Research Note

Enhancement of Resistance to Potato Leafroll Virus Multiplication in Potato by Combining the Effects of Host Genes and Transgenes

H. Barker, K. D. Webster, C. A. Jolly, B. Reavy, A. Kumar, and M. A. Mayo

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, United Kingdom
Received 20 December 1993. Accepted 21 March 1994.

Four potato clones with host gene–mediated resistance to potato leafroll virus (PLRV) multiplication were transformed with the PLRV coat protein (CP) gene. Plants of lines expressing high levels of transcript were highly resistant to PLRV multiplication; virus concentration was only 20–40 ng/g of leaf, which is approximately 1% of the concentration reached in susceptible cultivars. The effects of the transgenic and host-derived resistance genes appear to be additive.

Additional keywords: host gene resistance, transformation, transgenic resistance

Transformation with DNA which encodes a virus coat protein (CP) can result in transgenic plants that are resistant to infection by that virus (reviewed by Beachy *et al.* [1990]). This "coat protein–mediated resistance" (CP-MR) is effective against many viruses, including potato leafroll luteovirus (PLRV), an aphid-borne virus which causes a damaging disease in potato (Kawchuk *et al.* 1990, 1991; van der Wilk *et al.* 1991; Barker *et al.* 1992). CP-MR to PLRV induces some resistance to virus multiplication, but not immunity. Nevertheless, this resistance is useful because it is expressed throughout the life of an infected plant, even in plants with secondary (tuber-borne) infection, which are potent sources of inoculum for further spread of the virus (Barker *et al.* 1992).

Host gene–mediated resistance (host-MR) to PLRV multiplication has been found in a number of potato clones (Barker and Harrison 1985). Green peach aphids (*Myzus persicae*), the most important vector of PLRV, acquire little PLRV from infected plants with this type of resistance, which greatly diminishes aphid-borne spread of virus (Barker and Harrison 1986; Barker and Woodford 1992). This form of resistance to PLRV multiplication is being exploited in the Scottish Crop Research Institute (SCRI) breeding program, but it is unlikely to eliminate virus spread in environments in which inoculum pressure is high (Solomon-Blackburn and Barker 1993).

The PLRV concentrations in infected leaves of some commonly grown susceptible cultivars are compared in Table 1 with those reached in clones of *Solanum tuberosum* with CP-MR or various forms of host-MR and in *S. brevidens,* a highly resistant wild potato species (Jones 1979; Valkonen 1992). One strategy for improving resistance could be to diminish PLRV multiplication still further by combining CP-MR with host-MR. This could be a cheaper and more rapid method than attempting to combine sources of host-MR. In this paper we report that a combination of CP-MR and host-MR can give a greater level of resistance to PLRV than either form does alone.

Petiole and stem pieces of *S. tuberosum* cv. Pentland Crown and SCRI clones G8107(1), G7445(1), and G7032(5) were transformed as described by Barker *et al.* (1992). The vector contained the PLRV CP gene with 111 nucleotides of 5′ untranslated leader and 227 nucleotides of CP-readthrough protein coding sequence under the transcriptional regulation of a cauliflower mosaic virus 35*S* promoter. Each independent transformed line was propagated *in vitro* before well-rooted plantlets were transferred to potting compost and grown in an aphid-proof glasshouse at 20° C.

Table 1. Effect of host genes and transgenes on accumulation of potato leafroll virus (PLRV) in potato genotypes

Potato genotype	Mean PLRV titer (ng/g of leaf)[a]	References[b]
Susceptible clones		
Désirée	3,200	3, 5
Maris Piper	2,400	2, 4, 7, 8
Pentland Squire	980	3, 5
Resistant clones		
Désirée transgenic line B1	415	3, 5
Pentland Squire transgenic line C4	330	3, 5
Pentland Crown	265	4, 5, 7, 9
G7032(5)	175	4, 5, 7–9
G7445(1)	165	4, 5, 7–9
G8107(1)	120	4, 5, 7, 9
Solanum brevidens	20[c]	1, 6

[a] PLRV titer measured in fully expanded leaves of plants with secondary infection. Figures are the means of estimates given in the references.

[b] 1, Barker 1988; 2, Barker and Solomon 1990; 3, Barker *et al.* 1992; 4, Barker and Woodford 1992; 5, Derrick and Barker 1992; 6, Valkonen 1992; 7, Solomon-Blackburn and Barker 1993; 8, Barker *et al.*, in press; 9, this study.

[c] Level of PLRV accumulation in *S. brevidens* varies in different accessions, over a range of 4–40 ng/g of leaf.

MPMI Vol. 7, No. 4, 1994, pp. 528-530
©1994 The American Phytopathological Society

In previous studies, we found that the lines of transformed potato or tobacco most resistant to PLRV were usually those that produced the most CP gene transcript (Barker *et al.* 1992, 1993). Therefore, we compared the relative amounts of CP gene transcript produced in these lines and those in line C4 of potato and line F4 of tobacco, which produce substantial amounts of transcript and are highly resistant to PLRV multiplication (Barker *et al.* 1992, 1993), by Northern blotting as described by Barker *et al.* (1993). CP gene transcript was detected in 26 of 32 lines of G8107(1), 30 of 31 lines of G7032(5), 24 of 33 lines of G7445(1), and 21 of 30 lines of Pentland Crown. Potato lines which produced as much or more transcript than lines C4 and F4 were tested for virus resistance.

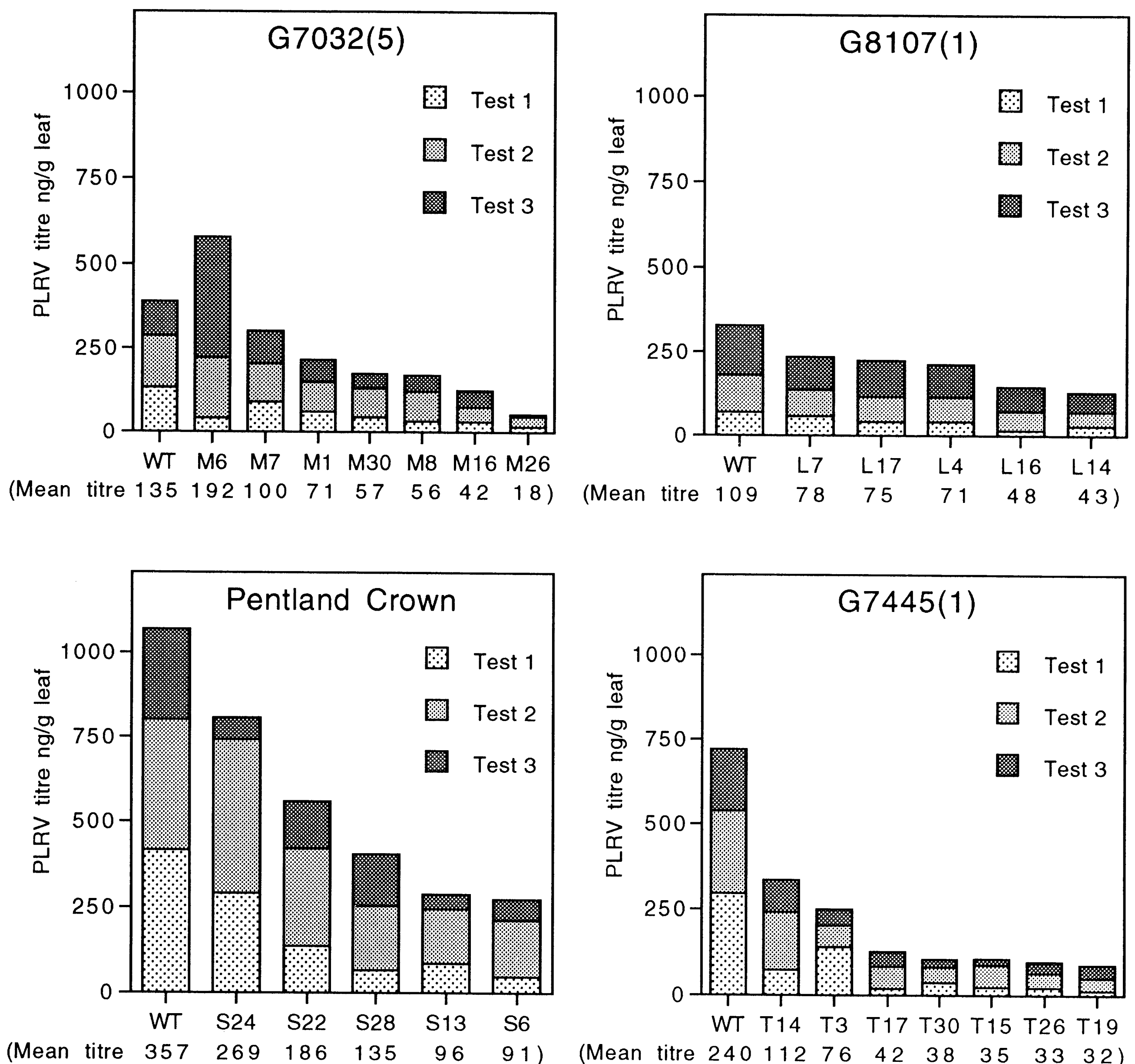

Fig. 1. Accumulation of potato leafroll virus (PLRV) in plants of transgenic lines of potato clones G7032(5), G8107(1), Pentland Crown, and G7445(1) and in nontransformed control plants (WT) of the four clones. The height of the bar represents the cumulative estimate of PLRV concentration in three tests made at 7- to 9-day intervals. The virus concentrations in each test are the means estimated from all the plants of that line. Every plant was infected. An analysis of variance of the data of virus titer estimates made on leaves from individual plants gave least significant differences (5%) of 76.1, 30.2, 139.0, and 63.0 for G7032(5), G8107(1), Pentland Crown and G7445(1), respectively. PLRV-containing plants were grown from infected tubers obtained from three plantlets of each transformed line, which were graft-inoculated with scions from PLRV-infected plants of the cultivar Maris Piper as described by Barker and Harrison (1985). Daughter tubers obtained from the infected primary transformants were retained and, after storage at 4° C for about 7 months, were used to grow plants with secondary infection. Between three and six infected plants of each transformed line and six plants of the nontransformed control parent clones were grown together with an equal number of virus-free plants of each line. Samples comprised leaf tissue from two or three fully expanded leaves per plant, taken from approximately halfway up the stem on three occasions at 7- to 9-day intervals during plant growth. The concentration of PLRV in leaves was estimated in nanograms of PLRV per gram of leaf (fresh weight) by comparing samples with known concentrations of purified virus particles added to extracts of virus-free leaves taken from the nontransformed control plants.

When PLRV titers were estimated in infected leaves of CP-transgenic lines of clones with host-MR at intervals during plant growth, several lines always contained substantially less virus than nontransformed control plants (Fig. 1), although no lines were immune. Only in line M6 was the PLRV titer greater than in control plants. An analysis of variance of the virus titer estimates in individual plants showed that many lines contained significantly less virus than the nontransformed control plants; in some transgenic lines this reduction was up to eightfold (Fig. 1). Although many transgenic lines were significantly more resistant to PLRV multiplication than the nontransformed parent clones, the transgene was more effective in some clones than others (e.g., compare G8107(1) and G7445(1) in Fig. 1). Further decrease in PLRV titer may be possible, but more extensive tests with a larger set of transformed lines would be required to determine if this is so.

PLRV coat protein was not detected by enzyme-linked immunosorbent assay in virus-free plants of any of the transgenic lines. The tests were able to detect 0.5 ng of PLRV CP per gram of leaf tissue. We have only detected PLRV CP occasionally and with difficulty in transgenic lines from our previous transformations (Barker *et al.* 1992, 1993).

Most commonly grown potato cultivars are susceptible to PLRV and accumulate large concentrations of PLRV, in contrast to a few cultivars and SCRI breeding clones which are partially resistant to multiplication (Table 1). In a field trial, Barker and Woodford (1992) found that there was less aphid-borne spread of PLRV from infected potato clones with host-MR than from susceptible clones, probably because aphid vectors were unable to acquire sufficient virus for efficient transmission (Barker and Harrison 1986). We have assessed PLRV spread from two CP-MR potato lines, obtained by Barker *et al.* (1992), in a small trial in a screenhouse. PLRV spread from lines B1 and C10 to 7% and none, respectively, of the neighboring test plants but spread to 22 and 11%, respectively, of nontransformed plants. The extra reduction in PLRV titer in the CP-transgenic lines we describe here may be sufficient to eliminate the spread of aphid-borne PLRV under field conditions.

Our results clearly demonstrate that if potato clones with host-MR are transformed with the PLRV CP gene, the extent of PLRV multiplication in secondarily infected plants of some transgenic lines is substantially decreased. Thus, in infected plants of the most resistant lines described in this paper containing both CP-MR and host-MR, the PLRV titer was approximately 1% of that in susceptible clones such as Maris Piper (Table 1). This concentration of PLRV is similar to that estimated to accumulate in leaves of the highly resistant *S. brevidens* (Barker 1988; Valkonen 1992).

This is the first report of combining resistances mediated by viral transgenes and host genes in potato. The success of this work should encourage other efforts to improve the resistance of crop plants by using similar combinations of resistance.

ACKNOWLEDGMENTS

This work was financed by a grant from the Overseas Development Administration in conjunction with the International Potato Centre, Lima, Peru, and by the Scottish Office Agriculture and Fisheries Department (SOAFD). The work was carried out under license GM/7/1990 issued by SOAFD in accordance with current legislation.

LITERATURE CITED

Barker, H. 1988. Restriction of potato leafroll luteovirus (PLRV) multiplication in *Solanum brevidens*. Page 187 in: Annu. Rep. Scottish Crop Res. Inst. 1987.

Barker, H., and Harrison, B. D. 1985. Restricted multiplication of potato leafroll virus in resistant potato genotypes. Ann. Appl. Biol. 107:205-212.

Barker, H., and Harrison, B. D. 1986. Restricted distribution of potato leafroll virus antigen in resistant potato genotypes and its effect on transmission of the virus by aphids. Ann. Appl. Biol. 109:595-604.

Barker, H., and Solomon, R. M. 1990. Evidence of simple genetic control in potato of ability to restrict potato leaf roll virus concentration in leaves. Theor. Appl. Genet. 80:188-192.

Barker, H., and Woodford, J. A. T. 1992. Spread of potato leafroll virus is decreased from plants of potato clones in which virus accumulation is restricted. Ann. Appl. Biol. 121:345-354.

Barker, H., Reavy, B., Kumar, A., Webster, K. D. , and Mayo, M. A. 1992. Restricted virus multiplication in potatoes transformed with the coat protein gene of potato leafroll luteovirus: Similarities with a type of host gene–mediated resistance. Ann. Appl. Biol. 120:55-64.

Barker, H., Reavy, B., Webster, K. D., Jolly, C. A., Kumar, A., and Mayo, M. A. 1993. Relationship between transcript production and virus resistance in transgenic tobacco expressing the potato leafroll virus coat protein gene. Plant Cell Rep. 13:54-58.

Barker, H., Solomon-Blackburn, R. M., McNicol J. W., and Bradshaw, J. E. 1994. Further evidence that resistance to potato leaf roll virus multiplication is under major gene control. Theor. Appl. Genet. (In press.)

Beachy, R. N., Loesch-Fries, S., and Tumer, N. E. 1990. Coat-protein mediated resistance against virus infection. Annu. Rev. Phytopathol. 28:451-474.

Derrick, P. M., and Barker, H. 1992. The restricted distribution of potato leafroll luteovirus antigen in potato plants with transgenic resistance resembles that in clones with one type of host gene–mediated resistance. Ann. Appl. Biol. 120: 451-457.

Jones, R. A. C. 1979. Resistance to potato leaf roll virus in *Solanum brevidens*. Potato Res. 22:149-152.

Kawchuk, L. M., Martin, R. R., and McPherson, J. 1990. Resistance in transgenic potato expressing the potato leafroll virus coat protein gene. Mol. Plant-Microbe Interact. 3:301-307.

Kawchuk, L. M., Martin, R. R., and McPherson, J. 1991. Sense and antisense RNA-mediated resistance to potato leafroll virus in Russet Burbank potato plants. Mol. Plant-Microbe Interact. 4:247-253.

Solomon-Blackburn, R. M., and Barker, H. 1993. Resistance to potato leafroll luteovirus can be greatly improved by combining two independent types of heritable resistance. Ann. Appl. Biol. 122:329-336.

Valkonen, J. P. T. 1992. Characterization of virus resistance in *Solanum brevidens* Phil. Ph.D. thesis, University of Helsinki, Helsinki, Finland.

van der Wilk, F., Posthumus-Lutke Willink, D., Huisman, M. J., Huttinga, H., and Goldbach, R. 1991. Expression of the potato leafroll luteovirus coat protein gene in transgenic potato plants inhibits viral infection. Plant Mol. Biol. 17:431-439.