MEETING

ABSTRACTS OF PRESENTATIONS ON PLANT PROTECTION ISSUES AT

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*Part 2**
Plenary Lectures

X· ANTIVIRAL AGENTS FROM PLANT SOURCES (PLANT EXTRACTS; PROTEINS) (continued)

Efficacy of Traditional Herbal Medicines on Herpes Virus Infection *in vitro* and *in vivo*

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Traditional herbal medicines have been used for the treatment of various human diseases since the time of the ancient Aztecs. We selected several herbal extracts (*Mentha piperita, Cinnamomum zeylanicum, Amaranthus leucocarpus, Aloe barbadensis, Euphorbia pulcherrima, Euphorbia cotnifolia, Fragaria* aff. *vesca, Sedum dendroideum, Ocimum basilicum, Celosia argentea, Solanum nigrum, Solanum mammosum, Jacobinia spicigera*) to detect antiviral activity *in vitro* against herpes simplex virus HSV-1 and HSV-2 The herbal extracts which showed antiviral effect *in vitro* against HSV were *M piperita, C. zeylanicum, O basilicum*, and *F.* aff. *vesca* The efficacy of the three first herbal extracts was studied on a cutaneous Balb/c mice HSV-2 infection The herbal extract was administered orally at doses corresponding to human use Animals treated with *O. basilicum* extract showed less loss of weight than mock-treated animals (4.9% *vs* 14.4%), also fewer and less severe cutaneous lesions, and lower virus titer from the brain (log 3.0 *vs* log 4 38 $TCID_{50}$/g of brain tissue). However, the herbal extract did not affect the mortality rate. *(P)*

XI: MOLECULAR CHARACTERIZATION OF ALPHA AND PICORNA-LIKE PLANT VIRUSES

Novel Overlapping Gene Encoded by the Cucumoviruses

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Viruses from the Bromoviridae contain a tripartite RNA genome that typically encodes four proteins. Proteins 1a and 2a, translated from RNAs 1 and 2, respectively, are components of the viral RNA polymerase. RNA 3 encodes 3a and coat protein (CP), of which CP is translated through a subgenomic mRNA, RNA 4. We have found that the cucumoviruses encode an overlapping gene (2b) which is absent in the bromoviruses and alfalfa mosaic virus. The 2b gene is located at the 3' portion of RNA 2 and expressed in infected plants by transcribing a new subgenomic mRNA, RNA 4A Both RNA 4A and protein 2b have been detected for two of the three known cucumoviruses, cucumber mosaic and tomato aspermy cucumoviruses (CMV and TAV) An efficient system for plant infection based on infectious plasmid DNAs has recently been established for CMV and TAV. Mutational analyses indicated that protein 2b of CMV is required for systemic spread in cucumber and for disease induction in *Nicotiana glutinosa* Transferring the 2b gene from TAV to CMV produced a highly virulent hybrid virus which spread faster and accumulated to a much higher level in infected plants than either CMV or TAV, indicating a synergistic interaction between CMV and the TAV 2b protein. This result also supported a role for 2b in virus movement/symptom expression. Transgenic tobacco plants containing the 2b gene from CMV or TAV have now been generated These transgenic plants as well as a tobacco protoplasts system established recently are being used to investigate further the function of the 2b gene. *(L)*

Cucumber Mosaic Virus 3a Gene: Analysis of Site-Directed Mutants that Affect Movement, RNA Binding and Host Range

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Alanine-scanning mutagenesis of the 3a gene of cucumber mosaic virus (CMV) yielded nine mutants. Three mutants could not promote the cell-to-cell movement of the mutated virus, but two of these mutant viruses could be complemented for movement in transgenic tobacco expressing the CMV 3a gene, and *in vitro*-expressed 3a protein encoded by the corresponding mutated 3a genes showed a reduced affinity for viral RNA. A fourth mutant was temperature-sensitive for movement at 34° C, and a fifth mutant, capable of promoting virus movement, showed a reduced ability to bind viral RNA co-operatively *in vitro*. Two mutants that were competent for movement in tobacco and squash showed differences in infectivity in tomato and *Zinnia elegans*; one mutant did not infect *Chenopodium quinoa*. The locations of the nine mutants were given, as well as their effects on RNA binding, GTP (guanosine triphosphate) binding, pathology and host range. *(L)*

Characterization of Cucumber Mosaic Virus (CMV) Defective RNA 3 Variants Generated in Tobacco Transgenic for the 3a Gene

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L = lecture sessions; *P* = poster (market place) sessions.

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Passage of CMV mutants containing small out-of-frame deletions within the 3a gene in tobacco transgenic for the 3a gene, led to the complementation of movement, and the generation of in-frame defective (D) RNA 3 molecules, with various intermediate sized D RNAs observed in earlier passages. The size of the final, stable deletion in the D RNA 3 molecules and the location of the deletion depended on the nature of the initial 3a gene mutation, and appeared to be selected by packaging constraints. Co-infection of the D RNA 3 variants with wildtype CMV led to the loss of the D RNAs in a single passage. In contrast, a naturally arising D RNA 3 could co-exist with the wildtype RNA 3. The individual D RNA 3 variants also showed a hierarchy of selection when co-inoculated with each other. When extracts from individual 3a transgenic plants inoculated with the same frameshift construct were passaged, several classes of different deletion variants were obtained in different plants, suggesting that pooling extracts from several plants may lead to selection of the dominant D RNA 3 variant. *(P)*

Cucumber Mosaic Virus (CMV) 3a Gene Sequences Controlling Systemic Infection in Squash

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Restriction in the long-distance movement of CMV in squash (*Cucurbita pepo*) was mapped to RNA 3 of CMV, using two strains, Fny-CMV and Sny-CMV, which showed differential infection: Fny-CMV infected squash systemically, whereas infection by Sny-CMV was restricted to the inoculated cotyledons. Western, Northern and leaf-press blot analyses showed that virus containing Sny-CMV RNA 3 spread slowly in inoculated cotyledons, but was absent from true leaves, whereas virus with Fny-CMV RNA 3 showed fast local and systemic spread throughout squash plants. Chimeras generated between RNA 3 cDNA clones of Fny- and Sny-CMV were used to demonstrate that specific sequence changes in the CMV 3a protein affected virus spread in squash. Specific amino acids at both positions 51 and 240 of the 3a protein were required for restriction of movement in squash. *(P)*

Cucumber Mosaic Cucumovirus (CMV-Um) Isolated from *Prunus mume* Belongs to Subgroup 1 but is Distinct in Serology

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CMV-Um was isolated from Japanese apricot (*Prunus mume* Sieb. et Zucc.) showing leaf-edge necrosis and asperny syndrome, together with prunus necrotic ringspot like ilarvirus. Serotype of CMV-Um was shown as neither subg. I nor II by both agar gel double diffusion and ELISA tests. Nucleotide sequence in the RNA3 coat protein (CP) coding region of CMV-Um was determined and compared with the earlier reports of other CMV-RNA3. Sequence homologies of nucleotides and putative amino acids between Um and subg I isolates showed 91–93% and 94–95%, respectively. On the other hand, homology between Um and subg. II isolates showed similarly 75–76% and 80%. Hydrophilicity analysis of amino acid sequence at the regions of high average hydrophilicity of CMV-Um CP indicated four amino acids changes in both subgroups in common, suggesting that a few amino acids changes cause variation of CMV serotype. In conclusion, CMV-Um belongs to subg I in sequence homology but is different from subg. I and II in serotype. *(P)*

Structural Analysis of Peptides Presented on the Surface of a Plant Virus

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We have shown previously that it is possible to express epitopes on the surface of cowpea mosaic virus (CPMV) particles without compromising virus viability. In order to compare the structure of a peptide as presented on the surface of CPMV with that which it adopts in its native environment, we have determined the structure of a CPMV-based chimera containing a 14 amino acid epitope from human rhinovirus 14 (HRV-14). The structure revealed that the inserted sequence is well-ordered but is proteolytically cleaved at its C-terminus. This results in the polypeptide adopting a conformation distinct from that found in its native environment and probably explains why antibodies raised against the chimera, although capable of binding to HRV-14 particles, are non-neutralizing. The influence of the precise site of insertion of an epitope on its structure and immunological properties is being determined. Using the CPMV-based system it will be possible to correlate the binding and immunogenic properties of polypeptides with their 3-dimensional presentation. In this way it should be possible to design CPMV-based chimeras which are optimized for their biological function. *(L)*

Expression of a Foot-and-Mouth Disease Virus (FMDV) Epitope on the Surface of Cowpea Mosaic Virus (CPMV) Causes Particle Aggregation and Limits Long-Distance Movement of the Virus

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The S protein of CPMV can be modified to express the immunodominant site from VP1 of FMDV serotypes O and C without abolishing viral infectivity. However, in contrast to the situation with wild-type CPMV, the infections are largely restricted to the inoculated leaves, systemic movement occurring only sporadically. The virus particles, although clearly present in large quantity in infected cells, are difficult to purify using standard techniques. Ultra-structural analysis of infected tissue revealed that the presence of the FMDV-specific sequence caused aggregation of the virus particles, resulting in extensive crystalline arrays being formed within infected cells. To investigate the cause of this behavior, portions of the FMDV-specific region of the serotype O chimera were deleted. Analysis of the phenotypes of the resulting deletion mutants revealed that the sequence derived from amino acids 150–159 of VP1 was responsible for particle aggregation. In each case where aggregation occurred, systemic spread was also debilitated, indicating a causal relationship between the two phenomena. *(P)*

Potyviral Recombination Using Particle Bombardment of Plants

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Recently we have developed an efficient method for infecting cucurbit plants with a cDNA clone of zucchini yellow mosaic virus (ZYMV), under the control of the 35S promoter, through particle bombardment. We have constructed truncated versions

of the ZYMV full-length infectious clone, with deletions of either part of the coat protein (δCP), or the helper component (δHC) genes. Each construct alone is not infectious through particle bombardment. However, approximately two-thirds of the squash plants show typical ZYMV symptoms when bombarded with a mixture of the two truncated constructs. The recombination was confirmed by the introduction of a six histidine tag sequence in the helper component of the δCP construct, a region deleted from the δHC construct. Progeny virions included both the tag and the coat protein, verified by RT (reverse transcriptase)-PCR and sequencing. Several truncated clones were made to study the genes involved with the recombination process. *(L)*

The Nucleotide Sequence of the Coat Protein Gene of Pachouli Mild Mosaic Virus (PaMMV)

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PaMMV belongs to the fabaviruses. Its genome is composed of two single-stranded RNA molecules, RNA 1 and RNA 2, which are 6.0 kb and 3 9 kb, respectively The molecular weight of coat proteins (CP) was 26K and 42K, as determined by SDS-PAGE. The cDNA clones of RNA 2 were expressed in *Escherichia coli*, and the product was detected by antibodies specific for the CP of PaMMV. The sequence of the 3-terminal 3581 nucleotides of RNA 2 was determined to identify the CP gene using cDNA clones. This sequence contained single open-reading frame (ORF) 3189 nucleotides encoded 118K protein. From amino acid sequences of N-terminal regions of CPs, the 118K protein is cleavaged at Gln/Gly and Gln/Ala and three proteins, 52K, 44K and 22K, are produced. The 44K and 22K proteins are CPs. These values are in good agreement with those estimated from SDS-PAGE analysis The 3-terminal untranslated region of PaMMV RNA 2 was 193 nucleotides *(P)*

Molecular Analysis of Alfalfa Mosaic Virus (AlMV) RNA 3 of Strain VRU

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Strain VRU of AlMV has been reported to form unusually long virus particles and it was shown that the length of the RNA molecules was not changed in comparison with other AlMV strains. Therefore, it was concluded that the coat protein (CP) is responsible for the formation of these long tubular particles. AlMV consists of a tripartite single-stranded positive-sense RNA genome. RNA 1 and 2 encode the viral replicase subunits. RNA 3 of AlMV is bicistronic and encodes the movement protein P3 and the CP which is expressed from a subgenomic RNA, RNA 4. To analyze the possible role of the CP in the formation of longer virus particles, RNA 3 of AlMV-VRU has been cloned and sequenced. Pairwise sequence comparisons of the known complete nucleotide sequences of AlMV RNA 3 (AlMV strain YSMV, S, Madison, 425 and VRU) have revealed a higher degree of sequence diversity for the strain VRU. This is shown in overall nucleotide sequence similarities of 94–95% in the alignments with strain VRU, whereas among the four other AlMV strains overall nucleotide homologies of 97–98% were determined. Furthermore, the amino acid comparisons of the P3 as well as of the CPs displayed higher amino acid identities (97–98%) among the strains YSMV, S, Madison and 425 than in the alignments with strain VRU (94–95%). Among several additional amino acid alterations in the CP coding region of the strain VRU, the non-conserved amino acid changes were chosen and introduced into an infectious clone of RNA 3 of strain 425. The effect of these mutations is currently under investigation. *(P)*

Studies of the Interaction of the RdRp of Turnip Yellow Mosaic Virus (TYMV) with the 3' Terminus of TYMV RNA

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The 3' terminus of the plus-stranded RNA genome of TYMV is folded into a tRNA- like structure. The 'aminoacyl acceptor' arm is formed by stacking of three stem regions, two of which are part of a pseudoknot structure. To investigate the interaction of the RNA-dependent RNA polymerase (RdRp) with the 3' terminus of TYMV RNA, various RNA constructs were made, starting with a 83n-fragment, containing the complete tRNA-like structure. By decreasing the length of this fragment from the 5' end on, a 44n-fragment, (G+30)-fragment and 28n-fragment were synthesized, all containing the pseudoknot structure, and a (G+20)-fragment containing only the 3' terminal hairpin. The RNA fragments were tested for their ability to function as templates in an *in vitro* RdRp assay. In comparison with the 83n-fragment, all fragments containing the pseudoknot structure showed a transcription efficiency of ~100% In the case of the 3' terminal hairpin (G+20) a reduction of transcription efficiency to 50% was observed. Besides these RNA constructs, unmodified yeast $tRNA^{Phe}$ was tested. A transcription efficiency of 57% was observed This finding corresponds with the result obtained with the 3' terminal hairpin (G+20), indicating that no specificity can be assigned to the stem or loop sequence as present in the 3' hairpin (G+20). The very 3' terminus of TYMV RNA consists of a non-basepaired ACC(A)-end When basepairing this part of the 3' terminal hairpin (G+20), transcription efficiency dropped to ~2%. This means that the 3' ACC(A)-end has to be single stranded for the recognition by the RdRp. By means of mutation studies it was shown that the A-residue of the ACC(A)-end is not important for this interaction However, when substituting this A-residue of the (G+20)-fragment into a C-residue and thereby creating the formation of a basepair with the 5' G, a decrease of transcription efficiency to 31% was observed. Substitution of the loop sequence by a UUCG sequence to stabilize the hairpin structure, had no effect on the transcription efficiency. *(L)*

Cloning and Sequencing of the 3' Terminal Region of Watermelon Mosaic Virus-Morocco (WMV-Morocco) and Its Relationship to Other Cucurbit Potyviruses

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WMV Moroccan isolate, WMV-Morocco, is one of a number of potyviruses which infect cucurbits worldwide. Historically, classification of cucurbit potyviruses based on serology has been confusing, due to cross reactivity of antisera with conserved core regions of the potyviral coat protein (CP). WMV-Morocco was originally classified as a strain of WMV based on serological cross reactivity with antisera to a WMV isolate which had not been characterized as either WMV I, now papaya ringspot virus type W (PRSV-W), or WMV II Based on tryptic peptide analysis of five peptides from the core region of the WMV-Morocco CP, McKern *et al.* reported that WMV-Morocco was a distinct potyvirus. We have cloned and sequenced the 3' terminal region,

including the 3' untranslated region, CP and part of the Nlb gene of a WMV-Morocco isolate from South Africa. Comparison of the nucleotide sequence with other potyvirus sequences showed that WMV-Morocco was most similar to PRSV-W (approximately 66%). Comparison of regions of nucleotide sequence in the N-terminus of the CP gene and 3' untranslated region suggests a possible evolutionary relationship between PRSV-W and WMV-Morocco WMV-Morocco appears to be a distinct potyvirus (based on current taxonomic criteria for classification of potyviruses) and therefore should be renamed accordingly. *(P)*

Molecular Characterization of Narcissus Latent Virus (NLV) and Maclura Mosaic Virus (MacMV) Demonstrates that They Should Form a New Genus of the Potyviridae

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NLV and MacMV have been classified previously as members of the carlavirus group due to their particle morphology. However, unusual properties of NLV and MacMV have been reported which have raised questions as to their classification. Reports of their coat protein size and the presence of cytoplasmic inclusion bodies in *Nicottana clevelandii* cells infected with NLV or MacMV are uncharacteristic of the carlavirus group. To ascertain the classification of these two viruses, we have re-examined their coat protein sizes and partially cloned and sequenced their 3' terminal regions. Sequence data from the capsid protein open-reading frame (ORF) and part of the nuclear inclusion body b (Nlb) ORF, show that they are highly related to each other, and are homologous to the bymoviruses. However, NLV and MacMV differ morphologically and biologically from the bymoviruses and thus we propose that they form a new genus of the Potyviridae, the macluraviruses. *(P)*

Ryegrass Mosaic Virus (RgMV) – Its Sequence Based Classification

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Ryegrass mosaic virus (RgMV) is thought to be the type member of the genus *Rymovirus* within the family of Potyviridae. From this genus only the nucleic acid sequences of brome streak mosaic virus (BrSMV) and of the 3'-terminus of wheat streak mosaic virus (WSMV) have been published. We cloned and sequenced the complete genome of a Danish isolate of RgMV. A CLUSTAL analysis of the deduced amino acid (aa) sequences of all so-far-published coat protein (CP) genes of potyviruses shows that RgMV is more closely related to other Potyviridae than are BrSMV and WSMV The homology of RgMV with viruses of the genus *Potyvirus* is 35%. For BrSMV and WSMV, the degree of homology with all other potyviruses, including RgMV, is only 8%. These relationships are true for the entire genomes too. Comparing the electrophoretically estimated size of the CP of RgMV with the deduced aa sequence, it was not possible to detect one of the common protease cleavage motifs (Q/A, S, R) which would result in a CP of the expected size. Sequencing the N-terminal aa of the CP of a Netherlands isolate of the virus, it was demonstrated that the cleavage site Nlb/CP has the unusual motif ANVT/AASST This aa motif is conserved for six other so-far-sequenced isolates of RgMV. Consequently, the protease NIa must have aa that differ in some of the conserved aa from those of other potyviruses. The aa of the catalytic center of NIa, Asp, His and Cys are all present, but 11 of the highly conserved 44 aa of the NIa of potyviruses are missing Some of them should be responsible for the different proteolytic activity of NIa. From our sequence data it is concluded that RgMV cannot be the type member of a genus *Rymovirus* to which WSMV and BrSMV also belong. *(P)*

Pathogenicity Analysis of Recombinant Hybrids between a Severe Strain of Papaya Ringspot Potyvirus and Its Mild Mutant

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Nineteen recombinant hybrids were constructed by exchanging the restriction fragments of a full-length infectious cDNA clone of a severe strain (Hawaii, HA) with the corresponding cDNA fragments of its nitrous-acid-induced mutant HA 5-1 that caused symptomless infection in papaya. Biologically functional transcripts *in vitro* synthesized from these hybrids were mechanically applied onto papaya seedlings for pathogenicity analysis. Twelve of the recombinant transcripts caused type 1 symptoms with mosaic and distortion on papaya leaves, similar to those induced by the severe HA strain. The replaced fragments of these hybrids were within the regions of CIP, 6K, NIa, NIb, CP, and part of the P3 proteins. Apparently, the functions of these genes of HA 5-1 were similar to those of HA The hybrids that had the replacement in the region of the C terminal half of the P1 protein induced type II symptoms, with mild mosaic and less distortion on leaves. Two recombinants with the exchange within the regions of the complete HC-Pro protein, a bit of P1 and part of P3 proteins, showed type III symptoms of mild mottling on leaves and yellowing along leaf veins. Four recombinant transcripts infected papaya plants without conspicuous symptoms, as did the mild mutant HA 5-1, and their reactions were classified as type IV. The type IV symptoms resulted from the interaction of both type II and type III exchanges, including the replacement of the C terminal half of the P1 protein, the complete HC-Pro protein and a small part of the P3 protein of HA 5-1 cDNA. The actual locations of the mutations and their coordinated effects on diminishing the pathogenicity of PRSV were discussed. *(P)*

Infectivity Assays of *in vitro* and *in vivo* Transcripts of Papaya Ringspot Potyvirus (PRSV)

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A full-length cDNA with nucleotide sequence reflecting the genomic RNA of a Hawaii strain (HA) of PRSV (PRSV HA) was constructed downstream from a bacteriophage T3 promoter in an *in vitro* transcription vector. The plasmid was able to generate an *in vitro* transcript corresponding to PRSV RNA (10326 nt) with one extra guanosine residue at the 5' terminus and 12 nonviral nucleotides at the 3' end following a poly (A36) tract. *In vitro* translation products and immunoprecipitation analysis with antiserum to PRSV verified the correctness of the gene expression of the transcript. When the capped transcript was mechanically introduced into the systemic host papaya and the local lesion host *Chenopodium quinoa*, typical symptoms of PRSV HA appeared at almost the same time as on those host plants inoculated with native PRSV DNA Western blotting and serologically specific electron microscopy with PRSV antiserum confirmed the infection. Uncapped *in vitro* transcript or transcript with longer nonviral nucleotides (64 nt) at the 3' end was not infectious. The full-length cDNA was also constructed with a cauliflower mosaic virus

(CaMV) 35S promoter and a nopaline synthase (NOS) terminator in an *in vivo* expression vector. Purified plasmids were applied directly onto host plants either mechanically or by bombardment with a particle delivery system to analyze their infectivity. The plasmid without extra nucleotides between the 35S promoter and the 5' end of PRSV sequence and with 12 residues and a *Not*I site at its 3' end was infectious, as evident from symptom development, and from ELISA, immunodiffusion, and serologically specific electron microscopy analyses with PRSV antiserum. The construct with 33 nonviral nucleotides at the 5' end of the PRSV sequence and more than 64 nonviral nucleotides at the 3' end was not infectious *(P)*

5' Non-Coding Sequences of Plant Viral RNAs as an Alternative Initiation of Translation in *Escherichia coli*

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In prokaryotes such as *Escherichia coli*, binding of ribosomal 30S subunit to mRNAs is strongly influenced by the nucleotide sequence (approximately ten nucleotides) 5' to the initiator codon (AUG). The interaction is known to be promoted by complementary base pairings between a purine-rich sequence (known as Shine-Dalgarno sequence, S/D) with the 3' end of the 16S ribosomal RNA (rRNA) This interaction, together with the distance of S/D sequence from the start codon, strongly influences the efficiency of initiation of translation. Recently, it was shown that the 5' untranslated region of tobacco mosaic virus RNA genome (known as omega sequence), which does not resemble the S/D sequence, is also capable of initiating translation in *E. coli*. We have previously illustrated that omega sequence initiates translation through binding 16S rRNA at a site different from the classical S/D binding site To investigate further the existence of other sites in the 16S rRNA capable of binding mRNA, we have constructed specific sequences complementary to different sites in the 16S rRNA Using an *E. coli* expression system, we obtained preliminary evidence suggesting that these sequences are capable of initiating translation Hence, two possible alternative mRNA binding regions in the 16S rRNA are identified. We have also obtained evidence suggesting that the 5' untranslated regions of clover yellow mosaic potexvirus and papaya mosaic potexvirus RNA genomes are both capable of initiating the translation in *E. coli (P)*

Banana Bract Mosaic Potyvirus (BBMV): A New and Widespread Virus of Bananas

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BBMV is a recently described potyvirus infecting bananas. Symptoms of BBMV were first noted in the Philippines in 1979 and include the characteristic discontinuous streaks on the bract of the banana inflorescence. We have cloned and sequenced the coat protein and 3' untranslated region of BBMV from several countries including the Philippines, India, Vietnam and Western Samoa Up to 5% variability at both nucleotide and amino acid levels occurs between the two most distantly related isolates. The Vietnam, Western Samoan and Indian isolates of BBMV have been isolated from banana plants showing symptoms of cucumber mosaic virus (CMV) infection and do not produce the mosaic symptom on the bract of the banana inflorescence. Our results provide evidence that BBMV has a much wider distribution than originally thought and that CMV-like symptoms on banana may indicate the presence of BBMV *(P)*

Comparison of Nucleic Acid Sequences of German Isolates of Sugarcane Mosaic Potyvirus (SCMV)

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SCMV appears to be the prevalent potyvirus infecting maize in Germany, in contrast to other European countries where maize dwarf mosaic potyvirus (MDMV) is more important. To compare the relationship of the German with isolates of other regions, first two German isolates of SCMV were cloned and the nucleic acids corresponding to the 3' ends of the RNA, including the 3' NTR, the coat protein gene (CP gene) and part of the NIb, were sequenced. One of the isolates was a mixture of two isolates with different sizes of the CP genes (33.5 and 34 kD). Among 12 German isolates compared by Western blot, this was the only case that an isolate had a 34 kD (CP) All three isolates showed some differences in nucleic acid (na) sequences, which were located especially in the 3' NTR. Using the sequence information, specific primers for amplification of the CP genes by IC-RT-PCR were synthesized. They were used successfully to amplify these genes of further isolates. The cDNAs were cloned The sequence data for the CP were compared with so-far-published data of other isolates of SCMV and MDMV by CLUSTAL analysis. Comparing the na sequences, the German isolates group together with SCMVI and, less closely, with MDMV (approx 70% homology). If only the deduced amino acid sequences are compared, the German isolates form a separate cluster with 67% homology to SCMVI, MDMV and a Spanish isolate of MDMV. According to the criteria established by Shakla, the German isolates should be considered as a group of different potyviruses and may be the result of viral adaptation to climatic conditions in Germany. *(P)*

Complete Sequence of Two Lettuce Mosaic Virus (LMV) Isolates Differing in Their Seed-Transmissibility and in Their Resistance-Breaking Properties

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LMV is probably the most damaging virus on lettuce crops worldwide. Its ability to be efficiently seed-transmitted in this host is one of the key factors of its epidemiology, together with the efficient transmission by aphids typical of potyviruses. Currently, control of LMV is based on employing both virus-free seed lots and resistance varieties containing one of two recessive resistance genes, $mo1^1$ or $mo1^2$. As a preliminary step in determining the molecular bases of resistance breaking and seed transmissibility in lettuce, the complete sequence of the genomic RNAs of two LMV isolates, LMV-O and LMV-E, was determined from cDNA clones. LMV-O is a seed-transmissible isolate against which the resistance genes are effective, whereas LMV-E is able to overcome both $mo1^1$ and $mo1^2$ but is not seed-transmitted in susceptible cultivars. The data obtained indicate that these two isolates are remarkably close, being over 97% identical at the protein level *(P)*

Functional Domains of the Helper Component-Proteinase (HC-Pro) Required for Genome Amplification

and Long-Distance Movement of Tobacco Etch Potyvirus (TEV)

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The HC-Pro of TEV is required for aphid transmission, long-distance movement (LD) in plants, genome amplification and polyprotein processing. Alanine-scanning mutagenesis was performed to define the domains of HC-Pro required for genome amplification and LD movement. Four different classes of mutants were identified. Two-thirds of the mutations had no effect on amplification or movement. Four mutants were amplification-debilitated, accumulating to 9–22% the level of parental virus, and moved long distances in plants; three mutants were debilitated in both amplification and LD movement; one mutant was proteolytic-processing and replication-defective. The mutations causing amplification defects spanned the central and C-terminal proteolytic regions, whereas those inducing LD movement defects were confined to the central region. The LD movement defects were complemented by HC-Pro supplied in transgenic plants, whereas the amplification defects were not rescued. These data suggest that overlapping regions of HC-Pro are required for amplification and LD movement functions, but that these functions are genetically separable. Finally, HC-Pro was localized in live, infected cells using a green fluorescent protein fusion strategy. HC-Pro was detected within large cytoplasmic vesicles and in association with a filamentous network. *(L)*

Evidence for Myristoylation of a Plant Viral Protein: Myristoylation of Potato Virus Y VPg

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The small nuclear inclusion protein, NIa, from the plant potyvirus potato virus Y, was found to be myristoylated by yeast N-myristoyltransferase (NMT1) using a dual plasmid *Escherichia coli* expression system and labeling with ^{3}H-myristate. The N-terminal half of the protein, containing the putative genome-linked protein VPg, was the predominant form of NIa labeled. A much smaller amount of full-length NIa was also labeled. Site-directed mutagenesis was performed on the NIa myristoylation consensus sequence, changing glycine 1 to alanine, serine 5 to proline, or serine 5 to glutamine. The serine 5 to glutamine mutation was designed to mimic the sequence at the N-terminus of tobacco etch virus NIa, while the two other changes were chosen with the expectation that they would not be able to be myristoylated. None of these mutants was myristoylated by yeast NMT1. We are currently examining the ability of these constructs to be myristoylated in tobacco protoplasts. This is the first report of a myristoylated plant viral protein, suggesting that common signals for myristoylation exist between plants and animals *(L)*

Tobacco Veinal Necrosis Determinants and Group-Specific Epitopes in the Potato Virus Y (PVY) Genome

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Three PVY isolates, representatives of distinct PVY groups, were submitted to immunological and molecular analysis Two isolates, PVY^{N}-Wi and PVY^{N}-Ny, belong to the necrotic strain and the third (PVY^{o}-LW) belongs to the common strain. Surprisingly, PVY^{N}-Wi did not react with MAbs directed against necrotic strain isolates that precipitated PVY^{N}-Ny. Coat protein (CP) genes and 3' NTR (non-translated regions) of the three isolates were sequenced The necrotic PVY^{N}-Wi isolate showed 99% amino acid homology with the common one PVY^{o}-LW, and differed significantly from the second necrotic isolate (PVY^{N}-Ny) 3'NTR sequences were identical for PVY^{N}-Wi and PVY^{o}-LW and differed from that of PVY^{N}-Ny Homology matrix and phylogenetic analysis led to classification of PVY^{N}-Ny into group I, whereas PVYB-Wi fell into group II. The sequence analysis allowed for identification of putative group I – specific epitopes not present in necrotic strains belonging to group II isolates. The near identity of 5'NTR, P1 gene, CP gene, 3'NTR of common (PVY^{o}-LW) and necrotic (PVY^{N}-Wi) isolates suggests that the tobacco necrosis determinant is carried on by the central PVY genome region. *(P)*

Genome Organization and Translational Strategies of the Cocksfoot Mottle Virus (CfMV)

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CfMV has a positive-sense ssRNA genome of 4082 nucleotides, as revealed by sequencing the entire genome. *In vitro* translation of CfMV genomic RNA revealed four major protein products of 100, 71, 34 and 16 kDa. The coding region contains four open-reading frames (ORFs). We suppose that ORF 1-encoded protein has a movement protein function. In contrast to other sobemoviruses, CfMV lacks the continuous large ORF 2 that codes for the putative Vpg, protease and replicase. Instead it has two overlapping ORFs, ORF 2a and 2b. We have revealed that the putative replicase, encoded by ORF 2b, is translated *in vitro* as a part of a polyprotein by -1 ribosomal frameshifting. This is a unique feature among characterized sobemoviruses. We are currently examining the possible regulatory mechanisms affecting the expression of the putative CfMV replicase. The 3'-terminal ORF 3 encodes the coat protein (CP), as deduced from the N-terminal amino acid sequence of CfMV CP. We consider that 1.2 kb CfMV-specific RNA molecule, present in CfMV-infected barley plants, is the subgenomic RNA for CP synthesis. *(L)*

Expression of Tomato Ringspot Nepovirus (TomRSV) RNA-1 Proteins by Intramolecular Proteolytic Processing

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TomRSV is a member of the picorna supergroup of viruses. Viral proteins involved in the replication and present in the replication complex are likely to include the putative NTP-binding protein, VPg, protease, and polymerase – which are all contained in the polyprotein encoded by RNA-1 (P1). We were interested in studying the expression of those proteins by proteolytic processing and in determining their involvement in viral replication. Recently we have raised monoclonal and polyclonal antibodies against the VPg, the protease and the polymerase. Using these antibodies to immunoprecipitate *in vitro* translation products of RNA-1 cDNA constructs, we could demonstrate that the protease recognizes P1 cleavage sites intramolecularly but not intermolecularly. Cleavage is detected *in vitro* between the putative NTP-binding protein and the VPg,

and between the protease and the polymerase, but not between the VPg and the protease. We are currently using those antibodies to study P1 cleavage *in vivo. (P)*

PCR Evidence for a Mitochondrial Origin of Satellite Tobacco Ringspot Virus (TobRV)

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Tobacco ringspot virus (TobRV) is the type member of the nepovirus group After serial passages of TobRV in plants, viral symptoms decrease dramatically and a small satellite RNA can be detected in TobRV capsids. This ~360 base satellite RNA (sTobRV) can account for over 90% of encapsidated RNA from infected tissue. Symptom reduction seems to be caused by preferential replication of sTobRV RNA by the TobRV replicase. sTobRV RNA shares little sequence homology with TobRV RNA, and is therefore not a defective interfering particle. In investigating potential origins for sTobRV, we have found preliminary evidence of sTRobRV-like sequences in mitochondrial DNA isolated from tobacco (*Nicotiana tabacum* L. cv. BY-2) cells employing the polymerase chain reaction. Using 19 base primers corresponding to the ends of (+) sense sTobRV RNA, we have amplified a ~400 base pair sequence. This indicates a potential mitochondrial origin for sTobRV RNA, and supports our previous hybridization evidence to this effect. *(P)*

Evolutionary Relationship in the Ilarviruses: Nucleotide Sequence of Prunus Necrotic Ringspot Virus (PNRSV) RNA 3

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PNRSV is the causal agent of several diseases affecting most cultivated stone fruits, including cherry, sour cherry, almond, peach, apricot and plum Ilarvirus and alfalfa mosaic virus are unique among plant viruses in that they require the coat protein (CP) or its mRNA to initiate infection. We have determined the complete nucleotide sequence of an isolate of PNRSV RNA 3 from cloned cDNAs and direct sequencing of the 5'-terminal RNA region. A transmembrane domain preceded by a basic region in the movement protein of all ilarviruses sequenced so far has been observed. An Arg-rich domain that resembles one of the RNA-binding proteins that belongs to the Arg-rich motif family is present at the N-terminus of the CP of all ilarviruses sequenced so far. Evolutionary relationships based both on the movement and CPs were presented. *(P)*

PCR Primers for the Rapid Identification of Carlaviruses, Bymoviruses and Macluraviruses

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Although cowpea mild mottle virus (CMMV), narcissus latent virus (NLV) and Maclura mosaic virus (MacMV) share many physiochemical properties with the carlavirus group, each has one or more features which are non-typical of that group. CMMV is not transmitted by aphids but by whiteflies (*Bemisia tabaci*), and it has been observed that MacMV- and NLV-infected tissue contains cytoplasmic inclusion bodies. To ascertain the classification of these viruses, a PCR primer was designed to a unique sequence found in the carlavirus 11K open-reading frame and used to identify CMMV as a member of the carlavirus group. When used on samples of NLV and MacMV, this carlavirus-specific primer gave a negative result, suggesting that these viruses were not members of the group. Sequence data from cDNA clones of MacMV and NLV showed that they were in fact related to the bymovirus group, a genus of the Potyviridae A primer was designed to a region of sequence unique to the NLV, MacMV, and three bymoviruses This primer gave a positive result on a sample from rice necrosis mosaic virus. The resulting fragment was sequenced to confirm its classification as a bymovirus. *(P)*

XII: POST-TRANSLATIONAL PROCESSING

Processing of the N-Terminus of Rice Tungro Spherical Virus (RTSV) Polyprotein

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RTSV, a plant picornavirus, belongs to the Sequiviridae family. It has a polyadenylated genome of ~12 kb which contains one large open-reading frame (ORF) and possibly two small ORFs expressed from subgenomic RNAs. The large ORF encodes a polyprotein of 393 kDa. The N-terminal part of the polyprotein (from amino acid 645 to approximately 1380) contains the capsid protein region, which is processed into three proteins, CP1, CP2 and CP3. The regions of the polyprotein upstream and downstream of the CPs have no known function. A putative NTP binding protein region is located around amino acid 1700. N-terminal sequences of CPs have been determined previously but little is known about the processing of them, particularly of the C-terminus of CP3, which is the C-terminus of capsid protein region. The region of polyprotein upstream of the CPs can potentially produce a polypeptide of ~75 kDa, if the first in-frame AUG initiates translation. Western blots of proteins from infected plants using polyclonal antisera to different parts of polyprotein as well as *in vitro* transcription/translation systems were used to study the processing of the CP region and the upstream and downstream regions. The processing of the N-terminal part of the RTSV polyprotein was compared with that of other picornaviruses and possible functions of different polyprotein processing products were discussed. *(L)*

XIII: VIRAL MEMBRANE PROTEINS

Localization of Tomato Spotted Wilt Virus (TSWV) Glycoproteins during Infection of Plant Tissues and Protoplasts

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TSWV is the type species of the genus *Tospovirus* within the large family of arthropod-borne Bunyaviridae. TSWV is able to replicate both in its insect vector (thrips) and in a broad range of host plants. Like all bunyaviruses, the enveloped TSWV particle contains two species of glycoproteins. We investigated how the glycoproteins of TSWV mature in a plant cell

context. Considering the knowledge about animal-infecting bunyaviruses, the defined involvement of the endoplasmic reticulum (E.R.) and the golgi apparatus during this maturation of glycoproteins and also during the budding process of virus particles is of particular interest In this study we utilized a number of specific antibodies directed against viral proteins as well as against cellular organels in both immunofluorescent-light microscopy and EM studies. We conclude that both the E R. and the golgi apparatus play a prominent role during processing of the viral glycoproteins and the formation of *de novo*-produced virus particles The evidence available seems to indicate that the golgi apparatus is the site of particle budding during infection of plant cells. To confirm this, we are currently studying transiently expressed glycoprotein which we expect to accumulate in the golgi apparatus due to a possible retention signal. All this would indicate that the maturation process of TSWV glycoproteins and particles in plant cells at least partially resembles that of animal-infecting bunyaviruses. *(P)*

XIV: RESISTANCE TO PLANT VIRUSES

Transgenic Strategies to Obtain Resistance to Cucumber Mosaic Virus (CMV) in Tobacco and Tomato Plants

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CMV is one of the most important plant viruses, causing considerable to dramatic yield losses in the cultivation of many important vegetable crops, including tomato, (sweet) pepper, melon, squash, cucumber, etc. In most crops, suitable resistance genes for use in breeding programs have so far not become available. For this reason we employed a number of transgenic strategies to obtain resistance against this plant pathogen. Transformation of host plants with viral sequences, including coat protein and replicase genes, engenders resistance in the transgenic plants to the homologous virus. To verify which viral sequences are capable of conferring resistance to CMV in transgenic plants, we constructed a number of plant expression vectors, containing CMV RNA 2 and RNA 3 sequences. The gene cassettes were transferred to tobacco and the levels of resistance were determined in the S1 and S2 progeny of the primary transformants. Besides these pathogen-derived approaches, we developed a novel strategy to gain CMV-resistance. Antisense RNA 3 molecules of CMV are constitutively expressed in transgenic plants, in which the viral movement protein gene is replaced by that of a gene encoding a hypersensitive defense response (HR) in plants carrying the matching resistance gene. It is assumed that the transgenic plants react rapidly with HR, only upon infection with CMV. The manifestation of the engineered resistance and the prospects of the different DNA constructs to confer resistance against CMV infections were discussed in more detail. *(P)*

Tobacco and Tomato Plants Transformed with Different Genes of Potato Virus Y (PVY) and Cucumber Mosaic Virus (CMV) are Resistant to Virus Infection, Respectively

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PVY and CMV are known to cause considerable damage in a broad range of plants. In the absence of natural resistance sources of *Lycopersicon esculentum* and its wild relatives, transgenic resistance methodology has been used. Structural and non-structural clones of PVY genes (CP, NIa, NIb, HC), and CMV CP and carna-5 cloned genes were used to prepare tobacco and tomato transgenic plants We have selected R1 generation plants that show a high level of resistance after virus inoculation in the greenhouse. Field tests will be conducted using the already prepared tomato R2 and R3 kanamycine-resistant plants *(P)*

Genotype-Dependent Coat Protein-Mediated Protection in Tobacco against Potato Virus Y (PVY)

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Nicotiana tabacum cv Burley BB16 or cv. Xanthi XHFD8 plants expressing the coat protein (CP) gene from lettuce mosaic virus (LMV) were analyzed for resistance to PVY. In Burley and Xanthi plants, the LMV CP was easily detectable by Western blot analysis. R1 plants were mechanically inoculated and virus accumulation was assessed by symptoms and ELISA tests. Two out of five Xanthi lines showed immunity to PVY infection, which was confirmed in R2, R3 and R4 progenies. 100% of the plants escaped the infection under all conditions tested. In these plants heteroencapsidation was observed when the plants were challenged with other potyviruses. In contrast, no Burley lines were completely immune to PVY. Instead, a tolerance to PVY infection was observed. This protection was similar in all ten lines tested. On R1 progeny, 30–60% of the plants escaped the infection. The remaining plants showed a similar resistance, characterized by an atypical gradient of low viral accumulation from the bottom leaves to the top ones, the three upper leaves remaining virus-free throughout the experiment. The growth of these infected plants was normal, as opposed to susceptible control plants, and the symptoms were mild and delayed. The differences in protection phenotypes observed in Burley and in Xanthi plants may suggest an effect of the genotype on the protection mechanism. *(P)*

Investigation into the Use of Virus-Induced Transgenes for Engineering Plant Virus Resistance

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Tobacco plants (SR1) have been transformed with a reverse-sense marker-gene construct. This construct has been linked to the 3' untranslated region (utr) of potato virus Y (PVY). The *in vivo* transcription of this construct results in the production of a reverse-sense luciferase message with a short viral sequence at the 3' end. During potyvirus replication negative-sense intermediates are produced, presumably involving a recognition event at the 3' end of the viral genome. The transgene message should be recognized by the viral replicase and a reverse-sense copy made, resulting in a translatable, positive-sense copy of the luciferase message being produced. The technique should allow the generation of versatile silent gene constructs which would be untranslatable until a viral infection event. One possible application is the development of plants which mimic the hypersensitive response by having a cytotoxic gene activated on viral infection and localizing the virus in an island of necrotic tissue. Data were presented on the testing of these transgenic plants, along with data on virus resistance which may be conferred by the viral sequences themselves. *(P)*

Resistance to Distinct Potyviruses PVY (Potato Virus Y) and ClYVV (Clover Yellow Vein Virus) in Transgenic Plants Expressing a Broad-Spectrum scFv

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The RNA sequences encoding the variable domains of a monoclonal antibody raised against Johnsongrass mosaic potyvirus and recognizing a broad spectrum of distinct potyviruses, including PVY, ClYVV, JGMV, MDMV, BYMV, PMV, PRSV and WMV2, have been cloned and ligated together to form a single chain antibody variable region (scFv). Two constructs have been produced: one containing additional 5' sequence encoding the natural Mab signal sequence, and the other lacking the signal sequence. These constructs have been cloned into a plant expression vector under the control of the CaMV 35S promotor and transfected into tobacco. Transgenic plants have been challenged with PVY and ClYVV, members of distinct potyviruses. Northern and Western blots show a correlation between mRNA and expressed protein levels, and also reveal that the level of signal-plus scFv is much greater than of signal-minus. Data from challenge experiments indicate that high protection levels (17% of transgenic plants were infected and had an average of one lesion/leaf 10 days post-inoculation with 20 μg/ml ClYVV, compared with 100% and 60 lesions for the non-transgenic controls, respectively) have been achieved for plants expressing the signal-plus protein. The data indicate that protection against a wide range of distinct potyviruses can be achieved in transgenic plants expressing single antibodies *(P)*

Enzymatic Activity of Pokeweed Antiviral Protein is Required for Its Antiviral Activity

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Pokeweed antiviral protein (PAP), a 29-kDa protein isolated from *Phytolacca americana*, inhibits translation by catalytically removing a specific adenine residue from the large rRNA of the 60S subunit of eukaryotic ribosomes. In addition to its ribosome-inactivating ability, PAP has potent antiviral activity against many plant and animal viruses, including HIV. We recently described the isolation and characterization of non-toxic PAP mutants by random mutagenesis and selection in yeast. One of the mutants isolated, NT123-2, has a point mutation (E176V) in the proposed active-site cleft which abolishes enzymatic activity. Another mutant, NT124-3, has a nonsense mutation at its C-terminus which results in deletion of the C-terminal 25 amino acids We expressed both the active-site mutant and the C-terminal deletion mutant in transgenic tobacco and showed that both mutants are non-toxic to transgenic plants. The active-site mutant expressed in transgenic plants did not have antiviral activity when applied exogenously to tobacco leaves in the presence of potato virus X (PVX). In contrast, the C-terminal deletion mutant was antiviral when applied exogenously Transgenic plants expressing very high levels of the active-site mutant PAP were not resistant to PVX, whereas plants expressing low levels of the wild-type PAP, the variant PAP (PAP-v) or the C-terminal deletion mutant PAP were resistant to PVX infection. These results demonstrate that ribosome-inactivating ability of PAP is required for its antiviral activity and suggest that the antiviral activity of PAP can be separated from its toxicity. *(P)*

Investigation of Silent Plant Virus Replicons as a Mechanism for Engineering Viral Resistance

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There has been much speculation on the possibility of using silent plant virus replicons to mimic the hypersensitive response and generate virus-resistant plants In this work we have carried out a detailed analysis of silent replicons derived from a number of plant viruses Tobacco plants were transformed with constructs containing viral sequences known to be involved in replication, linked to the marker gene luciferase and orientated with the CaMV 35S promoter so as to generate anti-sense RNA for luciferase. Luciferase expression from the constructs should remain silent until viral infection occurs, when the viral replicase should trans-activate the negative-sense RNA and luciferase will be expressed. If the marker gene is replaced with a gene for a cytotoxic protein, then upon virus infection the infected cell should ablate, thus halting replication and spread of the virus. Viral sequences involved in the replication of pea early browning virus (PEBV), tobacco rattle virus (TRV), potato virus X (PVX) and potato virus S (PVS) have been investigated. Data were presented on the levels of luciferase expressed in infected transgenic plants and the possible resistance conferred by the viral sequences present in these constructs. *(P)*

RNA-Mediated Resistance in Transgenic *Nicotiana benthamiana* Containing Modified Plum Pox Virus (PPV) Coat Protein (CP) Gene Constructs

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In order to limit biological risk when using transgenic plants expressing viral CP gene, three constructs of PPV CP gene were built and integrated into the *Nicotiana benthamiana* genome. One of them contains a punctual deletion of the nucleotides encoding for the transmission-associated DAG amino acid triplet. The second one bears a 420 nucleotides deletion in the 5' region of the CP gene The third harbours a stop codon downstream of the first initiation codon, in order to make a transgene mRNA untranslatable. Following the Ro primary transformants characterization, transgenic lines containing each type of construct were selected and challenged against PPV Resistant lines displayed two kinds of phenotype: recovery or immunity The steady state level of mRNA transgene is very low or undetectable in the immune lines and is down-regulated in the upper systemic, symptomless leaves of the recovered plants These results and nuclear run-off assays showed that this PPV resistance mechanism is thus similar to the widespread phenomenon in eukaryotic cells known as 'sense suppression'. *(P)*

Functional and Structural Destructions of Chloroplasts in Virus-Infected Tobacco Plants

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Tobacco (*Nicotiana benthamiana*) plants infected with three different viruses, representing three different forms of susceptibility or resistance, were studied Tobacco necrosis virus (TNV) caused local hypersensitive necroses in inoculated leaves Plum pox virus (PPV) caused systemic mosaic symptoms and the plants survived the infection Tomato spotted wilt virus (TSWV) infection resulted in slightly chlorotic spots on the leaves and after 2 weeks the plants suddenly wilted and died

Electron microscope findings revealed that TSWV caused the strongest destruction of chloroplast structures, but only at the latest stage of symptom development. All three viruses changed the relative 77 K fluorescence intensities of the chlorophyll-protein complexes. Fluorescence induction measurements proved that TNV and PPV partially inhibited the electron transfer between Q_A and Q_B in the PS II complex in early stages, before visible symptoms of the infection. TSWV, however, started to inhibit the above processes only in wilting leaves. *(P)* [1]

Resistance in Transgenic Tobacco Plants to Tomato Spotted Wilt Virus (TSWV)

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Oriental and Virginia types of 12 tobacco cultivars were transformed with two different constructs containing the Np gene of TSWV. The resistance to the virus was detected after mechanical inoculation in the greenhouse and under field conditions in southwest Bulgaria. The lines were obtained from T1 to T6 generations. Full 100% resistance was observed in eight cultivars in T2 and T3 progenies. The resistance remains stable in the further tested generations. No correlation between the accumulation of Np and the resistance was observed. In some of the cultivars the Np level remains unchanged during the vegetation season. In others this level changes from negative to positive absorption after inoculation or in the form of inoculation with water. This happens as a stress response. Dihaploid homozygous lines were produced from five transgenic cultivars. Fully resistant plants to the local homologous strains showed some resistance also to tomato chlorotic spot virus (BRO-3). *(P)*

RNA-Mediated Resistance to Tomato Spotted Wilt Virus (TSWV) in Transgenic Tobacco Plants

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Previous experiments have shown that immunity to TSWV can be obtained by expressing the nucleoprotein (N) gene in transgenic tobacco and tomato. Expression of a translation-deficient mutant revealed that protection is based on the expression of the viral RNA sequence. However, no correlation could be made between steady state RNA-levels and the degree of protection Evidence was presented that resistance in these plants is caused by a mechanism similar to co-suppression. To test whether any genomic sequence of TSWV could induce RNA-mediated resistance, 375 transgenic lines, expressing various viral sequences covering over 70% of the entire genome, were screened for resistance. Remarkably, only plants that expressed N or NSm gene sequences, either sense or anti-sense, coding or non-coding, displayed immunity to the virus, whereas the other lines were completely susceptible. A possible explanation for the sequence-specificity of RNA-induced resistance was presented. In addition, the RNA-mediated resistance against TSWV obtained in commercial tomato hybrids was compared with a natural TSWV resistance gene (SW-5) in tomato in terms of broadness and durability. *(P)*

Geminivirus Resistance in Transgenic Tobacco Expressing Mutated BC1 Protein

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Tobacco explants were transformed with the tomato mottle geminivirus (TMoV) movement and symptom-inducing protein gene (BC1) by *Agrobacterium*-mediated transformation. Transgenic tobacco lines expressing the BC1 protein had phenotypes ranging from severe stunting and mottling, to no visible symptoms. Western blot analyses revealed similar levels of BC1 protein in all phenotypes. Non-symptomatic plants remained free of virus symptoms during a continuous challenge with viruliferous (TMoV) whiteflies over a 5-month period. These plants also showed resistance to an RNA virus when mechanically inoculated with tobacco mosaic virus. The geminivirus resistance and the non-symptomatic phenotype were associated with mutations in the amino terminus of the BC1 protein in the transgenic tobacco. Segregation of non-symptomatic lines revealed some progeny with symptoms. Apparently in transgenic tobacco with multiple BC1 copies, the mutated BC1 protein suppressed the symptom induction by wild-type BC1 protein in transgenic plants containing both forms We identified transgenic, progeny variations in the phenotype and in virus resistance with unexpected mutations in the transgene. These variations during *Agrobacterium*-mediated transformation are similar to those reported in other studies involving transgene expression and function. *(L)*

Functional Analysis of Tobacco Basic β-1,3-Glucanase Gene Promoter Activity in Transgenic Tobacco Plants

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Tobacco basic β-1,3-glucanase is implicated in plant development and in plant defense responses against pathogens. We examined the functional structure of a basic β-1,3-glucanase promoter using tobacco plants transformed with a 1.6 kb promoter region and its deletion mutants linked to the β-glucuronidase (GUS) gene. Significant GUS activity was detected in leaves, roots and minor activity in stems. Deletions carrying 1436 promoter fragments directed maximal GUS activity in leaves and roots, whereas the intact 1606 bp promoter was far less active, suggesting the presence of a strong silencing regulatory element between positions -1606 and -1436 Further 5' deletions resulted in a considerable reduction in activity. Induction of promoter activity by virus infection reached the maximal GUS level with the 1436 and 1233 promoters using tobacco mosaic virus (TMV), potato virus Y and cucumber mosaic virus (CMV). Salicylic acid treatment had only a marginal effect on promoter activity. Two from another set of four promoter mutants, based on the parental 1606 construct and carrying an internal deletion, were significantly activated by TMV. These results, supported by the relevant DNA bend shift, suggested that a site for viral induction resides between 88 and 131 first bp of the promoter. That induction could be mediated without the two copies of the AGC-box located between -1233 and -1038. Histochemical analysis of TMV-induced expression in leaves revealed intense staining in cells around the viral lesion, whereas infection with CMV resulted in diffuse staining. Expression in roots was confined to the root tips and cortex. *(P)*

Study of Molecular Mechanisms of RNA-Mediated Virus Resistance

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Tobacco plants transformed with the open-reading frame (ORF) encoding the 54 kDa protein (putative component of the replicase complex) of TMV-U1 were resistant to subsequent infection by a very high concentration of virus or viral RNA inoculum. In an attempt to discover whether 54K-mediated resistance is dependent on sequence homology of the inoculated virus and the transgene, the 54K cDNA of TMV-U1 (54K U1), and the corresponding cDNA of a crucifer-infecting tobamovirus, cr-TMV (54K cr), were cloned into a potato virus (PVX) vector, respectively. The resistance was effective against PVX-54K.U1 but not against PVX-54K.cr This finding indicated that the resistance mechanism requires nucleotide sequence homology of the transgene and the inoculated virus. In order to assess the minimal length of homology needed to trigger the suppression of viral replication in transgenic plants, several PVX constructs were made that contained a set of 54K U1 PCR products (spanning the 5' end, central region and 3' end of the 54K gene) Chimeric PVX constructs which contained the 3' region of 54K.U1 overcame the resistance in these transgenic plants. A model is proposed to account for these observations. *(P)*

Characterization of Plant Genes Correlated with Systemically Acquired Virus Resistance

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Infection of *Nicotiana tabacum* cv Samsun NN with tobacco mosaic virus (TMV) results in a hypersensitive response in the plant Necrotic lesions are formed on the inoculated leaves and a systemic resistance is induced against a variety of pathogens, such as bacteria, fungi and viruses. This systemically acquired resistance (SAR) is accompanied by the induction of many plant genes, *e.g.* the genes for the pathogenesis-related (PR) proteins. Some of these PR proteins have shown antifungal activity, but proteins correlated with acquired resistance against viruses have not been identified so far. The aim of our study was to identify plant genes functional in inducing resistance against viruses, either in the signaling pathway or in actual defense mechanisms. To this aim mRNA was isolated from the non-inoculated leaves of TMV-inoculated tobacco plants showing SAR and from mock-inoculated plants, and cDNA libraries were constructed. Differential display was performed with 32 primer combinations, resulting in 50 fragments specific for leaves showing SAR. So far 25 of these fragments have been cloned and used to probe RNA blots. However, we could not confirm for any of the fragments that they were indeed derived from induced mRNAs. Therefore, an alternative strategy was adopted and a subtracted cDNA library enriched for systemically induced genes was constructed. cDNAs of known PR genes were eliminated from the subtracted library. Northern blot analysis was performed to confirm differential expression and full length cDNA clones were obtained from the cDNA library. The results obtained were presented. *(P)*

Expression and Biological Effects of Various Tobacco Mosaic Virus (TMV)-Specific Recombinant Antibodies in Transgenic Plants

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Transgenic plants producing recombinant full-size (rAbs) or single chain antibodies (scFvs) targeted to the apoplasm or cytoplasm were analyzed to investigate antibody-mediated protection against a plant virus. Cloned cDNAs of TMV-specific rAbs and scFvs were used for *Agrobacterium*-mediated transformation of *Nicotiana tabacum* cv. Xanthi-nc. Transgenic lines were analyzed by Northern blot, Western blot and ELISA for expression and reactivity of rAbs and scFvs in different plant cell compartments Depending on the constructs being expressed, a correlation between transcript and production levels of recombinant antibodies was found. T_1-progenies producing various antibodies showed different levels of necrotic lesion number reduction upon inoculation with TMV-*vulgare*, indicating a dependence of antibody valency and localization in the plant cell This finding was further supported by systemic inoculation assays of transgenic *N. tabacum* cv. Xanthi-nc lines at elevated temperatures as well as TMV-replication and assembly studies using protoplasts. *(P)*

Evaluation of Viral Resistance in Transgenic Tobacco Plants that Contain Both a Defective Movement Protein and the Coat Protein of Tobacco Mosaic Virus (TMV)

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Transgenic tobacco plants that express either a gene encoding a defective mutant of the tobacco mosaic virus (TMV) movement protein (dMP) or the TMV coat protein (CP) gene were sexually crossed, and progeny were analyzed with respect to their resistance to viral infection The F1 hybrids which express both transgenes were inoculated with TMV, TMV-RNA and SHMV. An increase in resistance to viral infection was observed in the F1 hybrid plants, indicating that both the CP and the dMP can confer resistance when co-expressed. There was no indication to negative or positive interaction between the two transgenes. Moreover, a gene dosage effect of the CP gene on the degree of CP-mediated resistance was observed. Transgenic plants heterozygous for the CP gene exhibited a lower level of resistance when compared with plants homozygous for the CP gene. However, our results indicate that the hybrid transgenic plants that express both the CP and dMP exhibit a higher level of resistance than that exhibited by the plants that express single copies of the transgenes. *(L)*

A Comparison of Different Pathogen-Derived Resistances to Virus Infection

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Plants resistant to viral infections can be developed utilizing a number of strategies. Originally, the coat protein (CP) of a virus was introduced into a plant to protect it from infection by the homologous virus. We have introduced the CP and several nonstructural genes of peanut stripe potyvirus (PStV) into *Nicotiana benthamiana* to develop resistance to PStV and to investigate the mechanism(s) involved in the resistance. We have identified two types of resistance, constitutive and inducible, with constructs engineered to express the CP, CI, NIa and NIb of PStV. These resistant phenotypes involve an increase in transgene mRNA turnover that we believe results in the resistance phenotype. We have also identified a third resistant phenotype using CP gene constructs that lack the original translation initiation site This resistance does not involve a rapid turnover of the mRNA and

appears to correlate with the highest accumulation of transgene mRNA The different types of pathogen-derived resistance were compared *(P)*

Coat Protein Gene-Mediated Resistance to Raspberry Bushy Dwarf Virus (RBDV) in Tobacco

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Raspberry bushy dwarf virus (RBDV) causes a serious disease in many countries where raspberry crops are grown. Transgenic resistance could help overcome a new threat from resistance-breaking isolates of RBDV. DNA corresponding to the coat protein gene of RBDV has been inserted into a variety of plant expression vectors, which have been used to transform *Nicotiana tabacum* and *N benthamiana* Plants were grown from seeds harvested from transgenic plants and were challenged by manual inoculation with RBDV. Preliminary results indicate that some lines of plants were resistant to RBDV accumulation. This resistance is being evaluated and the effective construct is being modified to improve the resistance obtained *(P)*

Analysis of a Ribozyme Targeted Towards Banana Bunchy Top Virus (BBTV) Replicase Associated Protein Gene

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BBTV is a persistently aphid-transmitted virus with a multicomponent ssDNA genome We have attempted to determine the effectiveness of ribozyme-mediated resistance against the replicase associated protein (RAP) gene of BBTV. A hammerhead ribozyme was designed around a central GUC sequence and included 20 nucleotides either side of this sequence which were conserved among all previously sequenced isolates of the virus. *In vitro* analysis of the ribozyme targeted towards an *in vitro*-produced transcript of the RAP gene indicated that the ribozyme cleaved the RAP transcript After 24 h at ratios of 1.1.4 (substrate· ribozyme), the ribozyme cleaved over 43% of the RAP transcript at 23°C and 86% at 37°C Elevating the temperature to 50°C enabled cleavage of over 83% of the RAP transcript in 2 h Effects of increasing the ribozyme transcript ratio improved the cleavage *In vivo* activity of the ribozyme was also examined by determining the effects of the ribozyme in several RAP-transformed tobacco plants. *(P)*

Molecular Analysis of Tobacco Plants Transformed with a Mouse 2.5A Synthetase Gene

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The 2.5A pathway found in mammalian systems is an interferon-induced RNA-degradation pathway which is activated by double-stranded RNA (dsRNA). One of the roles of this system is as a defense mechanism against viral infection. It is yet to be established conclusively whether a similar 2 5A pathway exists in plants, but it has been shown that expression of a rat 2 5A synthetase gene leads to elevated levels of viral resistance in transgenic plants. In this work we have further investigated the possibility of utilizing the 2 5A pathway to generate transgenic plants with multiple virus resistance, by transforming tobacco plants with a mouse 2 5A synthetase gene Unlike the rat 2.5A synthetase gene, this mouse gene has been shown to be functional, as its expression in mammalian cells protects these cells from viral infection. Our results suggest that this mouse 2.5A synthetase gene may also protect tobacco plants from infection by RNA plant viruses In an attempt to determine a possible molecular mechanism for this resistance, we transformed tobacco with a mutant form of the mouse 2.5A synthetase gene which encodes a truncated protein retaining the dsRNA binding activity but abolishing the catalytic activity of this enzyme. If these plants are resistant to viral infection, this would suggest resistance is not due to the mouse 2 5A synthetase triggering an already existing 2 5A pathway in plants, but may be due to the 2 5A synthetase binding to the dsRNA replicating intermediates of RNA viruses and inhibiting replication *(P)*

Mechanisms of Replicase-Mediated Resistance to Cucumber Mosaic Virus (CMV)

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Resistance to CMV engendered in transgenic tobacco expressing a defective polymerase gene involves several separate mechanisms: One mechanism affects virus replication, and the extent of inhibition is determined by the overall level of sequence identity between the transgene and RNA 2 of the challenge virus, but does not map to specific sequences. Another component of resistance inhibits virus (cell-to-cell and systemic) movement and is affected by the level of sequence identity between the transgene and the central third of RNA 2 of the challenge virus The resistance to movement is epistatic to the resistance to replication. Steady-state transgene mRNA levels in several tobacco lines exhibiting different degrees of inoculum concentration-dependent resistance correlated positively with the degree of resistance, which is inconsistent with a sense-suppression mechanism of resistance. *(L)*

A Defective Replicase Gene from Cucumber Mosaic Virus (CMV) Induces Immunity in Tomato

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Leaf or cotyledonary explants from commercial tomato lines were transformed with *Agrobacterium tumefaciens* LB 4404 harboring the pCMV N/B-23 Ti-binary vector, which carries a defective replicase gene from RNA-2 of CMV A total of 139 transformants from five tomato lines were isolated Individual R_0 plants were selfed and R_1 progeny was screened by a kanamycin-sensitivity assay Transgenic seedlings were subsequently tested for resistance to CMV by mechanical inoculation with the CMV-Fny strain at 0 5–1.0 mg/ml, or by aphid transmission. The responses of R_1 populations fitted into three categories: (i) fully susceptible lines, (ii) fully resistant lines; and (iii) intermediate-type (mixture of susceptible and resistant seedlings at variable proportions) No virus was detected in non-inoculated leaves from resistant plants in categories ii or iii Lines classified as totally immune by mechanical inoculations, show immunity also in aphid transmission assays Transcription of the viral transgene was verified in immune lines; however, translation products were not detectable Homozygous R_2 plants from resistant lines are being incorporated in current breeding programs. *(P)*

High Resistance to Cucumber Mosaic Virus (CMV) Conferred by Viral Coat Protein in Transgenic Tomato Plant

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For the production of broad commercial resistance to CMV infection, tomato plants, var UC82B, were transformed with two coat protein (CP) genes, one from each subgroup. The CP genes were cloned from two Italian CMV isolates which produce severe disease symptoms CMV-22 of subgroup I and CMV-PG of subgroup II. Four plant transformation vectors were constructed. pMON18774 and pMON18775 (CMV- 22/D CP), pMON18831 (CMV-PG CP) and pMON18833 (CMV-22CP and CMV-PG CP). All together 178 transformed R_0 plants were produced. Lines were selected based on CMV-CP gene expression, resistance to CMV infection in a growth chamber, and single gene segregation in R_1 progeny. Over the last 3 years, R_2 homozygous lines transformed with all CMV-CP-expressing plasmids were evaluated under field conditions in Italy. The results indicate that all vector constructs generated plants resistant to CMV infection and that the UC82B agronomic characteristics were maintained. The double gene vector construct produced plants with broad resistance against strains of CMV from both subgroups I and II Homozygous lines contain only one copy of transgenes, allowing future backcrossing into commercial tomato varieties and hybrid variety development *(P)*

Transgenic Tomato Plants Expressing Dysfunctional Geminivirus Movement Proteins Display Heterologous Protection

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Bipartite geminiviruses encode two movement proteins, BC1 and BV1, which are needed for efficient systemic infection Microinjection studies with *Escherichia coli*-expressed BC1 and BV1 proteins of bean dwarf mosaic geminivirus (BDMV) have established that the BC1 protein is the plasmodesmal cell-to-cell movement protein, whereas the BV1 protein is involved in DNA export out of the nucleus (Noueiry *et al.*, 1994) Both proteins also have DNA-binding properties Single amino acid substitutions made in highly conserved regions of both proteins disrupted their transport function, but not their DNA-binding properties To test the hypothesis that one or both of these mutated proteins could provide dominant negative mutants for crop protection against geminiviruses, transgenic tomato plants expressing functional and dysfunctional BDMV BC1 and BV1 proteins were produced. Agroinoculation with tomato mottle geminivirus (ToMoV), a close relative of BDMV and an important tomato pathogen, was used to challenge the transgenic plants Several T1 lines failed to become infected or showed a delay in symptom development, suggesting heterologous protection These lines are being further characterized and tested for resistance to other geminiviruses. *(P)*

Protein Induction in PVY^{NTN} Virus-Infected Plants of Different Potato Cultivars (*Solanum tuberosum*)

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Pathogen infection of plants causes the induction of numerous genes encoding defense proteins. We compared the protein composition of PVY^{NTN}-infected and healthy control plants from different cultivars of potato. In order to elucidate the biochemical basis for different reactions to the virus infection, we tested cultivars 'Igor', 'Pentland Squire', 'Désirée' and 'Sante', which differ in their susceptibility or resistance to the virus The total amount of plant proteins increased significantly in cultivars which are more susceptible to the virus infection. Special attention was paid to the proteinase inhibitors which are induced in leaves of plants attacked by insects or pathogens They may also be involved in the resistance mechanism of plants against viruses. Protein analysis was performed by SDS electrophoresis and isoelectric focusing. The increase in aspartic and cysteine proteinase inhibitors after virus infection was checked by Western blot analysis. *(P)*

Characterization of the P1-Gene-Mediated Resistance in Transgenic Potato Plants

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The cloned P1 gene sequence of PVY-O was transferred both in sense and in antisense orientation into the Finnish potato cultivar 'Pito' by *Agrobacterium tumefaciens* mediated transformation system. Transgenic potato plants were tested for their resistance against PVY-O by sap- and graft-inoculation Three sense transformed and five antisense transformed P1 transgenic lines showed resistance against PVY-O both in sap and in graft inoculation, 3 and 5 weeks after inoculation, respectively. The copy number of the P1 gene in these resistant lines varied from one to five copies. Northern analysis confirmed the expression of the P1 mRNA, but in Western analysis no P1 protein expression could be detected in the resistant lines In a second graft-inoculation experiment, resistant and wild-type Pito plants were used both as stock and scion, and subsequently sap-inoculated with PVY-O onto two leaves of the stock. In both cases, no virus could be detected in ELISA in the scion, 3 and 5 weeks after inoculation. To study the possible mechanism of the P1-gene-mediated resistance we are currently making an untranslatable version of the P1 gene in order to transform it into potato. Furthermore, studies of the expression levels of P1 mRNA in susceptible and resistant plants are being continued. *(P)*

Russet Burbank Potato Engineered for Virus and Colorado Potato Beetle Resistance

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'Russet Burbank' potato was transformed by an *Agrobacterium*-mediated method with double gene constructs. potato leafroll virus (PLRV) replicase gene and *cryIIIA* gene from *Bacillus thuringiensis* var. *tenebrionis* (*Btt*) or potato virus Y (PVY) coat protein gene and *Btt*. Initially, newly transformed potato lines were micropropagated and screened by an insect assay for resistance to Colorado potato beetle (CPB) CPB-resistant lines were challenged with PLRV or PVY to select lines with high

virus resistance. Selected lines were extensively field tested for CPB and virus resistance. Lines with the highest *Btt* expression and which were field immune to the homologous virus were selected. Additional tests with diverse virus strains and field isolates confirmed broad resistance of practical value. Potato lines which appear identical to nontransformed Russet Burbank and with the highest yield, are propagated for possible future commercial use. *(P)*

Tissue Damage in Potato Leaves Triggers Programmed Cell Death in Tissue Surrounding Injured Area and Along Vascular Bundles

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Inoculation by potato A potyvirus (PVA) causes formation of necrotic lesions in plants with a hypersensitive response (HR) gene for PVA. Plants without the HR gene are susceptible to PVA and do not form lesions. Samples from both types of plants as well as from plants in which tissue damage was inflicted by freezing or crushing portions of leaf were inspected. Cryosections prepared from plant leaf samples were stained by the Apoptag detection kit to visualize nuclei with extensive DNA fragmentation, a hallmark of programmed cell death. In healthy plants and PVA-infected susceptible plants, fluorescing nuclei were observed only sporadically and with a low intensity of fluorescence. In those plants that display necrotic lesions as a result of HR or had sustained other damage, strong, specific fluorescence was observed in the nuclei in the immediate vicinity of injured tissue Transfer cells surrounding secondary vascular bundles centimeters from the site of damage, also showed extensive DNA fragmentation in their nuclei. Our results indicate that potato plants respond to different types of tissue damage by triggering programmed cell death in cells directly in contact with the vascular bundles only centimeters away from the injury. In the context of plant defense, this can be seen as an effort to deny transportation to possible pathogens *(L)*

Some Factors that Contribute to Durable Resistance against Okra Viruses in Okra (*Abelmoschus esculentus*)

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Since the use of resistant cultivars is the cheapest and safest way of controlling plant viruses, a long-term screening program was initiated in Nigeria to locate sources of resistance to okra mosaic virus (OMV) and okra leaf curl virus (OLCV) in okra, one of the most important vegetables grown in Nigeria. Disease reaction of 265 okra accessions from 12 countries was assessed in replicated field trials. Of the accessions initially screened, 66 were resistant to both viruses Sources of resistance to OMV were identified in varieties showing no significant yield differences between inoculated and uninoculated treatments. The OLCV infection rate was slower in some varieties than others, indicating the presence of horizontal resistance, a more durable resistance since it is polygenic. Mixed infections by the two viruses reduced yield more than did single infections. Thus it is desirable to breed for combined resistance to both viruses. If OLCV symptoms developed after fruiting, yield was not significantly affected, unlike in lines in which disease developed either before or during flowering. This is a potentially useful trait which could be bred into commercial cultivars. *(P)*

Functional Characterization of Pathotype-Specific Recessive Resistances to Potato Virus Y (PVY) in Pepper

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PVY, the type member of the potyviruses, is an important pathogen of cultivated solanaceous crops, such as potato, tomato or pepper. Several sources of resistance to PVY have been found in pepper. The resistance conferred by the *pr-2* gene is characterized by the absence of any phenotype of virus-induced disease, no virus accumulation detectable by standard means and specificity of different alleles towards different virus pathotypes. The resistance trait is recessive. We have studied the mechanism(s) underlying the mode of action of the *pr-2* gene by immunofluorescence techniques on protoplasts of resistant and susceptible pepper cultivars using resistance-sensitive and resistance-breaking PVY pathotypes. The results obtained were presented and discussed. *(P)*

The Tobamoviral Coat Protein of Pepper Mild Mottle Virus (PMMoV-I) Acts as Elicitor of the *Capsicum* L^3 Gene-Mediated Resistance

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The *Capsicum* L^3 gene confers resistance to most of the tobamoviruses except for certain strains of PMMoV-I. In previous work, using chimeric viral genomes between PMMoV-I and PMMoV-S, we have shown a strain able to elicit the hypersensitive reaction, whose coat protein (CP) is required for the elicitation of the host response. To determine that the CP is the elicitor of the host response, we introduced the CP of the PMMoV-S into a PVX-based vector. The viral transcripts inoculated directly onto L^3 plants were able to induce necrotic local lesions indistinguishable from those induced by PMMoV-S. No PMMoV-related sequences or the CP in the upper non-inoculated part of the plant, were detected. The data prove that the CP of the PMMoV-S is sufficient to induce the host response in the absence of related viral sequences, thus showing that it acts as the elicitor of the host defense reaction. *(P)*

Post-Transcriptional Transgene Silencing and Consequent Tospovirus Resistance in Transgenic Lettuce are Affected by Transgene Dosage and Plant Development

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Transgenic lettuce plants expressing the nucleocapsid (N) protein gene of the lettuce isolate of tomato spotted wilt virus (TSWV-BL) were protected against TSWV isolates *via* transgenic N protein when the protein accumulated at high levels or *via* an N transgene silencing mechanism activated by its overexpression. Resistant reactions of lines D-TSW-1, D-TSW-6 and M-TSW-4 are correlated with levels of N protein accumulation, whereas line M-TSW-3 shows high resistance with low N protein accumulation. Upon further analysis we found post-transcriptional gene silencing was activated at a relatively earlier

developmental stage in homozygous than in hemizygous progenies of line M-TSW-3 As a result, the homozygous progenies generally showed a uniform suppression of N protein accumulation and consequently high levels of virus resistance in all leaves of the silenced plants In contrast, N protein accumulated at high levels in the lower leaves of the hemizygous progenies and at much reduced levels (due to transgene silencing) in the successive leaves, resulting in moderate levels of virus resistance. We also observed that the timing of the N transgene silencing in both homozygous and hemizygous plants was affected by environmental factors *(L)*

Severe Epidemic Caused by Virus Diseases in Egyptian Corn

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A severe stunting and yellowing phenomenon invaded most corn fields in Middle Egypt (Giza, Beni Sweif, Fayium and Minia) in the 1991 growing season. Several fields were overturned because of the severe infection in Fayium and Beni Sweif. The cause was identified as: (i) maize yellow stripe persistently leafhopper-transmited tenuivirus (MYStV); and (ii) barley yellow dwarf persistently aphid-transmitted luteovirus (BYDV). MYStV was identified and detected by visual examination for the external symptoms as well as insect transmission. BYDV was identified and detected by ELISA and visual examination as well. Developmental stages of MYStV-symptom types were followed up by bioassay The severe-infection type (coarse stripe) increased gradually for 50–60 days. The mild-infection type (fine stripe) decreased gradually for 50–60 days. The bright yellow-infection type was the dominant type in epidemic-diseased fields. Environmental factors, *i.e.*, a warm winter (positive temperature deviation from normal), a high population density of insect vectors, a high percentage of viruliferous vectors and the susceptible host influenced the occurrence and the prediction of severe epidemics in Egypt. *(P)*

A New Approach to Non-Conventional Viral Resistance and Tissue Culture of Tropical Maize

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The geminivirus maize streak virus (MSV) causes crop losses throughout sub-Saharan Africa The recalcitrance of maize cell cultures to regeneration has hampered its genetic manipulation towards virus resistance. We report an efficient method for regeneration of plants from multiple shoot clumps obtained from the apical meristem of Zimbabwean maize varieties, using a published technique. This has enabled us to use particle bombardment for genetic manipulation of the tissues. Currently, we are investigating new, non-conventional approaches to MSV resistance as our previous work using sense and antisense copies of the coat protein (CP) gene produced only partial resistance. We are making a suicide construct which will express the type 1 ribosome inactivating protein, dianthin, from the MSV CP gene promoter. Since geminivirus CP gene promoters are transactivated by the virus complementary sense gene products, virus replication will lead to death of the infected cell. Our studies defining the MSV CP promoter region involved in transactivating were presented. Our program also includes the genetic engineering of maize for insect resistance. Data on the toxicity of modified insecticidal proteins to virus vectors and other insects were reported. *(P)*

Genetically Engineered Protection against the Rice Tungro Spherical Virus in Transgenic Rice

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Rice tungro, a potentially devastating rice disease in Southeast Asia, is caused by two different viruses: rice tungro bacilliform badnavirus (RTBV), a dsDNA virus, responsible for the severity of symptoms; and rice tungro spherical waikavirus (RTSV), a ssRNA(+) virus which controls the transmission of both viruses by leafhoppers. The RTSV genome encodes a large polyprotein that is subsequently cleaved by the viral protease(s) to produce 8 to 10 proteins. The three coat proteins (CP1, 2, 3) and the polymerase (Pol) have been chosen to genetically engineer rice in pathogen-mediated resistance strategies against RTSV. A total of 71 CP and 46 Pol (sense and antisense) independent transgenic plant lines has been produced. A limited number of CP transgenic lines that express the CP gene have been challenged by virus inoculation. The challenged line that contains CP1 was found to exhibit poor resistance. However, one line expressing CP2 (controlled by the maize ubiquitin promoter) and two expressing CP3 (under the maize ubiquitin and the rice actin promoters) showed significant levels of resistance. As the CP-mediated resistance strategy gave promising results, more lines expressing a single CP or the Pol are currently being tested for resistance. In addition, 139 transgenic plant lines that contain genes encoding two or three different CPs have been produced and are also under analysis The molecular characterization of transgenic plants as well as resistance data were presented. *(P)*

Cloning and Preliminary Sequence Analysis of the Sugarcane Fiji Disease Virus Genome

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Sugarcane Fiji disease, caused by sugarcane Fiji disease reovirus (FDV), is one of the most important diseases affecting sugarcane in Queensland. FDV is the sole member of serogroup 1 within the genus *Fijivirus* of the family Reoviridae. It has a genome consisting of ten segments of dsRNA (~30 kbp) and is transmitted by the planthopper *Perkinsiella* spp. We have generated a random-primed cDNA library from purified viral dsRNA. Segment-specific clones were identified by Northern blot analysis and selected clones were sequenced. We have currently generated sequence data for approximately 90%, 50%, 50%, 90%, 30% and 95% of FDV genome segments 1 (4.4 kbp), 2 (3.7 kbp), 6 (2.8 kbp), 7 (2.2 kbp), 8 (1.9 kbp) and 10 (1.7 kbp), respectively. Preliminary analyses of these sequences have revealed (i) a genome organization similar to that of other Fijiviruses, and (ii) significant homology at both the nucleotide and amino acid level with maize rough dwarf, rice black-streaked dwarf and *Nilaparvata lugens* reoviruses. These sequences are being used to develop constructs for genetic-engineering of FDV-resistant sugarcane. *(P)*

Sequence Variability in Sugarcane Mosaic Virus (SCMV) Strain A

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SCMV is an important pathogen of sugarcane in Australia. Pathogen-derived resistance to plant viruses has been achieved using viral replicase (NIb) or coat protein (CP) genes as transgenes in a number of crops. However, sequence variability in these genes can lead to loss of resistance. Before designing constructs to generate SCMV-resistant sugarcane it was necessary to establish the extent of SCMV variation in the field. Eight SCMV-infected leaf samples were collected from four different locations in Australia Total nucleic acids were extracted and the CP and NIb genes were cloned and sequenced. The variability in the CP nucleotide sequences was approximately 6% and the translated amino acids sequences showed approximately the same level of variability. The NIb sequences showed slightly less variation (approximately 3%), with the amino acid sequences being greater than 99% homologous These SCMV sequences have allowed us to target the isolate most homologous to all others to design resistance constructs with either the CP or the NIb genes. *(P)*

Resistance in Oilseed Rape against Turnip Yellows Luteovirus (TuYV)

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In several years very high rates of infection by TuYV (syn. beet western yellows luteovirus) were detected on winter oilseed rape crops (*Brassica napus* L.) in Germany Yield losses of 12% to 34% were recorded on two cultivars in a 3-year experiment We investigated the resistance of 652 genotypes to TuYV infection under glasshouse conditions. All of the rape genotypes were susceptible to TuYV In DAS-ELISA, the average absorbance value was in the range of 1.18 to 2.13, indicating high virus concentration in infected plants. The only exception was the resynthesized rapeseed R 54 with some selected plants, which were immune to TuYV After selfing, the S1 progenies of four plants tested were TuYV-resistant at ratios of 4.9:1, 8.7:1, 1·2.3 and 2 4:1, respectively. In the S2, progenies yielded resistant as well as susceptible plants in different ratios. Crossing between several susceptible cultivars and the TuYV-resistant progeny of R 54 resulted in F_1 populations susceptible in a uniform manner. In F_2 populations, a 1:3 ratio of TuYV-resistant to TuYV-susceptible plants was detected, indicating for the first time the successful transmission of TuYV resistance in modern oilseed rape cultivars. *(P)*

Transgenic White Clover with Immunity against Alfalfa Mosaic Virus (AMV)

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White clover is the most important pasture legume to the Australian dairy industry and is a major component of improved pastures in the temperate zones of the world. AMV is a major pathogen of white clover worldwide, causing reported yield reductions of up to 40% in infected plants. The objective of this project is to produce AMV-resistant white clover plants by *Agrobacterium*-mediated gene transfer methods. Several gene constructs comprising the AMV coat protein (CP) gene from either a subgroup I or a subgroup II AMV, driven by either the cauliflower mosaic virus 35S or the *Arabidopsis* small subunit (ASSU) promoter, and expressing the CP open-reading frame in either plus or minus sense, were transferred into white clover. Transgenic plants were tested for resistance to mechanical inoculation with AMV under glasshouse conditions Three field isolates of AMV, including representatives of both subgroups of AMV, and mild and severe isolates from white clover, were used at inoculum levels of up to 200 μg/ml. Over 130 independent transgenic lines of white clover of the cvs. 'Haifa' and 'Waverley' were challenged The results with cv. .Haifa showed that when the CP is expressed in the plus sense, only one out of 13 (7.7%) lines was immune to all three AMV isolates when using the 35S promoter but 11 out of 33 (33%) were immune when using the ASSU promoter However, when the minus sense CP was expressed using the ASSU promoter, no immune lines were obtained out of 28 lines tested Similar results were obtained with cv. Waverley. In contrast, all the non-transgenic lines were susceptible, with average infection rates of ~85% Southern, Northern and Western blot analyses showed that there is a direct correlation between immunity and CP expression *(P)*

Molecular Studies of the Bean Yellow Mosaic Potyvirus (BYMV) NIa Gene for Pathogen-Derived Resistance

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BYMV infects a wide range of leguminous and non-leguminous plants. In Western Australia BYMV is a serious pathogen of cultivated lupins, especially narrow leafed and yellow lupins (*Lupinus angustifolius, L. luteus*). Over 1 million ha of narrow leafed lupin are cultivated in Western Australia annually. There is no naturally occurring resistance to BYMV, so pathogen-derived resistance is one approach for protection of the crop. Resistance to viral pathogens of plants has been demonstrated when a gene derived from the virus has been transformed into the genome of the host. This phenomenon can occur through expression of the gene at the RNA or protein level. In this study constructs derived from the non-structural viral gene NIa, encoding for the VPg and a serine-like protease of BYMV, were used to transform subterranean clover (*Trifolium subterraneum*) Constructs include translatable and non-translatable genes, and truncated or mutated genes Analysis to determine the degree of resistance afforded by the transgenes is in progress and to determine if the resistance is mediated at the RNA or protein level. The most promising of these constructs will be used to generate BYMV-resistant transgenic lupins. *(P)*

Use of the Bean Yellow Mosaic Virus (BYMV) Coat Protein and Replicase NIb Genes for Virus Resistance in Lupins

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In this work gene expression and virus resistance in transformed lupins are being examined. Genes studied include (i) the complete BYMV-MI isolate coat protein (CP) gene; (ii) the BYMV CP gene in reverse orientation with 3' part of the NIB gene, (iii) the BYMV replicase (NIb) gene; (iv) the binary plasmic pTAB16 containing the Basta®resistance (*bar*) and reporter (*gus*) genes Lupin species *Lupinus angustifolius* (cvs 'Merrit', 84A:473, 84L:439, 85A·479 and 85L·460), *L. luteus* (cvs Teo, Teo 102, Teo 105, Juno, Motiv, Popiel, WDT-6174, WDT-6172, WDT-6194) and *L. albus* (cv. 'Kiev Mutant') have been transformed using *Agrobacterium tumefaciens* with the above genes, using selection on Basta® Presence of the BYMV CP and *bar* genes in putative transgenic shoots has been confirmed by polymerase chain reaction The efficiency of transformation of *L. angustifolius, L. luteus* and *L. albus* is influenced by genotype. The plant regulator thidiazuron (TDZ) has been used to enhance multiple shoot

production to improve the regeneration efficiency after co-cultivation with *A. tumefaciens* (*L. luteus* and *L. albus*), and to increase the number of transgenic plants obtained after selection. Transformed shoots (R_0) were acclimatized under aseptic conditions before grafting onto glasshouse rootstocks or micrografting onto *in vitro* rootstocks. R_1 seeds from grafted plants have been sown Experiments are in progress to improve the efficiency of the transformation system and to analyze transformed tissue (R_1) seedlings. *(P)*

Application of Recombinant Antibodies for Disease Resistance in Plants

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Bean yellow mosaic virus (BYMV) is a serious pathogen of commercial lupin crops in Western Australia. Unfortunately, there are no natural BYMV resistance genes found in modern lupin cultivars or their wild relatives. Classical breeding strategies are therefore of little use for providing resistance to BYMV A number of molecular approaches have been employed to confer resistance to plant viruses These usually involve the constitutive expression of viral genes in transgenic plants A more recent novel approach has been to express single-chain antibodies (scFv) or $F(ab)_2$ fragments that have been targeted against viral proteins. We are in the process of developing both of these types of constructs to assess their effectiveness for providing resistance to plant viruses. *(P)*

Coat Protein-Mediated Resistance to Wheat Streak Mosaic (WSMV) and Barley Yellow Dwarf (BYDV) Viruses in Soft White Winter Wheats

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Immature embryogenic calli of commercial cultivars of soft white winter wheat (*Triticum aestivum* cvs 'Daws' and 'Lambert') were used for microprojectile bombardment and, in addition to the *bar* gene for resistance to phosphinothricin (PPT), transformed with the coat protein (CP) genes of either BYDV-PAV or WSMV. Putative T_0 transformants were screened by bioassay for PPT resistance and selected plants then assayed by polymerase chain reaction for the *bar* gene and either CP gene. Further verification was accomplished by Western blot analysis of WSMV CP transformed lines T_0, T_1 or T_2 lines with BYDV CP were challenge-inoculated using viruliferous aphids and evaluated for symptoms and virus titer 20 days post-inoculation. Similarly, lines with the WSMV CP were challenge-inoculated mechanically A limited number of lines in these segregating populations were obtained that showed clear resistance to BYDV. Many lines were obtained that exhibited either a delay in symptom development or no symptoms of WSMV. Test crosses and analysis of T_1 progeny indicated that the selectable marker and CP transgenes segregated independently, enabling removal of the herbicide resistance gene while retaining the virus resistance gene. *(P)*

Barley Yd2, a Naturally Occurring Gene that Provides Resistance against Barley Yellow Dwarf Virus (BYDV)

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The Yd2 gene of barley confers resistance to specific isolates of BYDV Our aim is to isolate and characterize the gene and to characterize the molecular basis of the host–pathogen interaction. In the absence of any measurable activity for the Yd2 gene product, we are using a map-based approach and have generated a high resolution map of barley chromosome 3H around the Yd2 locus using molecular and morphological markers from a wide variety of sources. Additional markers are being generated by AFLP (amplified fragment length polymorphism) analysis of DNA from appropriate crosses. Further, we have been able to establish the extent of synteny around the Yd2 region of barley and the corresponding portion of rice chromosome 1. Rice is the nearly ideal cereal species from which to isolate genes by positional cloning and this synteny makes it highly probable that we will be able to base future efforts towards the physical placement of Yd2 in rice rather than in barley A marker protein, YLP, that varies in isoelectric point between barleys with and without Yd2, has been characterized as a putative subunit of a vacuolar proton-translocating ATPase from barley. The 1040 nt mRNAs of this protein have been cloned and sequenced. So far, the YLP marker co-segregates with Yd2 in all crosses examined. *(P)*

Survey and Screening of Cereal Viruses for Resistance in Egypt

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Barley yellow dwarf luteovirus (BYDV) was first reported in 1980. BYDV serotypes (MAV, PAV, RPV, RMV, SGV) occur in Egypt because of the incidence of the four aphid species vectoring the virus. Virus detection was done by tissue blot indirect ELISA for BYDV, barley stripe mosaic hordeivirus (BSMV), maize yellow stripe tenuivirus (MYSV), maize streak geminivirus (MSV) in Middle Egypt (Giza, Beni Suife, Fayoum, Minia, Assiut) wheat fields in 1995. BYDV incidence ranged from 1.9% to 6.8%, gradually decreasing from Giza to Assiut. The highest incidence (80–95%) was recorded at the ARC, Giza. BSMV is reported here for the first time The incidence ranged from 0.3% to 1.8%. MYSV incidence was 0.8%. Eighteen entries from ICARDA and 24 from Egypt were inoculated and screened against BYDV; all entries are susceptible. 246 Egyptian entries were screened against natural BYDV infection; all entries are susceptible A scoring scale was initiated for disease intensity: (percentage of infection x degrees of severity) log. *(P)*

Genetics of Resistance to Gemini Viruses in *Vigna* Crops

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Mungbean yellow mosaic (MYMV) and cowpea golden mosaic (CGMV) gemini viruses cause serious losses in mungbean (*Vigna radiata*) and cowpea (*V. unguiculata*) crops Induction of resistance is an important means of controlling these virus diseases. Resistant parents of mungbean and cowpea were identified after 3 years of rigorous field and greenhouse screening under heavy inoculum pressure. In mungbean, the crosses between S × R and R × S gave variable reaction to MYMV in the F_1 generation, where dominance and recessiveness of resistance (R) and susceptibility (S) appeared to differ from cross to cross.

The presence of different resistant genes in resistant parents was clear from the F_2 generation of cross R × R. Analysis of the F_1 generation along with the F_2 strongly supported the view that resistance was dominant over susceptibility. Two major genes appeared to govern the resistance with complementary and inhibitory gene interaction, except in one cross of S × R, where the role of a single dominant gene was found when data of F_1, F_2 and backcross (BC) generations were analyzed. In cowpea, the F_1s of R × S crosses were completely resistant to CGMV, indicating that resistance was dominant over susceptibility. This was further confirmed by backcrosses. The segregation pattern gave a good fit of 15 R : 1 S. This has suggested that two recessive genes are responsible for expression of disease. Analysis of data of BC generations further confirmed that two recessive genes controlled the susceptibility to yellow mosaic. *(P)*

Transgenic RNA-Mediated Virus Resistance: Role of Repeated Transgenes and Fate of Heterologous RNAs Containing Small Insertions of the Transgene

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The replication of each of the two RNA segments of cowpea mosaic virus (CPMV) can be specifically blocked in transgenic plants expressing sequences derived from the RNA-segment. A post-transcriptional gene-silencing mechanism, which can also eliminate incoming homologous viral RNA-molecules, is proposed to be at the base of the process. The resistance mechanism can also affect heterologous potato virus X (PVX) RNAs that contain the sequence of the CPMV transgene. Specifically, sequences at the 3' region of the transgene direct elimination of recombinant PVX genomes. The efficiency of inhibition of the replication of recombinant PVX-RNAs is dependent on both origin and size of the CPMV-derived insertion, which can be as small as even 60 nucleotides. Remarkably, we observed different responses of individual plants within one homozygous line upon inoculation with several PVX-recombinants, which can be interpreted as a variation in the efficiency of the resistance mechanism. The arrangement of the transgenes in the plant genome plays an important role in establishing resistance, since upon transformation with a tandemly repeated transgene the frequency at which resistant lines arise is significantly increased. Deliberate integration of repeated transgenes thus might provide a general strategy for inducing virus resistance or silencing of endogenous genes. In all the transgenic plants containing a tandemly repeated transgene, strong methylation occurred. In sensitive lines specifically the promoter region was found methylated, whereas in resistant lines only transgene sequences were heavily methylated. This observation suggests an essential role for transgene-methylation in obtaining RNA-mediated viral resistance *(L)*

Immunity of Transgenic *Prunus domestica* to Plum Pox Virus (PPV) Inoculation

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Sharka is one of the most destructive viral diseases of *Prunus*. There are a number of research efforts underway by pathologists and breeders to control PPV. With the paucity of natural resistance, some attempts to control PPV are based on genetic engineering. Following successful transfer of the PPV coat protein (CP) gene to plums (Scorza *et al.*, 1994), transgenic plants were inoculated with PPV through chip-budding or aphid feeding. Infection was monitored through observations of virus symptoms, DAS-ELISA and Western-blotting assays. One transgenic clone, C-5, was found to be highly resistant. Immunocapture/PCR indicated that PPV was not present in inoculated plants and that the virus could not pass from inoculated shoots of clone C-5 into a susceptible rootstock. Molecular analysis of this clone prior to PPV inoculation reinforced the observations made by Smith *et al.* (1994) about the mechanisms of sense-suppression. *(P)*

Resistance of Apricots and Peaches to Plum Pox Virus (PPV): Problems of Detection and Evaluation

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Cultivars and hybrids of apricots and peaches were tested for degree of resistance to PPV by grafting onto young artificially infected trees of PPV-susceptible varieties. *Prunus armeniaca* cv. 'Vegama' was used for testing of apricots, and *Prunus persica* var. 'Siberian C' for testing of peaches. Both species were infected with a local isolate of PPV. The level of relative PPV concentration in leaves, flowers and fruits of investigated trees was repeatedly determined during 3 years by ELISA. Results were verified by IC PCR Characterization of plant material was completed by visual evaluation of symptoms. Research on resistance of apricots to PPV certified cv. 'Harlayne' as immune and cvs. 'Goldrich' and 'Stark Early Orange' as resistant. Although PPV moved from infected susceptible rootstocks, multiplication of the virus in the cultivars mentioned was found to be limited. In preliminary work, no peach cultivar showed high resistance. Susceptible cultivars of apricots and peaches showed PPV concentration similar to infected susceptible rootstock already in the year of grafting Results obtained indicate a different type of resistance mechanism in apricots and peaches. Both plant species will be infected with PPV-M and PPV-D strains to eliminate the influence of virus strain on the level of resistance. *(P)*

Application of L and M RNA-Derived Viral Products for Pathogen-Derived Resistance against Tospoviruses

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Tospoviruses can cause severe loss of yield in a broad variety of economically important crop species However, only a limited number of natural resistance genes is available for commercial breeding programs. Using an alternative approach, high levels of resistance have been obtained. This approach exploits induced RNA-mediated resistance in transgenic host plants that express the viral nucleoprotein (N) gene. Still, the potential application of this strategy is limited, since resistance is effective only against the virus from which the transgene originates. Two new lines of research were initiated to engineer more widely applicable resistance genes. The first approach is based on the M RNA-encoded putative viral movement protein (NS_M). Both protein- and RNA-mediated resistance strategies were investigated. The second line of work is directed to transgenic expression of defective interfering (DI) L RNA molecules. Expression of truncated L RNAs in host plants would potentially decrease the rate of viral replication and result in an increase in tolerance to tospoviruses. Eventually, a combination of successful approaches can be

applied to maximize the probability of maintaining the tolerance to the virus. The current state of knowledge in the development of resistance based on the transgenic expression of NS_M gene and L RNA-derived DI sequences was presented. *(P)*

An Alternative to the Threshold Model of Gene Silencing and Virus Resistance in Transgenic Plants

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It is now well established that virus resistance in transgenic plants may act at the RNA level and does not necessarily require a protein encoded by the transgene. This mechanism is related to the process of post-transcriptional gene silencing in transgenic plants and is dependent on homology of the virus and the transgene at the nucleotide sequence level. It has been suggested that the homology-dependent resistance mechanism is activated when accumulation of sense RNA from the transgene accumulates above a threshold limit (Lindboo *et al.*, 1993). However, data from several transgenic lines are inconsistent with this model. A more tenable alternative hypothesis invokes an aberrant RNA as the crucial factor of homology-dependent resistance and post-transcriptional gene silencing (English *et al.*, 1996). In many instances the production of aberrant RNA may be stimulated by methylation of the resistance/silencing transgene in the transcribed region. The correlation of homology-dependent resistance and post-transcriptional gene silencing with increased transgene dosage can be explained as an indirect consequence of methylation changes. Lines with multiple transgenes are more likely to confer virus resistance and post-transcriptional silencing because ectopic pairing of homologous DNA leads to methylation of the repeated sequences. *(L)*

2'–5' Oligoadenylate-Binding Protein(s) in Higher Plants

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A pathogen-derived resistance approach for the construction of virus-tolerant transgenic plants has enabled us to obtain plants protected from one specific plant virus or in some cases from a group of closely related viruses. The aim of creating plants displaying a broad-spectrum virus resistance remains to be achieved. We have demonstrated that plants expressing the mammalian 2'–5' oligoadenylate (2-5A) synthetase are protected against plant virus infections in the greenhouse as well as under field conditions. In order to understand better the mechanisms responsible for this protection, we analyzed the influence of 2-5A on plant cell-free translation and have shown that, similarly to the situation in mammals, 2-5A are translation-inhibiting agents in plant systems *in vitro*. This is due to their ability to induce rapid degradation of mRNA. We have also analyzed the presence of 2-5A-binding protein(s) in plants using chemical cross-linking, ligand blotting and immunoaffinity techniques (using antibodies against mammalian 2-5A-dependent ribonuclease). We have shown that the periodate oxidation method is the most effective for detecting plant 2-5A-binding protein(s). Our current aim is to clone and characterize plant cDNA encoding 2-5A-binding protein(s), which might act as novel plant resistance gene(s) against RNA virus infections. In order to achieve this we are: (i) screening tomato cDNA libraries with rat RNase L probe; (ii) screening tomato expression libraries with ^{32}P-labeled 2-5A; and (iii) purifying potato 2-5A-binding protein(s) on 2-5A-agarose affinity chromatography columns in order to determine the N-terminal amino acid sequence of the protein(s). The current state of art of the work was discussed. *(P)*

XV: VIRUS–HOST PROTEIN INTERACTIONS

Evidence for Binding of the Small Putative Protein Encoded in the Area Overlapping Transport Protein and Coat Protein Genes of Tobacco Mosaic Virus (TMV) with the Elongation Factor EF-1α

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We have identified a new gene coding for a putative small, positively charged protein (p4) in TMV (U1, U2 and L strains) genomes. In plant and animal protein-synthesizing systems this protein forms a very stable specific complex (54 kDa) with a protein of 50 kDa. The p4 protein purified from cell-free translational system efficiently binds to the crude preparations of eukaryotic translation factors. Among fractionated translation factors p4 specifically interacts with elongation factor EF-1α. Polyclonal antiserum against p4-DHFR fusion protein and plant factor EF-1α were prepared. Further characterization of the 54 kDa complex was presented and the putative role of p4 in the TMV infection cycle was discussed. *(P)*

Two-Hybrid System Screen for Interacting Plant and Potyviral Proteins

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We used the LexA two-hybrid system to identify proteins interacting with the VPg-proteinase and capsid protein of turnip mosaic potyvirus. The viral proteins were fused to the DNA-binding domain of LexA; immunodetection and repression assays indicated that the proteins were expressed in yeast and transported into the nucleus. Yeast cells harboring the above fusions were transformed with an *Arabidopsis thaliana* cDNA library; this library was fused to the B42 acid patch trx activation domain encoding gene. Interacting pairs were selected on media lacking leucine. Several clones showing galactose-dependence growth on selective plates and activating the *LacZ* reporter gene were isolated. Control experiments showed that the isolated cDNA clones were specific for the viral proteins. *(P)*

XVI: SATELLITE VIRUSES

Structural Analysis of *Cucumovirus* Satellite RNAs *in Planta*, in Virions and *in Vitro*

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The *Cucumovirus* genus includes cucumber mosaic virus (CMV), peanut stunt virus (PSV) and tomato aspermy virus (TAV). Satellite RNA (sat RNA) molecules have been found in co-infection with CMV and PSV strains. These small RNAs (335–393 nt) are able to alter the accumulation of helper virus genomic RNAs and the symtomatology. No functional open-reading frames have been detected, and sequence modification experiments suggest secondary interactions may be essential for the biological activity of sat RNAs. Base-paired regions of the sat RNA molecules *in planta* were analyzed. A correlation was

found between the hypervariable regions of CMV D-sat RNA, and bases not involved in Watson-Crick interactions. Also, regions highly conserved appeared to be less susceptible to chemical modification, suggesting interactions with other RNA or protein molecules Comparison of PSV P6-sat RNA molecules chemically modified *in planta* and in purified virions showed different patterns of accessibility to DMS. Several adenosine and cytidine residues located near the 3' end of the molecule appeared to be reacting with RNAs or proteins inside the virus particles *(L)*

In Vivo Mutation and Selection of a Pathogenic Satellite RNA of Cucumber Mosaic Virus (CMV) by Passage in Tobacco

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The B5-satellite RNA (B5-sat) of CMV ameliorates CMV-induced symptoms on tobacco Passage of clonally derived B5-sat RNA in tobacco with either the LS- or Fny-strains of CMV led to mutation of the B5-sat RNA at position 149, and selection of the B5*-sat RNA variant. B5*-sat RNA induces a severe yellow chlorosis in tobacco. In different passage trials, the B5*-sat RNA was selected for at different rates. When CMV containing B5-sat RNA or B5*-sat RNA was co-inoculated in a 100:1 ratio, respectively, the ratio of the two satellite RNAs in viral progeny was reversed within three passages. These data show that pathogenic satellite RNAs can be generated from non-pathogenic satellite RNAs, demonstrating a biological risk associated with the use of satellite RNAs for the biocontrol of CMV. *(P)*

Conformational Analysis of Satellite RNAs of Cucumber Mosaic Virus (CMV) by Thermal-Gradient Gel Electrophoresis (TGGE)

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TGGE was used to analyze conformational structures in satellite RNAs of CMV and chimeric satellite constructs generated from cDNA clones The data showed that conformational structures are influenced by separate parts of the satellite RNA molecule Thus, multiple co-existing structures present at ambient temperatures occur in WL47-sat RNA and chimeras containing the 5' half of WL47-sat RNA, whereas the D4-sat RNA and chimeras that contain the 5' half of the D4-sat RNA do not show the presence of co-existing conformers at ambient temperatures. However, satellites containing D4-sat RNA nucleotides 191-279 show bifurcations during thermal denaturation The number of species in multiple transitions is influenced by the nature of the flanking sequences These data indicate that satellite RNAs of CMV may have multiple possible secondary and tertiary structures. *(P)*

Structural Conservation is Essential for the Replication of a Sobemovirus Satellite RNA

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The Canadian strain of lucerne transient streak sobemovirus (C-LTSV) has an associated 322-nucleotide (nt) circular satellite RNA (sat-RNA) Up to 70% of nt in this sat-RNA is internally complementary, giving this RNA a mostly double-stranded rod-like structure under nondenaturing conditions. Similar structures are predicted for other small circular RNA plant pathogens (viroids and sat-RNAs) as well as the hepatitis delta agent. The importance of maintaining this overall structure *via* appropriate base-pairing has been reported for viroids, and is presumed to be the case also for other circular sat-RNAs, with analogous replicative functions likely involved in all these RNAs To investigate the requirement of rod conservation for the replication of a circular sat-RNA, we have constructed a series of C-LTSV sat-RNA mutants that produce a range of structural disruptions. Infectivity tests indicated that mutations causing significant disruptions (*i.e*, over 3 nt inserted/deleted) were lethal, whereas mutations having little effect on the overall rod (1–2 nt inserted/deleted at several positions) were not. A large (9 nt) palindromic insertion that preserved the rod was also not lethal This work indicates that rod-like structural conservation is essential not only to viroids but also to circular sat-RNAs associated with plant viruses. *(L)*

Replication of RPV Barley Yellow Dwarf Virus Satellite (satRPV) RNA in Monocot and Dicot Protoplasts and Plants: Its Effect on Symptoms and Accumulation of Two Helper Viruses

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SatRPV RNA depends on RPV barley yellow dwarf luteovirus for replication and encapsidation. It replicates *via* a rolling circle with hammerhead self-cleavage structures. The (+) strand hammerhead exists in an equilibrium with a slowly cleaving pseudoknot To determine roles of these alternative structures, we constructed mutants that altered this equilibrium. Mutations that favored the hammerhead increased self-cleavage, but decreased replication in oat protoplasts Mutations that restored the pseudoknot also restored replication. Oat plants infected with RPV and satRPV RNA had milder symptoms and accumulated less RPV RNA than those infected with RPV alone. However, satRPV RNA did not affect severe symptoms caused by the mixture of RPV and PAV. Beet western yellows luteovirus (BWYV) supported replication of satRPV RNA in *Nicotiana tabacum* protoplasts. BWYV and RPV encapsidated different conformations of satRPV RNA SatRPV RNA was transmitted to *Capsella bursa-pastoris* plants only in the presence of BWYV-associated ST9a RNA ST9a RNA does not support satRPV RNA replication but increases accumulation of BWYV and ST9a RNA symptoms. These results show a remarkably wide host and helper virus range for a satellite RNA. *(L)*

Determinants of Satellite RNAs Affecting the Genomic RNA Replication and Symptom Expression of Bamboo Mosaic Virus (BaMV)

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BaMV satellite RNA (satBaMV) is a RNA molecule with 836 nts and encodes a 20 kDa protein. Two full-length infectious cDNA clones, pBSF4 and pBSL6, 7% difference in nucleotide sequence, exhibit distinct phenotypes in the interference with BaMV RNA replication, modification of symptom expression caused by BaMV RNA, and the ability for systemic movement in *Nicotiana benthamiana* Six chimeric satRNAs were constructed by *in vitro* recombination of full-length cDNA clones between

the 5'-end untranslated region, open-reading frame and 3'-end untranslated region of pBSF4 and pBSL6. Northern blot analysis of total RNAs from infected barley protoplasts and *N. benthamiana* showed that the determinants involved in genomic RNA replication, symptom expression and systemic movement reside in the 5'-end untranslated region of pBSL6. *(L)*

Genome Organization and Function of Beet Necrotic Yellow Vein Virus (BNYVV) RNA-5

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The genome of BNYVV consists generally of four RNA species: RNA-1 and RNA-2 are required for viral RNA replication, assembly and virus movement, whereas RNA-3 and RNA-4 are needed for disease development and efficient infection of sugar-beet roots under field conditions. However, some Japanese isolates contain RNA-5 which is present together with RNA-3 and RNA-4. The sequence of RNA-5 was 1347 nucleotides in length and contained a single open-reading frame encoding a polypeptide of 26 kDa. No sequence identity was found between RNA-5 and either RNA-3 or RNA-4, except for the 5'-terminal nine residues and for about the 3'-terminal 200 residues. RNA-5 induced more severe symptoms in sugar-beet roots than did isolates lacking RNA-5. The differences in yield loss were paralleled with varietal differences in the susceptibility. However, laboratory isolates containing RNA-5 but lacking RNA-3 caused much more severe damage to sugar-beet roots of partially resistant varieties than to those of susceptible varieties. Thus, BNYVV RNA-5 is involved in disease development of sugar-beet roots, which differs from symptom expression responsible for RNA-3. It seems likely that RNA-5 may play an important role in the natural infection process, although it behaves as a satellite-like RNA. *(L)*

Functional Domains in the Satellite RNA of Groundnut Rosette Virus (GRV)

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Isolates of GRV support satellite RNAs of 895 to 903 nucleotides (nts), which contain several short open-reading frames (ORFs). We have constructed cDNA clones of several satellite variants, from which biologically active transcripts can be obtained. Deletion of all the ORFs or alteration of each AUG initiation codon did not impair replication of the satellites in *Nicotiana benthamiana*. Thus, no satellite-coded proteins are required for satellite replication. However, deletion of nts 47-281 prevented satellite replication. Although most satellite variants do not alter the symptoms of GRV infection in *N. benthamiana*, satellite YB3 induces brilliant yellow symptoms. Deletion of either nts 282-474 or nts 629-797 abolished the symptoms, but they were produced in plants inoculated with a mixture of the two mutants. Thus, symptom production in *N. benthamiana* involves two satellite domains, which can complement each other. A chimeric satellite, consisting of nts 282-474 from YB3 and nts 1-281 and 475-903 from the symptomless variant NM3, produced symptoms typical of YB3, but the converse construct did not. Satellite NM3 decreases the replication of GRV genomic RNA, and this property is determined by nts 47-281. *(L)*

A Viroid-Like RNA from Cherry with Hammerhead Ribozymes in Its Strands of Both Polarities

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A small circular RNA isolated in Italy from cherry trees affected by an unknown transmissible disease was characterized. Sequencing showed that it is a new viroid-like RNA of 451 nt which adopts a branched conformation of lowest free energy and that also contains in both polarity strands the conserved sequence and structural elements characteristic of hammerhead structures. Plus and minus transcripts of the 451-nt RNA self-cleaved *in vitro* during transcription and after purification as predicted by these hammerhead structures. Although the viroid or satellite nature of this small RNA remains to be convincingly established by bioassays in cherry, two lines of evidence favor the second alternative: (i) the 451-nt RNA is accompanied in the symptomatic tissue by a series of ds-RNAs probably corresponding to the genomic or replicative forms of an RNA virus; and (ii) the size and sequence of the cherry small circular RNA, and the characteristics of its hammerhead structures, resemble more some viroid-like RNAs than viroids. Northern blot hybridizations revealed that both polarity strands of the 451-nt RNA accumulate *in vivo* to similar levels, probably forming a complex. *(L)*

XVII· GENE EXPRESSION IN DNA VIRUSES

Downstream Enhancer Elements in Plant Pararetroviruses

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Two classes of plant pararetroviruses are known, the icosahedral caulimoviruses and the bacilliform badnaviruses. More is known about caulimoviruses, but interest in badnaviruses is growing, since several of its members cause severe crop diseases in subtropical and tropical countries. We are studying one member of each class, cauliflower mosaic virus (CaMV) and rice tungro bacilliform virus (RTBV). The active TATA box of the RTBV promoter was defined and it was shown that this promoter does not contain strong enhancer elements in the protoplasts tested. In fact, considerable promoter activity is still observed even with only 30 bp of upstream sequence. In contrast, sequences located within the first 90 bp of the RTBV leader sequence (downstream element, DE) were shown to have a dramatic effect on expression. Analysis of sequences downstream of the transcription start site that control expression is complicated by the multitude of levels at which such sequences might exert their effect. In fact, pararetrovirus leader sequences are involved in translation, splicing, polyadenylation and reverse transcription. In our case, the element in question acts in a promoter-specific manner, suggesting that post-transcriptional processes are not involved. The DE of RTBV consists of at least two elements, which have to act together to exert a full effect on the RTBV promoter, but which can act independently on a truncated CaMV promoter. One of these elements is a position- and orientation-independent DNA element recognized by a nuclear protein. The other element is position-dependent and contains a protein binding site in its RNA form.

Encouraged by these data we also searched for a DE in the already extensively characterized CaMV 35S promoter and identified a candidate DE in the first 60 bp of the CaMV leader sequence. Whereas in the protoplast systems tested the CaMV DE is masked by the strong upstream enhancer sequences, its effect becomes readily visible when some of these upstream enhancer sequences are deleted. *(L)*

Development of a Simple Baculovirus Multigene Expression System

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The flexibility of the insect baculovirus expression system has allowed the system to be used for the simultaneous expression of multiple foreign genes in a single cell. We have previously reported vectors that can synthesize 2 to 5 proteins simultaneously. However, construction of such recombination transfer vectors and generation of the recombinant viruses synthesizing multigene proteins are time-consuming and laborious, as many steps are needed to achieve the final products. To shorten the procedure and to simplify the system, we have modified both the baculovirus DNA as well as the multigene plasmid vectors. We have generated several baculoviruses, containing four different marker genes (β-galactosidase, alkaline phosphatase, glucuronidase and fluorescent green protein) in three discrete loci of viral DNA, which have facilitated the isolation of recombinants significantly. In each case a unique restriction site was inserted within the marker sequence, so that upon co-transfection of linearized viral DNA and plasmid, only the recombinant viruses would yield plaques that are screened by specific substrate. Moreover, for better efficiency a number of non-essential baculovirus genes (*e.g.* p10, p26, p74, EGT, DA26) were deleted. These modified viral DNAs have allowed us to generate recombinants either by co-transfection with PCR products or by ligation of foreign genes with baculovirus DNA. For expression of multigenes, we have designed the vectors such that some of the steps for obtaining recombinant viruses are avoided. Two vectors were constructed, each of which consisted of only one baculovirus sequence, late promoters, restriction sites for insertion of foreign genes and additional unique restriction site *NotI*. After cloning the foreign genes into each vector, the vectors were digested with *NotI*, ligated together and the mixture used directly for co-transfection. Viruses expressing up to seven foreign genes were generated using the new virus-vector system. *(L)*