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Genetic engineering of potato for resistance to tuber moth

A report prepared for the
Potato Industry Research and Development Grants Committee of the New Zealand Vegetable and Potato Growers Federation

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1 EXECUTIVE SUMMARY

A collection of potato lines transgenic for a *cry*1Ac gene known to confer resistance to potato tuber moth larvae have been grown in a containment greenhouse. Of the original 120 transgenic lines, 75 lines were identified with the *cry* gene and with significantly improved resistance to the larvae while not exhibiting an off-type appearance. Amongst these were 20 transgenic lines of Iwa, 13 of Ilam Hardy, 17 of Red Rascal, 10 of Karaka, 9 of Russet Burbank, 4 of Pacific and 1 each of Rua and White Delight. These new lines supplement the previous collection of potato lines transgenic for the *cry*1Ac gene and the *cry*9Aa2 gene. The latter also confers resistance to potato tuber moth larvae.

A successful application was made to the Environmental Risk Management Authority to field test potato lines transgenic for the *cry*1Ac and *cry*9Aa2 genes. On 22 December 1998 approval was granted to proceed with a field test for a five year period through to 2003. A small-scale field trial involving 10 plants in 3 m plots for each of 87 transgenic potato lines was planted on 24 December 1998. These lines included the 75 new lines identified with good appearance and improved resistance to potato tuber moth in the containment greenhouse, plus another 3 Ilam Hardy and 9 Iwa high performing lines identified from the 1997/98 field trial.

During the night of 10-11 March 1999 the trial was vandalised by a group calling itself "The Wild Greens". Of the 1120 plants established in the trial, 421 were destroyed (38%). At the time of harvest the number of missing plants had risen to 508 (45% destroyed). Too much damage occurred to the control plots to allow meaningful comparisons with the few transgenic plots left intact. Furthermore, many of the harvested plants were partially damaged and some of their tubers were missing which resulted in high variability associated with the measurements. This severely compromised the statistical analysis of the yield results, although a few lines with very poor performance could be eliminated.

Bioassays against larvae had been completed on foliage from 62 of the transgenic lines prior to the act of vandalism. Fifty-six of these lines (90%) exhibited improved resistance to potato tuber moth larvae in the field. Five transgenic lines identified in the 1997/98 field trial with high resistance against tuber moth larvae continued to exhibit a high degree of resistance in the 1998/99 field trial. Thirteen of the transgenic

lines field tested for the first time also had a high level of resistance to tuber moth larvae.

Two transgenic lines of Iwa and one of Ilam Hardy that exhibited high peformance in the 1997/98 field trial performed in a similar manner in the 1998/99 field trial. Two transgenic lines from each of Red Rascal and Russet Burbank had high field resistance to potato tuber moth larvae and high yield potential from the 1998/99 field trial. These seven lines are all suitable candidates for future scale-up field trials.

2 INTRODUCTION

Potato tuber moth is the major insect pest of potato crops in New Zealand. It is especially prevalent in dry seasons, and is a field and postharvest problem. The resulting physical damage to potato tubers makes them unmarketable and can also result in the development of secondary disease infections. Current control methods for potato tuber moth in potatoes include cultural management practices, biological control and the use of broad spectrum insecticides. Some cultural practices, such as planting time, planting depth, irrigation, re-moulding and hilling, pre-harvest removal of foliage with herbicide applications, crop hygiene and immediate removal of tubers to cold storage facilities, assist in reducing damage by potato tuber moth larvae. However, heavy infestations of potato tuber moth still require the use of repeated applications of organo-phosphates, synthetic pyrethroids, and/or carbamates for effective control.

The use of genetic engineering to clone *cry* 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. Genetic engineering of New Zealand potato cultivars with *cry* genes for resistance to potato tuber moth offers an important component of an integrated pest management system against this pest. It will assist in reducing the reliance on pesticides to control this insect pest as well as the production of high quality tubers without insect damage and with minimal pesticide residues.

The objectives of this project were to:

- complete the characterisation of a further 100 independently derived transgenic lines of additional potato cultivars transformed with *cry* genes known to target potato tuber moth,
- apply to the Environmental Risk Management Authority for permission to conduct a small-scale field trial on the existing transgenic potato lines with resistance to potato tuber moth, and
- establish the field trial and assess the performance of these existing transgenic potato lines.

3 EXPERIMENTAL METHODS

3.1 Gene transfer to potato

The general methods for gene transfer to potato are outlined in Conner et al. (1996). Leaf segments from *in vitro* cultured potato plants were dipped in cultures of *Agrobacterium* strains containing binary vectors with *cry* 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 *cry* gene permitted transformed cells to be selected by incorporating kanamycin into the culture medium. Sequential changes in the content of plant growth regulators in the culture media allowed the growth and regeneration of the transformed cells into complete plants (Conner et al. 1991).

The resulting plants were clonally multiplied via micropropagation and transferred to a containment greenhouse using our standard procedures (Conner et al. 1994) for observations on general plant appearance and tuber production, as well as bioassays against potato tuber moth larvae.

3.2 Field trial

Rows of the potato plants were spaced 75 cm apart, with 30 cm between plants within rows. Ten tubers of each line were planted as a single plot within a row, with a 1 m gap between plots. The trial involved the planting of up to 29 transgenic lines from eight cultivars. Plots of these transgenic lines were interspersed with plots of the appropriate non-transgenic control. The experimental plots were completely surrounded by 3 buffer rows of non-transgenic potato to prevent "edge effects" during the trial. These buffer rows were planted with a potato genotype that had purple tubers to allow them to be readily distinguished from the white or pink-red tubers of the transgenic potato lines.

Observations were made throughout the trial on the general appearance of the transgenic lines. At various times leaves were excised and used for feeding bioassays with larvae of potato tuber moth. At harvest the number of tubers and weight of tubers per plot were recorded.

3.3 Tuber moth bioassays

The growth of potato tuber moth larvae feeding on the field-grown plants was determined on excised leaves on moistened filter paper in 250 ml plastic containers. Five leaves were placed in each container with 10 pre-weighed neonate larvae. The larvae were transferred to fresh leaves after 5 days. Three replicate containers for each line were maintained at room temperature for 10 days, after which the larvae were individually reweighed. A growth index (GI) of the larvae was calculated as:

GI = ln final weight - ln initial weight.

This index provides a measure of the mean relative growth rate of the larvae.

3.4 Statistical analysis

ANOVA and REML analyses were carried out using Genstat 5 (1998).

4 RESULTS

1. We have produced genetically engineered lines in 10 cultivars of potato with a vector containing the *cry*1Ac gene from *Bacillus thuringiensis*. These include: Iwa, Ilam Hardy, Red Rascal, Rua, Russet Burbank, Karaka, Kaimai, Pacific, White Delight, and Summit.

2. DNA analysis of 120 transgenic potato lines has confirmed the presence of the *cry*1Ac gene in 76% of these lines.

3. These 120 transgenic potato lines have been grown in a containment greenhouse to allow assessment of plant appearance. Approximately 20% of these lines showed marked off-type appearance, usually evident as malformed or very slow growing plants with abnormal development of foliage and/or small knobbly tubers.

4. Bioassays against potato tuber moth were performed on greenhouse-grown foliage from 120 transgenic lines. Significantly reduced growth rates of potato tuber moth larval were recorded for 70% of these lines.

5. Following assessment in the containment greenhouse, 75 lines were identified with significantly improved resistance to potato tuber moth larvae and not exhibiting an off-type appearance. These include 20 transgenic lines of Iwa, 13 of Ilam Hardy, 17 of Red Rascal, 10 of Karaka, 9 of Russet Burbank, 4 of Pacific and 1 each of Rua and White Delight.

6. An application was made to the Environmental Risk Management Authority (ERMA) to field test the lines with potato tuber moth resistance. Responses were prepared to the ERMA report and public submissions on the field trial application. Following a public hearing in Wellington on 11 November 1998 and after responding to several requests for further information, approval to proceed with a field test for a five year period was granted by ERMA on 22 December 1998.

7. A field trial involving 87 transgenic potato lines was planted on 24 December 1998. These lines included the 75 new lines identified with good appearance and improved resistance to potato tuber moth in the containment greenhouse, plus another 3 Ilam Hardy and 9 Iwa lines identified from the 1997/98 small-scale field trial as having improved field resistance to tuber moth and no reduction in tuber yield. Seven of the Iwa lines were engineered with the *cry*9Aa2 gene, whereas all others were engineered with the *cry*1Ac gene. Plots

of these transgenic lines were interspersed with 1-7 plots of the appropriate non-transgenic control. In total the trial consisted of 112 plots, each 3 m long with 10 plants.

8. During the night of 10-11 March 1999 the trial was vandalised. A group calling itself "The Wild Greens" claimed responsibility for the damage. The initial assessment of the damage documented those plants that had been totally removed. Since it was very difficult to distinguish between many partly damaged and largely undamaged plants, all plants maintaining some viability after 2 weeks were recorded as survivors. The resulting damage occurred:

Number of transgenic lines in trial	87
Number of transgenic lines damaged	73
Number of control plots in trial	25
Number of control plots damaged	23
Total number of transgenic plants in trial	870
Total number of transgenic plants destroyed	317
% of transgenic plants destroyed	36%
Total number of control plants in trial	250
Total number of control plants destroyed	104
% of control plants destroyed	42%

Of the 1120 plants established in the trial, 421 were destroyed (38%). At the time of harvest the number of missing plants had risen to 508 (45% destroyed). No further use could be made of this field trial since too much damage was sustained by the control plots to allow meaningful comparisons with the few transgenic plots left intact. Nevertheless, some general observations could be made on the performance of individual transgenic lines.

9. Fifteen transgenic lines developed marked changes in the appearance of the foliage of field-grown plants and exhibited characters such as stunted plants, reduced plant vigour and/or leaf puckering. These plants were not used for bioassays against tuber moth larvae since any inhibition of insect growth could be due to factors others than the expression of the *cry* gene.

The tuber yields from the individual transgenic and control lines are presented in Table 1. However, the yield estimates from the harvested tubers are of little value due to the high variability associated with the measurements. Many of the harvested plants were partially damaged and some of their tubers were

missing. In some cases tubers were recovered from points where foliage had been completely removed, often making it difficult to ascertain whether they arose from one plant or two. Consequently, high error terms associated with the calculated mean values are to be expected. For this reason measurement of yield data on an individual plant basis, as originally intended, was not appropriate. Instead, data were collected on a plot basis. Therefore, the only estimate of variability available was that which could be obtained from the replicated control plots. This estimate was obtained using analysis of variance. Although this analysis was not very powerful, it did allow a few lines with very poor performance to be eliminated (Table 1). From our past experience with field trials on transgenic potato lines, more lines would have been expected to have a statistically inferior yield performance. While many lines appeared to have yield performance statistically equivalent to the controls, a more thorough yield assessment, as originally intended, would have allowed the identification of additional lines with inferior performance.

10. Prior to the act of vandalism on the trial some information was gained from the field assessment of tuber moth resistance. Bioassays against larvae had been completed on foliage from 62 of the transgenic lines. Statistical analysis of the bioassay data using REML established that lines could be compared using the variation between the plastic containers for each line (ignoring the original field plots). The analysis indicated that 56 lines (90%) exhibited significantly improved resistance to potato tuber moth larvae in the field (Table 2). Five transgenic lines identified in the 1997/98 field trial with high resistance against the tuber moth larvae (lines 32, 41, 52, 92 and 107) continued to exhibit a high degree of resistance in the 1998/99 field trial. Among the transgenic lines field tested for the first time, 13 had a high level of resistance to tuber moth larvae. These included D11 and D67 (Ilam Hardy); D18, D48, T18, T22 and T48 (Iwa); D02, D53 and T76 (Red Rascal); D61 and D63 (Russet Burbank); and D30 (White Delight), all of which supported a larval growth index of about three or less. From our previous studies on transgenic potatoes, such low growth indices are associated with a very low incidence of larval survival to pupation and an even lower emergence of adult moths.

Table 1: Summary of the yield data from field-grown transgenic potato lines.

Cultivar and line	Number of tubers per plant	Weight of tubers per plant (g)	Mean weight per tuber (g)
Ilam Hardy			
Control	6.3	684	109
107	5.4	616	114
113	6.3	731	116
D06[a]	7.3	468	64
D07[a]	9.0	590	66
D09	9.0	587	65
D11	8.5	378	44
D15	7.5	400	53
D22	8.7	732	84
D49	12.0	806	67
D57	7.6	456	60
D62[a]	4.0	310	78
D65[a]	3.0	72	24
D67	7.0	601	86
D68[a]	7.4	313	42
LSD[b] (df=23)	6.4	737	135
Iwa			
Control	7.2	1058	147
02	8.0	786	98
32	3.8	995	262
41	7.2	1658	230
43	9.7	1807	186
52	5.5	965	175
74	4.3	949	221
75	4.7	807	172
76	3.3	311	94
92	4.5	1061	236
D18	9.6	816	85
D47[a]	10.3	642	62
D48	8.6	667	78
D54	9.3	655	70
T18	9.2	784	85
T20[a]	8.3	718	87
T22	11.0	838	76
T24[a]	8.3	771	93
T31[a]	0.8	4	5

T33[a]	6.0	268	45
T48	8.0	873	109
T49	12.5	1222	98
T50	9.3	981	105
T51	5.9	564	96
T53	11.1	705	64
T54	10.6	815	77
T68	8.5	979	115
T69	10.2	1056	104
T70	10.0	867	87
T73	9.8	744	79
LSD[b] (df=23)	6.1	705	129
Karaka			
Control	6.5	492	76
T02[a]	4.8	279	58
T04[a]	3.5	310	89
T05[a]	7.4	669	90
T37	8.0	768	96
T38	7.4	651	88
T39	7.0	389	56
T57	9.2	649	71
T59	7.3	420	58
T83	2.6	198	76
T84	9.8	675	69
LSD[b] (df=23)	6.6	761	40
Pacific			
Control	6.7	303	45
T15	5.0	377	75
T16	5.5	439	80
T17	6.7	603	90
LSD[b] (df=23)	8.1	932	171
Red Rascal			
Control	11.2	896	80
D02	6.0	601	100
D20	13.0	1255	97
D53	7.7	649	84
T09	6.7	608	91
T12	8.5	666	78
T13	11.0	1147	104
T35	6.3	453	72

T62	3.8	180	47
T64[a]	3.0	126	42
T65	4.6	177	38
T75	3.3	130	39
T76	9.0	476	53
T78	7.7	477	62
T86	10.7	365	34
LSD[b] (df=23)	6.4	737	135
Rua			
Control	7.8	796	102
D01	11.3	930	82
LSD[b] (df=23)	8.1	932	171
Russet Burbank			
Control	7.2	976	136
D50	8.4	776	92
D60	10.1	1060	105
D61	8.8	643	73
D63	6.1	756	124
D64	9.5	875	92
D69	7.0	739	106
D72	7.0	856	122
D73	8.2	838	102
D74	9.0	954	106
LSD[b] (df=23)	6.6	761	140
White Delight			
Control	2.6	148	57
D30	3.7	205	55
LSD[b] (df=23)	8.1	932	171

[a]Lines exhibiting marked phenotypic changes in the appearance of the field-grown foliage such as stunted plants, reduced plant vigour and/or leaf puckering.

[b]Least significant difference at 5% level to compare any line with the control.

Note: For six lines no intact surviving plants were left at harvest time following the earlier vandalism of the field trial. These included 109 and D56[a] (Ilam Hardy), T41 (Pacific), D23, T11 and T74 (Red Rascal).

Table 2: **Summary of resistance of field grown transgenic potato lines to potato tuber moth larvae.**

Cultivar and line	*cry* gene	Mean larval growth index
Ilam Hardy		
Control	-	5.47
107	1Ac	1.30
109	1Ac	2.93
113	1Ac	2.86
D11	1Ac	2.52
D22	1Ac	3.92
D49	1Ac	5.31
D57	1Ac	4.02
D67	1Ac	2.43
LSD[a] (df=104): Min	0.67	
Max	1.24	
Iwa (batch 1)		
Control	-	5.14
02	9Aa2	3.86
32	9Aa2	3.50
41	9Aa2	3.18
43	9Aa2	4.93
52	1Ac	3.31
74	9Aa2	3.68
75	1Ac	3.84
76	9Aa2	2.30
92	9Aa2	2.65
D18	1Ac	3.09
D48	1Ac	1.76
D54	1Ac	3.25
LSD[a] (df=104): Min	0.53	
Max	1.11	
Iwa (batch 2)		
Control	-	5.81
T18	1Ac	3.07
T22	1Ac	2.67
T48	1Ac	3.17
T49	1Ac	3.88
T50	1Ac	4.03

T51		1Ac	3.54
T53		1Ac	3.58
T69		1Ac	4.46
T73		1Ac	4.34
LSD[a] (df=69):	Min	0.61	
	Max	0.70	

Karaka

Control		-	5.80
T37		1Ac	4.70
T38		1Ac	4.34
T39		1Ac	4.83
T57		1Ac	4.30
T59		1Ac	4.20
T84		1Ac	4.54
LSD[a] (df=69):	Min	0.56	
	Max	0.79	

Pacific

Control		-	6.14
T15		1Ac	4.11
T16		1Ac	5.41
T17		1Ac	4.50
LSD[a] (df=69):		0.73	

Red Rascal (batch 1)

Control		-	5.04
D02		1Ac	2.09
D20		1Ac	4.26
D23		1Ac	3.45
D53		1Ac	2.60
LSD[a] (df=104):	Min	0.85	
	Max	1.09	

Red Rascal (batch 2)

Control		-	5.93
T09		1Ac	3.31
T12		1Ac	3.24
T13		1Ac	3.71
T35		1Ac	5.24
T62		1Ac	4.20
T65		1Ac	4.23
T74		1Ac	4.42

T78		1Ac	4.45
LSD[a] (df=69):	Min	0.58	
	Max	0.63	
Rua			
Control		-	6.29
D01		1Ac	6.37
LSD[a](df=104):		0.79	
Russet Burbank			
Control		-	5.61
D50		1Ac	3.76
D60		1Ac	5.29
D61		1Ac	2.86
D63		1Ac	2.68
D64		1Ac	3.44
D69		1Ac	4.03
D72		1Ac	3.88
D73		1Ac	3.55
D74		1Ac	3.49
LSD[a] (df=104):	Min	0.68	
	Max	1.15	
White Delight			
Control		-	5.37
D30		1Ac	2.87
LSD[a] (df=104):		0.76	

[a] Least Significant Difference at 5% level to compare any line with the control. Because of the varying number of pottles containing larvae varied between lines, the LSD varies for each cultivar. Thus, the smallest (Min) and the largest (Max) LSD for each group are presented.

Note: Of the 87 transgenic potato lines, 25 were not tested in bioassays against potato tuber moth. Bioassays were not performed on 15 lines (D06, D07, D47, D56, D62, D65, D68, T02, T04, T05, T20, T24, T31, T33, and T64) due to marked phenotypic changes in the appearance of the field-grown foliage such as stunted plants, reduced plant vigour and/or leaf puckering. For 10 lines (D09, D15, T11, T41, T54, T68, T70, T75, T83 and T86) bioassays were not completed prior to the field trial being vandalised.

5 CONCLUSIONS

The results of this study further establish the value of genetic engineering for developing potato lines with improved resistance to larvae of potato tuber moth. Transgenic lines engineered with improved resistance to potato tuber moth larvae in the field have now been produced for Ilam Hardy, Iwa, Karaka, Pacific, Red Rascal, Russet Burbank, and White Delight.

In the 1997/98 field trial two transgenic lines of Iwa (line 32, engineered with the *cry*9A2 gene; and line 52, engineered with the *cry*1Ac gene) and one transgenic line of Ilam Hardy (line 107, engineered with the *cry*1Ac gene) were identified with high field resistance to potato tuber moth larvae and high yield potential. These lines performed in a similar manner in the 1998/99 field trial. Four additional lines engineered with the *cry*1Ac gene, two of Red Rascal (D02 and D53) and two of Russet Burbank (D61 and D63), with similar performance were identified in the 1998/99 field trial. These seven lines are all suitable candidates for future scale-up field trials.

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