight}) / \ln(\text{initial weight}).$$

4 RESULTS

1. Over 2000 independently derived transformed cell colonies of potato have been selected in tissue culture from 3000 leaf explants co-cultivated with appropriate *Agrobacterium* strains (1900 colonies of Ilam Hardy and 120 of Iwa).

2. Some form of plant regeneration was obtained for 36% of the Ilam Hardy cell colonies and 79% of the Iwa cell colonies.

3. As expected, the majority of the regenerated shoots obtained were malformed or very slow growing. These were discarded, leaving 14 lines of Ilam Hardy and 16 lines of Iwa transgenic for the *cry*IAc gene from *Bacillus thuringiensis*.

4. After repeated micropropagation, a further four lines of Ilam Hardy and one line of Iwa were discarded due to poor plant appearance.

5. *In vitro* plants from nine transgenic Ilam Hardy lines were challenged with larvae of potato tuber moth. The weight of the *in vitro* plants and their number of leaves were determined one week after placing five to six one-day-old larvae of potato tuber moth on each plant. All nine transgenic lines showed improved resistance to potato tuber moth larvae (Table 1).

6. The growth of potato tuber moth larvae feeding on the transgenic plants was significantly reduced, compared to control plants, on 12 of the 15 transgenic Iwa (Table 2). Similarly nine of the 10 transgenic Ilam Hardy lines also supported significantly reduced growth of potato tuber moth larvae (Table 2).

7. Four of the transgenic Iwa lines exhibited off-type appearance when grown in the containment greenhouse. This ranged from slow, stunted growth to abnormal appearance of leaves (small leaves with a twisted and puckered surface). These plants also showed reduced tuber production (number and/or size of tubers), or many small tubers, often with a "knobbly" appearance. Such plants are not unusual following regeneration from tissue culture, especially for Iwa. The remaining nine Iwa lines and all of the Ilam Hardy lines had a "normal" appearance with respect to shoot morphology and tuber production.

Table 1: **Response of *in vitro* Ilam Hardy transgenic lines to potato tuber moth larvae.**

Plant line	Mean number of leaves per plant	Mean plant weight (g)
Control	0.38	0.10
107	11.0	0.33
108	10.0	0.29
109	7.25	0.21
110	9.75	0.27
112	7.25	0.23
113	10.5	0.37
114	7.75	0.19
115	9.25	0.37
116	7.25	0.21

Table 2: **Growth Index of potato tuber moth larvae and tuber appearance for the various control and transgenic potato lines.**

Plant line	Larval Growth Index	Statistical difference[1]	Tuber appearance[2]
Iwa			
Control	5.3	na	normal
30	3.8	*	not evaluated
35	2.1	*	small tubers
39	2.9	*	normal
45	4.4	*	normal
52	2.8	*	normal
57	3.1	*	normal
60	4.8	=	small tubers
67	3.8	*	normal
75	2.9	*	normal
77	4.5	=	not evaluated

78	2.6	*	small tubers
87	3.6	*	normal
89	3.0	*	very poor
94	2.7	*	normal
97	6.0	=	normal
Ilam Hardy			
Control	6.0	na	normal
107	2.3	*	normal
108	3.1	*	normal
109	3.8	*	normal
110	2.9	*	normal
111	2.5	*	normal
112	4.1	*	normal
113	5.0	*	normal
114	4.4	*	normal
115	3.3	*	normal
116	4.3	=	normal

[1] na, =, and * represent not applicable, not significantly different from the control and significantly different from the control at the 5% probability level, respectively, for the Growth Index of potato tuber moth larvae.

[2] as evaluated from plants grown in PB5 Planta bags in a containment greenhouse.

5 CONCLUSIONS

The results from this study have conclusively established the value of genetic engineering for developing transgenic potato lines with improved resistance to the larvae of potato tuber moth. From a large collection of transformed cell colonies of Ilam Hardy and Iwa, completed potato plants with adequate growth were regenerated for 10 independently derived lines of Ilam Hardy and 15 independently derived lines of Iwa. As expected from our earlier research results, the genetic engineering of Ilam Hardy was less efficient than that achieved for Iwa.

All of the transgenic Ilam Hardy lines and 12 of the 15 transgenic Iwa supported significantly reduced growth of potato tuber moth larvae. As expected, some of these lines had poor plant appearance and tuber formation. Overall, nine Ilam Hardy lines and eight Iwa lines with improved resistance to potato tuber moth larvae and "normal" plant appearance and tuber production have been identified as worthy of field testing next summer.

6 ACKNOWLEDGEMENT

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