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Short communication

Some coat protein constituents from strawberry latent ringspot virus expressed in transgenic tobacco protect plants against systematic invasion following root inoculation by nematode vectors

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Abstract

The coding sequences in RNA2 for the coat proteins (CP) of strawberry latent ringspot virus (SLRSV) were modified and amplified using polymerase chain amplification reactions (PCR) to facilitate their expression in *Agrobacterium tumefaciens*-transformed *Nicotiana tabacum* Xanthi-nc. The coding sequences for the smaller capsid protein (S, 29kDa) and that for the theoretical precursor of L and S (P, 73kDa) had ATG 'initiation' codon sequences added at the 5′-proximal Ser/Gly (S/G) cleavage site in the unmodified sequence. The sequence coding for the larger of the two proteins of mature SLRSV capsids (L, 44kDa) had an ATG codon added at its 5′ S/G site and a TAG 'stop' codon sequence added at the 3′-proximal S/G site. The P, L and S proteins were expressed *in planta* to a maximum concentration of 0.01% of total extractable proteins but did not assemble into virus-like particles. When challenged by mechanical inoculation with virus particles or viral RNA, and compared with control plants, tobacco plants (primary transgenic clones or S1 and S2, kanamycin-resistant seedlings) expressing the virus capsid subunits separately, or their precursor, decreased the accumulation of SLRSV particles in inoculated leaves and fewer plants became invaded systemically. In experiments in which the roots of seedlings were exposed to SLRSV-carrying vector nematodes (*Xiphinema diversicaudatum*), SLRSV was detected in the roots of non-transformed control tobacco plants (6/20) and in transgenic tobacco expressing the L protein (7/40), but not in any of 25 tobacco plants expressing the S protein or in 35 expressing the P protein. This is the second example of CP-mediated resistance to virus inoculation by nematode vectors.

Introduction

There are numerous reports of pathogen – derived resistance [*sensu* Sanford and Johnston, 1985] based on the expression of virus-derived sequences in plants [for reviews *see* Harrison, 1992; Fitchen and Beachy, 1993]. Such transgenic plants tend to be tolerant of disease and/or resistant to systemic invasion [Cooper and Jones, 1983]. In some systems, correlations have been made between the amounts of expressed protein in transformed plants and the amount of disease amelioration but there has been no consistent relationship [*vis* Stark and Beachy, 1989; Powell *et al.*, 1990; Kaniewski *et al.*, 1990]. In some instances transgenic plants expressing virus-derived genes or products were also protected against vector-borne inoculation [e.g. Van der Wilk *et al.*, 1991, Quemada *et al.*, 1991, Van der Vlugt and Goldbach, 1993] but this is also not a consistent property [cf Lawson *et al.*, 1990]. For example, constituents of tobraviruses

elicited protection against mechanical challenge inoculation but not against inoculation by virus-carrying trichodorid nematodes [Ploeg *et al.*, 1993; MacFarlane and Davies, 1992]. By contrast, when seedlings of transgenic tobacco expressing the capsid protein of arabis mosaic nepovirus (ArMV) were exposed for 8 weeks to field populations of *X. diversicaudatum* (10/100g soil) carrying ArMV [Bertioli *et al.*, 1991, 1992; Cooper *et al.*, 1994] none were infected, whereas a large proportion of nontransformed control seedlings challenged in parallel became systemically invaded. Similar results were obtained when handpicked nematodes from glasshouse cultures were used in laboratory tests (A.T. Jones and D.J.F. Brown, unpublished information).

Strawberry latent ringspot virus (SLRSV), like ArMV, has isometric particles, is transmitted by longidorid nematodes [*Xiphinema diversicaudatum*; Lister, 1964] and has a bipartite genome comprised of two positive sense single-stranded RNA molecules [Murant, 1974]. These and other properties are similar to those of many nepoviruses [Harrison and Murant, 1977]. However, unlike definitive nepoviruses which have particles that contain a single polypeptide of M_r *c.* 54 kDa, particles of SLRSV contain two polypeptides, L and S, of M_r 44 kDa and 29 kDa respectively [Mayo *et al.*, 1974; Gallitelli *et al.*, 1982]. In this property they resemble more closely comoviruses [Bruening, 1978]. Comparative analyses of SLRSV RNA2 sequences (containing the coat protein genes) with those published for the RNA2 of nepoviruses and comoviruses showed insufficient homologies for the unambiguous classification of SLRSV as either a nepovirus or a comovirus [Kreiah *et al.*, 1994; Everett *et al.*, 1994a; Van Wezenbeek *et al.*, 1983; Shanks *et al.*, 1986]. Presently, therefore, SLRSV remains a tentative nepovirus [Harrison and Murant, 1977] in the *Comoviridae* [Mayo and Martelli, 1993].

In vitro translation of the genomic RNA2 of SLRSV yields two polyproteins of *Mr* 99kDa and 96kDa [Hellen *et al.*, 1991]: their cleavage by one or more RNA1-encoded protease(s) releases the precursor to each capsid protein but they are not assembled into virus particles. We were interested to determine the relative efficacy of the different capsid coding sequences as transgenes for resistance to infection by SLRSV. For this purpose we have generated three different genotypes of transgenic tobacco (*Nicotiana tabacum* L. cv. Xanthi-nc) plants expressing either the L or S capsid protein subunits or, their 73kDa precursor (P). Representatives of each transgenic genotype have been screened for reaction to challenge with SLRSV by mechanical inoculation of infective plant sap and viral RNA and by viruliferous *X. diversicaudatum* vector nematodes.

Materials and methods

The SLRSV isolate, originally obtained from strawberry (culture T39; Scottish Crop Research Institute), was propagated in *Chenopodium quinoa* and virus particles purified from infected plants as described by Kreiah *et al.* [1993]. The N- and C-termini of the SLRSV coat protein genes were identified in the sequence of RNA2 [EMBL X77466; Kreiah *et al.*, 1994]. Using polymerase chain amplification (PCR), each coat protein-coding sequence was provided with a 5′ initiation codon: the sequence coding for the large coat protein was further provided with a 3′ stop codon. Four primers were used for the PCR amplification of the three sequences encoding the coat proteins or their precursor; the template was cloned DNA representing the capsid coding sequence. The primers (1–4) contained regions complementary to residues 1399–1428, 2534–2562, 2593–3310 and 3201-3342 of the SLRSV RNA2 sequence, respectively. Each primer also contained a region of non-complementary residues (indicated as capitals below) to facilitate the introduction of a restriction site for cloning (BamHI) followed by an initiation codon (primers 1 + 3) or a stop codon (TAG).

primer 1 5′
GGGGATCCATGgggcttcacgaagagctggttcctgcatcc 3′
primer 2 5′
GGGGATCCCTAtgaagcaactaaagtagttgccggattaa 3′
primer 3 5′
GGGGATCCATGgggggctcctgtagttcaagttggaacttctg 3′
primer 4 5′
GGGGATCCCTAgtaaaagcccatcctgggccagggatattc 3′

Primers 1 + 2, 3 + 4 and 1 + 4 were used to amplify the sequences encoding the L, S or P proteins respectively prior to digestion with BamHI and ligation into the BamHI-cut and dephosphorylated intermediate vector pMJD-86 (provided by Dr M. Dowson-Day of the IPSR Nitrogen Fixation Laboratory, University of Sussex, Brighton, UK). The transformation of *N. tabacum* L. cv. Xanthi-nc using *Agrobacterium tumefaciens* LBA 4404 was substantially as described by Bertioli *et al.* [1991; 1992].

The SLRSV transgenic Xanthi-nc tobacco were examined for virus-like shells using antiserum-coated grids viewed in an electron microscope as described by Bertioli *et al.* [1991].

Experiments to assess the reaction of plants to challenge with SLRSV by mechanical inoculation were done both in Oxford (UK) and in Dundee (UK). Nematode transmission inoculation tests were done in Dundee. For mechanical inoculation of SLRSV transgenic plants in Oxford, the following lines were tested: 6 (a total of 41 clonal replicates) expressing the S subunit, 6 (a total of 34 replicates) expressing the L subunit and 5 (a total of 61 replicates) expressing the precursor (P). The controls used were; 37 non-transformed Xanthi-nc seedlings and 37 lines in total of CP-negative transgenic tobacco plants expressing either an *E. coli*-derived beta-glucuronidase gene [10 lines, GUS; Jefferson *et al.*, 1986], the vector plasmid pBin19 only [6 lines; Bevan, 1984] or, a satellite-RNA from ArMV [21 lines; Liu *et al.*, 1991]. For all experiments in Dundee, a single line from each of the 3 transgenic genotypes was used. The choice of these lines was based on a combination of characters, the most important being, good expression of the respective CP gene, satisfactory plant growth and adequate seed production.

To assess the reaction of plants to the challenge of mechanical inoculation, two leaves were dusted with either carborundum or corundum and inoculated with undiluted sap from SLRSV-infected *Nicotiana clevelandii*. In some instances, plants were also inoculated with purified virus particle preparations or RNA (15μg/ml) from purified virus preparations. To detect virus in challenge inoculated plants, the inoculated and the later-formed uninoculated leaves of the tobacco plants were bioassayed separately in *C. quinoa* plants 2 and 4 weeks after inoculation: tip leaves of all *C. quinoa* plants with virus-like symptoms were tested for SLRSV by enzyme-linked immunosorbent assays (ELISA). ELISA was as described by Edwards and Cooper [1985] using polyvalent rabbit antiserum, supplied by SCRI, and prepared against purified intact virus particles of SLRSV (T39). As the products of transgenes under the control of the 35S promoter from cauliflower mosaic caulimovirus are not uniformly distributed in plants [Hensgens *et al.*, 1992; Nilsson *et al.*, 1992] and, because SLRSV tends to be discontinuously distributed systemically in infected tobacco, assays of challenged plants were made on pooled samples of (uninoculated) leaves from the top, middle and bottom of plants.

For challenge inoculations with viruliferous vector nematodes, the procedures were as described by Brown and Trudgill [1983]. Three-week old seedlings of *N. clevelandii* and/or *Petunia hybrida* in plastic pots of 25 cm^3 were manually inoculated with sap from SLRSV-infected *N. clevelandii* and kept in temperature controlled cabinets at 20 °C. Two days later, groups of 30–40 virus-free *X. diversicaudatum* nematodes from a glasshouse culture maintained under strawberry and raspberry plants at SCRI were added to each pot. After a 4 week acquisition access on these virus source plants, the nematodes were recovered from the pots and counted, and the number of galls induced by nematode feeding on the source plant roots counted. Groups of 5 or 10 hand-picked nematodes were then added to 25 cm^3 pots containing 3–4 week old transgenic or nontransformed seedlings of *N. tabacum* cv. Xanthi-nc. After a 4 week transmission access on these test plants, the nematodes were recovered and counted, the root systems of the plants thoroughly washed in running water, and the number of galls on the roots counted. The roots were then detached, triturated in a mortar and pestle and the sap extract mechanically inoculated to corundum dusted leaves of *C. quinoa*. These indicator plants were observed for 2–3 weeks and all those that developed virus-like symptoms were tested by ELISA for SLRSV.

Results

Total RNA preparations from leaves of tobacco transformants rooted in kanamycin (100 g/ml) hybridised with a ($-^{32}$P)-dATP nick-translated probe specific for the region of SLRSV RNA2 which encoded the precursor to the capsid proteins. As judged by spot intensity in relation to standards, the transcription product occurred at a concentration in the range 1–5 ng/mg of total RNA. Most (70%) kanamycin-resistant transformants, in which the relevant transcripts were detected by RNA dot blot hybridisation or northern transfer, contained one protein of the expected size which reacted with the SLRSV antiserum on Western blots. No protein reacting with the SLRSV antiserum was detected in the other transcript-containing, kanamycin-resistant plants. Product abundance, as judged against a dilution series of standardised purified SLRSV virions, varied to a maximum of *c.* 1 μg/g of fresh leaf which is within the range reported with other virus-derived coding sequences [Beachy *et al.*, 1990]. Nevertheless, ELISA failed to detect SLRSV proteins

Table 1. Response to SLRSV inoculation by groups of viruliferous *Xiphinema diversicaudatum* nematodes of Xanthi-nc tobacco plants expressing the L or S coat proteins of SLRSV or their precursor (P) and non-transformed plants

Experiment no.	Genotype designation	No. nemas added	Mean no. nemas recovered	Mean no. root galls/ plant	No. plants infected/no. inoculated
Experiment 1					
	S3A (S)	nt	nt	nt	nt
	L1A (L)	10	8.4	8.2	0/10
	W3A (P)	10	9.3	6.6	0/10
	untransformed*	10	10	9.4	10/10
Experiment 2					
	S3A (S)	10	8.3	5.3	0/10
	L1A (L)	10	8.0	4.9	5/15
	W3A (P)	10	7.4	5.1	0/10
	untransformed	10	7.1	4.3	3/10
Experiment 3					
	S3A (S)	5	3.7	2.3	0/15
	L1A (L)	5	3.3	2.5	2/15
	W3A (P)	5	3.2	2.7	0/15
	untransformed	5	3.4	2.6	3/10

nt = not tested; * = *Nicotiana clevelandii*

in any of the three different SLRSV-CP transgenic tobacco genotypes and no product was revealed when leaf extracts analysed in SDS-PAGE gels were stained with Coomassie Brilliant Blue. Subsequent tests on products from SLRSV-CP transfected *S. fugiperda* cells used as another source of SLRSV-derived capsid protein, indicated that these proteins were sensitive to constituents of tobacco leaf sap. A similar phenomenon was reported in assays for the CP of potato leaf roll luteovirus in potato plants [Kawchuck *et al.*, 1990; Van der Wilk *et al.*, 1991].

Using antiserum – coated grids, isometric virus-like shells were not seen in foliar extracts from plants expressing any of the P, L or S proteins in circumstances when VLPs were observed in *C. quinoa* or tobacco systemically invaded with SLRSV.

In ELISA, the $A405_{nm}$ values for 12 uninoculated CP-negative tobacco plants ranged between 0.01 and 0.03 and that for 30 SLRSV-infected CP-negative tobacco plants ranged between 0.53 and 3.62 (mean = 2.5). However, the average $A405_{nm}$ values for 14 SLRSV-infected CP+ lines was only 0.5 (range, 0.5–1.20).

Following mechanical inoculation with SLRSV in sap of infected *N. clevelandii* or RNA from purified SLRSV particles at Oxford, UK all transformed and nontransformed tobacco seedling lines were infected in their inoculated leaves. However, in bioassays 4 weeks after mechanical inoculation, the frequency of systemic invasion for plants inoculated with SLRSV in sap was; 39% (16/41) in 6 lines expressing the S protein, 32% (11/34) in 6 lines expressing the L protein, and 24% (15/61) in 5 lines expressing the P protein. By contrast, the frequency of systemic invasion was 70% (26/37) in nontransformed tobacco seedlings, 75% (16/21) in seedlings expressing the sat-RNA of ArMV, 80% (8/10) in seedlings expressing GUS, and 66% (4/6) in seedlings containing the vector plasmid (pBin 19); an overall frequency in CP-negative plants of about twice that of the CP+ plants. For plants inoculated with SLRSV-RNA, the frequency of systemic invasion was, 37% (7/19) for lines expressing the S protein, 42% (5/13) for lines expressing the L protein, and 20% (3/15) in lines expressing the P protein when 54% (7/13) non-transformed plants were systemically invaded.

In similar mechanical inoculation experiments (in Dundee, UK) on a single line of each of the three SLRSV transgenic tobacco, all plants were infected with SLRSV in the inoculated leaves. However, none

of the plants which expressed either the S (0/7) or the P (0/6) proteins were invaded systemically whereas systemic invasion was detected in 2 of 7 of the plants expressing the L protein and in 2 of 4 of the non-transformed control plants. When the same lines of the three transgenic genotypes were challenged with virus-carrying *X. diversicaudatum*, Table 1 shows that over three experiments, SLRSV was detected in only the non-transformed plants (30% of *N. tabacum* Xanthi-nc and 100% of *N. clevelandii*) and in plants expressing the L protein (17.5% overall). In these tests, none of 25 tobacco plants expressing the S protein and none of 35 plants expressing the precursor (P) became infected despite the fact that root galls on these plants indicated that nematodes had fed on them.

Discussion

The 73 kDa protein (P), expressed in tobacco was not proteolytically processed and did not assemble into virus-like particles. In this respect, our findings are similar to those of Nida *et al.* [1992] with the capsid precursor protein (60 kDa) of cowpea mosaic comovirus. Purified preparations of SLRSV [Mayo *et al.*, 1974] and other nepo – and como-viruses typically contain 'empty' virus-like protein shells [e.g. Bertioli *et al.*, 1992; Bruening, 1978]. The absence of such virus-like particles (VLPs) in our transgenic plants indicates possibly that there is a stabilising process before the assembly of SLRSV particles occurs, as is reported for picornaviruses which infect vertebrate animals [cf. Burns *et al.*, 1989; Compton *et al.*, 1990]. However, the location or the abundance of expressed protein might also explain the observation. By controlled cross pollination we have produced seedlings which express both the S and the L proteins but no VLPs have been observed in extracts from such plants. However, the progeny have yet to be rigorously screened to identify individuals in which each of the SLRSV-derived proteins are expressed efficiently in the same cell.

Notwithstanding the modest systemic infectibility of nontransformed Xanthi-nc tobacco with SLRSV, in leaves manually inoculated with SLRSV there was a reproducible difference in virus concentration (as assessed by ELISA) of genotypes expressing the coat proteins L or S or their precursor, P, compared with CP-negative plants. In addition, transgenic plants expressing the S, L, or P proteins of SLRSV were each largely indistinguishable in their infectibility by mechanical inoculation with SLRSV as either virus particles or, viral RNA; a result similar to that observed for plants expressing the CP gene of ArMV [Bertioli *et al.*, 1992], potexviruses [Hemenway *et al.*, 1988] and carlaviruses [MacKenzie and Tremaine, 1990]. However, this contrasts with the observations that CP protection of alfalfa mosaic virus [AlMV; Van Dun *et al.*, 1987] and tobacco mosaic virus are not effective against viral RNA [TMV; Powell *et al.*, 1986]. One likely explanation for this difference in resistance between viruses in these respective taxa is that they differ in the ways in which they invade plants. TMV and AlMV move between adjoining cells as RNA rather than virus particles whereas virions have been implicated in the local dispersal of nepoviruses and comoviruses. In tobacco etch potyvirus, CP has been shown to have separable functions for virion assembly, cell-cell movement and "long distance" systemic invasion [Dolja *et al.*, 1994]. Even though few experiments were done, it is tempting to speculate that the different parts of the SLRSV capsid protein have analagous distinct roles in the movement processes and that these were reflected in the differences in the resistance associated with the S, L, and P proteins.

Our experiments indicate a decreased frequency, or absence, of systemic invasion of SLRSV in CP+ plants compared with CP-negative ones. In those CP+ plants that became systemically invaded, 75% of such plants were invaded 3 weeks later than the CP-negative plants. Furthermore, in the 3 SLRSV transgenic lines examined in nematode transmission studies, this decreased frequency of systemic invasion seemed to provide complete protection against infection with SLRSV via nematode inoculation in plants expressing the S or the P protein, but not the L protein (Table 1). This is the second example of the efficacy of CP-mediated resistance to virus inoculation by a nematode vector; the previous example being collaborative ArMV studies by us [Bertioli *et al.*, 1991; 1992; Cooper *et al.*, 1994; A.T. Jones and D.J.F. Brown, unpublished information].

Our results provide new opportunities to address the underlying mechanisms of SLRSV invasion in plants and offer the possibility of using transgenic protection based on the expression of capsid subunits in commercial crop plants. However, because most of the economically important plants infected with nepoviruses are long-lived woody perennials, such forms of resistance will need to be very durable. Very few serological variants of SLRSV are known in Europe, and therefore there is the prospect that genet-

ically engineered resistance will be suitable. However, it is necessary to determine whether protection is afforded against challenge inoculations by the range of SLRSV isolates occurring in Europe and against a recently described isolate from flowering cherry in New Zealand [Everett *et al.*, 1994a, b].

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References

Beachy RN, Loesch-Fries S and Tumer N (1990) Coat protein mediated resistance against virus infection. Annual Review of Phytopathology 28: 451–474

Bertioli DJ, Cooper JI, Edwards ML and Hawes WS (1992) Arabis mosaic nepovirus coat protein in transgenic tobacco lessens disease severity and virus replication. Annals of Applied Biology 120: 47–54

Bertioli DJ, Harris RD, Edwards ML, Cooper JI and Hawes WS (1991) Transgenic plants and insect cells expressing the coat protein of arabis mosaic virus produce empty virus-like particles. Journal of General Virology 72: 1801–1809

Bevan M (1984) Binary Agrobacterium vectors for plant transformation. Nucleic Acids Research 12: 8711–8721

Brown DJF and Trudgill DJ (1983) Differential transmissibility of arabis mosaic and strains of strawberry latent ringspot viruses by three populations of *Xiphinema diversicaudatum* (Nematoda, Dorylaimida) from Scotland, Italy and France. Revue de Nematologie 6: 229–238

Bruening G (1978) Comovirus group. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses. No. 199, 5 pp

Burns CC, Lawson MA, Semler BL and Ehrenfeld E (1989) Effects of mutations in poliovirus 3Dpol on RNA polymerase activity and on polyprotein cleavage. Journal of General Virology 63: 4866–4874

Compton SR, Nelson B and Kirkegaard K (1990) Temperature-sensitive poliovirus mutant fails to cleave VP40 and accumulates provirions. Journal of General Virology 64: 4067–4075

Cooper JI, Edwards ML, Rosenwasser O and Scott NW (1994) Transgenic resistance genes from nepoviruses: efficacy and other properties. New Zealand Journal of Crop and Horticultural Science 22: 129–137

Cooper JI and Jones AT (1983) Responses of plants to viruses: proposals for the use of terms. Phytopathology 73: 127–128

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

Edwards ML and Cooper JI (1985) Plant virus detection using a new form of ELISA. Journal of Virological Methods 11: 309–319

Everett KR, Milne KS and Forster RLS (1994a) Nucleotide sequence of the coat protein genes of strawberry latent ringspot virus: lack of homology to the nepoviruses and comoviruses. Journal of General Virology 75: 1821–1825

Everett KR, Milne KS and Forster RLS (1994b) A new host record: strawberry latent ringspot virus isolated from flowering cherry. Australasian Plant Pathology 23: 11–15

Fitchen JH and Beachy RN (1993) Genetically engineered protection against viruses in transgenic plants. Annual Review of Microbiology 47: 739–763

Gallitelli D, Savino V and Martelli GP (1982) The middle component of strawberry latent ringspot virus. Journal of General Virology 59: 169–172

Harrison BD (1992) Genetic engineering of virus resistance: a successful genetic alchemy. Proceedings of the Royal Society of Edinburgh 99B: 66–77

Harrison BD and Murant AF (1977) Nepovirus group. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses. No. 185, 4 pp

Hellen CUT, Liu YY and Cooper JI (1991) Synthesis and proteolytic processing of arabis mosaic nepovirus, cherry leaf roll nepovirus and strawberry latent ringspot nepovirus proteins in reticulocyte lysate. Archives of Virology 120: 19–31

Hemenway C, Fang R, Kaniewski W, Chua NH and Tumer N (1988) Analysis of the mechanism of protection in transgenic plants expressing the potato virus X coat protein or its antisense RNA. EMBO Journal 7: 1273–1280

Hensgens LAM, Fornerod MJW, Rueb S, Winkler AA, van der Veen S and Schilperoort RA (1992) Translation controls the expression level of a chimaeric reporter gene. Plant Molecular Biology 20: 921–938

Jefferson RA, Burgess SM and Hirsch D (1986) Beta-glucuronidase from *Escherichia coli* as a gene-fusion marker. Proceedings of the National Academy of Sciences, U.S.A. 83: 8447–8451

Kaniewski W, Lawson C, Sammons B, Haley L, Hart J, Delannay X and 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

Kawchuck LM, Martin RR and McPherson J (1990) Resistance in transgenic potato expressing the potato leaf roll virus coat protein gene. Plant-Microbe Interactions 3: 301–307

Kreiah S, Cooper JI and Strunk G (1993) The nucleotide sequence of a satellite associated with strawberry latent ringspot virus. Journal of General Virology 74: 1163–1165

Kreiah S, Strunk G and Cooper JI (1994) Sequence analysis and location of capsid proteins within RNA2 of strawberry latent ringspot virus. Journal of General Virology 75: 2527–2532

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

Lister RM (1964) Strawberry latent ringspot: a new nematode-borne virus. Annals of Applied Biology 54: 167–176

Liu YY, Cooper JI, Edwards ML and Hellen CUT (1991) A satellite RNA of arabis mosaic nepovirus and its pathological impact. Annals of Applied Biology 118: 577–587

Mayo MA and Martelli GP (1993) New families and genera of plant viruses. Archives of Virology 133: 496–498

Mayo MA, Murant AF, Harrison BD and Goold A (1974) Two protein and two RNA species in particles of strawberry latent ringspot virus. Journal of General Virology 24: 29–37

MacFarlane SA and Davies JW (1992) Plants transformed with a region of the 201-kilodalton replicase gene from pea early browning virus RNA1 are resistant to virus infection. Proceedings of the National Academy of Sciences USA 89: 5829–5833

MacKenzie DJ and Tremaine JH (1990) Transgenic *Nicotiana debneyi* expressing viral coat protein are resistant to potato virus S infection. Journal of General Virology 71: 2167–2170

Murant AF (1974) Strawberry latent ringspot virus. Commonwealth Mycological Institute/Association of Applied Biologists Descriptions of Plant Viruses. No. 126, 4 pp

Nida DL, Anjos JR, Lomonossoff GP and Ghabriel SA (1992) Expression of cowpea mosaic virus coat protein precursor in transgenic tobacco plants. Journal of General Virology 73: 157–163

Nilsson O, Alden T, Sitbon F, Little CHA, Chulupa V, Sandberg G and Olsson O (1992) Spatial pattern of cauliflower mosaic virus 35S promoter-luciferase expression in transgenic hybrid aspen trees monitored by enzymic assay and non-destructive imaging. Transgenic Research 1: 209–220

Ploeg AT, Mathis A, Bol JF, Brown DJF and Robinson DJ (1993) Susceptibility of transgenic tobacco plants expressing tobacco rattle virus coat protein to nematode-transmitted and mechanically inoculated tobacco rattle virus. Journal of General Virology 74: 2709–2715

Powell AP, Nelson RS, De B, Hoffman N, Rogers SE, Fraley RT and Beachy RN (1986) Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738–743

Powell AP, Sanders PR, Turner N, Fraley RT and Beachy RN (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

Quemada HD, Gonsalves D and Sligholm JL (1991) Expression of coat protein gene from cucumber mosaic virus strain C in tobacco: protection against infections by CMV strains transmitted mechanically or by aphids. Phytopathology 81: 794–802

Sanford JC and Johnston SA (1985) The concept of pathogen-derived resistance – deriving resistance genes from the parasite's own genome. Journal of Theoretical Biology 113: 395–405

Shanks M, Stanley J and Lomonossoff GP (1986) The primary structure of red clover mottle virus middle component RNA. Virology 155: 697–706

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

Van Dun C, Bol JF and Van Vloting-Doting L (1987) Expression of alfalfa mosaic virus and tobacco rattle virus coat protein genes in transgenic tobacco plants. Virology 159: 299–305

Van der Vlugt RAA and Goldbach RW (1993) Tobacco plants transformed with the potato virus Y^N coat protein gene are protected against different PVY isolates and against aphid-mediated infection. Transgenic Research 2: 109–114

Van der Wilk F, Willink JDP-L, Huisman MJ, Huttinga H and Goldbach R (1991) Expression of the potato leaf roll luteovirus coat protein gene in transgenic potato plants inhibits viral infection. Plant Molecular Biology 17: 431–439

Van Wezenbeek P, Verver J, Harmsen J, Vos P and van Kammen A (1983) Preliminary structure and gene organisation of the middle component RNA of cowpea mosaic virus. EMBO Journal 2: 941–946