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Kathleen L. Hefferon · Houri Khalilian
Mounir G. AbouHaidar

Expression of the PVY^{O} coat protein (CP) under the control of the PVX CP gene leader sequence: protection under greenhouse and field conditions against PVY^{O} and PVY^{N} infection in three potato cultivars

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Abstract Coat protein-mediated resistance (CPMR), resistance conferred as a result of the expression of viral coat proteins in transgenic plants, has been illustrated to be an effective way of protecting plants against several plant viruses. Nonetheless, consistent protection has not been achieved for transgenic plants expressing the coat protein of potato virus Y (PVY), the type member of the potyvirus family. In this report, three different potato cultivars were transformed with a chimeric construct consisting of the capsid protein (CP) coding sequences of PVY flanked by the AUG codon and the translational enhancer from the coat protein gene of potato virus X (PVX). These cultivars were shown to express high levels of PVY CP and confer a high degree of protection against PVY^{o} and PVY^{N} under both greenhouse and field conditions. In addition, transgenic plants infected with potato virus A (PVA), a related potyvirus, exhibited a delay in virus accumulation, which could be easily overcome with increasing virus concentrations.

Key words Potato virus Y · Potato virus X · Transgenic plants · Translational enhancer · CPMR

Introduction

Potyviruses belong to one of the largest plant virus groups and are of considerable economic importance (Robaglia et al. 1989; Riechmann, 1992). The necrotic strain of potato virus Y (PVY^{N}), the type member of the potyviruses, has been responsible for substantial damage to the potato industry in North America over the past few years (McDonald and Kristjannson 1993). Coat protein-mediated resistance (CPMR) has been established as an effective means of protection against viral infection and the prevention of crop loss (for review, see Beachy et al. 1990). A number of groups have used this technology to generate transgenic plants which express the coat protein of PVY^{N}, as well as other potyviral coat protein genes. However, unlike transgenic plant systems previously studied which express other viral coat proteins, it appears that the level of potyviral capsid protein (CP) which accumulates in these transgenic plants bears no relation to the degree of protection that these plants exhibit against infection with similar or related viruses (Lawson et al. 1990; Van der Vlugt et al. 1992; Farinelli et al. 1992; Farinelli and Malnoe 1993).

Since PVY is expressed as a single large polypeptide, it does not contain its own initiation codon and cannot be expressed independently (Riechmann et al. 1992). To circumvent this problem we provided an artificial initiation codon so that PVY coat protein expression could take place.

In this study, transgenic plants derived from the cultivars 'Russet Burbank', 'Shepody' and 'Norchip' were generated by the construction of a PVX/PVY chimeric molecule in which the N-terminus of the PVY coat protein was substituted for a region containing the initiation codon and translational enhancer of the PVX coat protein gene. The correlation between the amount of PVY coat protein expressed in transgenic plants and the protection against the virus under greenhouse and field conditions is presented.

Materials and methods

Virus purification

PVY^{o} (from the American Type Culture Collection), PVY^{N} and PVA (both obtained from infected potato fields grown in New Brunswick, Canada) were passaged and amplified in the systemic host *Nicotiana glutinosa* and *N. tabacum* cv Samsun, respectively. Purified PVY^{N} and PVA were kindly provided by Dr. R.P. Singh, Agriculture Canada, Fredricton, New Brunswick. PVY^{o} was purified using the method of Singh and McDonald (1981).

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K. L. Hefferon · H. Khalilian · M. G. AbouHaidar (✉)
Department of Botany, University of Toronto, Toronto, Ontario, Canada, M5S 3B2

RNA extraction and cDNA synthesis

Viral RNA was extracted and cDNA synthesis carried out as described by Leclerc et al. (1992). cDNAs were subcloned into the *Pst*1 site of pBS+. The coat protein gene of PVY was identified by nucleotide sequence analysis.

Engineering of the PVX/PVY construct and potato transformation

A 1-kbp *Cla*I DNA fragment encoding the coat protein gene was excised from the cDNA PVY-56, gel-purified and inserted into the plasmid pBS+. This cDNA lacked the first four amino acids from the designated cleavage site of PVY coat protein. A 450-bp *Xba*I-*Hin*dIII fragment containing regions of the initiation codon and ribosome binding site of the PVX CP was excised and inserted adjacent to the PVY coat protein gene. The *Sal*I site generated by this ligation was filled in with DNA polymerase I (Klenow fragment) to produce a single open reading frame. This PVX/PVY "chimeric" cDNA construct was inserted into the plant expression vector PGA643 (Fig. 1). The resulting (PVX/Y) clone was transformed into *Agrobacterium tumefaciens* strain LBA4404 by the procedure of An et al. (1989). Potato tuber discs of cvs. 'Shepody', 'Norchip' and 'Russett Bur-bank' were then transformed as described by Horsh et al. (1985) and Xu et al. (1995). Transformants were selected for resistance to kanamycin (100 µg/ml).

Western blot analysis of PVY coat protein in transgenic plants

Protein samples were prepared according to the protocol of Laemmli et al. (1970). Samples were added to loading dye (5% sodium dodecyl sulfate, 30% sucrose and 0.1% bromophenol blue) and loaded onto a 12.5% polyacrylamide gel. Following electrophoresis, proteins were electro-transferred onto nitrocellulose filters and immunoblotted using antibodies generated against the PVY coat protein (generously provided by Dr. R. P. Singh, Agriculture Canada, Fredericton, New Brunswick).

Challenge of transgenic plants with PVY^O, PVY^N, PVX and PVA

Eight to fifteen transgenic potato plants of each line (generated by cuttings) were challenged with 0.5 mg/ml and 5.0 mg/ml of each virus type. Three or four leaves were mechanically inoculated with a virus suspension in 0.1 *M* sodium-phosphate buffer, pH 7.0. Plants were maintained in a growth chamber set for an 8-h dark and a 16-h light photoperiod at a light intensity of 50 $mEm^{-2}\,s^{-1}$. Five leaves were randomly collected weekly from each plant for a period of 8 weeks postinoculation.

Challenge with PVY^O under field conditions

R112, one of the 'Russet Burbank' lines, was planted on a plot of land leased by the Ontario Ministry of Food and Agriculture in Alliston, Ontario. The R112 line was arranged in alternate rows with nontransformed 'Russet Burbank' plants used as controls. Several leaves of each plant were inoculated with 25 µl of purified PVY^o at 0.5 mg/ml when the plants were approximately 15 cm in height. Four to eight young, mature leaves were collected from each plant every week postinoculation, combined together and then tested for virus accumulation for a period of 6 weeks.

Preparation of leaf samples from inoculated plants

Leaf tissue samples (0.15–0.3 g each) were taken from infected or healthy plants and ground in 2 ml extraction buffer containing 0.1 *M* TRIS-HCl, pH 7.5, 1 m*M* DIECA (diethyldithiocarbamic acid), 5 m*M* EDTA and 1% Triton X-100. The suspension was centrifuged at 12 000 *g* for 15 min at 4°C and immediately frozen at –70°C. Samples were thawed at room temperature for 1 h, and 5 ml of each was dotted in a series of twofold dilutions onto a nitrocellulose filter presoaked in 6SSC (1×SSC = 150 mM NaCl, 15 mM sodium citrate, pH 7.0) for 2 min and dried under a heat lamp.

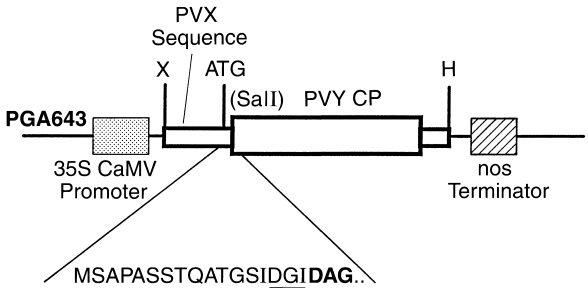

Fig. 1 Schematic representation of plant expression vector PGA643 containing the PVX/Y chimeric construct. A 1-kbp *Cla*I cDNA fragment corresponding to the coat protein gene of PVY was inserted adjacent to a 450-bp cDNA fragment containing the initiation codon and surrounding sequences of the gene encoding the CP of PVX. The amino acid sequence of the N-terminus of the PVX CP fused to the PVY CP is depicted *below*. Amino acids corresponding to the PVY CP are in *bold* type. New non-PVX, non-PVY amino acids are *underlined*. *X Xba*I, *H Hin*dIII, *Sal*I: modified *Sal*I site

Dot blot hybridization

Filters were prehybridized for 4 h in 1 *M* NaCl, 5 m*M* EDTA, 100 m*M* TRIS-HCl buffer, pH 7.5 and 0.1% sodium dodecyl sulfate containing 100 mg/ml Homomix I (100 mg/ml Homomix I: 2 g yeast tRNA, 0.3 *M* KOH, 8.4 g urea in 20 ml total volume). They were then hybridized overnight at 56°C with a denatured cDNA probe corresponding to the coat protein gene of PVY (or PVA). The probe was labelled with [^{32}P]-dATP (specific activity 24 TBq/mmole) by random priming (Feinberg and Vogelstein, 1978). The filters were washed twice for 15 min at room temperature in 6×SSC and 1% SDS, then twice for 30 min at 55°C in 1×SSC, 0.1% SDS and then were dried and exposed to X-ray film (Amersham RPN 30) for 1–3 days.

Results

Engineering of a PVX/PVY chimeric construct

Since the PVY CP is proteolytically cleaved from a larger polyprotein, both an independent translation initiation codon and a ribosome binding site are absent from the corresponding gene. These elements were added by the fusion of a 450-bp fragment containing the AUG codon and upstream sequences of the PVX CP gene to a fragment containing the PVY CP gene. The first 4 amino acids following the cleavage site of the sequences encoding the PVY CP (GNDT) were removed and replaced with the first 14 amino acids of the PVX CP gene (MSAPASSTQATGSI). This chimeric construct was then inserted between the CaMV 35S promoter and the Nos terminator of the plant expression vector PGA643 to generate the clone PVX/Y (Fig. 1).

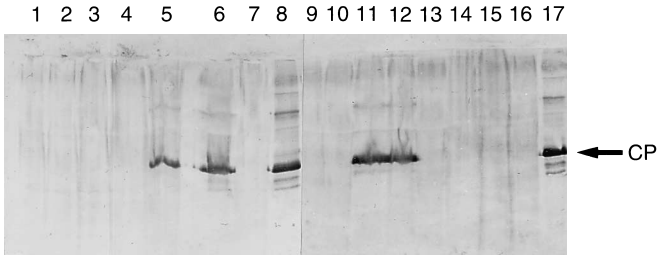

Fig. 2 Western blot depicting protein extracted from leaf tissue of potential transgenic potato plants. PVY coat protein (*CP*) was detected by immunoblotting with PVY-specific antibodies (*arrow*). *Lanes 1* and *9* represent protein extracted from nontransformed plants, *lanes 8* and *17* contain 60 ng of purified PVY CP control. *Other lanes* represent protein extracts of putative transgenic potato cultivars: *lanes 2–5* are derived from 'Norchip', *lanes 6* and *7* from 'Shepody', and *lanes 10–16* from 'Russet Burbank'

Expression of PVY^O coat protein in transgenic potato plants

Fifty putative transgenic plants generated from the transformation of potato vs. 'Shepody', 'Norchip' and 'Russet Burbank' were tested for coat protein expression by Western blot analysis using PVY CP specific antisera. A 30K protein, corresponding in size to the PVY CP used as a positive control, was detected in several plants of each cultivar, as illustrated in Fig. 2. Protein bands reacting with CP antisera were not detected in the nontransgenic plants used as negative controls. The level of coat protein expression was estimated to be 0.1% of the total soluble protein extracted from leaf tissue.

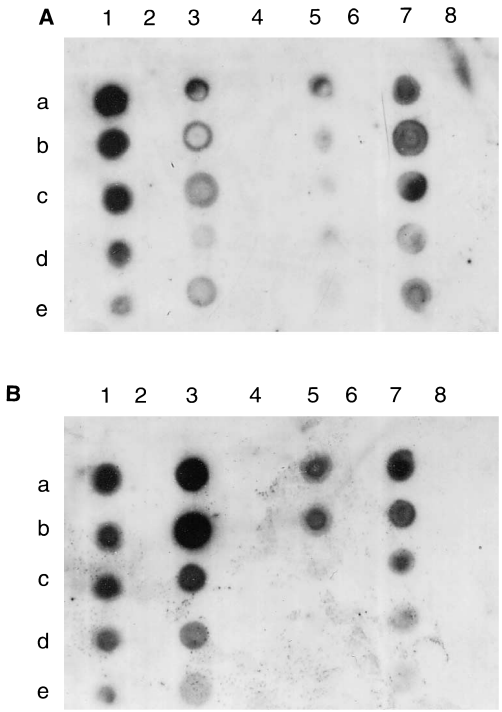

Fig. 3A,B Dot blot hybridization analysis of plants infected with PVY^N and PVY^o. Extracts taken from leaf tissue samples of potato plants inoculated with 5.0 m g/ml PVY^o (**A**) and PVY^N (**B**) 3 weeks postinoculation, dotted onto nitrocellulose filters and hybridized with a [^{32}P]-labelled cDNA probe corresponding to the CP gene of PVY^o. *Lane 1* purified PVY^o at 100 ng (in *a*) *lane 2* noninfected potato, *lane 3* nontransformed line 'Norchip', *lane 4* transgenic line 'Nor1', *lane 5* nontransformed line 'Shepody', *lane 6*: transgenic line She25, *lane 7* nontransformed line 'Russet Burbank'; lane 8: transgenic 'Russet Burbank' R112. All samples consisted of five leaves randomly picked from 8–15 potato plants. Dilution factors were *a*=1:2, *b*=1:4, *c*=1:8, *d*=1:16, *e*=1:32

PVY accumulation in infected transgenic and control plants

Eight to 15 individual plants (generated by cuttings) from each transgenic line of the three cultivars were challenged with either PVY^o or PVY^N by mechanical inoculation with increasing concentrations of each virus (0.5 and 5.0 mg/ml). Nontransformed potato plants infected with virus were used as controls. Samples of leaf tissue were taken from each plant on a weekly basis postinoculation, and the level of virus accumulation was determined by dot blot hybridization analysis over a period of 7 weeks. No virus could be detected in any of the transgenic plant lines tested (Fig. 3), indicating that complete protection took place. High levels of both PVY^o and PVY^N accumulated in the nontransformed plants used as negative controls.

PVX accumulation in infected transgenic plants

To determine whether the PVX sequence introduced in front of the PVY CP sequence played a role in resistance, we challenged 8–15 transgenic plants from each of the three lines R112, She25 and Nor1 with 0.5 mg/ml, 1 mg/ml and 5 mg/ml of PVX. No detectable differences in PVX levels were found in either the transgenic or nontransformed control lines, indicating that these transgenic plants remain susceptible to PVX infection (data not shown).

Resistance of transgenic plants to PVY infection under field conditions

Plants from the transgenic line R112 were tested for resistance to PVY^o infection under field conditions. The level of PVY^o accumulation was determined for a period of 6 weeks. Four to five leaves from each of 18 individual plants were ground together to constitute each sample. Virus accumulated steadily in the nontransgenic control line; however, no virus could be detected in samples taken from the transgenic plant line (Fig. 4A).

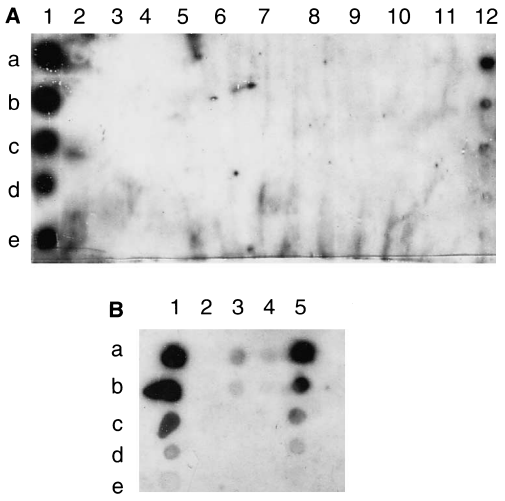

Fig. 4 Dot blot hybridization analysis of plants infected with PVY^{o} in the field and plants infected with PVA in the greenhouse. **A** Extracts were taken from samples of potato plants inoculated with 0.5 mg/ml PVY^{o} 5 weeks postinoculation in the field and spotted onto nitrocellulose filters. Filters were hybridized with a [^{32}P]-labelled cDNA probe corresponding to the CP of PVY^{o}. *Lane 1* purified PVY^{o} [a serial twofold dilution starting with 100 ng (*a*)]; *lanes 2–11* samples from transgenic line R112, *lane 12* nontransformed potato. All samples consisted of virus extracted from five leaves randomly picked from 18 potato plants. **B** Extracts were taken from leaf tissue samples inoculated with 0.5 mg/ml or 5.0 mg/ml PVA 3 weeks postinoculation and maintained under greenhouse conditions. Samples were spotted onto nitrocellulose and hybridized as described above with a cDNA probe corresponding to the CP gene of PVA. *Lane 1* nontransformed potato infected with 0.5 mg/ml PVA, *lane 2* noninfected potato, *lanes 3* and *4* transgenic line R112 infected with 0.5 mg/ml PVA, *lane 5* transgenic line R112 infected with 5.0 mg/ml PVA. All samples were taken from five leaves randomly picked from 10 plants

Delay in accumulation of PVA in transgenic plants

Line R112 was also challenged with increasing concentrations of PVA, a closely related potyvirus. A delay in virus accumulation of approximately 1 week compared to that in nontransformed plants used as controls was observed in transgenic plants infected with 0.5 mg/ml of PVA (Figure 4B). However, when transgenic plants were challenged with a 5.0 mg/ml inoculum of virus, resistance was lost completely (Fig. 4B).

Discussion

The work presented here demonstrates that the PVY CP is expressed in transgenic plants at high levels and that it provides a high degree of protection for the virus concentrations tested. The relative levels of PVY CP found by Western blot analysis represent about 0.1% of total soluble leaf protein. These levels were consistent throughout various plant lines (Fig. 2). Variability in expression among 'Russet Burbank', 'Norchip' and 'Shepody' cultivars was also for practical purposes indistinguishable.

In this study no virus could be detected in the transgenic lines challenged with either PVY^{o} or PVY^{N}, while the level of virus increased steadily in the nontransformed plants used as controls (Fig. 3). This high level of protection was exhibited in all three cultivars of the transgenic potato plants tested. A number of studies comparing the relationship between PVY coat protein expression in transgenic plants and the ability of these plants to protect against viral infection indicated that resistance is greatest in lines where CP levels are low or undetectable (Lawson et al. 1990; Farinelli et al. 1992). Low CP levels are frequently accompanied by proportionately higher transcript levels, implying that resistance is mediated by RNA alone. Recently, Dougherty et al. (1994) demonstrated that certain lines of transgenic plants expressing transcripts corresponding to the tobacco etch virus (TEV) CP gene are totally resistant or able to recover from infection. A working model of resistance was proposed, and states that the accumulation of both challenging viral RNA and transgenic plant viral transcripts activates an RNA-specific degradation pathway particularly for untranslatable RNAs (Dougherty et al. 1994). Further studies using tobacco plants transformed with the replicase gene of PVX have also shown a delayed resistance phenotype upon which a homology-dependent gene silencing mechanism of resistance, involving the degradation of RNA homologous to the transgene, has been proposed (Mueller et al. 1995).

The high level of coat protein expression found in this study could have resulted from the ability of the PVX sequence to initiate expression of the coat protein more efficiently. Transgenic potato plants containing the same upstream PVX sequence have been generated and shown to express the PVX CP at high levels as well (Xu et al. 1995). A 101-nucleotide fragment located immediately upstream of the coat protein of the carlavirus potato virus S (PVS), a relative of the potexviruses, has been demonstrated to possess translational enhancer-like activities (Turner et al. 1994). A translational enhancer has also been identified in the 5′-nontranslated region of PVX (Tomashevskaya et al. 1993; Pugin et al. 1994). Transgenic plants generated by Pugin et al. (1994) were shown to express increased PVY CP and mRNA levels upon addition of a PVX 5′-leader sequence upstream of the initiation codon, whereas plants expressing constructs which lacked the leader sequence did not accumulate CP. It is possible that the PVX sequences upstream of the CP used in the present study play a similar role in enhancing translation.

Since PVY CP was not expressed at detectable levels in transgenic plants used in other studies, it is possible that the replacement of the N-terminal amino acids of the PVY CP (4 amino acids) with those of PVX (14 amino acids) has assisted in maintaining CP stability. Since the PVY CP is highly expressed in our transgenic plants, it is likely that the CP mRNA which contains the PVX sequence at its 5′ end is translatable and presumably capped. Consequently, this mRNA must be stable and is not degraded by the cellular surveillance system proposed by Dougherty

et al. (1994). Recently, Leclerc and AbouHaidar (1995) reported that a truncated form (46 amino acids from the N-terminus) of the capsid protein gene (CP) of potato aucuba mosaic potexvirus (PAMV) loses its ability to protect the plant against the virus. However, a shorter form of the capsid protein gene (86 amino acids deleted from the C-terminus) preserves the ability, like the full-length CP, to provide protection against the virus. Consequently, the resistance mechanism may involve the capsid protein rather than the transgenic mRNA. Sokolova et al. (1994) have demonstrated that transgenic plants containing the PVX 5′-leader sequence and expressing higher levels of PVY mRNA and CP are more sensitive to virus infection than plants which lacked the leader sequence. Further experiments are necessary to determine the precise role of the PVY CP or its mRNA in the resistance process.

Since these transgenic plants contain PVX sequences, they were tested for their ability to protect against infection with PVX. The accumulation of virus in transgenic plants infected with PVX indicated that expression of these sequences in transgenic plants offered no protection against PVX infection (data not shown).

Studies by other groups have indicated that fewer transgenic plant lines retain the ability to confer resistance to PVY infection when tested under field conditions (Hoekema et al. 1989; Kaniewski et al. 1990). However, the study described here demonstrates that when transgenic line R112 was tested for its ability to resist PVY infection under field conditions, protection against PVY^O infection in the R112 line was absolute, as was found under greenhouse conditions (Fig. 4A). It is worth mentioning that the field study was conducted under ideal environmental conditions, with adequate sunshine and rainfall. The results are comparable to those completed with PVX, in which high levels of expression are accompanied by a high level of resistance to the virus (Jongedijk et al. 1992; Xu et al. 1995).

The ability of these transgenic plants to exhibit mild heterologous protection against PVA in the form of a delay in virus accumulation was also demonstrated (Fig. 4B). The PVA CP possesses only 58.5% amino acid sequence identity to PVY^N (Collins et al. 1993). This might explain the slight protection against this virus. Resistance was overcome completely with a tenfold increase in inoculum concentration. It appears, therefore, that these plants can offer a certain degree of protection to related potyviruses but not to unrelated virus groups such as PVX.

Although the precise mechanism by which these transgenic plants confer resistance to potyviral infection remains unclear, the ability of these plants to offer slight protection against a related potyvirus is reminiscent of studies carried out with transgenic plants which express detectable levels of soybean mosaic virus, papaya ringspot virus, zucchini yellow mosaic virus and watermelon mosaic virus II coat protein and exhibit varying degrees of resistance to related potyviruses (Stark and Beachy 1989; Ling et al. 1991; Namba et al. 1992). It is likely that the coat protein itself plays a direct role in mediating resistance against related potyviruses, thereby differing from the RNA-mediated or homology-dependent cellular degradation resistance pathways described earlier. The coat protein may act by disrupting the balance of virus disassembly/reassembly which exists in the cell or by interacting with an unknown ‘receptor’ molecule present in the plant that is necessary for virus infection and/or spread (Dolja et al. 1994; 1995).

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References

An G, Ebert PR, Mitra A, Ha SB (1989) Binary vectors. In: Gelvin SB, Scilperoot RA, Verma DPS (eds) Plant molecular biology manual. Kluver Publ. Dordrecht, The Netherlands. A-3, pp 1–19

Beachy RN, Loesch-Fries S, Tumer NE (1990) Coat protein-mediated resistance against virus infection. Annu Rev Phytopathol 28:451–474

Collins RF, Leclerc D, AbouHaidar MG (1993) Cloning and nucleotide sequence of the capsid protein and the nuclear inclusion protein of potato virus A. Arch Virol 128:135–142

Dolja VV, Haldeman R, Robertson NL, Dougherty WG, Carrington JC (1994) Distinct functions of capsid protein in assembly and movement of tobacco etch potyvirus in plants. EMBO J 13: 1482–1491

Dolja VV, Haldeman-Cahill R, Montgomery AE, Vandenbosch KA, Carrington JC (1995) Capsid protein determinants involved in cell-to-cell movement of tobacco etch potyvirus. Virology 206: 1007–1016

Dougherty WG, Lindbo JA, Smith HA, Parks TD, Swaney S, Proebsting WM (1994) RNA-mediated virus resistance in transgenic plants: exploitation of a cellular pathway possibly involved in RNA degradation. Mol Plant-Microbe Interact 7:544–552

Fang G, Grumet R (1993) Genetic engineering of potyvirus resistance using constructs derived from the zucchini yellow mosaic virus coat protein gene. Mol Plant-Microbe Interact 6:358–367

Farinelli L, Malnoe P (1993) Coat protein gene-mediated resistance to potato virus Y in tobacco: examination of the resistance mechanisms – is the transgenic coat protein required for protection? Mol Plant-Microbe Interact 9:284–292

Farinelli L, Malnoe P, Collet GF (1992) Heterologous encapsidation of potato virus Y strain o (PVY^O) with the transgenic coat protein of PVY strain N (PVY^N) in *Solanum tuberosum* cv ‘Bintje’. Bio/Technology. 10:1020–1025

Feinberg AP, Vogelstein B (1978) A technique for labeling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Hoekema A, Huisman MJ, Molendijk L, van den Elzen PJM, Cornelissen BJC (1989) The genetic engineering of two commercial potato cultivars for resistance to potato virus X. Bio/Technology 7:273–278

Horsh RB, Fry JE, Hoffman NL, Eichholtz D, Rogers SG, Frayley RT (1985) A simple and general method for transferring genes into plants. Science 127:1229–1231

Jongedijk E, Schutter A, Stolte T, van den Elzen PJ, Cornelisson BJC (1992) Increased resistance to potato virus X and preservation of cultivar properties in transgenic potato under field conditions. Bio/Technology 10:422–429

Kaniewski W, Lawson C, Sammons B, Haley L, Hart J, Delanney X, Tumer NE (1990) Field resistance of transgenic ‘Russet Burbank’ potato to effects of infection by potato virus X and potato virus Y. Bio/Technology 8:750–754

Laemmli, UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature 227:680–685

Lawson C, Kaniewski W, Haley L, Rozman R, Newell C, Sanders P, Tumer NE (1990) Engineering resistance to mixed virus infection in a commercial potato cultivar: resistance to potato virus Y in transgenic 'Russet Burbank'. Bio/Technology 8:127–134

Leclerc D, AbouHaidar MG (1995) Transgenic tobacco plants expressing a truncated form of the PAMV capsid protein (CP) gene show CP-mediated resistance to potato aucuba mosaic virus. Mol Plant-Microbe Interact 8:58–65

Leclerc D, Eweida M, Singh RP, AbouHaidar MG (1992) Biotinylated DNA probes for detecting potato virus Y and aucuba mosaic virus in leaves and dormant tubers of potato. Potato Res 35:173–182

Ling K, Namba S, Gonsalves C, Slighton J, Gonsalves D (1991) Protection against detrimental effects of potyvirus infection in transgenic tobacco plants expressing the papaya ringspot virus coat protein gene. Bio/Technology 9:752–758

McDonald JG, Kristjannson GT (1993) Properties of strains of potato virus Y^N in North America. Plant Dis 77:87–89

Mueller E, Gilbert J, Davenport G, Brigneti G, Baulcombe DC (1995) Homology-dependent resistance: transgenic virus resistance in plants related to homology-dependent gene silencing. Plant J 7:1001–1013

Namba S, Ling K, Gonsalves C, Slighton JL, Gonsalves D (1992) Protection of transgenic plants expressing the coat protein gene of watermelon mosaic virus II or zucchini yellow mosaic virus against six potyviruses. Phytopathology 82:940–946

Pugin MM, Sokolova MA, Shul'ga OA, Skryabin KG (1994) Effect of potato virus X 5′-leader on expression of potato virus Y coat protein gene in transgenic *Solanum tuberosum* plants. Mol Biol 28:752–760

Riechmann JL, Lain S, Garcia JA (1992) Review article: Highlights and prospects of potyvirus molecular biology. J Gen Virol 73: 1–16

Robaglia C, Durand-Tardif M, Tronchet M, Boudazin G, Astier-Manifacie S, Casse-Delbart F (1989) Nucleotide sequence of potato virus Y (N strain) genomic RNA. J Gen Virol 70:935–947

Singh RP, McDonald JG (1981) Purification of potato virus Y and its detection in potato by enzyme-linked immunosorbant assay (ELISA). Am Potato J 50:180–186

Sokolova MA, Pugin MM, Shul'ga OA, Skryabin KG (1994) Construction of transgenic potato plants resistant to potato virus Y. Mol Biol 26:646–649

Stark DM, Beachy RN (1989) Protection against potyvirus infection in transgenic plants: evidence for broad spectrum resistance. Bio/Technology 7:1257–1262

Tomashevskaya OL, Solovyev AG, Karpova OV, Fedokin ON, Rodionova NP, Morozov SYU, Atabekov JG (1993) Effects of sequence elements in the potato virus X RNA 5′ non-translated $\alpha\beta$-leader on its translation enhancing activity. J Gen Virol 74:2717–2724

Turner B, Bate N, Twell D, Foster GD (1994) *In vivo* characterization of a translational enhancer upstream from the coat protein open reading frame of potato virus S. Arch Virol 137:123–132

Van der Vlugt RA, Ruiter RK, Goldbach R (1992) Evidence for sense RNA-mediated protection to PVY^N in tobacco plants transformed with the viral coat protein cistron. Plant Mol Biol 20:631–639

Xu H, Khalilian H, Eweida M, AbouHaidar MG (1995) Genetically engineered resistance to potato virus X in four commercial cultivars. Plant Cell Rep 15:91–96