Proc. Indian natn. Sci. Acad.(PINSA) **B67** No.1&2 pp53-80 (2001)

Transgenic Approaches for Producing Virus Resistant Plants

PALLOB KUNDU and R K MANDAL
Department of Biochemistry, Bose Institute, Calcutta 700 054

(Received on 14 December 1998; after 2nd revision 16 January 2001; Accepted on 23 March 2001)

Loss of productivity of crops due to viral damage is enormous. Control measures available to date are inadequate and costly. Moreover, the indiscriminate use of insecticides to control virus vectors not only causes health problem but also affects the ecosystem. There are only a few tolerant varieties available for some crops which have been developed by conventional breeding programs. The advent of genetic engineering and plant transformation techniques has opened up the possibility of introduction of resistant gene to many different crop species. Application of this was boosted up when it was found that expression of viral genes in transgenic plant could bring the resistance in the plant. Later several other genes were also found, transgenic expression of which gave resistance. These include the gene of mammalian interferon system as well as virus resistance genes of some plant species. In this review all these biotechnological approaches for production of virus resistant plants are described under three categories: transgenic expression of genes or sequences derived from the pathogens' own genome, induced expression of the genes that kill the host cell to restrict the virus in initially infected cells and expression of some foreign genes which interfere with the viral particle or genome.

Key Words : Biosafety, Coat protein, Plant virus, Transgenic, Virus resistance

Introduction

Almost all economically important crops may become infected with viruses. Severity of the infection and extent of economic loss depend both on the virus strain and the host species infected. In most cases, virus infection causes a reduction in yield or quality of the crop. In some cases even 100% loss in crop production has been reported in many tropical and subtropical areas (Kunik et al. 1994). Virus infection in vegetatively propagated plants and perennial crops such as trees and plantation crops are often more serious than the seed generated crop plants, as all clones obtained from the plant part will carry the infection in case of the latter. Once a tree gets infection, it will remain so for life long with its impaired yield and quality.

Viruses that infect plants differ in their particle morphology, type of genome and several other characteristics, based on which they are classified. The most important characteristic feature of a virus is its genome. There are examples of viruses containing double stranded DNA (dsDNA) (e.g. Caulimoviruses), single stranded DNA (ssDNA) (e.g. Geminiviruses), double stranded RNA (dsRNA) (e.g. Reoviridae), and single stranded RNA (ssRNA) with positive sense or negative sense genome (e.g. Potyvirus). However majority of plant virus groups have a genome consisting of RNA (Brunt et al. 1996). Viruses use differential strategies for replication of their genome and production of infectious particles inside the cell. Despite these differences, there are some basic commonness followed by viruses in their life cycle. These are : (i) entry of the virus particle inside the host cell via biological vectors or through mechanical damage of the cell wall (ii) exposure of the viral nucleic acid to the cellular milieu and (iii) production of viral mRNA for translation by host system. The transcription of mRNA is done by virus coded enzymes in case of RNA viruses, whereas DNA viruses enter the nucleus and using host enzymes, produce their own mRNA.

In most of the viruses, a protein is required along with coat protein for cell to cell as well as long distance systemic movement. This is called movement protein (MP). Therefore, three functions are common in the plant viruses except satellite viruses. These are the coat proteins, replicase function protein(s) and movement protein. Along with these another factor is required for attachment and transmission by invertebrate vectors.

With the above mentioned insight gained in the molecular biology and genetics of plant viruses, plant genetic engineering strategies have been developed for producing virus resistant plants. This review attempts to present an uptodate state of the art and extends to comment on the future prospects of the various approaches. Several excellent reviews are available on different aspect of the topic (Wilson 1993, Scholthof et al. 1993, Fitchen & Beachy 1993, Zaitlin & Palukaitis 2000) some of which may not be available to general readers and particularly to young investigators and students interested to take up this area of plant biotechnology as their research topic. This updated review is expected to be useful as a ready reference to such readers.

Approaches to Control Viral Damage

The control of diseases caused by viruses is very important for maximising plant productivity. But no direct control measures are presently available to farmers. A few conventional methods of control which include eradication of infected crop, avoidance of the source of infection or prevention of disease spread are practised. All these methods have their disadvantages such as poor effectiveness, high cost and lack of reliability. Conventional breeding programmes used to develop some resistant or tolerant varieties are also expensive and time consuming. Moreover, virus variability is able to circumvent the resistance. Another type of protective measure used, was the cross protection. In this method, a crop is intentionally infected with a mild symptomless or attenuated strain of virus so that the former develops resistance against further infection with a related severe strain (Fulton 1986). Although this approach raised serious ecological concern about conversion of the mild strain to a pathogenic form or potential of the mild strain to cause serious damage to other crops, the approach was a promising one and investigators tried to find out the mechanism of this cross protection. Investigations on this line led to the development of the concept of pathogen-derived resistance (PDR) (Sanford & Johnston 1985). In this concept, it was predicted that the excess amount of expression of any of the viral genes in inappropriate time would disrupt the normal balance between viral products, thereby some step of viral life cycle could be perturbed.

The development of genetic engineering and plant transformation techniques has made it possible to explore these possibilities. Potential application of PDR was first reported by Powell-Abel et al. (1986) who demonstrated that transgenic expression of TMV coat protein gene in tobacco produced resistance against TMV. Effectiveness of this approach was extended by the use of other viral genes like replicase gene, defective movement protein gene, viral helper component gene, viral protease gene, sequences of defective interfering nucleic acids, sequences incapable of encoding proteins, ameliorating satellite RNA sequences etc. Other approaches include expression of foreign gene which prevent virus multiplication and spread by interacting with the incoming viral genome or their expression products or by killing the host cell. These also include transfer of natural virus resistant gene from one species to another via plant transformation techniques. Al these approaches are summarized in table 1.

Control of Viruses by Transgenic Expression of Genes or Sequences Derived From Pathogen's Own Genome.

Replicating Viral Genome

There have been a few attempts on the expression of replicating RNA viral genome in transgenic plants as an alternative to cross protection. In the first one, tobacco plants expressing the cDNA copy of the TMV genomic RNA did not develop any severe symptoms upon inoculation with a virulent TMV strain (Yamaya et al. 1988). In other reports, transgenic expression of RNA1 and RNA2 components of the genome conferred resistance to BMV (Brome mosaic virus) and CMV (Cucumber mosaic virus). In case of BMV the resistance was predicted as the result of homology de pendent resistance mechanism (Kaido et al. 1995) whereas

Table 1 Types of transgene used to control viral damage

Genes and/or sequences	Examples of resistance against virus and host used	
	Virus	Host
1. Pathogen derived genes or sequences		
Defective replicon	TMV, BMV, CMV	Tobacco
Satellite RNAs (SatRNA)	CMV, TRSV	Tobacco, Tomato, Pepper
Defective interfering nucleic acid (DI-DNA/RNA)	ACMV, BCTV, BMV	*N. benthamiana*, Barley
Antisense RNA (AsRNA)	TGMV, PLRV	Tobacco, Potato
3'-Untranslated region (3'-UTR)	TYMV	*Brassica napus*
Replicase protein	TMV, PEBV, PVX	Tobacco, *N. Benthamiana*
Defective movement protein (MP)	TMV, BMV, PLRV	Tobacco, Potato
Helper component protein (HC-Pro)	PVY	Tobacco
Proteinase	TVMV, PVY, TEV	Tobacco
Coat protein (CP)	TMV, TVMV, PVX	Tobacco, Tomato, Potato
2. Genes which are expressed to kill the host cell to limit virus spread		
Antiviral compounds (Ribosome inhibiting proteins)	TMV, PVX	Tobacco, Potato
2'-5' Oligoadenylate synthetase and ribonuclease (2-5 Aase and RNAse)	PVX, TMV, TEV, AlMV	Potato, Tobacco
Natural resistance gene	TMV, PVX	Tomato
3. Genes targeted directly to the viral particle or genome		
Ribozymes	TMV, PSTVd	Tobacco, Potato
Ribonuclease	PSTV	Potato
Plantibodies	TSWV, TMV, AMCV	*N. Benthamiana*

Abbreviations used are same as described in table 2.

in case of CMV in tobacco, the mechanism was thought to be different from homology dependent resistance (Suzuki et al. 1996). In the latter case the plants were more resistant against CMV RNA inoculum rather than virion and was also resistant against related tomato aspermy virus (TAV).

Ameliorating Satellite RNAs

These are small satellite RNAs (satRNAs) dependent on a helper virus for replication and get encapsidated in particles of the helper virus (Roossinck et al. 1992). They do not code for coat protein and can increase the severity or ameliorate the disease symptoms along with the helper virus infection. The transgenic expression of satRNAs of a number of viruses has decreased virus symptoms and/or titers in many systems (Scholthof et al. 1993). Tobacco plants transformed with the DNA-copy of symptom-ameliorating satRNA of cucumber mosaic virus under the transcriptional control of the CaMV35S RNA promoter contained small amounts of transcribed satRNA and on infection with a satellite-free inoculum of CMV produced large amount of satRNA. In these plants the replication of CMV was highly inhibited and symptom development was largely suppressed. Although the replication of tomato aspermy virus, which is closely related to CMV, was not inhibited in those plants but symptom development was suppressed (Harrison et al. 1987). The latter

phenomenon was explained by the ability of ameliorating satRNAs to prevent helper virus CP from entering the chloroplasts of infected cells (Wilson et al. 1993).

Similar kind of results obtained in transgenic tomato and hot pepper plants expressing satRNA of CMV (McGarvey et al. 1994, Kim et al. 1997). This kind of resistance phenotype was also observed in transgenic tobacco plants expressing full length satellite of tobacco ringspot virus (TRSV) or its complementary sequence as RNA transcripts, when infected with TRSV (Gerlach et al. 1987). Satellite of TRSV also interfered with the replication and disease development by another nepovirus, cherry leaf roll virus, in cowpea plant even though it was not a helper virus for satTRSV (Ponz et al. 1987). The resistance is thought to be the result of competitive inhibition of viral genome replication due to the chanelling of host and viral proteins for the production of satRNA (Baulcombe 1996). However, Kong et al. (1997) showed that satRNA-mediated resistance to turnip crinkle virus (TCV) in *Arabidopsis* was associated with a reduction in virus movement. This approach has been used in field tests in China with satRNAs as a prophylactic inoculum in a manner similar to the use of mild strains for cross protection (Baulcombe 1989).

Defective Interfering (DI) Nucleic Acid

These molecules are derived from a viral genome by substantial deletions of internal nucleotide sequences. They depend on the presence of the intact virus for replication and reduce the yield of helper virus. Their presence cause either milder disease symptoms or intensified symptoms of helper virus (Li et al. 1989, Jones et al. 1990). *Nicotiana benthamiana* plant was transformed with a single copy of a tandem repeat of subgenomic DNA B component of African cassava mosaic geminivirus (ACMV). The transgenic plants challenged with ACMV, showed reduced symptom severity in association with mobilization and amplification of subgenomic DNA, which interfered with the replication of both A and B components of ACMV DNA. But symptom severity by related geminiviruses like beet curly top virus (BCTV) and tomato golden mosaic virus (TGMV) was not reduced. Therefore, nature of the interaction was very specific (Stanley et al. 1990). DI RNA also conferred resistance to Cymbidium ringspot virus (Kollar et al. 1993) and BCTV in *N. benthamiana* plants (Frischmuth et al. 1997). A synthetic DI RNA was constructed using cloned deletion mutants of RNA-2 of brome mosaic virus, the expression of which in barley protoplasts effectively interfered with synthesis of native viral RNA (Marsh et al. 1991).

Therefore, DI nucleic acid and synthetic DI nucleic acid should provide potent sources of resistance. The transgenic DI RNA (or DNA) competes with the viral genome to redirect host or virus encoded proteins. As a result, replication of viral genome or spread of virion could be affected (Baulcombe 1996).

Antisense RNA

Antisense RNA, complementary to a target RNA, has been used to artificially suppress gene expression with high specificity in many systems including plants. Expression of RNA transcript complementary to the viral encoded replication associated AL1 gene of tobacco golden mosaic virus (TGMV), a ssDNA virus in tobacco, resulted in positive correlation between the accumulation of antisense RNA and reductions in symptom development in virus inoculated plants (Day et al. 1991). Antisense RNA against the tomato yellow leaf curl virus (TYLCV) replicase protein mRNA, was expressed in transgenic tomato plants. Some of the plants were totally symptomless when infected with TYLCV (Bendahmane & Gronenborn 1997). This technology was also successful against another gemini virus bean golden mosaic virus (BGMV) in beans (Aragao et al. 1998). The expression of antisense coat protein mRNAs of potato leaf roll virus (PLRV) in potato (Kawchuk et al. 1991), cymbidium mosaic virus (CyMV) in *Nicotiana occidentalis* (Lim et al. 1999), potato virus X (PVX), cucumber mosaic virus (CMV) and tobacco mosaic virus (TMV) in transgenic tobacco also provided protection against respective viruses (Hemenway et al. 1988, Cuozzo et al. 1988, Powell et al. 1989). In latter three cases, only limited protection against low inoculum concentrations was observed. The reason of low level of protection was either due to the hybridization of transgenic RNA with cis-acting elements of viral genome and thereby

neutralization or the transgenic RNA might serve as a decoy molecule. The high level of resistance was attributed to the homology-dependent post-transcriptional gene silencing (Baulcombe 1996).

Thus this technology may be particularly useful for those restricted to particular tissues, such as phloem limited PLRV and to viruses that replicate in the nucleus, such as the ssDNA containing gemeini viruses where replication probably occurs in close proximity to the transcription of antisense gene (Scholthof et al. 1993).

3'-Untranslated Region

Very few attempts have been made to use the untranslated region of viral RNA. *Brassica napus* plant transformed with a chimeric gene construct containing (+) strand 3'-terminal 100 nucleotides of the noncoding region of turnip yellow mosaic tymovirus (TYMV) genome were shown to be partially protected against infection by TYMV RNA or virions. Protection was overcome when the virus inoculum concentration was increased (Zaccomer et al. 1993). This dose-dependent inhibition pointed to titration of some 3'-untranslated region (UTR) binding factor(s) essential for viral expression.

Replicase Protein

Integration of a complete or partial cDNA clone of the viral replicase gene into the plant genome and its expression can produce virus resistance and have great potential (Rubino & Russo 1995). Zaitlin's group demonstrated that expression of the 54 kD protein of TMV in transgenic tobacco offered higher levels of protection against a TMV infection than CPMR (Golemboski et al. 1990, Carr et al. 1992). Subsequently the same phenomenon was also observed in plants transformed with related sequences from pea early browning virus (PEBV) in *N. benthamiana* (MacFarlane & Davies 1992), potato virus X in tobacco (Braun & Hemenway 1992), cucumber mosaic virus in tobacco (Anderson et al. 1992, Hellwald & Palukitis 1995), Cymbidium ringspot virus (Rubino & Russo 1995) and pepper mild mottle virus (PMMV) in *N. benthamiana* plants (Tenllado et al. 1995), rice yellow mottle virus (RYMV) in rice (Pinto et al. 1999), wheat streak mosaic virus in wheat (Sivamani et al. 2000) and potato virus Y in potato (Russo & Slack 1998). Success was also achieved using the geminivirus replicase protein. Norris et al. (1996) transformed *N. benthamiana* plants with the truncated form (210 amino acid) of the C1 gene of tomato yellow leaf curl virus (TYLCV) which codes for the replicase protein. These plants showed resistance when infected with TYLCV using the agroinfection technique.

The resistance was thought to be RNA mediated. In plants carrying a transgene derived from the replicase gene of PVX, cowpea mosaic virus and pepper mild mottle tobamovirus, it was due to homology dependent gene silencing, whereas in other cases although it was assumed to be due to gene silencing, the data were not conclusive (Baulcombe 1996). In case of CMV replicase, the resistance was found to be both RNA mediated and protein mediated suppression of virus replication and virus movement (Carr et al. 1994, Hellwald & Palukaitis 1995, Wintermantel & Zaitlin 2000).

In two recent reports, resistance phenotype was positively correlated with low levels of transgene mRNA as well as viral RNA accumulation, suggesting that it was mainly due to a gene silencing mechanism. Progenies of the transformed *N. benthamiana* plants carrying plum pox potyvirus (PPV) nuclear inclusion a (NIa) and b (NIb) proteins and the N-terminus of the capsid protein were completely resistant to PPV infection irrespective of the allelic status of the parental plant (Guo et al. 1998). Similar phenomenon was also observed in transgenic pea lines carrying pea seed-borne mosaic potyvirus (PSbMV) replicase (NIb) gene (Jones et al. 1998). In this latter case, plants were found to be more consistently resistant to closely sequenced isolates of the PSbMV than distantly related one. Therefore, sequence identity was the primary importance, in inducing gene silencing. Transgenically expressed replication associated cylindrical inclusion (CI) protein of PPV also produced resistant plant lines (Wittner et al. 1998).

Movement Protein

Virus encoded movement proteins (MPs) increase the size exclusion limits of plasmodesmata in inoculated and neighbouring cells and facilitate the transport of viral genome to adjacent cells. There

are sequence similarities among MPs of different virus groups. Therefore, production of transgenic plant expressing dominant negative mutant form of MP might result in resistance to one or more viral diseases by interfering with the function shared by the different viral movement proteins (Cooper et al. 1995). The effectiveness of this strategy was illustrated by the transgenic expression of viral MP, which conferred resistance only when the transgene specified a dysfunctional MP of TMV (Lapidot et al. 1993, Cooper et al. 1995) or brome mosaic virus (BMV) (Malyshenko et al. 1993). Expression of mutant versions of potato leaf roll virus (PLRV) movement protein in transgenic potato plants conferred broad range protection against PVY, PLRV and PVX infections (Tacke et al. 1996). Similar kind of broad range protection was also observed in *N. benthamiana* plants carrying mutated form of 13 kD protein of the triple gene block (TGB) of white clover mosaic potex virus (WClMV) (Beck et al. 1994), in tobacco plants expressing the p24 protein (one of three movement associated proteins) of potato virus X (PVX), against TMV and Ob tobamovirus infections (Ares et al. 1998) and in tomato plants expressing bean dwarf mosaic virus (BDMV) mutated BV1 or BC1 movement proteins against tomato mottle virus (ToMV) (Hou et al. 2000). Probably the competition between mutant MP and wild type MP of inoculated virus in transgenic plant for plasmodesmatal binding site confer the resistance (Baulcombe 1996). Whereas in the resistance mediated by TSWV NSM gene (putative MP), it was found to be gene mediated and targeted towards virus mRNAs and not the virus genome (Prins et al. 1997).

Helper Component Protein

Expression of the first three PVY cistrons including the HC, in transgenic tobacco conferred resistance to PVY (Vardi et al. 1993). This type of resistance is mainly attributed to homology dependent post transcriptional gene silencing.

Viral Proteinases

Two different groups reported the expression of NIa proteinase of potyviruses in transgenic tobacco plant. This expression could provide resistance only against the virus from which the gene was derived. Maiti et al. (1993) first reported that the expression of NIa of TVMV produced resistant plant against TVMV but not to other potyviruses. When the cistron of PVY NIa protease (containing all but the first hundred 5' terminal nucleotides of NIa as well as the first 251 nucleotides of NIb) was introduced in transgenic tobacco, the plant became resistant to PVY infection (Vardi et al. 1993). With a aim of attaining resistance to more than one potyvirus in a single plant line by introducing a nonstructural protein gene, Fellers et al. (1998) introduced genes of NIa proteinase TVMV, PVY and TEV in combinations into tobacco. The published result of viral challenge to the transgenic tobacco plants constitutively expressing these genes showed specificity of resistance and differential pattern of resistance phenotype. The lines which carried the multiple NIa genes of different viruses were resistant or recovered after initial infection to the viruses from which the genes were derived. Similar kind of result was also obtained with potyviral P1 proteinase and P3 cofactor to proteinase, genes expression in transgenic plant. The plants showed a narrow spectrum of resistance to a high dose of inoculum (Moreno et al. 1998). The resistance was found to be RNA mediated. Presence of NIa inside the cell may alter the usual processing of viral polyprotein and the action of VPg. Involvement of RNA based silencing mechanism has also been proposed (Maki-Valkama et al. 2000).

Coat Protein Mediated Resistance (CPMR)

Background and Concept

Of the different approaches, CPMR has been most promising and most extensively studied. Development of CPMR is the extension of the work on understanding the mechanisms of cross protection. Earlier, it was believed that cross protection was the result of encapsidation of superinfecting virus RNA by the CP of the virus of original infection. Sarkar and Smitamana (1981) using the coat protein free and nonfunctional CP mutant of TMV showed that N. glutinosa plants were protected against challenging TMV. Contrary to this observation, Sherwood and Fulton (1982) showed the specific involvement of coat protein in TMV cross protection. Sherwood (1987) resolved

much of the controversy by showing that the CP minus TMV strain used by Sherwood and Fulton did indeed provide protection against TMV superinfection but greater protection was observed with CP plus strain as a protecting virus. Disassembly and simultaneous translation of the exposed viral mRNA were found to be inhibited in vitro by the addition of free coat protein.

Based on earlier studies on mechanism of cross protection, Sequeira (1984) proposed that insertion and expression of virus *cp* gene in plants might result in protection. Beachy et al. (1985) discussed about the use of *cp* gene for virus protection. With the availability of method of expressing *cp* gene constitutively under CaMV35S promoter in transgenic tobacco (Beachy et al. 1985, Bevan et al. 1985), Powell Abel et al. (1986) and Beachy et al. (1990), showed delay in disease development in these plants after infection with TMV. This dominant trait can be easily transferred to other nontransformed plant by conventional breeding.

Phenotypes and Levels of CPMR

CPMR is characterized by one or more phenotypes. First, it results in fewer number of sites of infection after inoculation, as was the case with TMV, AlMV, PVX CPMR (Nelson et al. 1987, Loesch-Fries et al. 1987, Hemenway et al. 1988). Secondly, fewer plants become systemically infected and a third manifestation of resistance is a lesser accumulation of virus in infected plants (Murry et al. 1993, Nelson et al. 1987, Cuozzo et al. 1988 etc.). As a result of these a reduction in severity of disease symptoms in infected plants occur. Sometimes the expression of CP is not even required for resistance but the CP RNA can give resistance (Lindbo & Dougherty 1992). It has been also observed that expression of CP of PVY in potato can prevent aphid transmission of that virus (cited in Nelson et al. 1990). Not all examples of CPMR exhibit all these phenotypes but some of them are exhibited.

Variable levels of resistance have been observed in different plants transgenic for different viral coat proteins. In general resistance level was correlated with the level of CP expression in transgenic tissue. The CP expression level was found to be as low as 0.001 to 0.01% of total soluble leaf protein in TEV- tobacco CPMR (Lindbo & Dougherty 1992) or as high as 1% of that in maize. In all these cases, CP expressing plants showed resistance to low inoculum concentration. But this resistance was overcome by higher levels of inoculum (Murry et al. 1993). Resistance to quite high level of inoculum (40-50 mg/ml) was also observed (Nelson et al. 1988, Cuozzo et al. 1988). There are examples (Lindbo & Dougherty 1992) where a truncated form of CP of TEV expression provided significant delay in symptom appearance but the expression of full length CP showed only slight attenuation to no delay of symptom appearance when challenged with TEV.

The ability of CPMR to protect plants against inoculation with viral RNA is variable. TMV or AlMV CP expressing lines were susceptible to disease development when inoculum was applied in the form of virion naked RNA (Nelson et al. 1987, Loesch-Fries et al. 1987). By contrast, plants transgenic for the coat proteins of a number of other viruses, such as PVX (Hemenway et al. 1988) and PVS (Mackenzie & Tremaine 1990) were equally resistant to infection with virions or RNA. CPMR is most effective against the virus from which the *cp* gene was derived. It is also effective against related strain of that virus and to other related virus of the same group. For example tomato plants expressing the coat protein of tobacco vein mottling virus (TVMV) showed high degree of resistance against infection with tobacco etch virus (TEV) of the same virus group (Kundu & Mandal, unpublished).

Examples of CPMR

The majority of viruses against which CPMR has been achieved containing the single stranded positive sense RNA genome. These include Tobamo-, Cucumo-, Potex-, Poty-, Alfalfa mosaic-, Luteo-, Carla-, Ilar-, Tobra-, Tenui-, Nepo-, Bromo- and Furo virus group. Expression of viral nucleoprotein (N) gene of negative strand RNA virus tomato spotted wilt virus (TSWV) of Tospovirus group in transgenic tobacco also provided high level of protection (Gielen et al. 1991, Mackenzie & Ellis 1992). Report on extending the concept against ssDNA virus tomato yellow

leaf curl geminivirus came only recently (Kunik et al. 1994). Resistance has been observed in a number of different crop plants, some examples of which are listed in table 2. The CPMR approach has been reviewed in detail by Nelson et al. (1990), Beachy et al. (1990) and Beachy (1993).

Mechanism of Protection

The protection could be protein mediated or RNA mediated. In case of TMV, it has been definitely proved that expression of protein is required for resistance (Powell et al. 1990). In subsequent experiments with plants transgenic for coat proteins of AlMV, PVX, TVMV, ToMV, etc. (Loesch-Fries et al. 1987, Hemenway et al. 1987, Maiti et al. 1993, Sanders et al. 1992) only the protein expressor lines were examined. The lack of correlation between protein expression level and resistance in cases of PLRV and PVY CPMR initially indicated the possible involvement of CP mRNA in protection. This was indicated in experiments of Lindbo & Dougherty (1992) with untranslatable CP sequence of TEV in transgenic tobacco. Similar experiments were also performed with TSWV nucleocapsid gene (deHaan et al. 1992).

The protein mediated resistance operates in two stages. Firstly, in the initially infected cell where virion disassembly might be inhibited and secondly, the local and systemic spread of the virus could be inhibited. The inhibition of virion disassembly hypothesis was proposed based on the observations with TMV-CPMR (Register & Beachy 1988) in which plants were more resistant to TMV virion than to TMV RNA and presence of large amount of CP in initially infected epidermal cells was required for greater protection (Clark et al. 1990, Reimann-Philipp & Beachy 1993). Experiments on GUS pseudovirion disassembly (Osbourn et al. 1989) and the resistance against TMV in transgenic plant expressing assembly defective mutant of CP(Clark et al. 1995), strongly supported the inhibition of disassembly hypothesis. Grafting experiment to test the influence of CPMR on systemic virus spread through vascular system indicated that this process could also be suppressed. Additional experiments with AIMV and PVX CPMR proposed that transgenic CP might interfere with viral origin of assembly and with the viral nucleic acid necessary for replication. In another experiment, mutant TMV-CPs that were incapable of helical aggregation or unable to interact with viral RNA were expressed in *N. benthamiana* plants using PVX vector. These plants did not delay the accumulation of TMV. On the other hand, expression of mutant TMV-CP with enhanced intersubunit interactions that favored helical aggregation delayed TMV accumulation for significantly greater time compared to wild type control. These observations suggest that the protecting CP recoats the challenging virus RNA as it disassembles (Lu et al. 1998). It is also possible that CPMR inhibits cell-to-cell movement of PVX, for which CP is an essential cofactor (Spillane et al. 1997).

The proposal of RNA mediated protection was based on the observation of inverse correlation between RNA accumulation level and resistant phenotype. There may be a cellular RNA degradation mechanism that gets activated when unacceptably high level of a particular transcript accumulates within the cell leading to the degradation of that specific transcript or cosuppression with viral genes (Prins & Goldbach 1996). Therefore, the homology of viral sequence with the transgene makes it susceptible to degradation (Lomonossoff 1995, Wassenegger & Pelissier 1998). These findings also led to the possibility of using truncated form of coat protein. Use of truncated CP reduces the chance of heteroencapsidation associated risk and multiple CP sequences can be introduced by this method. Recent works with TSWV N-gene has showed that a fragment as small as 110 base pair when fused with a nontargeted gene could confer resistance (Pang et al. 1997). A fusion gene containing this 110 base pair N gene and full length CP gene of Turnip mosaic virus (TuMV) conferred resistance to both the viruses in *N. benthamiana* plant (Jan et al. 2000). The work of Ingelbrecht et al. (1999) with the sorghum mosaic potyvirus untranslatable coat protein gene in transgenic sugarcane, extended the observations on

Table 2 *Examples of CPMR.*

CP gene	Virus group	Challenge virus	Transgenic plant	References
AlMV	Alfamo	AlMV	Tobacco Tomato Alfalfa	Tumer et al. 1987 Van Dun et al. 1987 Loesch-fries et al. 1987 Tumer et al. 1987 Hill et al. 1991
BMV	Bromo	BMV	Rice protoplast	Huntley and Hall et al. 1987
PVS	Carla	PVS	Potato *N. debneyii*	Mackenzie et al. 1991 Mackenzie et al. 1990
GLRaV	Clostero	GLrAV	*N.benthamiana*	Ling et al. 1997
SqMV	Como	SqMV	Squash	Pang et al. 2000
CMV	Cucumo	CMV	Tobacco Cucumber Squash Tomato	Quemada et al. 1991 Cuozzo et al. 1988 Namba et al. 1991 Gonsalves et al. 1992 Tricolli et al. 1995 Xue et al. 1994 Fuchs et al. 1996 Tomassoli et al. 1999
CMV+ZYMV+ WMV2	Cucumo+ Poty	CMV, ZYMV, WMV2	Squash	Tricolli et al. 1995
PEMV	Enamo	PEMV	Pea	Chowrira et al. 1998
BNYVV	Furo	BNYVV	Sugarbeet	Kallerhoff et al. 1990
PMTV	Furo	PMTV	*N.benthamiana*	Reavy et al. 1995
TYLCV	Gemini	TYLCV	Tomato	Kunik et al. 1994
TSV	Ilar	TSV	Tobacco	Van Dun et al. 1988b
PLRV	Luteo	PLRV	Potato	Kawchuk et al. 1990 Vander Wilk et al. 1991 Presting et al. 1995
ArMV	Nepo	ArMV	Tobacco	Bertoli et al. 1992
GCMV	Nepo	GCMV	Tobacco	Brault et al. 1993
GFLV	Nepo	GFLV	*N.benthamiana*	Bardonnet et al. 1994
PAMV	Potex	PAMV	Tobacco	Leclerc & Abouhaider 1995
PVX	Potex	PVX	Tobacco Potato	Hemenway et al. 1988 Hoekama et al. 1989 Jogedijk et al. 1992 Kaniewski et al. 1990 Lawson et al. 1990
MDMV	Poty	MDMV, MCMV	Maize	Murry et al. 1993
PPV	Poty	PPV	*N. clevelandii* *N. benthamiana* Plum	Regner et al. 1992 Jacquet et al. 1998 Ravelonandro et al. 1998
PRV	Poty	TEV,PeMV,PVY PRV	Tobacco Papaya Plum	Ling et al. 1991 Fitch et al. 1992 Tennant et al. 1994 Scorza et al. 1995
PStV	Poty	PStV	*N. benthamiana*	Cassidy & Nelson 1995
PVY	Poty	PVY	Potato Tobacco Potato	Malonoe et al 1994 Smith et al. 1994 Smith et al. 1995 Hassairi et al 1998

Table 2 *(Contd..)*

PVY+PVX	Poty	PVY, PVX	Potato	Lawson et al. 1990
SCMV	Poty	SCMV, SrMV	Sugarcane	Mirkov et al. 1997
SMV	Poty	TEV, PVY	Tobacco	Stark & Beachy 1998
SrMV	Poty	SrMV	Sugarcane	Ingelbrecht et al. 1999
SPFMV	Poty	SPFMV-S	*N. benthamiana*	Sonoda et al. 1999
TEV	Poty	TEV	Tobacco	Lindbo & Dougherty 1992
TuMV	Poty	TuMV	*N. benthamiana*	Jan et al. 1999
TVMV	Poty	TVMV, PVY, TEV	Tobacco	Maiti et al. 1993 Xu et al. 1997a
WMV2	Poty	WMV2	Squash *N. benthamiana*	Fuchs & Gonsalves 1995 Namba et al. 1992
ZYMV	Poty	ZYMV	Squash *N. benthamiana*	Fuchs & Gonsalves 1995 Namba et al. 1992
ZYMV+WMV2	Poty	ZYMV, WMV2	Cantaloupe Squash	Fang et al. 1993 Fuchs & Gonsalves 1995
RSV	Tenui	RSV	Rice	Hayakawa et al. 1992 Yan et al. 1998
TMV	Tobamo	TMGMV, PPMV, ORSV	Tobacco	Nejidat &Beachy 1990
		TMV PV230/U1	Tobacco	Nelson et al. 1988
		TMV U1	Tobacco	Powell-Abel et al. 1986
		ToMV	Tobacco	Nelson et al. 1987
			Tomato	Nelson et al. 1988
ToMV	Tobamo	ToMV	Tomato	Sanders et al. 1992
TRV	Tobra	TRV, PEBV	Tobacco, *N. benthamiana*	Van Dun et al. 1988a
TSWV (Nucleocapsid)	Tospo	TSWV	Tobacco	Gielen et al. 1991 deHaan et al. 1992 Mackenzie et al. 1992
			Peanut	Li et al. 1997
		TSWV, INSV	*N. benthamiana*	Pang et al. 1994
TSWV+TCSV+ GRSV (Nucleocapsid	Tospo	TSWV, TCSV, GRSV	Tobacco	Prins et al. 1995
GVA	Tricho	GVA	*N. benthamiana*	Radian-Sade et al. 2000
PhMV	Tymo	PhMV	Tobacco Tomato	Ranjith-Kumar et al. 1999 Vidya et al. 2000

Abbreviations: *AlMV*, Alfalfa mosaic virus; *ArMV*, Arabis mosaic virus; *BMV*, Brome mosaic virus; *BNYVV*, Beet necrotic yellow vein virus; *CMV*, Cucumber mosaic virus; *GCMV*, Grapevine chrome mosaic virus; *GFCV*, Grapevine fan leaf virus; *GLRaV*, Grapevine leaf roll associated virus 3; *GRSV*, Groundnut ringspot virus; *GVA*, Grapevine A; *INSV*, Impatiens necrotic spot virus; *MCMV*, Maize chlorotic mosaic virus; *MDMV*, Maize dwarf mosaic virus; *ORSV*, Odontoglossum ringspot virus; *PAMV*, Potato aucuba mosaic virus; *PEBV*, Pea early browning virus; *PeMV*, Pepper mottle virus; *PEMV*, Pea enation mosaic virus; *PhMV*, Physalis mottle virus; *PLRV*; Potato leaf roll virus; *PMMV*, Pepper mild mottle /mosaic virus; *PMTV*, Potato mop top virus; *PPV*, Plum pox virus; *PRV*, Papaya ring spot virus; *PStV*, Peanut stripe virus; *PVS*, Potato virus S; *PVX*, Potato virus X; *PVY*, Poato virus Y; *RSV*, Rice stripe/streak virus; *SCMV*, Sugarcane mosaic virus; *SMV*, Soybean mosaic virus; *SPFMV*, Sweet potato feathery mottle virus; *SqMV*, Squash mosaic virus; *SrMV*, Sorghum mosaic virus; *TCSV*, Tomato chlorotic spot virus; *TEV*, Tobacco etch virus; *TMGMV*, Tobacco mild green mosaic virus; *TMV*, Tobacco mosaic virus; *ToMV*, Tomato mosaic virus; *TRV*, Tobacco rattle virus; *TSV*, Tobacco streak virus; *TSWV*, Tomato spotted wilt virus; *TuMV*, Turnip mosaic virus; *TYLCV*, Tomato yellow leaf curl virus; *TVMV*, Tobacco vein mottling virus; *WMV2*, Watermelon mosaic virus 2; *ZYMV*, Zucchini yellow mosaic virus.

homology dependent post transcriptional gene silencing in virus resistance also to a monocot crop. From what has been said above, it appears that CPMR operates through more than one mechanism. Mechanisms of CPMR has been also reviewed in detail by Reimann-Philipp (1998).

Factors Involved in Vector Transmission and Seed Transmission

Cauliflower mosaic virus and most of the potyviruses are insect transmitted. Potyviral encoded two proteins are involved in this process: CP and HC-Pro. HC-Pro can recognize viral CP and a surface receptor in insect mouthpart (Blanc et al. 1993). Thus it acts as a bridge to attach the virus in insect stylet. A conserved amino acid triplet, Asp-Ala-Gly (DAG) in the N terminus of the CP and a lysine motif (KITC) in HC-Pro seemed to be important for this purpose in most of the cases (Maia et al. 1996, Pirone and Blanc 1996). Another motif in HC-Pro called PTK also have some role. Mutations in ZYMV PTK-motif and TVMV motif prevented vector transmission of these viruses (Peng et al. 1998, Pirone and Blanc 1996). One possibility is the production of dominant negative mutants of these gene products in transgenic plant as a method of developing resistance.

Several host genes and multiple viral determinants are involved in seed transmission process. However, use of hybrid pea seed borne mosaic virus between seed transmissible and nontransmissible, indicated that viral determinants are 5'-NTR, HC-Pro and CP regions of the genome. These regions are also important for virus replication and movement. Therefore, further studies on viral and host factors would open up the possibility of limiting the virus spread by prevention of seed transmission.

Approaches to Kill the Host Cell to Restrict the Virus

Antiviral compounds

Use of ribosome-inhibiting proteins (RIPs), such as pokeweed antiviral protein (PAP), trichosanthin, dianthin etc. have been explored recently for developing a broad spectrum resistance in transgenic plant. Due to their high phytotoxic effect a constitutive expression in transgenic plant produced abnormal plants (Lodge 1993, Lam 1996). Therefore, alternative strategies were developed. In these strategies transgenes were used as latent suicide gene, would be activated only after virus infection. Diphtheria toxin A fragment, ricin or PAP was expressed at low level from a constitutive promoter in transgenic plant cell along with a minus-sense plant viral subgenomic RNA promoter at its 3' end. During the infection by cognate virus the nonsense RNA is transcribed into mRNA, allowing expression of the phytotoxin and killing the cell, thus virus spread was limited. As PAP is phytotoxic so recovery of transgenic plants were difficult. Pokeweed antiviral protein II (PAPII) is comparatively less toxic. PAPII inhibit eukaryotic translation by catalytically removing a specific adenine residue from the large rRNA of the 60 S subunit of eukaryotic ribosome. Transgenic plants constitutively expressing PAPII were resistant to TMV and potato virus X (Wang et al. 1998). Salicyclic acid was not accumulated after infection so the resistance mechanism was independent of salicylic acid mediated pathway. Broadspectrum of resistance was also observed in PIP expressing (*Phytolacca insularis* antiviral protein) potato plants against PVX, PVY and potato leaf roll virus (Moon et al. 1997).

In another novel strategy, a ribozyme was designed to trans-splice the coding sequence of diptheria toxin A chain in frame with the cucumber mosaic virus coat protein mRNA. The ribozyme was designed based on the self splicing group I introns. Diptheria toxin inhibit eukaryotic protein translation. When Yeast was used as the model system, it was found that growth of the cells expressing the viral mRNA was specifically inhibited. Therefore, this strategy would be highly useful to develop resistance against viruses in plant with reduced illegitimate toxicity in vivo (Ayre et al. 1999). By the use of the PVX subgenomic RNA promoter and diphtheria toxin mRNA, transgenic tobaccoes showed a 20 fold reduction in PVX concentration (Wilson 1993).

Virion sense genes of ACMV and TGMV are transcriptionally transactivated by the complementary sense gene AC2. Therefore, dianthin gene was cloned under virion sense promoter and was expressed in the tissue of transgenic *N. benthamiana* plants only after virus infection, thus a targeted phytotoxic effect was obtained and systemic symptom development was attenuated (Hong et al. 1996, 1997). Although these approaches may not be successful under field condition but can be used as a probe for studying transcriptional transactivation.

Mammalian 2'-5' oligoadenylate Synthetase and RNase

Use of enzymes of mammalian interferon system is the latest in the development of virus resistant transgenic plants effective against a broad range of plant viruses. Interferons are proteins secreted by mammalian cells during viral infection or in other occasions. They induce the synthesis of other proteins which ultimately lead to the inhibition of viral multiplication. One of these is the RNA degradation pathway referred as 2'-5' oligoadenylate synthetase (2-5 Aase) system. This pathway is activated by dsRNA, the usual replicating intermediate of RNA viruses (poorly activated by cellular dsRNAs). The synthesized 2'-5' oligoadenylates (2-5 A) in turn activate ribonuclease L (RNAseL), a single stranded RNAse, which degrade viral and cellular RNAs. Degradation of cellular RNAs results in cell death and thus it limits virus replication and spread (Beachy 1996).

Although existence of this pathway in higher plants is yet to be established, Truve et al. (1993), first reported the constitutive expression of 2-5 Aase in transgenic potato plants that were protected against PVX infection under field conditions and in some clones, level of virus accumulation was lower than in potatoes expressing PVX CP.

Two independent research works using the same enzyme (2-5 Aase) but of human origin were reported in 1996. Mitra et al. (1996) reported that the virus was contained only in the initial infected leaves of transgenic tobacco, when relatively low levels of inoculum were applied, but very high levels of inoculum resulted in limited systemic spread. The plants developed were resistant to TMV, alfalfa mosaic virus and tobacco etch virus (TEV). In the second case (Ogawa et al. 1996) a double transgenic line was produced by crossing of two tobacco lines containing 2-5 Aase and RNase L. The plants were totally resistant to TMV, alfalfa mosaic virus and tobacco etch virus (TEV). In the second case (Ogawa et al. 1996) a double transgenic line was produced by crossing of two tobacco lines containing 2-5 Aase and RNase L. The plants were totally resistant to CMV infection and survived upto 20 days after PVY infection. As a result of cell death necrotic local lesions were formed, reduced number of which also restricted virus movement through out the plant. Thus this approach is better than pathogen derived resistance in that, there is no chance of genetic recombination or transencapsidation between foreign gene or protein with incoming virus (Beachy 1996).

Natural Resistance Gene

Some plants show natural resistance to some viruses. This resistance to viruses may be the result of inhibition of virus replication or virus movement or hypersensitive reaction (Baulcombe 1989). In hypersensitive reaction the virus is restricted within the cells of primary infection, due to the necrotic death of the cells around the primary infection site and the resistance is encoded by single dominant genes in most of the cases. There are several examples and most common is the *N* gene of tobacco (Whitham et al. 1994). It confers gene for gene resistance to TMV and other tobamoviruses by localizing the viral pathogens in the necrotic lesions. These resistance genes can be transferred from resistant to susceptible species using plant transformation techniques. Whitham et al. (1996), took the initiative to test this proposal by the transformation of TMV susceptible tomato cultivar with the *N* gene. Transgenic tomato plants expressing the *N* gene, showed hypersensitive response mediated necrotic local lesions similar to the *N* gene carrying tobacco

plants when inoculated with TMV U1 strain, TMV Cg strain or tomato mosaic virus. This kind of response was absent in nontransformed plant as well as in transgenic plants which were mock inoculated and the response was temperature sensitive as is observed in TMV. Moreover, in the transgenic plant systemic movement of the virus was also not observed. Therefore, this result showed that the components required for *N* mediated resistance are present in tomato and has opened up the possibility of transferring the resistance to more distantly related species.

In the gene-for-gene interaction when a pathogen infects a plant, the avirulence (*avr*) gene products of the pathogen are recognised by the receptor molecules of plant resistance system. This recognition event activate the signal transduction pathway that lead to the induction of a number of plant defences which ultimately inhibit the pathogen's replication or restrict it in the initially infected cells by activating hypersensitive response. In tomato, the *Pto* resistance gene confers resistance against *Pseudomonas syringae* pv. *tomato* (*Pst*) carrying the avirulence gene *avrpto*. *Pto* and *avrPto* directly interact to trigger the resistance. Two other gene products *pti1* and *prf* which also interact with *Pto* take part in this signal transduction cascade. To address the questions like whether the plant recognition of avirulence component would lead to the activation of all plant defenses independent of the nature of the infecting pathogen, two components of this pathway were overexpressed in plant. The overexpression of *prf*, thought to be involved in bacterial recognition, led to resistance to both bacterial and viral pathogens (Oldroyd & Staskawicz 1988). Expression of *avrPto* in tomato plant using PVX-based vector could activate the defense pathway which effectively prevented virus proliferation (Tobias et al. 1999). Therefore, expression of genes involved in signal transduction pathway of plant defenses would lead to a broad spectrum resistance against pathogen infection in plant which is effective against viral pathogens.

Other Approaches Targeted to the Viral Particle or Genome

Ribozymes and ribonuclease genes

Ribozymes are small RNA molecules which are capable of highly specific catalytic cleavage of RNA. It is possible to target a ribozyme to a particular sequence by modifying its catalytic as well as flanking antisense arms. deFeyter et al. (1996) used this approach targeted towards TMV genome. They cloned a ribozyme containing three catalytic hammerhead domains within a 1 kb antisense RNA to TMV. Transgenic tobacco plants homozygous for that gene were found to be highly resistant to TMV virion or RNA and related tomato mosaic virus. But same type of resistance was also observed in the tobacco plants transgenic for only the anti sense construct. Moreover, the resistant phenotype was not changed when the homozygous plants were challenged with the TMV strain modified in the ribozyme-target sites. Therefore, they suggested that in this case the resistance was developed by antisense mechanisms. But the ribozyme alone was also found to be effective in controlling diseases. In turnip, an active *cis*-hairpin ribozyme cloned into cauliflower mosaic virus genome was shown to delay systemic viral symptom development for at least one week when compared with a nonfunctional ribozyme control (Borneman et al. 1995). Ribozyme is also effective in suppressing the viroid in undetectable level in plant. Transgenic potato plants expressing a hammerhead ribozyme directed against PSTVd minus strand RNA developed a high resistance against PSTVd replication (Yang et al. 1997). To detect the activity of ribozyme in vivo the ratio of ribozyme/target RNA should be between 100:1 and 1000:1. Therefore, attempts were taken to exploit high copy number viral replicon (TMV or PVX vectors) to produce high levels of cytoplasmic ribozyme targeted against viruses (Wilson et al. 1993).

In another novel approach, resistance was conferred in potato plants to potato spindle tuber viroid (PSTV) by expressing double stranded RNA-specific ribonuclease (Hammond 1997, Sano et al. 1997). The single stranded circular RNA of

the viroid form a base paired double stranded RNA like structure and during replication it goes through a intermediated dsRNA structure which are target for this type of ribonuclease.

Plantibodies

As the plant cells can synthesize and assemble functional monoclonal antibodies, there is a potential of expressing an appropriate IgG, Fab2 fragment or single-chain Fv (scFv) antibody against viral structural or catalytic proteins to control viral diseases. This approach has been tried with TSWV nucleoprotein or TMV CP. A high molar excess of antibody is required to inactivate a virus invasion (Wilson 1993). Initially a hybridoma was developed secreting monoclonal antibody, able to recognize an epitope of the glycoprotein G1 conserved among a large number of tospoviruses. From that cell the scFv fragment was engineered in PVX vector or plant transformation vector to transform *N. benthamiana* plants. The plants were able to produce functional antibodies. These plants were used to genetically immunize against tomato spotted wilt virus (Franconi et al. 1999). The expression of single-chain Fv (scFv) antibody in transgenic R_0 *N. benthamiana plants* raised against artichoke mottled crinkle virus (AMCV) delayed the disease symptom development (Tavlardoraki et al. 1993). The R_1 plants also showed lower virus accumulation, a highly reduced incidence of infection and a marked delay in symptom appearance. But the mechanism of action of the intracellular plantibody against the virion is not well defined. Plasma membrane display of anti-TMV ScFv conferred resistance to TMV in tobacco (Schillberg et al. 2000).

In another approach, the full size monoclonal antibody raised against TMV was constitutively expressed in tobacco plants (Voss et al. 1995). The resulting transgenic plants were expressing the heavy and light chains which were assembled and secreted into the intercellular space. The transgenic plants when challenged with TMV showed a reduction of necrotic lesion numbers which was correlated with the amount of antibody produced in transgenic plants.

Biosafety of Transgenic Plants

Introduction of a selection marker gene along with the foreign gene desired to be introduced in a plant is the common practice in transgenesis. When an agronomically important trait like virus resistance is thus introduced into a crop plant and the question of releasing the plant for cultivation and for human consumption or other uses arises, the biosafety assessments are to be done from different points of concern (Stewart et al. 2000). In a commercially important crop transformed with gene of a viral origin, the biosafety considerations should deal with the viral gene as well as the marker gene. There are many marker genes currently in use (for a list, see Yoder & Goldsbrough 1994) but risk assessments to date focused largely on the most commonly used selectable marker gene, neomycin phosphotransferase (*nptII*). The major points analysed are : the possibility of *nptII* gene product to be toxic or allergenic when consumed, the *nptII* gene being horizontally transferred to other plants or microorganisms, the increase in the number of drug resistant pathogenic microorganisms in the human or animal gut and the impact of gene flow from transgenic plant to other organisms on ecosystem. It was found that plant expressed NPTII protein was equivalent to microbially expressed NPTII protein (Fuchs et al. 1993a). Administration of a high dose of bacterially expressed NPTII protein got rapidly degraded in gastric and intestinal fluids (Fuchs et al. 1993b). Furthermore, NPTII requires ATP in order to catalyze the inactivation of kanamycin or neomycin. ATP is present in the digestive system at extremely low concentration because it is unstable at low *p*H. So the consumption of NPTII will not compromise the efficacy of oral kanamycin or neomycin. In mammalian cells, NPTII is not toxic *in vitro*. There are as many as 10^{12} kanamycin or neomycin-resistant bacteria already present in gut. Therefore, chance of transformation with plant expressible *npt*II (if it reaches the gut) is very very low. Even if the transgenic plants are extensively cultivated, it has been estimated

that the kanamycin resistant bacterial population in soil will not increase by more than 0.0001 per cent (assuming that the gene could be transferred from plants to microbe) (Flavell et al. 1992). Horizontal gene transfer via pollen to other plants, if occurs at all will be also at very low rate (unless the transgene confers a significant selective advantage) and the latter plant will not be any different than the transgenic plant itself (Gupta 1996, Scott & Wilkinson 1998). However, in a recent study using a highly selfing species such as *Arabidopsis thaliana* transgenic for the chlorsulphuron resistance trait, an increase in the outcrossing to the wild type mother has been observed compared to the mutant control plants (Bergelson et al. 1998).

Regarding the risks on introduction of genes of viral origin, four important questions arise. Firstly, whether the benign form of satellite RNA on mutation would be able to form a pathogenic form. Secondly, the effect of heteroencapsidation of viral RNAs by transgenically expressed CP on epidemiology of heterologous viruses. Thirdly, the potential impact of recombination between transcripts of viral genes and infecting viruses and finally, the effect of transgenically expressed viral proteins on the disease development by incoming virus (Tepfer 1993).

The satRNA of CMV is known to reduce the disease symptom in most of the crops. But a single point mutation could convert this non-necrogenic satRNA to a lethal necrogenic one on tomato and an outbreak of this disease have been observed in southern Italy during 1988-1989 growing season (Masuta & Takanami 1989, Sleat & Palukaitis 1990). In a recent study by Palukaitis and Roossinck (1996), after 10 passages in tobacco benign satRNA was spontaneously changed to a pathogenic variant. Based on this they expressed their worry that use of this satRNA for controlling virus diseases might pose an inherent risk.

Heteroencapsidation of viral RNA by transgenically expressed CP does occur and it is common in cases of closely related viruses but not for distantly related or for viruses of different groups. A good example is that the CP of plum pox potyvirus confer aphid-transmissibility on a non-transmissible isolate of zucchini yellow mosaic virus (cited in Wilson et al. 1993). However some efforts have been taken to prevent heteroencapsidation or to block aphid transmission of heteroencapsidated virions without affecting the effect of CPMR. For example, PPV-CP mutated constructs designed to prevent the heteroencapsidation or aphid-attachments were transgenically expressed in *N. benthamiana* plants. Performance of these plants was evaluated on infection with zucchini yellow mosaic virus (ZYMV-NAT) (Jacquet et al. 1998). The potential risk of other kinds of heteroencapsidation (cited in Tepfer 1993) and the effect on epidemiology of the virus has yet to be studied in details.

Genetic recombination between a transgene and an infecting virus and its potential risk has been described in many instances. Greene and Allison (1994), reported that genetic recombination between the parts of the capsid gene of cowpea chlorotic mottle virus expressed in transgenic *N. benthamiana* plant and the mutated virus could restore the systemic infectivity of the virus. In another report, *N. benthamiana* plants transgenic for the coat protein gene of tomato bushy stunt virus (TBSV) was inoculated with TBSV mutants containing defective (point mutation and deletion mutation) coat protein gene. It was found that a double recombination event between coat protein transgene and the defective virus could regenerate the wild type virus. Similar results were obtained with a chimeric cucumber necrosis virus (CNV) containing the defective TBSV coat protein gene. CNV chimeric RNA was obtained, generated by recombination with wild type TBSV-CP transgene (Borja et al. 1999). More serious concern has been raised with the use of movement proteins. These have been shown that a functional analog of MP of TMV increases susceptibility of several other viruses (Cooper et al. 1995), genetic recombination with the transgene increased the systemic movement of CaMV (Wintermantel & Scholez 1996) and a hybrid RNA virus between CMV and TAV for the movement function found to be more virulent (Ding et al. 1996). Another recent observation showed that the synergistic disease

development phenomena by PVX and PVY could be attributed only to the transgenic expression of the 5' proximal third (containing MP gene) of other potyviruses (Vance et al. 1995). So, the recombination can result in a more pathogenic virus. But under natural condition a recombination is common between similar sequences of virus on mixed infection and the mutation rate in viral genome replication is also high. Therefore, a new recombination with transgene will only be dangerous when that rate will overcome the natural frequency of occurrence. On the other hand, use of mutated or truncated form of CP in transgenic protection has been successfully reduced the risk of heterologous encapsidation or particle assembly with effective resistance (Varrelmann & Maiss 2000). Moreover as Falk and Bruening (1994) argued the potential benefits of engineered resistance genes far outweigh the infinitesimally small risk of creating new and harmful viruses in significant excess over those being created by natural process.

Future Prospects

Apart from the strategies described above, considerable potential exists in developing new strategies for virus resistance. Recently, reports are appearing on use of multiple forms of protection to elevate the resistance and for broader spectrum of resistance. Yie et al.(1992) found that transgenic tobacco plants expressing both coat protein gene of cucumber mosaic virus (CMV) and symptom attenuating satRNA provided twice the protection observed with expression of either construct. The cantaloupe line, containing multiple coat protein genes from CMV, zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus 2 (WMV2) was resistant to all these three viruses under field condition (Fuchs et al. 1997). This concept was further expanded by using a plant carrying the endogenous resistance gene. As was the case described by Xu et al. (1997b), they transformed tobacco plant carrying an endogenous resistance gene known as Virgin A mutant (VAM) with tobacco vein mottling (TVMV)-coat protein gene. The transgenic line had greater resistance and a broader spectrum of resistance against potyviruses than did transgenic lines without the VAM gene. Another important finding in the use of heterologous gene for controlling virus has been described by Dolja et al. (1997). They used tobacco etch virus (TEV) based expression system for the insertion of unrelated beet yellow virus leader proteinase (BYV) (L-Pro) open reading frame. It was found that BYV L-Pro severely impaired systemic transport and accumulation of recombinant TEV in tobacco but not in *N. benthamiana* plants. So the Closterovirus L-Pro can inhibit potyvirus infection in a host-dependent manner. Similar kind of result was also obtained using potato virus X (PVX) vector carrying coat protein of TMV. Both TMV CP and its RNA coding sequences when expressed by the PVX vector, conferred protection against subsequent TMV challenge inoculation (Culver 1996). The most surprising results were obtained in the resistance against potato leaf roll virus (PLRV) in transgenic potato plant (Presting et al. 1995). Where the plants transformed with control construct carrying the vector sequence and *nptII* gene, were highly resistant to PLRV infection and the resistance phenotype was heritable.

Tobacco plants expressing antisense RNA of uroporphyrinogen decarboxylase (UROD) or coproporphyrinogen oxidase (CPO) induces pathogen defense responses. As a result PR protein expression increased and salicylic acid accumulates in cell. In infection with TMV these plants showed reduced accumulation of viral mRNA (Mock et al. 1999). Reduced activity of UROD and CPO in plant leads to the accumulation of tetrapyrrole. Reactive oxygen species (ROS) are derived from these tetrapyrrole and trigger the pathogen defense responses. Lesion formation in TMV infected tobacco is attributed to the programmed cell death (PCD) and ambient oxygen level has a role in PCD. So, future research would correlate the role of tetrapyrroles in PCD and further developments in the field of controlling virus through PCD are expected. On the other hand, unfolding of the plant signal transduction pathway in the gene-for-gene interaction with the pathogen is indicating that activation of common defense

responses is the cause of resistance. Therefore, transfer of the genes of that pathway to susceptible hosts probably would lead to a broad spectrum of resistance against viruses.

During infection with necrotizing viruses a rapid accumulation of reactive oxygen species occurs. Among these ROS, the free hydroxyl radicals ($OH^{\cdot}$) are most harmful. Accumulation of this radical depends on the presence of free iron in the living cells. Free iron catalyzes oxidative reactions. Therefore, control of the accumulation of free iron would be a potential way of preventing oxidative damage on pathogen infection. There are some iron-binding proteins (ferritin) present in plant which are transcriptionally regulated upon oxidative stress. Deak et al. (1999) took the challenge of assessing this possibility by overexpressing alfalfa ferritin in transgenic tobacco. They inoculated the plants with tobacco necrosis virus (TNV) and found that transgenic plants developed reduced symptoms compared to the control. Although the exact nature of this protection remains to be established, still it opens a new possibility of controlling virus through manipulating a general mechanism of plant.

Use of plant derived gene in protection can carefully avoid the questions of effect of heterologous gene products on food quality. Therefore, recent report of using plant proteinase inhibitor against potyviral cysteine proteinase (NIa) is of a promising work (Gutierrez-Campos et al. 1999). Potyviral polyprotein is processed by cysteine proteinase NIa and constitutive expression of rice cysteine proteinase inhibitor in tobacco lines produced resistance against two potyviruses PVY and TEV. This finding can be used potentially also against other viruses which require a cysteine proteinase activity during replication.

The discovery of correlation between RNA-mediated virus resistance (RmVR) and post-transcriptional gene-silencing (PTGS) is paving the way of engineering plants for very specific and robust resistance against viruses (Bruening 1998). There are several mechanistic models of RmVR and PTGS available to date (Baulcombe 1999, Dalmay et al. 2000, Fagard & Vaucheret 2000, Meins 2000, VanHoudt et al. 2000, Wassenegger & Pelissier 1998, Waterhouse et al. 1999). It has been proposed that, initially small aberrant RNAs are produced from the transgene. These aberrant RNAs direct the synthesis of antisense RNA to the transgene by action of a RNA-dependent RNA-polymerase. The antisense RNA would then hybridize with complementary RNA sequences such as transgene mRNA and infecting viral RNAs, leading to formation of double-stranded RNA molecules. Sequence specific degradation of these hybrid molecules by dsRNA-specific RNAses would finally destroy the viral as well as the corresponding mRNA. In accordance with this model it has been observed that virus resistance and gene silencing in plants is inducible by simultaneous expression of sense and antisense RNA in the same plant (Waterhouse 1998). PTGS represents a natural mechanism for plant protection against viruses. Because it has been found there are certain suppressors of PTGS encoded by viral genome (Brignetti et al. 1998). It explains the synergistic effect of different virus infection in plant (e.g. PVX and PVY synergism). Where as the mechanism of cross protection could be explained by induction of PTGS by initial inoculum. Therefore, future strategies would include delivery of RNAs with the potential to form RNA-RNA hybrid for induction of gene silencing and virus resistance in transgenic plants.

There are possibilities of combining virus resistance with other pathogen or pest resistance genes. Recently a report on this approach has described the expression of Bt (*Bacillus thuringiensis*) delta toxin gene with cucumber mosaic virus coat protein (CMV-CP) gene in transgenic tobacco plant. The plant was protected from CMV infection and tolerant to feeding damage by *Manduca sexta* (Liang et al. 1994). Coexpression of PVY-CP and CryV-Bt genes in potato conferred resistance to both PVY and potato tuber moth (Li et al. 1999). All these examples are pointing that enough scope is still there for developing transgenic plants resistant to several simultaneous biotic stresses.

Most of the genes described in this review have been patented. Several coat protein mediated virus-resistant transgenic plants have

been commercially released. For example, Freedom II squash had been released by Asgrow which was the first disease resistant transgenic crop released for cultivation in commercial level. As mentioned earlier, this crop had coat protein genes of two viruses, Zucchini yellow mosaic virus (ZYMV) and Water melon mosaic virus 2 (WMV2) and was evaluated in the field under severe incidence of ZYMV and WMV2 (Fuchs & Gonsalves 1995). None of the transgenic plants developed severe symptoms when all the control plants were severely infected. Although this crop showed excellent resistance against both the viruses under experimental field trials, but Asgrow withdrew FreedomII from distribution after poor results from the commercially cultivated squash fields. Development of Papaya ring spot virus (PRSV) resistant UH Sun Up and UH Rainbow papaya cultivars is another success story of pathogen derived resistance. PRSV causes the most serious virus disease of papaya worldwide and in Hawaii it almost devastated the state's second largest crop industry. Scientists in University of Hawaii in collaboration with others attempted to develop papaya plants expressing the CP gene of PRSV. In 1992, Fitch et al (1992) reported that transgenic papaya plant line 55-1 expressing the CP was highly resistant to the PRSV strain isolated from Hawaii. Although this line did not show good resistance against strains of other geographical region, it was evaluated for its performance in Hawaii fields. Field trials were accomplished with two newly named varieties UH Sun Up, which was the original 55-1 line homozygous for the coat protein gene and UH Rainbow, which was a hybrid between transgenic UH Sun Up and a nontransgenic popular cultivar. Result was dramatic and most of the transgenic lines remained resistant even 35 months after initiating the trial. Fruit quality was also appreciable. All the official procedures for commercialization was completed by 1998 and in the same year farmers of Hawaii started growing the transgenic papaya crop (Gonsalves 1998). Transgenic seeds are being produced by the Hawaii Agriculture Research Center and by 2001 it will furnish about 27 million seeds. Resistant tomato, tobacco and capsicum are commercially available in China. Besides, a large number of transgenic virus-resistant crop plants are in the advanced stage of field trial and waiting for commercial release. A comprehensive list of these may be obtained in the reference of James (1999) and in World Wide Web (http://www. psrast.org/gexappr1298.htm).

Concluding Remarks

In this review we have illustrated the successful strategies employed so far to produce virus resistant transgenic plants. Developing broad and durable resistance is the main objective in transgenic virus resistant plant production. In addition, it is also desirable of limiting virus spread within a plant and from one plant to another. All the strategies described here have its specific advantage over other. But some strategies have fulfilled these above mentioned criteria. In CPMR, it has been shown that broad spectrum resistance was produced as well as virus spread was limited. Defective MP also gave broader resistance in some cases and it prevented virus movement within a plant thus a reduction in virus titer was possible. In field it is highly probable that a plant will be simultaneously infected with several different viruses. Therefore, for durable resistance introduction of multiple CP gene is desired. Continuing research on natural resistance genes and non-host factors has opened up the possibility of combining host encoded factor derived resistance with PDR as a strategy to ensure durability and enhanced resistance. Activation of PTGS pathway by introduction of viral genes although showed promising results for developing strong resistance but effectiveness of this approach has yet to be confirmed under large scale field trials. Similarly other approaches also needed to be evaluated under extensive field exposure with high inoculum. The rapidly accumulating knowledge on host genome and studies on mechanisms by which resistance is activated would help in further modification of these presently available strategies for engineering durable and broader spectrum disease resistance in transgenic plants.

References

Anderson J M, Palukaitis P and Zaitlin M 1992 A defective replicase gene induces resistance to cucumber mosaic virus in transgenic tobacco plants; *Proc. Natl. Acad. Sci.* USA **89** 8759-63

Aragao F J L, Ribeiro S G, Barros L M G, Brasileiro A C M, Maxwell D P, Rech E L and Faria J C 1998 Transgenic beans (*Phaseolus vulgaris* L.) engineered to express viral antisense RNAs show delayed and attenuated symptoms to bean golden mosaic geminivirus; *Mol. Breeding* **4** 491-499

Ares X, Calamante G, Cabral S, Lodge J, Hemenway P, Beachy R N and Mentaberry A 1998 Transgenic plants expressing potato virus X ORF2 protein (p24) are resistant to tobacco mosaic virus and Ob tobamoviruses; *J. Virol.* **72** 731-738

Ayre B G, Kohler U, Goodman H M and Haseloff J 1999 Design of highly specific cytotoxins by using trans-splicing ribozymes; *Proc. Natl. Acad. Sci.* USA **96** 3507-3512

Bardonnet N, Hans F, Serghini M A and Pinck L 1994 Protection against virus infection in tobacco plants expressing the coat protein of grapevine fanleaf nepovirus; *Plant Cell Rep.* **13** 357- 360

Baulcombe D 1989 Strategies for virus resistance in plants; *Trends Genet.* **5** 56-60

Baulcombe D 1999 Viruses and gene silencing in plants; *Arch. Virol. Suppl.***15** 189-201

Baulcombe D C 1996 Mechanisms of pathogen-derived resistance to viruses in transgenic plants; *Plant Cell* **8** 1833-1844

Beachy R N 1993 Virus resistance through expression of coat protein genes; *Biotechnology in Plant Disease Control* pp 89-104 ed I Chet (Wiley-Liss Inc.)

_____1996 The 2'-5' A antiviral system in plants : A dose of mammalian medicine; *Nat. Biotechnol.* **14** 1538-1539

_____, Abel P, Oliver M J, De B, Fraley R T and Rogers S G 1985 Potential for applying genetic transformation to studies of viral pathogenesis and cross-protection; *Biotechnology in Plant Science: Relevance to Agriculture in the Nineteen Eighties* pp 265-275 eds M Zaitlin, P Day and A Hollander (A.P. Inc.)

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

Beck D L, Dollewerd C J V, Lough T J, Balmari E, Voof D M, Anderson M T, O'Brien I E W and Forster R L S 1994 Disruption of virus movement confers broad-spectrum resistance against systemic infection by plant viruses with a triple gene block; *Proc. Natl. Acad. Sci.* USA **91** 10310-10314

Bendahmane M and Gronenborn B 1997 Engineering resistance against tomato yellow leaf curl virus (TYLCV) using antisense RNA; *Plant Mol. Biol.* **33** 341-357

Bergelson J, Purrington C B and Wichmann G 1998 Promiscuity in transgenic plants; *Nature* **395** 25

Bertoli D J, Cooper J I, Edwards M L and Hawes W S 1992 Arabis mosaic nepovirus coat protein in transgenic tobacco lessens disease severity and virus replication; *Ann. Appl. Biol.* **120** 47- 54

Bevan M W, Mason S E and Goelet P 1985 Expressioon of tobacco mosaic virus coat protein by a cauliflower mosaic virus promooter in plants transformed by Agrobacterium; *EMBO J.* **4** 1921-1926

Blanc S , Cerutti M, Usmany M, Valk J M and Hull R 1993 Biological activity of cauliflower mosaic virus aphid transmission factor expressed in a heterologous system; *Virology* **192** 643-650

Borneman J, Tritz R, Hampel A and Altschuler M 1995 Detection of cleavage products from an *in vitro* trascribed cis hairpin ribozyme in turnips using the CaMV plant virus; *Gene* **159** 137-142

Borza M, Rubio T, Scholthof H B and Jackson A O 1999 Restoration of wild type virus by double recombination of tombusvirus mutants with a host transgene; *Mol. Plant Microbe Interact.* **12** 153-162

Braun C J and Hemenway C L 1992 Expression of amino-terminal portions or full length viral replicase genes in transgenic plants confers resistance to potato virus X infection; *Plant Cell* **4** 735-744

Brault V, Candresse T, Le Gall O, Delbos R P, Lanneau M and Dunez J 1993 Genetically engineered resistance against grapevine chrome mosaic nepovirus; *Plant Mol. Biol.* **21** 89-97

Brigneti G, Voinnet O, Li W X, Ji L H, Ding S W and Baulcombe D C 1998 Viral pathogenecity determinants are suppressors of transgene silencing in *Nicotiana benthamiana*; *EMBO J.* **17** 6739-6746

Brunt A A, Crabtree K, Dall Witz M J, Gibbs A J and Watson L 1996 *Viruses of plants : Description and lists from the VIDE database* (CAB International)

Bruening G 1998 Plant gene silencing regularized; *Proc. Natl. Acad. Sci.* USA **95** 13349-13351

Carr J P, Marsh L E, Lomonossoff G P, Sekiya M E and Zaitlin M 1992 Resistance to tobacco mosaic virus induced by the 54 kDa gene sequence requires expression of the 54 kDa protein; *Mol. Plant Microbe Interact.* **5** 397-404

_____, Gal on A, Palukaitis P and Zaitlin M 1994 Replicase-mediated resistance to cucumber mosaic virus in transgenic plants involves suppression of both virus replication in the inoculated leaves and long-distance movement; *Virology* **199** 439-447

Cassidy B G and Nelson R S 1995 Differences in protection phenotypes in tobacco plants expressing coat protein genes from peanut stripe potyvirus with or without an engineered ATG; *Mol. Plant Microbe Interact.* **8** 357-365

Chowrira G M, Cavileer T D, Gupta S K, Lurquin P F and Berger P H 1998 Coat protein-mediated resistance to pea enation mosaic virus in transgenic *Pisum sativum* L.; *Transgenic Res.* **7** 265-271

Clark W G, Fitchen J H and Beachy R N 1995 Studies of coat protein-mediated resistance to TMV : the PM2 assembly defective mutant confers resistance to TMV; *Virology* **208** 485-491

_____, Register III J C, Nejidat A, Eichholtz D A, Sanders P R, Fraley R T and Beachy R N 1990 Tissue specific expression of the TMV coat protein in transgenic tobacco plants affects the level of coat protein mediated virus protection; *Virology* **179** 640-647

Cooper B, Lapidot M, Heick J A, Dodds A and Beachy R N 1995 A defective movement protein of TMV in transgenic plants confers resistance to multiple viruses whereas the functional analog increases susceptibility; *Virology* **206** 307-313

Culver J N 1996 Tobamovirus cross protection using a potexvirus vector; *Virology* **226** 228-235

Cuozzo M, O'Connell K M, Kaniewski W K, Fang R K, 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; *Biotechnology* **6** 549-557

Dalmay T, Hamilton A, Mueller E and Baulcombe D C 2000 Potato virus X amplicons in arabidopsis mediate genetic and epigenetic gene silencing; *Plant Cell* **12** 369-379

Day A G, Bejarano E R, Buck K W, Byrrell M and Lichtenstein C P 1991 Expression of an antisense viral gene in transgenic tobacco confers resistance to the DNA virus tomato golden mosaic virus; *Proc. Natl. Acad. Sci.* USA **88** 6721-6725

Deak M, Horvath G V, Daveletova S, Torok K, Sass L, Vass I, Barna B, Kiraly Z and Dudits D 1999 Plants ectopically expressing iron-binding protein, ferritin, are tolerant to oxidative damage and pathogens; *Nat. Biotechnol.* **17** 192-196

De-Feyter R, Young M, Schroeder K, Dennis ES and Gerlach W 1996 A ribozyme gene and an antisense gene are equally effective in conferring resistance to tobacco mosaic virus on transgenic tobacco; *Mol. Gen. Genet.* **250** 329-338

deHaan P, Gielen J J L, Prins M, Wijkamp I G, Schepen A V, Peters D, Grinsven M Q J M V and Goldbach R L 1992 Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants; *Biotechnology* **10** 1133-1137

Ding S W, Shi B J, Li W X and Symons R H 1996 An interspecies hybrid RNA virus is significantly more virulent than either parental virus; *Proc. Natl. Acad. Sci.* USA **93** 7470-7474

Dolja V V, Hong J, Keller K E, Martin R R and Peremyslov V V 1997 Suppression of potyvirus infection by coexpressed closterovirus protein; *Virology* **234** 243-252

Fagard M and Vaucheret H 2000 Systemic silencing signal(s); *Plant Mol. Biol.* **43** 285-293

Falk B H and Bruening G 1994 Will transgenic crops generate new viruses and new diseases ? *Science* **263** 1395-1396

Fang G and 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

Fellers J P, Collins G B and Hunt A G 1998 The NIa-proteinase of different plant potyviruses provides specific resistance to viral infection; *Crop Sci.* **38** 1309-1319

Fitch M M M, Manshardt R M, Gonsalves D, Slightom J L and Sandford J C 1992 Virus resistant papaya plants derived from tissues bombarded with the coat protein gene of papaya ringspot virus; *Biotechnology* **10** 1466-1472

Fitchen J H and Beachy R N 1993 Genetically engineered protection against viruses in transgenic plants; *Annu. Rev. Microbiol.* **47** 739-763

Franconi R, Roggero P, Pirazzi P, Arias F J, Desiderio A, Bitti O, Pashkoulov D, Mattei B, Bracci L, Masenga V, Milne R G and Benvenuto E 1999 Functional expression in bacteria and plants of an scFv antibody fragment against tospoviruses; *Immunotechnology* **4** 189-201

Frischmuth T, Engel M and Jeske H 1997 Beet curly top virus DI RNA-mediated resistance is linked to its size; *Mol. Breeding* **3** 213-217

Flavell R B 1994 Inactivation of gene expression in plants as a consequence of specific sequence duplication; *Proc. Natl. Acad. Sci.* USA **91** 3490-3496

_____, Dart E, Fuchs R L, Fraley R J 1992 Selectable marker genes : Safe for plants ? *Biotechnology* **10** 141-143

Fuchs M and Gonsalves D 1995 Resistance of transgenic hybrid squash ZW-20 expressing the coat protein genes of zucchini yellow mosaic virus and watermelon mosaic virus 2 to mixed infections by both potyviruses; *Biotechnology* **13** 1466-1473

_____, Mcferson J R, Tricolli D M, Mcmaster J R, Deng R Z, Boeshore M L, Reynolds J F, Russell P F, Quemada H D and Gonsalves D 1997 Cantaloupe line CZW-30 containing coat protein genes of cucumber mosaic virus, zucchini yellow mosaic virus and watermelon mosaic virus-2 is resistant to these three viruses in the field; *Mol. Breeding.* **3** 279-290

Fuchs M, Provvidenti R, Slightom J L and Gonsalves D 1996 Evaluation of transgenic tomato plants expressing the coat protein gene of cucumber mosaic virus strain WL under field conditions; *Plant Dis.* **80** 270-275

Fuchs R L, Heeren R A, Gustafson M E, Rogan G J, Bartnicki D E, Leimgruber R M, Finn R F, Herhman A and Berberich S A 1993a Purification and characterization of microbially expressed neomycin phosphotransferase II (NPTII) protein and its equivalence to the plant expressed protein; *Biotechnology* **11** 1537-1542

_____, Ream J E, Hammond B G, Naylor M W, Leimgruber R M and Berberich S A 1993b Safety assesment of the neomycin phosphotransferase II (NPTII) protein; *Biotechnology* **11** 1543-1547

Fulton R W 1986 Practices and precautions in the use of cross protection for plant virus disease control; *Annu. Rev. Phytopathol.* **24** 67-71

Gerlach W C, Llewellyn D and Hareloff J 1987 Construction of a plant disease resistance gene from the satellite RNA of tobacco ringspot virus; *Nature* **328** 802-805

Gielen J J L, deHaan P, Kool A J, Peters D, van Grinsven M Q J M and Goldbach R W 1991 Engineered resistance to tomato spotted wilt virus,a negative-strand RNA virus; *Biotechnology* **9** 1363-1367

Golemboski D B, Lomonossoff G P and Zaitlin M 1990 Plants transformed with a tobacco mosaic virus nonstructural gene sequence are resistant to the virus; *Proc. Natl. Acad. Sci.* USA **87** 6311-6315

Gonsalves D 1998 Control of papaya ringspot virus in papaya: A case study; *Annu. Rev. Phytopathol.* **36** 415-437

Gonsalves D, Chee P, Provvidenti R, Seem R and Slightom J L 1992 Comparison of coat protein-mediated and genetically-derived resistance in cucumbers to infection by cucumber mosaic virus under field conditions with natural challenge inoculations by vectors; *Biotechnology* **10** 1562-1570

Greene A E and Allison R F 1994 Recombination between viral RNA and transgenic plant transcripts; *Science* **263** 1423-1425

Guo H S, Cervera M T and Garcia J A 1998 Plum pox potyvirus resistance associated to transgene silencing that can be stabilized after different number of plant generations; *Gene* **206** 263-272

Gupta P K 1996 Transgenic plant : some current issues; *Curr. Sci.* **70** 654-660

Gutierrez-Campos R, Torres-Acosta J A, Saucedo-Arias L J and Gomez-Lim M A 1999 The use of cysteine proteinase inhibitors to engineer resistance against potyviruses in transgenic tobacco plants; *Nat. Biotechnol.* **17** 1223-1226

Hammond J 1997 Repelling plant pathogens with ribonuclease; *Nat. Biotechnol.* **15** 1247

Harrison B D, Mayo M A and Baulcombe D C 1987 Virus resistance in transgenic plants that express cucumber moosaic virus satellite RNA; *Nature* **328** 799-802

Hassairi A, Masmoudi K, Albouy J, Robaglia C, Jullien M and Ellouz R 1998 Transformation of two potato cultivars 'Spunta' and 'Claustar'(*Solanum tuberosum*) with lettuce mosaic virus coat protein gene and heterologous immunity to potato virus Y; *Plant Sci.* **136** 31-42

Hayakawa T, Zhu Y, Itoh K, Kimura Y and Izawa T 1992 Genetically engineered rice resistant to rice stripe virus, an insect-transmitted virus; *Proc. Natl. Acad. Sci.* USA **89** 9865-9869

Hellwald K H and Palukaitis P 1995 Viral RNA as a potential target for two independent mechanisms of replicase-mediated resistance against cucumber mosaic virus; *Cell* **83** 937-946

Hemenway C L, 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

Hill K K, Jarvis-Eagan N, Halk E L, Krahn K J, Liaò L W, Mathewson r J, Merlo D J, Nelson S E, rashka K E and Loesch-Fries L S 1991 The development of virus resistant alfalfa, *Medicago sativa* L.; *Biotechnology* **9** 373-377

Hoekema A, Huisman M J, Molendijk L, van den Elzen P J M and Cornelissen B J C 1989 The genetic engineering of two commercial potato cultivars for resistance to potato virus X; *Biotechnology* **7** 273-278

Hong Y, Saunders K, Hartley M R and Stanley J 1996 Resistance to geminivirus infection by virus induced expression of dianthin in transgenic plants; *Virology* **220** 119-127

____, Saunders K, and Stanley J 1997 Transactivation of dianthin transgene expression by African-cassava-mosaic virus AC2; *Virology* **228** 373-377

Hou Y M, Sanders R, Ursin V M and Gilbertson R L 2000 Transgenic plants expressing geminivirus movement proteins: abnormal phenotypes and delayed infection by Tomato mottle virus in transgenic tomatoes expressing the Bean dwarf mosaic virus BV1 or BC1 proteins; *Mol. Plant Microbe Interact.* **13** 297-308

Huntley C C and Hall T C 1996 Interference with brome mosaic virus replication in transgenic rice; *Mol. Plant Microbe Interact.* **9** 164-170

Ingelbrecht I L, Irvine J E and Mirkov T E 1999 Posttranscriptional gene silencing in Transgenic sugarcane. Dissection of homology dependent virus resistance in a monocot that has a complex polyploid genome; *Plant Physiol.* **119** 1187-1198

Jacquet C, Delecolle B, Raccah B, Lecog H, Dunez J and Ravelonandro M 1998 Use of modified plum-pox virus coat protein genes developed to limit heteroencapsidation-associated risks in transgenic plants; *J. Gen. Virol.* **79** 1509-1517

Jacquet C, Ravelonandro M, Bachelier J C and Dunez J 1998 High resistance to plum-pox virus (PPV) in transgenic plants containing modified and truncated forms of coat protein gene; *Transgenic Res.* **7** 29-39

James C 1999 Global review of commercialization of transgenic crops *ISAAA BRIEFS* (ISAAA) Ithaca, NY

Jan F J, Fagoaga C, Pang S Z and Gonsalves D 2000 A single chimeric transgene derived from two distinct viruses confers multi-virus resistance in transgenic plants through homology-dependent gene silencing; *J. Gen. Virol.* **81** 2103-2109

_____, Pang S Z, Fagoaga C and Gonsalves D 1999 Turnip mosaic potyvirus resistance in *Nicotiana benthamiana* derived by post-transcriptional gene silencing; *Transgenic Res.* **8** 203-13

Jones A L, Johansen I E, Bean S J, Bach I and Maule A J 1998 Specificity of resistance to pea seed-borne mosaic potyvirus in transgenic peas expressing the viral replicase (NIb) gene; *J. Gen. Virol.* **79** 3129-3137

Jones R W, Jackson A O and Morris T J 1990 Defective-interfering RNAs and elevated temperatures inhibit replication of tomato bushy stunt virus in inoculated protoplasts; *Virology* **176** 539-545

Jongedijk E, de Schutter A A J M, Stolte T, vanden Elzen P J M and Cornelissen B J C 1992 Increased resistance to potato virus X and preservation of cultivar properties in transgenic potato under field conditions; *Biotechnology* **10** 422-429

Kaido M, Mori M, Mise K, Okuno T and Furusawa I 1995 Inhibition of brome mosaic virus (BMV) amplification in protoplasts from transgenic tobacco plants expressing replicable BMV RNAs; *J. Gen. Virol.* **76** 2827-2833

Kallerhoff J, Perez P, Bouzoubaa S, BenTahar S and Perret J 1990 Beet necrotic yellow vein virus coat protein-mediated protection in sugarbeet (*Beta vulgaris* L.) protoplasts; *Plant Cell Rep.* **9** 224-228

Kaniewski W, Lawson C, Sammons B, Haley L, Hart J, Delannay X and Tumer N E 1990 Field resistance of transgenic Russet Burbank potato to effects of infectioon by potato virus X and potato virus Y; *Biotechnology* **8** 750-754

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

_____, Martin R R and Mcpherson J 1991 Sense and antisense RNA- mediated resistance to potato leafroll virus in russet burbank potato plants; *Mol. Plant Microbe Interact.* **4** 247-253

Kim S J, Lee S J, Kim B D and Paek K H 1997 Satellite-RNA mediated resistance to cucumber mosaic virus in transgenic plant of hot pepper (*Capsicum annuum* cv. Golden Tower); *Plant Cell Rep.* **16** 825-830

Kollar A, Dalmay T and Burgyan J 1993 Defective interfering RNA- mediated resistance against cymbidium ringspot tombusvirus in transgenic plants; *Virology* **193** 313-318

Kong Q, Wang J and Simon A E 1997 Satellite RNA-mediated resistance to turnip crinkle virus in Arabidopsis involves a reduction in virus movement; *Plant Cell* **9** 2051-2063

Kunik T, Salomon R, Zamir D, Navot N, Zeidan M, Michelson I, GafniY and Czosnek H 1994 Transgenic tomato plants expressing the tomato yellow leaf curl virus capsid protein are resistant to the virus; *Biotechnology* **12** 500-504

Lam Y H, Wong Y S, Wang B, Wong R N S, Yeung H W and Shaw P C 1996 Use of trichosanthin to reduce infection by turnip mosaic virus; *Plant Sci.* **114** 111-117

Lapidot M, Gafny R, Ding B, Wolf S, Lucas W J and Beachy R N 1993 A dysfunctional movement protein of tobacco mosaic virus that partially modifies the plasmodesmata and limits virus spread in transgenic plants; *Plant J.* **4** 959-970

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; *Biotechnology* **8** 127-134

Leclerc D and Abouhaider M G 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

Li W B, zarka K A, Douches D S, Coombs J J, Pett W L and Grafius E J 1999 Coexpression of potato PVYo coat protein and cryV-Bt genes in potato; *J. Amer. Soc. Hort. Sci.* **124** 218-223

Li X, Heaton L A, Morris T J and Simon A E 1989 Turnip crinkle virus defective interfering RNAs intensify viral symptoms and are generated de novo; *Proc. Natl. Acad. Sci.* USA **86** 9173-9177

Li Z, Jarret R L and Demski J W 1997 Engineered resistance to tomato-spotted-wilt virus in transgenic peanut expressing the viral nucleocapsid gene; *Transgenic Res.* **6** 297-305

Liang X, Zhu Y, Mi J and Chen Z 1994 Production of virus resistant and insect tolerant transgenic tobacco plants; *Plant Cell Rep.* **14** 141-144

Lim S H, Ko M K, Lee S J, La Y J and Kim B D 1999 Cymbidium mosaic virus coat protein gene in antisense confers resistance to transgenic *Nicotiana occidentalis*; *Mol. Cells* **9** 603-608

Lindbo J A and Dougherty W G 1992 Pathogen-derived resistance to a potyvirus : Immune and resistant phenotypes in transgenic tobacco expressing altered forms of a potyvirus coat protein nucleotide sequence; *Mol. Plant Microbe Interact.* **5** 144-153

Ling K, Namba S, Gonsalves C, Slightom J L and Gonsalves D 1991 Protection against detrimental effects of potyvirus infection in transgenic tobacco plants expressing the papaya ringspot virus coat protein gene; *Biotechnology* **9** 752-758

Ling K S, Zhu H Y, Alvizo H, Hu J S, Drong R F, Slightom J L and Gonsalves D 1997 The coat protein gene of grapevine leafroll associated closterovirus-3: cloning, nucleotide sequencing and expression in transgenic plants; *Arch. Virol.* **142** 1101-1116

Lodge J K, Kaniewski W K and Tumer N E 1993 Broad resistance in transgenic plants expressing pokeweed antiviral protein; *Proc. Natl. Acad. Sci.* USA **90** 7089-7093

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 RNA4 in transgenic plant confers virus resistance; *EMBO J.* **6** 1845-1851

Lomonossoff G P 1995 Pathogen-derived resistance to plant viruses; *Annu. Rev. Phytopathol.* **33** 323-343

Lu B, Stubbs G and Culver J N 1998 Coat protein interactions involved in tobacco mosaic tobamovirus cross-protection; *Virology* **248** 188-198

Macfarlane S A and Davies J W 1992 Plants transformed with a region of the 201-kilodalton replicase gene from pea early browning virus RNA1 are resistant too virus infection; *Proc. Natl. Acad. Sci.* USA **89** 5829-5833

MacKenzie D J and Ellis P J 1992 Resistance to tomato spotted wilt virus infection in transgenic tobacco expressing the viral nucleocapsid gene; *Mol. Plant Microbe Interact.* **5** 34-40

_____ 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

_____, Tremaine J H and Mcpherson J 1991 Genetically engineered resistance to potato virus S in potato cultivar Russet Burbank; *Mol. Plant Microbe Interact.* **4** 95-102

Maia I G, Haenni A, Bernardi F 1996 Potyviral HC-Pro: a multifunctional protein; *J. Gen. Virol.* **77** 1335-1341

Maiti I B, Murphy J F, Shaw J G and Hunt A G 1993 Plants that express a potyvirus proteinase gene are resistant to virus infection; *Proc. Natl. Acad. Sci.* USA **90** 6110-6114

Maki-Valkama T, Valkonen J P, Kreuze J F and Pehu E 2000 Transgenic resistance to PVY(O) associated with post-transcriptional silencing of P1 transgene is overcome by PVY(N) strains that carry highly homologous P1 sequences and recover transgene expression at infection; *Mol. Plant Microbe Interact.***13** 366-73

Malnoe P, Farinelli L, Collet G F and Reust W 1994 Small-scale field tests with transgenic potato cv. Bintje, to test resistance to primary and secondary infections with potato virus Y; *Plant Mol. Biol.* **25** 963-975

Malyshenko S I, Kondakova O A, Nazarova J V, Kaplan I B, Taliansky M E and Atabekov J G 1993 Reduction of tobacco mosaic virus accumulation in transgenic plants prooducing non-functional viral transport proteins; *J. Gen. Virol.* **74** 1149-1156

Marsh L E, Pogue G P, Connell J P and Hall T C 1991 Artificial defective interfering RNAs derived from brome mosaic virus; *J. Gen. Virol.* **72** 1787-1792

Masuta C and Takanami Y 1989 Determination of sequence and structural requirements for pathogenicity of a cucumber mosaic virus satellite RNA (4-sat RNA); *Plant Cell* **1** 1165-1173

McGarvey P B, Montasser M S and Kaper J M 1994 Transgenic tomato plants expressing satellite RNA are tolerant to some strain of cucumber mosaic virus; *J. Am. Soc. Hortic. Sci.* **119** 642-647

Meins F 2000 RNA degradation and models for post-transcriptional gene silencing; *Plant Mol. Biol.* **43** 261-273

Mirkov T E, Yang Z N, Ingelbrecht I and Irvine J E 1997 Transgenic virus resistant sugarcane; *Phytopathology* **87** S114

Mitra A, Higgins D W, Langenberg W G, Nie H, SenGupta D N and Silverman R 1996 A mammalian 2-5 A system functions as an antiviral pathway in transgenic plants; *Proc. Natl. Acad. Sci.* USA **93** 6780-6785

Mock H P, Heller W, Molina A, Neubohn B, Sandermann H Jr and GrimmB 1999 Expression of uroporphyrinogende-carboxylaseor coproporphyrinogen oxidase antisense RNA in tobacco induces pathogen defense responses conferring increased resistance to tobacco mosaic virus; *J. Biol. Chem.* **274** 4231-4238

Moon Y H, Song S K, Choi K W and Lee J S 1997 Expression of a cDNA encoding *Phytolacca insularis* antiviral protein confers virus resistance on transgenic potato plants; *Mol. Cells* **7** 807-815

Moreno M, Bernal J J, Jimenez I and Rodriguez-Cerezo E 1998 Resistance in plants transformed with P1 and P3 gene of tobacco vein mottling potyvirus; *J. Gen. Virol.* **79** 2819-2827

Murry L E, Elliott L G, Capitant S A, West J A, Hanson K K, Scarafia L, Johnston S, Flaherty C D, Nichols S, Cunanan D, Dietrich P S, Mettler I J, Dewald S, Warnick D A, Rhodes C, Sinibaldi R M and Brunke K J 1993 Transgenic corn plants expressing MDMV strain B coat protein are resistant to mixed infections of maize dwarf mosaic virus and maize chlorotic mottle virus; *Biotechnology* **11** 1559-1564

Namba S, Ling K, Gonsalves C, Gonsalves D and Slightom J L 1991 Expression of the gene encoding the coat protein of cucumber mosaic virus (CMV) strain WL appears to provide protection to tobacco plants against infection by several different CMV strains; *Gene* **107** 181-188

_____, Ling K, Gonsalves C, Slightom J L and 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

Nejidat A and Beachy R N 1990 Transgenic tobacco plants expressing a coat protein gene of tobacco mosaic virus are resistant to some other tobamoviruses; *Mol. Plant Microbe Interact.* **3** 247-151

Nelson R S, McCormick S M, Delannay X, Dube P, Layton J, Anderson E J, Kaniewska M, Proksch R K, Horsch R B, Rogers S G, Fraley R T and Beachy R N 1988 Virus tolerance, plant growth and field performance of transgenic tomato plants expressing coat protein from tobacco mosaic virus; *Biotechnology* **6** 403-409

_____, Powell-Abel P and Beachy R N 1987 Lesions and virus accumula tion in inoculated transgenic tobacco plants expressing the coat protein gene of tobacco mosaic virus; *Virology* **158** 126-132

_____, Powell P A and Beachy R N 1990 Coat protein-mediated protection against virus infection; *Genetic Engineering of crop plants* pp 13-24 eds G W Lycett and D Grierson (Heidelberg: Springer Verlag)

Norris F, Accotto G P, Tavazza R, Brunetti A, Crespi S and Tavazza M 1996 Resistance to tomato yellow leaf curl geminivirus in *Nicotiana benthamiana* plants transformed with a truncated viral C1 gene; *Virology* **224** 130-138

Ogawa T, Hori T and Ishida I 1996 Virus-induced cell death in plants expressing the mammalian 2',5' oligoadenylate system; *Nat. Biotechnol.* **14** 1566-1569

Oldroyd G E D and Staskawicz B J 1998 Genetically engineered broad-spectrum disease resistance in tomato; *Proc. Natl. Acad. Sci.* USA **95** 10300-10305

Osbourn J K, Watts J W, Beachy R N and Wilson T M A 1989 Evidence that nucleocapsid disassembly and a later step in virus replication are inhibited in transgenic tobacco protoplasts expressing TMV coat protein; *Virology* **172** 370-373

Palukaitis P and Roossinck M J 1996 Spontaneous change of a benign satellite RNA of cucumber mosaic virus to a pathogenic variant; *Nat. Biotechnol.* **14** 1264-1268

Pang S Z, Bock J H, Gonsalves C, Slightom J L and Gonsalves D 1994 Resistance of transgenic Nicotiana benthamiana plants to tomato spotted wilt and impatiens necrotic spot tospoviruses : evidence of involvement of the N protein and N gene RNA in resistance; *Phytopathology* **84** 243-249

Jacquet C, Jan F J, Gonsalves D 1997 Nontarget DNA sequences reduce the transgene length necessary for RNA-mediated tospovirus resistance in transgenic plants; *Proc. Natl. Acad. Sci.* USA **15** 8261-8266

Jacquet C, Jacquet C, Tricoli D M, Russell P F, Carney K J, Hu JS, Fuchs M, Quemada H D and Gonsalves D 2000 Resistance to squash mosaic comovirus in transgenic squash plants expressing its coat protein genes; *Mol. Breeding* **6** 87-93

Peng Y H, Kadoury D, Gal-On A, Huet H, Wang Y, Raccah B 1998 Mutations in the HC-Pro gene of zucchini yellow mosaic potyvirus: effects on aphid transmission and binding to purified virions; *J. Gen. Virol.* **79** 897-904

Pirone T P, Blanc S 1996 Helper-dependent vector transmission of plant viruses; *Annu. Rev. Phytopathol.* **34** 227-247

Pinto Y M, Kok R A and Baulcombe D C 1999 Resistance to rice yellow mottle virus (RYMV) in cultivated African rice varieties containing RYMV transgenes; *Nat. Biotechnol.* **17** 702-707

Ponz F, Rowhani A, Mircetich S M and Bruening G 1987 Cherry leaf roll virus interactions are affected by a satellite RNA that the virus does not support; *Virology* **160** 183-190

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, 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

_____, 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

Presting G G, Smith O P and Brown C R 1995 Resistance to potato leaf roll in potato plants transformed with the coat protein gene or with vector control construct; *Phytopathology* **85** 436-442

Prins M, Haan P de, Luyten R, Veller M van, Grinsven M Q J M van and Goldbach R 1995 Broad resistance to tospoviruses in transgenic tobacco plants expressing three tospoviral nucleoprotein gene sequences; *Mol. Plant Microbe Interact.* **8** 85-91

_____, Kikkert M, Ismayadi C, deGraauw W, deHann W and Goldbach R 1997 Characterization of RNA-mediated resistance to tomato spotted wilt virus in transgenic tobacco plants expressing NSM gene sequences; *Plant Mol. Biol.* **33** 235-243

Prins M, and Goldbach R 1996 RNA-mediated virus resistance in transgenic plants; *Arch. Virol.* **141** 2259-2276

Quemada H D, Gonsalves D and Slightom J L 1991 Expression of coat protein from cucumber mosaic virus strain C in tobacco: protection against infection by CMV strains transmitted mechanically or by aphids; *Phytopathology* **81** 794-802

Radian-Sade S, Perl A, Edelbaum O, Kuznetsova L, Gafny R, Sela I and Tanne E 2000 Transgenic *Nicotiana benthamiana* and grapevine plants transformed with grapevine virus A (GVA) sequences; *Phytoparasitica* **28** 79-86

Ranjith-Kumar C T, Manoharan M, Prasad S K, Cherian S, Umashankar M, Sita G L and Savithri H S 1999 Engineering resistance against physalis mottle tymovirus by expression of the coat protein and 3' noncoding region; *Curr. Sci.* **77** 1542-1547

Ravelonandro M, Scorza R and Dunez J 1998 Characterization of phenotype resistance to plum pox of transgenic plums expressing plum pox virus capsid gene; *Acta Virol.* **42** 270-272

Reavy B, Arif M, Kashiwazaki S, Webster K D and Barker H 1995 Immunity to potato mop top virus in Nicotiana benthamiana plants expressing the coat protein gene is effective against fungal inoculation of the virus; *Mol. Plant Microbe Interact.* **8** 286-291

Register III 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

Regner F, da Camara Machado A, da Camara Machado M L, Steinkellner H, Mattanovich D, Hanzer V, Weiss H and Katinger H 1992 Coat protein mediated resistance to plum pox virus in *Nicotiana clevelandii* and *benthamiana*; *Plant Cell Rep.* **11** 30-33

Reimann-Philipp U and Beachy R N 1993 Coat protein protein mediated resistance in transgenic tobacco plants expressing the tobacco mosaic virus coat protein from tissue specific promoters; *Mol. Plant Microbe Interact.* **6** 323-330

_____, _____, 1998 Mechanism of Resistance. Expression of coat protein; *Methods Mol. Biol.* **81** 521-532

Roossinck M J, Sleat D and Palukaitis P 1992 Satellite RNAs of plant viruses : Structures and Biological effects; *Microbiol. Rev.* **56** 265-269

Rubino L and Russo M 1995 Characterization of resistance to cymbidium ringspot virus in transgenic plants expressing a full length viral replicase gene; *Virology* **212** 240-243

Russo P and Slack S A 1998 Tissue culture methods for the screening of putative virus resistant transgenic potato plants; *Phytopathology* **88** 437-441

Sanders P, Sammons B, Kaniewski W, Haley L, Layton J and Tumer N E 1992 Field resistance of transgenic tomatoes expressing the tobacco mosaic virus or tomato mosaic virus coat protein genes; *Phytopathology* **82** 683-690

Sanford J C and Johnston S A 1985 The concept of parasite-derived resistance : deriving resistance genes from the parasite's own genome; *J. Theo. Biol.* **113** 395-405

Sano T, Nagayama A, Ogawa T, Ishida I and Okada Y 1997 Transgenic potato expressing a double-stranded RNA-specific ribonuclease is resistant to potato spindle tuber viroid; *Nat. Biotechnol.* **15** 1290-1294

Sarkar S and Smitamana P 1981 A protein less mutant of tobacco mosaic virus : evidence against the role of a viral coat protein for interference; *Mol. Gen. Genet.* **184** 158-159

Schillberg S, Zimmermann S, Findlay K and Fischer R 2000 Plasma membrane display of anti-viral single chain Fv fragments confers resistance to tobacco mosaic virus; *Mol. Breeding* **6** 317-326

Scholthof K B G, Scholthof H B and Jackson A O 1993 Control of plant virus diseases by pathogen derived resistance in transgenic plants; *Plant Physiol.* **102** 7-12

Scorza R, Levy L, Damsteegt V, Yepes L M, Cordts J, Hadidi A, Slightom J and Gonsalves D 1995 Transformation of plum with the papaya ringspot virus coat protein gene and reaction of transgenic plants to plum pox virus; *J. Am. Soc. Hortic. Sci.* **120** 943- 952

Scott S E and Wilkinson M J 1998 Transgene risk is low; *Nature* **393** 320

Sequeira L 1984 Cross protection and induced resistance : their potential for plant disease control; *Trends Biotech.* **2** 25-29

Sivamani E, Brey C W, Dyer W E, Talbert L E and Qu R D 2000 Resistance to wheat streak mosaic virus in transgenic wheat expressing the viral replicase (NIb) gene; *Mol. Breeding* **6** 469-477

Sherwood J L 1987 Demonstration of the specific involvement of coat protein in tobacco mosaic virus (TMV) cross protection using a TMV coat protein mutant; *J. Phytopathol.* **118** 358-362

Sherwood J L and Fulton R W 1982 The specific involvement of coat protein in tobacco mosaic virus cross protection; *Virology* **119** 150-158

Sleat D E and Palukaitis P 1990 Site directed mutagenesis of a plant viral satellite RNA changes its phenotype from ameliorative to necrogenic; *Proc. Natl. Acad. Sci.* USA **87** 2946-2950

Smith H A, Swaney S L, Parks T D, Wernsman E A and Dougherty W G 1994 Transgenic plant virus resistance mediated by untranslatable sense RNAs : expression, regulation and fate of nonessential RNAs; *Plant Cell* **6** 1441-1453

Smith H A, Powers H, Swaney S, Brown C and Dougherty W 1995 Transgenic potato virus Y resistance in potato : evidence for an RNA-mediated cellular response; *Phytopathology* **85** 864-870

Sonoda S, Mori M and Nishiguchi M 1999 Homology-dependent virus resistance in transgenic plants with the coat protein gene of sweet potato feathery mottle potyvirus: Target specificity and transgene methylation; *Phytopathology* **89** 385-391

Spillane C, Verchot J, Kavanagh T A and Baulcombe D C 1997 Concurrent suppression of virus replication and rescue of movement-defective virus in transgenic plants expressing the coat protein of potato virus X; *Virology* **236** 76-84

Stanley J, Freschmuth T and Ellwood G 1990 Defective viral DNA ameliorates symptoms of geminivirus infection in transgenic plants; *Proc. Natl. Acad. Sci.* USA **87** 6291-6295

Stark D M and Beachy R N 1989 Protection against potyvirus infection in transgenic plants : evidence for broad spectrum resistance; *Biotechnology* **7** 1257-1262

Stewart C N Jr., Richards H A IV and Halfhill M D 2000 Transgenic plants and biosafety: science, misconceptions and public perceptions; *Biotechniques* **29** 832-843

Suzuki M, Masuta C, Takanami Y and Kuwata S 1996 Resistance against cucumber mosaic virus in plants expressing the viral replicon; *FEBS Lett.* **379** 26-30

Tacke E, Salamini F and Rhode W 1996 Genetic engineering of potato for broad spectrum protection against virus infection; *Nat. Biotechnol.* **14** 1597-1601

Tavladoraki P, Benvenuto E, Trinca S, Martinis D D, Cattaneo A and Galeffi P 1993 Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack; *Nature* **366** 469-472

Tenllado F, Garcia L I, Serra M T and Diaz-Ruiz J R 1995 Nicotiana benthamiana plants transformed with the 54 kD region of the pepper mild mottle tobamovirus replicase gene exhibit two types of resistance responses against viral infection; *Virology* **211** 170-183

Tennant P F, Gonsalves C, Ling K S, Fitch M, Manshardt R, Slightom J L and Gonsalves D 1994 Differential protection against papaya ringspot virus isolates in coat protein gene transgenic papaya and classically cross-protected papaya; *Phytopathology* **84** 1359-1366

Tepfer M 1993 Viral genes and transgenic plants : what are the potential environmental risks? *Biotechnology* **11** 1125-1131

Tobias C M, Oldroyd G E D, Chang J H and Staskawicz B J 1999 Plants expressing the *Pto* disease resistance gene confer resistance to recombinant PVX containing the avirulence gene *AvrPto*; *Plant J.* **17** 41-50

Tomassoli L, Ilardi V, Barba M and Kaniewski W 1999 Resistance of transgenic tomato to cucumber mosaic cucumo virus under field conditions; *Mol. Breeding* **5** 121-130

Tricolli D M, Carney K J, Russell P F, McMaster J R, Groff 1 D W, Hadden K C, Himmell P T, Hubbard J P, Bocshore M L and Quemada H D 1995 Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2 and zucchini yellow mosaic virus; *Biotechnology* **13** 1458-1465

Truve E, Aaspollu A, Houkanen J, Puska R, Mehto M, Hassl A, Teeri T H, Kelve M, Seppanen P and Saarma M 1993 Transgenic potato plants expressing mammalian 2'-5' oligoadenylate synthetase are protected from potato virus X infection under field conditions; *Biotechnology* **11** 1048-1052

Tumer N E, Fraley R T and Shah D M 1987 Expression of the alfalfa mosaic virus coat protein gene confers cross protection in transgenic tobacco and tomato plants; *EMBO J.* **6** 1181-1188

Vance V B, Berger P H, Carrington J C, Hunt A G and Shi X M 1995 5' proximal potyvirus sequences mediate potato virus X/potyviral synergistic disease in transgenic tobacco; *Virology* **206** 583-590

Vander Wilk F, Willinik D P L, Huisman M J, Huttinga H 1991 Expression of the potato leaf roll luteovirus coat protein gene in transgenic potato plants inhibit viral infection; *Plant Mol. Biol.* **17** 431-439

Van Dun C M P and Bol J F 1988a Transgenic tobacco plants accumulating tobacco rattle virus and pea early browning virus; *Virology* **167** 649-652

_____, _____, 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

_____, Overduin B, Van Vloten-Doting L and Bol J F 1988b 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 Houdt H, Van Montagu M and Depicker A 2000 Both sense and antisense RNAs are targets for the sense transgene-induced posttranscriptional silencing mechanism; *Mol. Gen. Genet.* **263** 995-1002

Vardi E, Sela I, Edelbaum O, Livneh O, Kuznetsova L and Stram Y 1993 Plants transformed with a cistron of a potato virus Y protease (NIa) are resistant to virus infection; *Proc. Natl. Acad. Sci.* USA **90** 7513-7517

Varrelmann M and Maiss E 2000 Mutations in the coat protein gene of plum pox virus suppress particle assembly, heterologous encapsidation and complementation in transgenic plants of *Nicotiana benthamiana*; *J Gen Virol.* **81** 567-76.

Vidya C S S, Manoharan M, Kumar C T R, Savithri H S and Sita G L 2000 Agrobacterium-mediated transformation of tomato (*Lycopersicon esculentum* var. Pusa Ruby) with coat-protein gene of Physalis mottle tymovirus; *J. Plant Physiol.* **156** 106-110

Voss A, Niersbach M, Hain R, Hirsch H J, Liao Y C, Kreuzaler F and Fischer R 1995 Reduced virus infectivity in *N. tabacum* secreting a TMV-specific full size antibody; *Mol. Breeding.* **1** 39-50

Wang P, Zoubenko O and Tumer N E 1998 Reduced toxivity and broad spectrum resistance to viral and fungal infection in transgenic plants expressing pokeweed antivaral protein II; *Plant Mol. Biol.* **38** 957-964

Wassenegger M and Pelissier T 1998 A model for RNA-mediated gene silencing in higher plants; *Plant Mol. Biol.* **37** 349-362

Waterhouse P M, Graham M W and Wang M B 1998 Virus resistance and gene silencing in plants can be induced by simultaneous expression of sense and antisense RNA; *Proc. Natl. Acad. Sci.* USA **95** 13959-13964

_____, Smith N A and Wang M B 1999 Virus resistance and gene silencing: killing the messenger; *Trends Plant Sci.* **4** 452-457

Wilson T M A 1993 Strategies to protect crop plants against viruses : pathogen derived resistance blossoms; *Proc. Natl. Acad. Sci.* USA **90** 3134-3141

Wintermantel W M and Schoelz J E 1996 Isolation of recombinant viruses between cauliflower mosaic virus and a viral gene in transgenic plants under conditions of moderate selection pressure; *Virology* **223** 156-164

_____, Zaitlin M 2000 Transgene translatability increases effectiveness of replicase-mediated resistance to cucumber mosaic virus; *J Gen Virol.* **81** 587-95

Wittner A, Palkovics L and Balazs E 1998 *Nicotiana benthamiana* plants transformed with the plum pox virus helicase are resistant to virus infection; *Virus Res.* **53** 97-103

Whitham S, Dinesh-Kumar S P, Choi D, Hehl R, Corr C and Baker B 1994 The product of the tobacco mosaic virus resistance gene *N* : similarity to Toll and the Interleukin-1 receptor; *Cell* **78** 1101- 1115

_____, McCormic S and Baker B 1996 The N gene of tobacco confers resistance to tobacco mosaic virus in transgenic tomato; *Proc. Natl. Acad. Sci.* USA **93** 8776-8781

Xu D M, Collins G B, Hunt A G and Nielsen M T 1997a Field resistance of transgenic burley tobacco lines and hybrids expressing the tobacco vein mottling virus coat protein gene; *Mol. Breeding* **3** 319-330

_____, _____, _____, and _____, 1997b Combining a host gene and a tobacco vein mottling virus coat protein gene for broad and effective resistance to potyviruses in tobacco (*Nicotiana tabacum* L.); *Mol. Breeding* **3** 331-339

Xue B, Gonsalves C, Provvidenti R, Slightom J L, Fuchs M and Gonsalves D 1994 Development of transgenic tomato expressing a high level of resistance to cucumber mosaic virus strains of subgroups I and II; *Plant Dis.* **78** 1038-1041

Yamaya J, Yoshioka M, Meshi T, Okada Y and Ohno T 1988 Cross protection in transgenic tobacco plants expressing a mild strain of tobacco mosaic virus; *Mol. Gen. Genet.* **215** 173-175

Yan Y T, Wang J F, Qui B S and Tien P 1997 Resistance to rice stripe virus conferred by expression of coat protein in transgenic Indica rice plants regenerated from bombarded suspension culture; *Virol. Sin.* **12** 260-269

Yang X , Yie Y, Zhu F, Liu Y, Kang L, Wang X and Tien P 1997 Ribozyme-mediated high resistance against potato spindle tuber viroid in transgenic potatoes; *Proc. Natl. Acad. Sci.* USA **94** 4861-4865

Yie Y, Zhao F, Zhao S Z, Liu Y Z, Liu Y L and Tien P 1992 resistance to cucumber mosaic virus conferred by satellite RNA and coat protein in transgenic commercial tobacco cultivar G-16; *Mol. Plant Microbe interact.* **5** 460-465

Yoder J I and Goldsbrough A P 1994 Transformation systems for generating marker free transgenic plants; *Biotechnology* **12** 263- 267

Zaitlin M and Palukaitis P 2000 Advances in understanding plant viruses and virus diseases; *Annu. Rev. Phytopathol.* **38** 117-143

Zaccomer B, Cellier F, Boyer J C, Haenni A L and Tepfer M 1993 Transgenic plants that express genes including the 3' untranslated region of the turnip yellow mosaic virus (TYMV) genome are partially protected against TYMV infection; *Gene* **136** 87-94