EVALUATION OF FIELD GROWN POTATO LINES TRANSGENIC FOR A *cry1Ac9* GENE CONFERRING RESISTANCE TO POTATO TUBER MOTH

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ABSTRACT

Phthorimaea operculella (Zeller), potato tuber moth (PTM), is an important pest of potatoes. The larvae of this insect are difficult to control with insecticides because they mine into foliage and tubers. Seven potato cultivars were genetically engineered with a *Bacillus thuringiensis cry1Ac9* gene to confer resistance to PTM larvae. Fifty-six transgenic lines were established in the field at Lincoln, Canterbury, as small plots of 10 plants along with replicate control plots of the same, but non-transgenic, cultivars. Forty-four of the transgenic lines were phenotypically normal. Foliage excised from these plants was challenged with PTM larvae in the laboratory. All but one line inhibited larval growth compared to non-transgenic plants ($P < 0.05$). For transgenic plants the growth indices of larvae fed field-grown foliage were similar to those fed greenhouse-grown foliage. Thirteen of the 43 resistant lines inhibited larval growth by $\geq 40\%$ and produced tuber yields comparable to the non-transgenic plants.

Keywords: potato tuber moth, transgenic potato plants, *cry1Ac9*, insect resistance, *cry* gene.

INTRODUCTION

Potato tuber moth (PTM) (*Phthorimaea operculella*) is an economically important pest of potato crops in warm temperate regions, such as New Zealand (Foot 1979), and subtropical climates (Raman & Palacios 1982; Siddig 1988). Larvae of this insect mine both foliage and tubers. Tubers of potato with larval mines are unmarketable. Larval mines also allow pathogens to enter the tubers (Plaisted et al. 1994). The mines afford the larvae a degree of protection from insecticide sprays, and cultural strategies do not always guarantee protection from infestation. While management of this pest could be augmented by the development of resistant cultivars, traditional breeding methods have failed to produce resistant cultivars (Arnone et al. 1998).

Genetic engineering could provide a viable alternative to traditional breeding methods for developing insect resistant potato plants. *Cry* genes from *Bacillus thuringiensis* have been the predominant source for developing PTM resistant potatoes, but most of the work has been limited to greenhouse experiments (Jansens et al. 1995; Li et al. 1999). The purpose of this study was to evaluate the phenotypic appearance and yield of potatoes transgenic for a *cry1Ac9* gene when grown in the field. Field grown foliage of transgenic lines was used in insect bioassays to determine resistance to PTM larvae.

METHODS

Genetically engineered potato plants were developed using an *Agrobacterium* – mediated transformation method to transfer a *cry1Ac9* gene under the transcriptional

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control of the CaMV 35S promoter (Takla et al. 1999; Davidson 2001). The transgenic lines were evaluated in a containment greenhouse to identify phenotypically normal lines and those with resistance to PTM larvae (inhibited larval growth). Tubers harvested from the transgenic lines and their parent cultivars, were planted in a field at Lincoln, Canterbury, on 23 December 1998. The field trial had been approved by the NZ Environmental Risk Management Authority (permit GMF98008). Ten seed tubers of each transgenic and non-transgenic line were planted 0.3 m apart in rows of small plots. Plots were separated by 1 m within a row and 0.75 m between rows. Lines derived from the same cultivar were planted within neighbouring plots. Replicated non-transgenic plots of a given parent cultivar were positioned randomly among the single transgenic plots. One non-transgenic plot was planted for every three to four transgenic lines belonging to that cultivar. A total of 75 plots were planted, of which 56 were transgenic lines and 19 were non-transgenic lines (Table 1). The foliage and growth of transgenic plants were evaluated 8 to 12 weeks after planting using the categories: phenotypically normal, marginal leaf curl, leaf wrinkling, reduced vigour, stunted growth, and/or spindly appearance of shoots.

Tubers were harvested 18 weeks after planting, in accordance with the field trial approval conditions. Vandalism to the trial on 11 March 1999 prevented tubers from each plant within a plot being harvested separately; instead tubers from all the plants within a plot were harvested together. The number of plants remaining per plot was determined and used to calculate the mean number of tubers per plant, mean weight of tubers per plant and mean weight per tuber.

Insect bioassays using field-grown foliage

Natural infestation levels of PTM were too low to assess the resistance of transgenic lines in the field. Therefore, foliage excised from phenotypically normal transgenic lines and non-transgenic plants in the field was challenged with PTM larvae in a laboratory using the method described in Davidson et al. (in press). Briefly, this involved weighing 10 neonate larvae together, then placing them on four terminal leaflets excised from plants in the field trial inside a 350 ml plastic container, the bottom of which was lined with dry filter paper (Whatman® No. 1, 75 mm diameter). Three containers were used for each small plot. Lids were placed on the labelled containers, which were kept in a controlled temperature room at 22°C (±3°C) with a 16:8 h light:dark cycle. Larvae were transferred to fresh leaflets of the same transgenic or non-transgenic line after 4 days, and after 10 days recovered larvae were weighed individually. A growth index (GI) was calculated as $\log_e$ (final weight / mean initial weight). Bioassays were conducted over a period of one month.

Statistical analysis

Data from the field trial were initially examined with residual maximum likelihood (REML) methods (Gilmour et al. 1995) to assess whether there were any statistically significant contributions to the variability in the data (variance components) between plots from the same line (larval GI and yield data) or containers from the same plot (larval GI data). REML was used because the unequal numbers of plots, plants and surviving larvae for each line made the data unbalanced. However, the variance components for the plots were not statistically significant ($P > 0.05$), so could be ignored. This resulted in balanced data sets, allowing ANOVA to be used for the final analyses. Yield data were transformed with logarithms before analysis. The growth indices and yield data of transgenic lines analysed here were part of a larger field trial that included additional transgenic lines not relevant to this study. All statistical analyses were carried out in Genstat 5 release 4.1.

RESULTS AND DISCUSSION

The 56 phenotypically normal *cry1Ac9*-transgenic potato lines that had inhibited larval growth when grown in the greenhouse (Takla et al. 1999; Davidson 2001) were established in the field. The foliage and growth of 79% of these lines were phenotypically normal in the field (Table 1). The plants considered abnormal (off-types) exhibited stunted growth,

reduced plant vigour, and/or leaf wrinkling. Off-types are typical of plants regenerated from cell cultures and are often observed among transgenic plant lines (Conner & Christey 1994). Off-types have often been attributed to somaclonal variation, which involves epigenetic or genetic changes. These changes become more evident under the fluctuating environmental conditions that occur during field trials (Conner et al. 1994).

TABLE 1: Numbers of transgenic potato lines and non-transgenic (control) plots planted for each cultivar in a field trial assessing plant growth and inhibition of potato tuber moth larval growth.

Parent cultivar	Total no. of transgenic lines planted	No. of control plots planted	No. of phenotypically normal lines	No. of lines to inhibit PTM larval growth (P<0.05)
Iwa	13	4	11	11
Ilam Hardy	10	3	4	4
Red Rascal	14	4	11	10
Russet Burbank	8	3	8	8
Karaka	7	3	6	6
Pacific	3	1	3	3
White Delight	1	1	1	1
TOTAL	56	19	44	43

The growth indices of larvae fed field-grown foliage were similar to those fed greenhouse-grown foliage (Takla et al. 1999; Davidson 2001). Foliage from all of the phenotypically normal lines grown in the field reduced larval growth (Fig. 1). For all but one line (D20, derived from Red Rascal) this reduction was statistically significant

FIGURE 1: Growth indices of PTM larvae fed transgenic foliage as a percentage of larval growth on control (non-transgenic) foliage. Foliage from all transgenic lines inhibited larval growth when compared to their corresponding controls (LSD, P<0.05) except D20 (derived from Red Rascal).

($P < 0.05$). The level of larval growth inhibition varied between the transgenic lines (Fig. 1). Such variation is common in independently derived plants transformed with the same transgene (e.g. Jansens et al. 1995; Beuning et al. 2001). It has often been ascribed to "position effects" that result from the random integration of transgenes into different sites of the plant genome (Conner & Christey 1994).

In a previous study *cry1Ac9*-transgenic potato lines that inhibited PTM larval growth by 35%, resulted in 83 - 97% mortality of PTM when the insects were allowed to develop through to adults (Davidson et al. in press). In this study, 13 of the resistant lines inhibited larval growth by $\geq 40\%$ and were, therefore, considered to have a high level of resistance to PTM larvae. The highly resistant lines also produced yields that were similar ($P > 0.05$) to their corresponding parental control yields, for all three parameters measured (Table 2).

TABLE 2: The transgenic lines that produced yields similar ($P > 0.05$) to their corresponding parental cultivar (control) for all three yield parameters and inhibited PTM larval growth by $\geq 40\%$.

Cultivar and line	Mean no. of tubers per plant	Mean wt of tubers per plant (g)	Mean wt per tuber (g)	Mean larval growth index
Iwa				
Control	7.2	1058	160	5.5
D18	9.6	816	85	3.1
D48	8.6	667	78	1.8
LSD ($P < 0.05$) ¹	-	-	-	1.2
T22	11.0	838	76	2.7
T48	8.0	873	109	3.2
LSD ($P < 0.05$)	6.1	705	129	0.7
Ilam Hardy				
Control	6.3	684	123	5.5
D67	7.0	601	86	2.4
LSD ($P < 0.05$)	6.4	737	135	1.4
Red Rascal				
Control	11.7	896	78	5.5
D02	6.0	601	100	2.1
D53	7.7	649	85	2.6
LSD ($P < 0.05$)	-	-	-	1.4
T09	6.7	608	91	3.3
T12	8.5	666	78	3.2
LSD ($P < 0.05$)	6.4	737	135	0.6
Russet Burbank				
Control	7.2	976	141	5.6
D61	8.8	643	73	2.9
D63	6.1	756	123	2.7
D64	9.5	875	92	3.4
LSD ($P < 0.05$)	6.6	761	140	1.6
White Delight				
Control	2.6	148	57	5.4
D30	3.7	205	56	2.9
LSD ($P < 0.05$)	8.1	932	171	0.8

¹The growth indices for the D and T lines were analysed separately, resulting in different LSDs for each group, within the cultivars. The yield data for both D and T lines was analysed together resulting in a single LSD for each cultivar.

Screening a relatively large number of new transgenic lines for the first time in the field is an effective way of eliminating off-types. The visual classification of foliage appearance gave a strong indication of potential yield. Severe off-types often produced extremely small (< 10 g/tuber) and/or knobbly tubers. All of the transgenic lines listed in Table 2 produced phenotypically normal tubers. The large LSDs calculated for the yield analysis is a reflection of the inherently high variability typically found between and within potato plants. However, this variability was further exacerbated by having to determine the number of plants remaining after the vandalism and having to combine the tubers of all the plants left in a plot at harvest. Despite this, we were able to identify elite transgenic lines worthy of further testing in larger-scale field trials.

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REFERENCES

- Arnone, S.; Musmeci, S.; Bacchetta, L.; Cordischi, N.; Pucci, E.; Cristofaro, M.; Sonnino, A. 1998: Research in *Solanum* spp. of sources of resistance to the potato tuber moth *Phthorimaea operculella* (Zeller). *Potato Res.* 41: 39-49.
- Beuning, L.L.; Mitra, D.S.; Markwick, N.P.; Gleave, A.P. 2001: Minor modifications to the *cry1Ac9* nucleotide sequence are sufficient to generate transgenic plants resistant to *Phthorimaea operculella*. *Ann. Appl. Biol.* 138: 281-292.
- Conner, A.J.; Christey, M.C. 1994: Plant breeding and seed marketing options for introduction of transgenic insect-resistant crops. *Biocontrol Sci. Technol.* 4: 463-473.
- Conner, A.J.; Williams, M.K.; Abernethy, D.J.; Fletcher, P.J.; Genet, R.A. 1994: Field performance of transgenic potatoes. *N.Z. J. Crop Hort. Sci.* 22: 361-371.
- Davidson, M.M. 2001: Management of genetically engineered potatoes resistant to potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae). PhD thesis, Lincoln University, Canterbury.
- Davidson, M.M.; Jacobs, J.M.E.; Reader, J.K.; Butler, R.C.; Frater, C.M.; Markwick, N.P.; Wratten, S.D.; Conner, A.J. in press: Development and evaluation of potatoes transgenic for a *cry1Ac9* gene conferring resistance to potato tuber moth. *J. Amer. Soc. Hort. Sci.* in press.
- Foot, M.A. 1979: Bionomics of the potato tuber moth, *Phthorimaea operculella* (Lepidoptera: Gelechiidae), at Pukekohoe. *N.Z. J. Zool.* 6: 623-636.
- Gilmour, A.R.; Thompson, R.; Cullis, B.R. 1995: Average information REML, an efficient algorithm for variance parameter estimation in linear mixed models. *Biometrics* 51: 1440-1450.
- Jansens, S.; Cornelissen, M.; de Clercq, R.; Reynaerts, A.; Peferoen, M. 1995: *Phthorimaea operculella* (Lepidoptera: Gelechiidae) resistance in potato by expression of the *Bacillus thuringiensis* CryIA(b) insecticidal crystal protein. *J. Econ. Entomol.* 88: 1469-1476.
- Li, W.B.; Zarka, K.A.; Douches, D.S.; Coombs, J.J.; Pett, W.L.; Grafius, E.J. 1999: Coexpression of potato PVY^o coat protein and *cry5-Bt* genes in potato. *J. Amer. Soc. Hort. Sci.* 124: 218-23.
- Plaisted, R.L.; Bonierbale, M.W.; Yencho, G.C.; Pineda, O.; Tingey, W.M.; Van den Berg, J.; Ewing, E.E.; Brodie, B.B. 1994: Potato improvement by traditional breeding and opportunities for new technologies. In: Belknap, W.R.; Vayda, M.E.; Park,

- W.D. *ed.* The Molecular and Cellular Biology of the Potato. CAB International, Wallingford, UK. Pp. 1-20.
- Raman, K.V.; Palacios, M. 1982: Screening potato for resistance to potato tuberworm. *Entomol. Soc. Amer.* 75: 47-49.
- Siddig, S.A. 1988: Cultural means of controlling potato tuber moth (*Phthorimaea operculella* (Zell)) and improvement of potato yield and quality in the Sudan. *Acta Hort./Trop. Hort.* 218 / XII: 281-288.
- Takla, M.F.G; Davidson, M.M.; Reader, J.K; Conner, A.J.; Wratten, S.D. 1999: Transformation of potato cultivars with a *cry1Ac* gene for improved resistance to potato tuber moth. *Forestry Research Bulletin* 213: 27.