MOLECULAR PLANT-MICROBE INTERACTIONS
Vol. 4, No. 3, pp. 247-253, 1991

Sense and Antisense RNA-Mediated Resistance to Potato Leafroll Virus in Russet Burbank Potato Plants

L. M. Kawchuk,[1] R. R. Martin,[1] and J. McPherson[2]

[1]Research Station, Agriculture Canada, Vancouver, British Columbia V6T 1X2, and [2]Department of Plant Science, University of British Columbia, Vancouver, British Columbia V6T 2A2, Canada.
Received 14 November 1990. Accepted 6 January 1991.

Three constructs of the potato leafroll virus (PLRV) coat protein gene were efficiently inserted into the commercial potato cultivar Russet Burbank via *Agrobacterium tumefaciens*-mediated gene transfer. One construct possessed 12 nucleotides of the untranslated leader sequence 5′ to the coat protein gene AUG, the second construct had 192 nucleotides from this leader sequence, and the third construct was the same as the second construct except that it was inserted in the reverse orientation. Introduced as chimeric genes under the control of the duplicated cauliflower mosaic virus 35S promoter, both positive- and negative-sense constructs showed high levels of transcription but coat protein was not detectable in any of the plants. Results show that significant levels of sustained resistance were obtained with each construct, resulting in PLRV titers as low as 1% of the level observed in controls. Both the pattern and level of protection were the same for constructs producing positive- and negative-sense RNA, suggesting a similar mechanism of resistance. Aphid transmission of virus from PLRV-inoculated transgenic Russet Burbank potato plants was reduced significantly when PLRV titers were reduced by at least 95%.

Additional keywords: luteovirus, *Solanum tuberosum*, genetically engineered resistance.

Genetically engineered resistance to a plant virus was first reported by Powell Abel *et al.* (1986), who observed a delay in symptom development when transgenic tobacco plants expressing the tobacco mosaic virus (TMV) coat protein gene were inoculated with TMV. Subsequently, it was shown that the TMV resistance was coat protein-mediated (Powell *et al.* 1990) and that the coat protein gene inhibited virus uncoating (Register and Beachy 1988). Resistance to other viruses has also been observed in transgenic plants expressing a specific coat protein gene, but different characteristics of resistance have been reported. Resistance to TMV was reduced in transgenic plants when lower coat protein levels were expressed (Beachy *et al.* 1990), whereas for potato virus Y the greatest protection occurred in plants with the lowest coat protein levels (Lawson *et al.* 1990). Resistance both to inoculation with RNA and with virus has been shown for potato virus X (PVX) (Hemenway *et al.* 1988) and potato virus S (MacKenzie and Tremaine 1990) but did not occur in transgenic plants resistant to TMV (Nelson *et al.* 1987), alfalfa mosaic virus (Loesch-Fries *et al.* 1987; Van Dun *et al.* 1987), or tobacco streak virus (Van Dun *et al.* 1988). In some cases, such as plants expressing cucumber mosaic virus (CMV) (Cuozzo *et al.* 1988) or soybean mosaic virus (Stark and Beachy 1989) coat protein, resistance to virus was not dependent on the level of virus inoculum. Recently, we have shown that insertion of the potato leafroll virus (PLRV, a member of the luteovirus group) coat protein gene into *Solanum tuberosum* L. cv. Desiree conferred sustained resistance to PLRV that was independent of the level of virus inoculum (Kawchuk *et al.* 1990a).

Expression of negative-sense RNA in transgenic plants has been effective in reducing polygalacturonase activity (Smith *et al.* 1988), chalcone synthase expression (van der Krol *et al.* 1988), and ethylene synthesis (Hamilton *et al.* 1990). Investigations to effect resistance to specific virus by expression of a negative-sense coat protein gene transcript have shown that for expression of PVX (Hemenway *et al.* 1988) or CMV (Cuozzo *et al.* 1988), protection occurred but only at low virus inoculum levels. Unlike PLRV, which occurs at low titer and is confined to the phloem of the host plant, PVX and CMV occur at very high titers. We used a negative-sense construct of the PLRV coat protein gene to determine if the negative-sense transcript would be more effective in providing resistance to a slowly accumulating low titer virus. Here we report that positive- and negative-sense constructs of the PLRV coat protein gene are equally effective in mediating PLRV resistance. A preliminary report of these findings has been published previously (Kawchuk *et al.* 1990b).

MATERIALS AND METHODS

The PLRV coat protein gene. DNA fragments encoding PLRV coat protein were excised from a cDNA clone of PLRV that was characterized previously (Kawchuk *et al.* 1989). Fragments were gel-purified and inserted into the intermediate expression vector pCDX1 (Kay *et al.* 1987), which possesses a duplicated cauliflower mosaic virus (CaMV) 35S promoter-enhancer. The orientation of insertion of each fragment was confirmed by restriction analysis. One construct (LCP12) possessed 12 nucleotides of the untranslated virus leader sequence 5′ to the coat protein gene AUG;

Address correspondence to R. R. Martin: Research Station, Agriculture Canada, 6660 N.W. Marine Drive, Vancouver, British Columbia V6T 1X2, Canada.

Permanent address of L. M. Kawchuk: Research Station, Agriculture Canada, P.O. Box 3000, Main, Lethbridge, Alberta T1J 4B1, Canada.

this construct was previously referred to as LCP31 (Kawchuk *et al.* 1990a). The second construct (LCP192) had 192 nucleotides of the 5′ leader sequence, and the third construct (LCP192R) was the same as the second construct except that it was inserted in the reverse orientation to produce negative-sense RNA (Fig. 1). Constructs were inserted into the disarmed octopine-type Ti plasmid pTiB6S3-SE of *Agrobacterium tumefaciens* (Smith and Townsend) Conn by homologous recombination as described by Rogers *et al.* (1986).

Russet Burbank transformation. A 1-mm axillary meristem from a greenhouse-grown plant of Russet Burbank was aseptically excised and transferred to an axenic culture. Plants were maintained via stem cuttings, each with one lateral meristem. Propagation was on Murashige and Skoog salts (Murashige and Skoog 1962) containing 30 g/L of sucrose and 0.6% (w/v) agar. Both leaf and internodal stem cuttings from axenic plants were inoculated with recombinant *A. tumefaciens* and cultured *in vitro* by the procedure of De Block (1988).

Transgenic plants that were to be challenged with PLRV were selected on the basis of Southern blot analysis and plant morphology. Transgenic Russet Burbank lines 7A and 7B, which possess the LCP12 construct (RB7A and RB7B, respectively), lines 1 and 25, which contain the LCP192 construct (RB1 and RB25, respectively), and lines 2 and 16B, which possess the LCP192R construct (RB2 and RB16B, respectively), were chosen for inoculation. Thirty plants

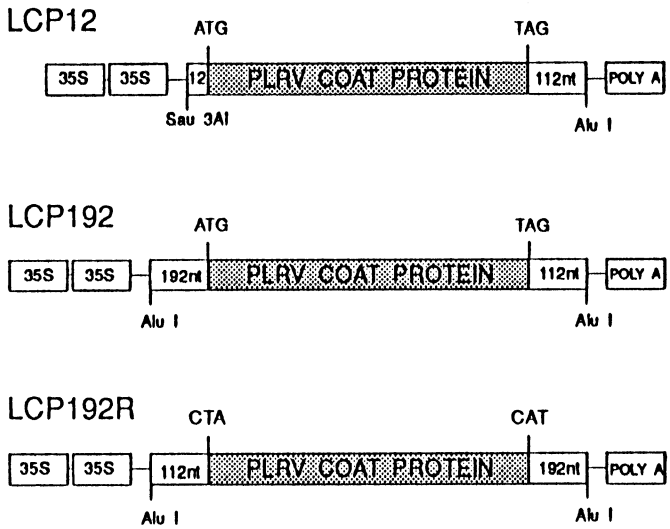

Fig. 1. Diagrammatic representation of the three chimeric genes that were constructed to produce transcripts of the potato leafroll virus (PLRV) coat protein gene. The PLRV cDNA fragment that was used in construct LCP12 consists of a 12-nucleotide (nt) viral leader sequence that precedes the 627-nucleotide coat protein gene open reading frame and the 112 nucleotides occurring beyond the coat protein gene amber codon. The other construct, LCP192, which was also inserted in the reverse orientation to produce negative-sense RNA from construct LCP192R, has 192 nucleotides from the viral leader sequence preceding the coat protein gene. Also shown are the duplicated 35S cauliflower mosaic virus (CaMV) promoter and the nopaline synthase (NOS) polyadenylation signal, which provide the control regions for the expression of the PLRV coat protein gene sequence. Fewer than 50 bases of the polylinker occur between the duplicated CaMV 35S promoter or the NOS sequence and the PLRV sequences.

from each transgenic line and a control consisting of Russet Burbank not transformed by *A. tumefaciens* were propagated in axenic culture by stem cuttings, transferred to 5-inch-diameter pots, and grown in a greenhouse with a 16-hr photoperiod.

Analysis of the PLRV coat protein gene in transgenic plants. Leaves frozen with liquid nitrogen were powdered with a mortar and pestle before suspension in a buffer of 100 mM Tris-Cl, pH 8.0, containing 100 mM NaCl, 10 mM EDTA, 0.2% (w/v) sodium dodecyl sulfate (SDS), and 0.5% (v/v) 2-mercaptoethanol. Nucleic acids were extracted twice with equal volumes of phenol and chloroform, then once with chloroform, and precipitated with ethanol containing sodium acetate. The pellet was dissolved in a buffer (10 mM Tris-Cl, pH 8.0, containing 10 mM NaCl and 1 mM EDTA), and RNA was precipitated by the addition of an equal volume of 5 M LiCl. Poly(A)$^+$ RNA was isolated by oligo(dT)-cellulose (Boehringer Mannheim, Laval, Quebec) column chromatography as described by Aviv and Leder (1972).

Southern analysis (Southern 1975) was performed on *Hin*dIII-digested genomic plant DNA (from 100 mg of plant tissue) that was transferred to Nytran membranes (Schleicher and Schuell, Keene, NH) and probed. Isolated plant poly(A)$^+$ RNA for the northern blots was run on a 1% (w/v) agarose gel containing 5 mM methylmercuric hydroxide, transferred to Nytran membranes, and probed according to the membrane manufacturer's instructions. Randomly primed ^{32}P-labeled DNA probes were prepared using a gel-purified *Alu*I restriction fragment of the cDNA clone LP79 (Kawchuk *et al.* 1990a), which contains the coat protein gene open reading frame. These probes, which are specific for the PLRV coat protein gene cistron, were used for probing both the Southern and northern blots.

Total phenol-soluble protein was extracted from plant tissue following the protocol of Van Etten *et al.* (1979). Protein was prepared as described previously (Kawchuk *et al.* 1990a) and assayed by the method of Bradford (1976) using the Bio-Rad (Richmond, CA) protein assay. Protein was resuspended to give a concentration of 15 μg/μl in 125 mM Tris-Cl, pH 6.8, containing 10% (v/v) 2-mercaptoethanol, 4% (w/v) SDS, 20% (v/v) glycerol, and 0.02% (w/v) bromophenol blue. Samples were placed in boiling water for 5 min, and 150 μg per lane was loaded onto a 12.5% SDS-polyacrylamide gel with a 4% stacking gel (Laemmli 1970). After electrophoresis, proteins from the polyacrylamide gels were electro-blotted at 100 V for 2 hr onto an Immobilon-P membrane (Millipore, Bedford, MA). The western blots were blocked, probed with PLRV monoclonal ascitic fluid from hybridoma 371A (Martin and Stace-Smith 1984) diluted 1:5,000, and incubated with 10^6 cpm/ml of ^{125}I chloramine T-labeled (Hunter and Greenwood 1962) goat anti-mouse antiserum as described previously (Kawchuk *et al.* 1990a). Western blots were also probed with PLRV polyclonal antiserum diluted 1:250 and incubated with 10^6 cpm/ml of ^{125}I chloramine T-labeled protein A.

Challenge of transgenic plants with PLRV. Each plant was challenged with PLRV by transferring 50 *Myzus persicae* Sulz. that had been given an acquisition access period of at least 5 days on *Physalis pubescens* L. infected with

PLRV. Approximately 50 viruliferous aphids were placed on various leaves of plants approximately 15 cm in height for an inoculation access period of 5 days. Plants were inspected during the inoculation period to ensure that the aphids were feeding. Leaf samples were collected individually from each plant before inoculation and then every 2 wk during a period of 56 days postinoculation. Inoculated leaves were avoided by taking two leaves, each approximately 1 cm^2, from tissue that had developed following inoculation and were at least 10 cm from the apical growing point. Sampled leaves were unblemished and from the primary stem, except for the last sampling when one leaf from each plant was from a secondary shoot.

Assay for PLRV. Accumulation of PLRV within the transgenic plants was assayed by enzyme-linked immunosorbent assay (ELISA) as described previously (Kawchuk *et al.* 1990a). Leaf samples were homogenized 1:10 (w/v) in phosphate-buffered saline (137 mM NaCl, 1.5 mM KH_2PO_4, 8.1 mM $Na_2HPO_4 \cdot 12H_2O$, 2.7 mM KCl, and 3.1 mM NaN_3, pH 7.4) containing 0.2% (w/v) nonfat dried milk, 0.05% (v/v) Tween 20, and 2% (w/v) polyvinylpyrrolidone (M_r 44,000). From the 30 individual extracts of each line, a 100 μl-aliquot was removed and combined, and a twofold dilution series was prepared using the grinding buffer. PLRV titers were determined for both the dilution series of each line and each of the individual plants. Each microtiter plate had both the negative control, which was uninoculated untransformed Russet Burbank, and the positive control, a twofold dilution series of quantitated purified PLRV in leaf sap from uninoculated Russet Burbank potato plants. These controls that were used at each time point provided an internal standard between testing at different time points. A Titertek Multiscan 8 channel plate reader (Flow Laboratories, McLean, VA) was blanked on a row containing the negative control. Absorbance values ($A_{405\ nm}$) were recorded after overnight incubations at 22° C and reported as virus titers by comparison to a standard curve that was prepared for each individual microtiter plate using purified quantitated virus in healthy plant extract.

Aphid transmission. Two individual leaves from the first eight plants of each Russet Burbank line that had been inoculated using 50 viruliferous aphids were selected as described above and collected 47 days postinoculation. They were placed in a petri plate with a moistened filter paper, and PLRV-free *M. persicae* were given a 3-day acquisition access period on these detached leaves. Four aphids, two from each leaf, were then transferred to healthy *P. pubescens* seedlings approximately 1 month of age and given a 5-day inoculation access period. One month later, ELISA was used to determine if these *P. pubescens* plants were infected with PLRV.

RESULTS

Transformation efficiency. Approximately half of the leaves and internodal stem segments produced calli, and 50% of these produced shoots within 3 wk of being transferred to shooting media. The transgenic plants with the exception of one line (RB1) showed no abnormalities as a result of the inserted PLRV coat protein gene. However, plants of the transgenic line RB1 appeared mottled, were spindly with extensive branching, and had an altered leaf morphology with pinnate leaflets being variably fused.

Southern analysis. Fragments of approximately 1,100 nucleotides (LCP12) or 1,300 nucleotides (LCP192 and LCP192R), which contain the 751 nucleotides and 931 nucleotides, respectively, of the inserted PLRV DNA sequence (Fig. 2), were observed on Southern blots probed with PLRV-specific probes. The remainder of the restriction fragments originated from the pCDX1 plasmid and polylinker.

Transcript analysis. Various levels of poly(A)$^+$ PLRV coat protein transcript were detected in northern blots (Fig. 3) of each of the six transgenic potato lines. Comparison of the signal intensity of known quantities of PLRV RNA to that of the detected transcript indicated that levels of PLRV transcript in the transgenic plants ranged from approximately 1 to 50 ng/μg of total polyadenylated RNA. The LCP12 transcripts were approximately 1,000 nucleotides, which is the expected length if the transcripts have a poly(A)$^+$ tail of approximately 250 nucleotides. The LCP192 and LCP192R transcripts were approximately 1,200 nucleotides long, again indicating a poly(A)$^+$ tail of approximately 250 nucleotides. The level of detected transcript differed significantly between transgenic lines carrying the same positive-sense construct. Differences of 20- to 50-fold were noted between transgenic lines RB7A and RB7B or RB2 and RB16B, respectively. Those transgenic lines carrying the negative-sense construct showed similar levels of transcripts, which were intermediate between the extremes of positive-sense constructs. Levels of transcript showed no direct relationship with the coat protein gene DNA content.

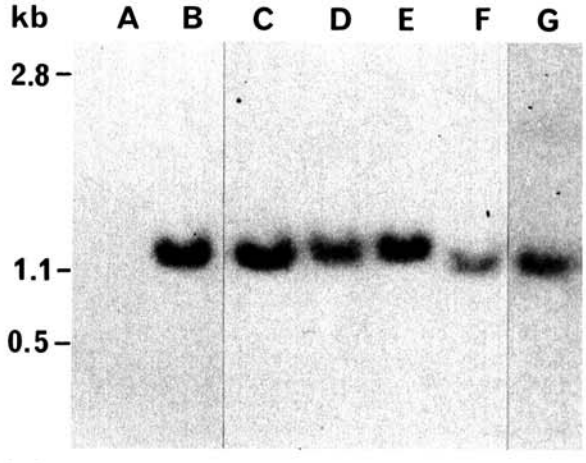

Fig. 2. Southern blot analysis of *Hin*dIII-digested DNA from plants transformed with the potato leafroll virus (PLRV) coat protein gene. The autoradiograph shows a *Hin*dIII fragment of approximately 1,100 base pairs in transgenic potato RB7A and RB7B that were transformed with construct LCP12 (lanes G and F, respectively). The *Hin*dIII fragment was approximately 1,300 base pairs in transgenic potato lines with the LCP192 construct, RB1 and RB25 (lanes E and D, respectively), and in those with the LCP192R construct, RB2 and RB16B (lanes C and B, respectively). No fragment was detected in untransformed Russet Burbank (lane A). The blot was probed with ^{32}P-labeled DNA that was prepared by random priming of a gel-purified *Alu*I fragment containing the PLRV coat protein gene.

Expression of coat protein. Western analysis of the transgenic potato plants indicated undetectable levels of PLRV coat protein in Russet Burbank. The presence of the untranslated leader sequence did not induce detectable changes in the transcription or translation of the coat protein gene. The level of coat protein produced by transgenic plants of Russet Burbank must, therefore, be below 0.01% of the total phenol-soluble leaf protein that was found to be the level of detection in transgenic Desiree (Kawchuk *et al.* 1990a). It had been shown previously (Kawchuk *et al.* 1990a) that the coat protein subunit was not detected by ELISA in transgenic plant extracts presumably because of the ability of the monoclonal antibody to discriminate between assembled virus particles and subunits. Thus, ELISA, as described previously, was used to determine virus levels in plants inoculated with PLRV.

PLRV resistance. Titers of PLRV in aphid-inoculated transgenic plants possessing any one of the three constructs (Fig. 4) were extremely low throughout a growth period of 56 days. At 56 days postinoculation, the average virus titers in the most and least protected transgenic lines, RB7A and RB7B, respectively, were 1 and 8% of the level observed in control plants. The pattern and level of resistance were similar for the different transgenic lines carrying either orientation of the coat protein gene.

An increase in virus titer occurred in all the transgenic lines and untransformed controls between 14 and 28 days postinoculation. However, the increase in the transgenic lines was less than 30% of the level observed in the untransformed controls. The increase in titer that occurred in untransformed controls throughout the growth period became more gradual between 28 and 42 days postinoculation. However, during this period a decrease of virus occurred in each transgenic line except in RB1, which was consistently low. Rapid plant growth occurring during this period may have resulted in the observed lowering of virus titer in the absence or reduction of virus replication. The average titers in the transgenic lines leveled off between 42 and 56 days postinoculation, while the virus titer in the untransformed controls proceeded to rise sharply.

All Russet Burbank untransformed controls became infected and each individual plant accumulated PLRV to high levels, whereas three different responses were observed among plants of each transgenic line. In the transgenic lines, with the exception of RB1, the majority of plants showed a slight increase in virus titer that was followed by a decrease occurring 28 and 42 days postinoculation to lower but still detectable levels. This profile did not occur for RB1 in which extremely low overall levels were maintained throughout. For a small proportion of plants in the transgenic lines, virus was never detected or decreased to undetectable levels.

The primary symptoms of PLRV infection are less severe than secondary symptoms, and greenhouse-propagated

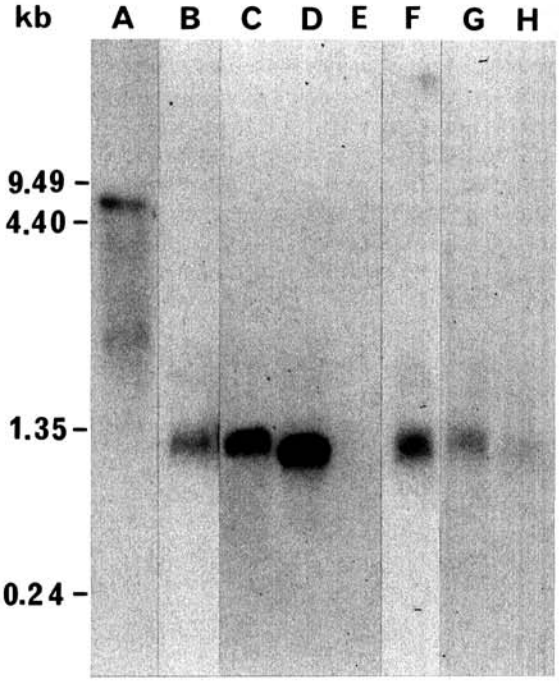

Fig. 3. Northern blot analysis of polyadenylated RNA from plants transformed with the potato leafroll virus (PLRV) coat protein gene. Blots were probed with ^{32}P-labeled DNA that was prepared by random priming of a gel-purified *Alu*I fragment containing the PLRV coat protein gene. A polyadenylated transcript with an estimated length of 1,000 nucleotides was detected in total polyadenylated RNA (0.9 μg) from transformed potato RB7A and RB7B (lanes D and H, respectively), which possess the LCP12 construct. Autoradiograms of total polyadenylated RNA (0.9 μg) from transgenic potato RB1 and RB25 (lanes C and G, respectively), which possess the LCP192 construct, and transgenic potato RB2 and RB16B (lanes B and F, respectively), which contain the LCP192R construct, revealed a polyadenylated transcript with an estimated length of 1,200 nucleotides. No homologous transcript was observed in total polyadenylated RNA (0.9 μg) of untransformed potato plants (lane E). Lengths of transcripts were estimated by comparison with an RNA ladder (not shown). Quantities of transcript were estimated from the signal intensity of 10 ng of purified PLRV RNA of which one sixth would be homologous to the coat protein gene probe (lane A).

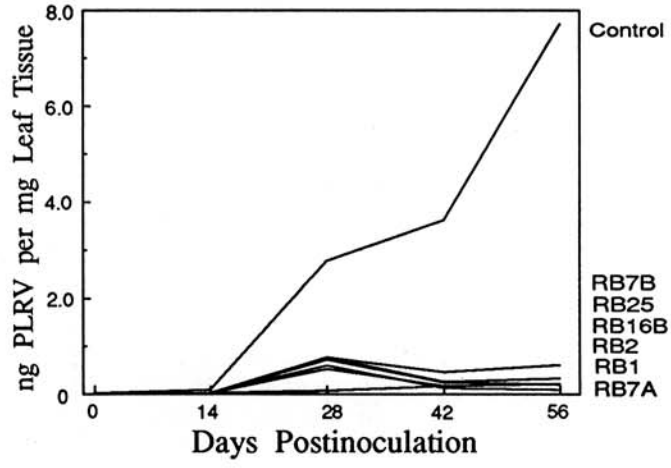

Fig. 4. Average potato leafroll virus (PLRV) titers of combined leaf extracts from 30 Russet Burbank potato plants each inoculated by 50 viruliferous aphids. Virus titers were determined from known quantities of purified PLRV. Leaf extracts from plants not inoculated with PLRV gave background levels of less than 0.03 ng of PLRV per milligram of leaf tissue. All standard deviations were less than 0.4 ng of PLRV per milligram of leaf tissue. Each transgenic Russet Burbank line contained one of the three coat protein constructs: transgenic lines RB7A and RB7B possess the LCP12 construct, RB1 and RB25 contain the LCP192 construct, and RB2 and RB16B have the LCP192R construct.

plants manifested few if any discernible symptoms. Thus, ELISA readings rather than symptoms were used to monitor the progress of the virus infection. Leaf samples that were taken from tissue above the site of inoculation represented postinoculation growth; thus, for detection, the virus must be capable of replication and systemic movement within the transgenic plants.

Some chlorosis and leaf curling were observed in PLRV-infected Russet Burbank untransformed controls and to a lesser degree in some inoculated transgenic individuals. However, these "symptoms" occurred late in the infection when the plants were large and could have been a result of the growing conditions. In any case, these putative symptoms were extremely mild and sporadic, and they could not be reliably scored.

Vector transmission. Aphid transmission of PLRV from inoculated transgenic plants (Table 1) was significantly reduced in transgenic lines in which the virus levels were less than 5% of that in the untransformed controls (transgenic lines RB7A, RB1, and RB2). Lines with higher levels of virus had reduced transmission efficiency but the levels varied considerably. The ability of the vector to transmit the virus from the transgenic plants indicates that the transgenic plants possess viable virus particles and not simply coat protein aggregates.

DISCUSSION

Sustained resistance to PLRV has been achieved in transgenic Russet Burbank potato plants containing high levels of the PLRV coat protein gene transcript in either the positive- or negative-sense orientation. In addition, a significant reduction of virus transmission by *M. persicae* from the transgenic plants occurred when PLRV titers had been reduced by at least 95% of those obtained in control plants. The presence of the untranslated leader sequence of the coat protein gene did not appear to affect the levels of the positive-sense transcript and failed to increase coat protein to detectable levels. In a previous study (Kawchuk *et al.* 1990a) in which the PLRV coat protein gene was expressed in cultivar Desiree, the detection of coat protein at levels of approximately 0.01% of total phenol-soluble protein in the leaves of the transgenic potato plants (this is the limit of detection) was reported. The absence of detectable coat protein in transgenic Russet Burbank leaves may reflect differences between cultivars in translational efficiency, protein stability, or response to extraction procedures. Since we were at the limit of detection for the coat protein, small changes in protein concentration could not be measured. In any event, the presence of the leader sequence did not affect the levels of resistance observed in the transgenic Russet Burbank potato plants.

Resistance toward PLRV differs from that observed with the other plant viruses examined in that it occurs at high levels with the expression of either positive- or negative-sense constructs of the coat protein gene. Negative-sense constructs of PVX (Hemenway *et al.* 1988) and CMV (Cuozzo *et al.* 1988) coat protein genes provided resistance to the respective virus but only at low virus inoculum levels and were much less effective than positive-sense coat protein gene constructs. Unlike these viruses, PLRV transmission occurs by aphid vectors in a persistent manner, virus accumulation occurs slowly, and titers remain low, which may provide a better opportunity for the antisense construct to interfere with viral replication. This combined with the higher activity of the CaMV 35S promoter in phloem cells as compared to epidermal or mesophyll cells may result in the resistance to PLRV with the antisense construct that was not observed with other plant viruses.

The level and pattern of protection for PLRV were similar in transgenic plants expressing either positive- or negative-sense transcripts, suggesting that a similar mechanism of protection may be operating. Positive- and negative-sense transcripts may interfere with virus replication by interacting with the opposite sense RNA strand of the virus. A model for sequestering complementary RNA has been suggested by Palukaitis and Zaitlin (1984) to explain the cross-protection phenomenon that has been observed with viruses, coat protein-defective viruses, and viroids. This would presumably require effective levels of transcription such as those obtained using the duplicated CaMV 35S promoter-enhancer construct (Kay *et al.* 1987). Since the levels of positive- and negative-sense viral RNA presumably differ during the course of PLRV infection, it appears that the resistance produced in the transgenic plants occurs at an early stage when both positive and negative RNA strands of the virus are at low levels. Such a situation would produce similar patterns of effective resistance for positive- and negative-sense constructs in transgenic plants. Possible competition by the expressed RNA for essential viral or host molecules, such as the replicase, may also affect the infection process. Transgenic plants that expressed RNA sequences complementary to those encoding the TMV coat protein with the 3′ untranslated region, which includes a putative replicase binding site, were protected from infection by TMV at low levels of inoculum (Powell *et al.* 1989).

Aphid transmission of PLRV from the transgenic Russet Burbank potato plants was considerably reduced when PLRV titers were less than 5% of those in the untransformed controls. Previous work had shown that a single aphid when given a sufficient acquisition and inoculation access

Table 1. Transmission of potato leafroll virus (PLRV) by four *Myzus persicae* from Russet Burbank potato plants previously inoculated with PLRV using approximately 50 viruliferous aphids

Line	Construct[a]	PLRV titers[b]	Transmission efficiency[c]
RB7A	LCP12	3.7	12
RB7B	LCP12	12.8	50
RB1	LCP192	4.8	0
RB25	LCP192	7.3	37
RB2	LCP192R	5.0	25
RB16B	LCP192R	6.8	75
Control	Untransformed	100.0	88

[a] Depicted in Figure 1.
[b] Titers are given as a percentage of the titer in the control plants. Average PLRV titers were determined 42 days postinoculation for the 30 transgenic plants of each line (Fig. 4), and these averages were used to calculate the percentage of virus in each line as compared to the control plants.
[c] Percentage of eight *Physalis pubescens* that were infected with PLRV following inoculation using *M. persicae*. Four aphids from each transgenic line were transferred to a single *P. pubescens* plant after they had an acquisition access period of 3 days. The transmission efficiency was determined 47 days postinoculation.

period on untransformed infected potato would not necessarily transmit the virus (Kawchuk *et al.* 1990a). Thus, significantly reduced virus titer in the transgenic plants would be expected to lower the frequency of transmission even further. This should affect the epidemiology of PLRV by reducing virus transmission within a field and the spread to other fields without the use of insecticides. Since Russet Burbank is the major potato cultivar grown in North America, genetic resistance to PLRV would have a substantial commercial impact.

There may be advantages in using the negative-sense transcript instead of the coat protein to achieve viral resistance. Since primary symptoms of PLRV are difficult to detect in a greenhouse, field trials will be required to determine whether the presence of the coat protein produces any symptoms of the potato leafroll disease in the absence of virus infection. Plants with the positive-sense constructs may also commit molecules and energy to the translation of the positive-sense viral transcripts. The possibility of transencapsidation, which has been shown to occur with luteovirus coat proteins (Waterhouse *et al.* 1988), may prove undesirable in transgenic plants. Such a phenomenon would not occur in transgenic plants that are protected by negative-sense RNA and would presumably give them an advantage.

This is the first report of high levels of antisense RNA-mediated resistance toward a plant virus. In addition, it has also been shown that a significant reduction in transmission efficiency by the vector, *M. persicae*, from transgenic plants occurred when virus titers were greatly reduced. For viruses that are transmitted by specific vectors, transmission efficiency is of considerable importance in practical aspects of resistance.

ACKNOWLEDGMENTS

We thank R. Stace-Smith for helpful discussions, Anita Quail for technical assistance, and Wes MacDiarmid for preparation of the photographs.

LITERATURE CITED

Aviv, H., and Leder, P. 1972. Purification of biologically active globin messenger RNA by chromatography on oligothymidylic acid-cellulose. Proc. Natl. Acad. Sci. USA 69:1408-1412.

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

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72:248-254.

Cuozzo, M., O'Connell, K. M., Kaniewski, W., Fang, R.-X., Chua, N.-H., and Tumer, N. E. 1988. Viral protection in transgenic tobacco plants expressing the cucumber mosaic virus coat protein or its antisense RNA. Bio/Technology 6:549-557.

De Block, M. 1988. Genotype-independent leaf disc transformation of potato (*Solanum tuberosum*) using *Agrobacterium tumefaciens*. Theor. Appl. Genet. 76:767-774.

Hamilton, A. J., Lycett, G. W., and Grierson, D. 1990. Antisense gene that inhibits synthesis of the hormone ethylene in transgenic plants. Nature (London) 346:284-287.

Hemenway, C., Fang, R.-X., Kaniewski, W. K., Chua, N.-H., and Tumer, N. E. 1988. Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. EMBO J. 7:1273-1280.

Hunter, W. M., and Greenwood, F. C. 1962. Preparation of iodine-131 labelled human growth hormone of high specific activity. Nature (London) 194:495-496.

Kawchuk, L. M., Martin, R. R., Rochon, D. M., and McPherson, J. 1989. Identification and characterization of the potato leafroll virus putative coat protein gene. J. Gen. Virol. 70:783-788.

Kawchuk, L. M., Martin, R. R., and McPherson, J. 1990a. 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. 1990b. Resistance in transgenic potato expressing the potato leafroll luteovirus coat protein gene. (Abstr.) Phytopathology 80:1035.

Kay, R., Chan, A., Daly, M., and McPherson, J. 1987. Duplication of CaMV 35S promoter sequences creates a strong enhancer for plant genes. Science 236:1299-1302.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) 227:680-685.

Lawson, C., Kaniewski, W., Haley, L., Rozman, R., Newell, C., Sanders, P., and Tumer, N. E. 1990. Engineering resistance to mixed virus infection in a commercial potato cultivar: Resistance to potato virus X and potato virus Y in transgenic Russet Burbank. Bio/Technology 8:127-134.

Loesch-Fries, L. S., Merlo, D., Zinnen, T., Burhop, L., Hill, K., Krahn, K., Jarvis, N., Nelson, S., and Halk, E. 1987. Expression of alfalfa mosaic virus RNA 4 in transgenic plants confers virus resistance. EMBO J. 6:1845-1851.

MacKenzie, D. J., and Tremaine, J. H. 1990. Transgenic *Nicotiana debneyii* expressing viral coat protein are resistant to potato virus S infection. J. Gen. Virol. 71:2167-2170.

Martin, R. R., and Stace-Smith, R. 1984. Production and characterization of monoclonal antibodies specific to potato leafroll virus. Can. J. Plant Pathol. 6:206-210.

Murashige, T., and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.

Nelson, R. S., Powell Abel, P., and Beachy, R. N. 1987. Lesions and virus accumulation in inoculated transgenic tobacco plants expressing the coat protein gene of tobacco mosaic virus. Virology 158:126-132.

Palukaitis, P., and Zaitlin, M. 1984. A model to explain the "cross-protection" phenomenon shown by plant viruses and viroids. Pages 420-429 in: Plant-Microbe Interactions: Molecular and Genetic Perspectives, Vol. 1. T. Kosuge and E. W. Nester, eds. Macmillan Co., New York.

Powell Abel, P., Nelson, R. S., De, B., Hoffmann, N., Rogers, S. G., Fraley, R. T., and Beachy, R. N. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.

Powell, P. A., Stark, D. M., Sanders, P. R., and Beachy, R. N. 1989. Protection against tobacco mosaic virus in transgenic plants that express tobacco mosaic virus antisense RNA. Proc. Natl. Acad. Sci. USA 86:6949-6952.

Powell, P. A., Sanders, P. R., Tumer, N., Fraley, R. T., and Beachy, R. N. 1990. Protection against tobacco mosaic virus infection in transgenic plants requires accumulation of coat protein rather than coat protein RNA sequences. Virology 175:124-130.

Register, J. C., and Beachy, R. N. 1988. Resistance to TMV in transgenic plants results from interference with an early event in infection. Virology 166:524-532.

Rogers, S. G., Horsch, R. B., and Fraley, R. T. 1986. Gene transfer in plants: Production of transformed plants using Ti plasmid vectors. Methods Enzymol. 118:627-640.

Smith, C. J. S., Watson, C. F., Ray, J., Bird, C. R., Morris, P. C., Schuch, W., and Grierson, D. 1988. Antisense RNA inhibition of polygalacturonase gene expression in transgenic tomatoes. Nature (London) 334:724-726.

Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. 98:503-517.

Stark, D. M., and Beachy, R. N. 1989. Protection against potyvirus infection in transgenic plants: Evidence for broad spectrum resistance. Bio/Technology 7:1257-1262.

van der Krol, A. R., Lenting, P. E., Veenstra, J., van der Meer, I. M., Koes, R. E., Gerats, A. G. M., Mol, J. N. M., and Stuitje, A. R. 1988. An anti-sense chalcone synthase gene in transgenic plants inhibits flower pigmentation. Nature (London) 333:866-869.

Van Dun, C. M. P., Bol, J. F., and Van Vloten-Doting, L. 1987. Expression of alfalfa mosaic virus and tobacco rattle virus coat protein genes in transgenic tobacco plants. Virology 159:299-305.

Van Dun, C. M. P., Overduin, B., Van Vloten-Doting, L., and Bol, J. F. 1988. Transgenic tobacco expressing tobacco streak virus or mutated alfalfa mosaic virus coat protein does not cross-protect against alfalfa mosaic virus infection. Virology 164:383-389.

Van Etten, J. L., Freer, S. N., and McCune, B. K. 1979. Presence of a major (storage?) protein in dormant spores of the fungus *Botryodiplodia theobromae*. J. Bacteriol. 138:650-652.

Waterhouse, P. M., Gildow, F. E., and Johnstone, G. R. 1988. Luteovirus group. No. 339 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, Surrey, England. 9 pp.