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Genetic engineering of Ilam Hardy for potato tuber moth resistance

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CONTENTS

1 EXECUTIVE SUMMARY

Plants of Ilam Hardy have been genetically engineered using vectors containing Bt genes known to code for insecticidal proteins that target potato tuber moth. These plants resulted from the co-cultivation of more than 2000 explants with modified *Agrobacterium* strains, from which more than 1400 transformed cell colonies were selected. Most of these colonies were discarded due to poor expression of the transferred genes or the regeneration of plants with abnormal appearance. To date, 10 genetically engineered Ilam Hardy lines with normal plant appearance in tissue culture have been successfully developed and will be tested for resistance to potato tuber moth.

2 INTRODUCTION

The use of genetic engineering is a well established method to confer resistance to insects. This is done by cloning Bt genes from *Bacillus thuringiensis*, and transferring and expressing these genes in plants. This approach is currently being attempted in many crops throughout the world to develop cultivars resistant to 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, transformed Russet Burbank plants with Colorado beetle resistance received FDA approval for commercialisation in the USA, and the first crops were recently planted.

Potato tuber moth is the major insect pest of potato crops in New Zealand. It is especially prevalent in drier seasons. The resulting physical damage to potato tubers makes them unmarketable. Ilam Hardy is the most widely grown 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 objective of this project was to develop 250 independently derived lines of Ilam Hardy transformed with *Agrobacterium*-based vectors containing Bt genes known to target potato tuber moth. In addition, these lines were to be screened to confirm their transformed status and to assess whether or not the regenerated plants had a normal appearance in tissue culture.

3 EXPERIMENTAL METHODS

Two binary vectors for *Agrobacterium*-mediated transformation of plants were obtained from HortResearch. These vectors, pART27IA(c)B and pART27IG'M14, contained two different Bt genes, both of which are known to result in effective resistance to larvae of potato tuber moth when transferred to tobacco. We transferred these vectors into two disarmed *Agrobacterium* strains (LBA4404 and AGL1).

Pieces of plant tissue (explants) from *in vitro* cultured Ilam Hardy potato plants were dipped in the *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. The presence of a kanamycin resistance gene on the same vector as the Bt gene permits the transformed cells to be selected in tissue culture and regenerated into plants.

The cultivar Iwa is highly amenable to *Agrobacterium*-mediated transformation. Therefore, in some experiments it was used as a control to check that the gene transfer method was working.

4 RESULTS

In total, 2113 explants of Ilam Hardy were co-cultivated with *Agrobacterium* and 1413 putatively transformed cell colonies were selected for transfer to shoot regeneration medium (Table 1). Some transformation experiments failed to produce transformed cell colonies, whereas others were highly successful. Clearly, the use of *Agrobacterium* strain AGL1 was more successful than LBA4404. The use of Iwa resulted in 70 transformed cell colonies from 260 explants (Table 1).

Upon transfer of the selected cell colonies to shoot regeneration medium, many showed regeneration potential (Table 2). For Ilam Hardy, regeneration was observed in only 32% of the cell colonies resulting from transformation using AGL1 strains, whereas 63% of the cell colonies from transformation using LBA4404 showed some form of regeneration. Following transformation of Iwa using LBA4404, 76% of the selected cell colonies exhibited regeneration.

As expected, many of the regenerated shoots from these cell colonies showed abnormal development. Others showed poor expression of the kanamycin resistance genes and failed to continue growth on the selection medium. All such colonies were, therefore, discarded. To date, complete transformed plants have been regenerated from 10 cell colonies of Ilam Hardy and 39 cell colonies of Iwa (Table 2). Further plants may be regenerated from some of the Ilam Hardy cell colonies still under evaluation.

Table 1: Summary of experiments to select transformed cell colonies of Ilam Hardy.

Experiment (date initiated)	*Agrobacterium* (strain vector)	No. of co-cultivated explants	No. of selected colonies
Ilam Hardy			
Expt 1 (11/9/95)	LBA4404/pART27IA(c)B	400 leaf explants	None
		60 stem explants	None
		60 root explants	None
Expt 2 (13/11/95)	LBA4404/pART27IA(c)B	300 leaf explants	59
		45 stem explants	34
		45 root explants	None
Expt 3 (27/11/95)	LBA4404/pART27IA(c)B	500 leaf explants	48
Expt 4 (12/1/96)	AGL1/pART27IA(c)B	240 leaf explants	350
	AGL1/pART27IG'M14	240 leaf explants	880
Expt 5 (5/2/96)	AGL1/pART27IA(c)B	21 leaf explants	None
Expt 6 (24/5/96)	AGL1/pART27IG'M14	48 leaf explants	42
Expt 7 (7/6/96)	AGL1/pART27IA(c)B	154 leaf explants	In progess
Total	-	2113 explants	1413 colonies
Iwa			
Expt 1 (27/11/95)	LBA4404/pART27IA(c)B	60 leaf explants	19
	LBA4404/pART27IG'M14	200 leaf explants	51
Total	-	260 explants	70 colonies

Table 2: Further characterisation of transformed cell colonies of Ilam Hardy.

Agrobacterium strain/vector	No. of selected colonies	% of colonies regenerating	No. of transformed lines discarded	No. of transformed plants retained
Ilam Hardy				
LBA4404/pART27IA(c)B	141	63	79	10
AGL1/pART27IA(c)B	350	32	95	In progess
AGL1/pART27IG'M14	922	32	270	In progess
Total	1413	35	444	10+[1]
Iwa				
LBA4404/pART27IA(c)B	19	74	12	2
LBA4404/pART27IG'M14	51	76	2	37
Total	70	76	12	39

[1] Research in progress involves the assessment of a further 47 transformed lines.

5 DISCUSSION

The selection of transformed cell colonies using *Agrobacterium* strain AGL1 was considerably more successful than we initially anticipated. However, cell colonies resulting from these transformation events were very difficult to regenerate into plants with normal appearance. Clearly, greater efficiency in genetic engineering can be achieved using strain LBA4404 with lower frequencies of cell colony selection, but much higher frequencies of normal plant regeneration. This has been a valuable finding for our ongoing research in potato genetic engineering.

The plants produced to date will be assessed using molecular techniques to determine whether they have the Bt genes inserted into their chromosomes. Leaves from these Bt-transformed plants will be fed to potato tuber moth larvae to confirm biologically active levels of resistance. These lines will be transferred to a containment greenhouse for further observations on their phenotypic appearance, and propagation of tubers for field trials.