The Plant Journal (2005) **42**, 392–405 doi: 10.1111/j.1365-313X.2005.02381.x

The *pvr1* locus in *Capsicum* encodes a translation initiation factor eIF4E that interacts with *Tobacco etch virus* VPg

Byoung-Cheorl Kang[1,†], Inhwa Yeam[1,†], James D. Frantz[1,†], John F. Murphy[2] and Molly M. Jahn[1,*]
[1]*Department of Plant Breeding and Genetics, Cornell University, Ithaca, NY 14853, USA, and*
[2]*Department of Entomology and Plant Pathology, Auburn University, Auburn, AL 36849-5409, USA*

Received 3 December 2004; revised 15 January 2005; accepted 28 January 2005.
[*]For correspondence (fax 607 255 6683; e-mail mmj9@cornell.edu).
[†]These authors contributed equally to this study.

Summary

Mutations in the eIF4E homolog encoded at the *pvr1* locus in *Capsicum* result in broad-spectrum potyvirus resistance attributed to the *pvr1* resistance allele, a gene widely deployed in agriculture for more than 50 years. We show that two other resistance genes, previously known to be *eIF4E* with narrower resistance spectra, *pvr2*[1] and *pvr2*[2], are alleles at the *pvr1* locus. Based on these data and current nomenclature guidelines, we have re-designated these alleles, *pvr1*[1] and *pvr1*[2], respectively. Point mutations in *pvr1*, *pvr1*[1], and *pvr1*[2] grouped to similar regions of *eIF4E* and were predicted by protein homology models to cause conformational shifts in the encoded proteins. The avirulence determinant in this potyvirus system has previously been identified as VPg, therefore yeast two-hybrid and GST pull-down assays were carried out with proteins encoded by the *pvr1* alleles and VPg from two different strains of *Tobacco etch virus* (TEV) that differentially infected *Capsicum* lines carrying these genes. While the protein encoded by the susceptible allele *pvr1*[+] interacted strongly, proteins translated from all three resistance alleles (*pvr1*, *pvr1*[1], and *pvr1*[2]) failed to bind VPg from either strain of TEV. This failure to bind correlated with resistance or reduced susceptibility, suggesting that interruption of the interaction between VPg and this eIF4E paralog may be necessary, but is not sufficient for potyvirus resistance *in vivo*. Among the three resistance alleles, only the *pvr1* gene product failed to bind m[7]-GTP cap-analog columns, suggesting that disrupted cap binding is not required for potyvirus resistance.

Keywords: recessive virus resistance, Solanaceae, potyvirus, pepper, pepper mottle virus, *Potato virus* Y.

Introduction

Potyviruses comprise approximately 30% of all known plant viruses and, as a group, are very destructive in agriculture (Ward and Shukla, 1991). The family Potyviridae is characterized by a monopartite single-stranded positive-sense RNA genome with a covalently bound viral-encoded protein (VPg) attached at the 5′ terminus and a 3′ poly-A tract (Riechmann *et al.*, 1992). The genome is approximately 10 kb in length and is translated as a polyprotein that is subsequently cleaved into smaller polypeptides by viral-encoded proteases. Based on similarities in genome structure, including conserved gene order and function among homologous replication proteins, potyviruses have been assigned to the proposed picorna-like superfamily of viruses, which includes many important human and animal pathogens, such as poliovirus and foot-and-mouth disease virus (Goldbach *et al.*, 1990; Riechmann *et al.*, 1992).

Potyvirus infection requires the interaction of host factors with viral proteins and RNA for replication and systemic spread (Carrington *et al.*, 1996). Although much is known regarding the functions of the individual potyvirus proteins and RNA structures in viral replication and movement (Revers *et al.*, 1999; Riechmann *et al.*, 1992; Urcuqui-Inchima *et al.*, 2001), less is known about the identity and function of host factors that are required for potyviral infection in plants. The ‘negative model’ of plant virus resistance predicts that a recessive resistance gene may represent a deleted or defective host protein that is essential for viral infection but is dispensable for the host (Fraser, 1992). Recessive resistance is especially prevalent for potyviruses, comprising approximately 40% of all known resistance genes (Provvidenti and Hampton, 1992). Many of these genes, including the *Capsicum*

resistance gene *pvr1*, the focus of this study, have been used successfully for decades in crop breeding programs as effective and stable sources of resistance (Greenleaf, 1986).

Several reports indicate that eukaryotic translation initiation factors, eIF(iso)4E and eIF4E, play critical roles in potyviral infection. In plants, the mRNA cap-binding protein, eIF(iso)4E and the scaffolding protein eIF(iso)4G interact to form the core of the eIF(iso)4F complex, which supports translation initiation predominantly from mRNAs with unstructured 5′ leaders (Gallie and Browning, 2001). In contrast, eIF4E and eIF4G comprise the eIF4F complex, which supports translation initiation of mRNAs with more complex 5′ structures and may promote translation under cellular conditions that inhibit cap-dependent translation such as viral infection (Gallie, 2001). In Arabidopsis, eIF(iso)4E and eIF4E show 52% amino acid sequence identity and show different patterns of expression (Rodriguez *et al.*, 1998), although both are strongly expressed in young tissues that support high levels of potyviral replication. Map-based cloning in Arabidopsis revealed that induced nonsense mutations in eIF(iso)4E resulted in broad-spectrum potyvirus resistance to *Tobacco etch virus* (TEV) and *Turnip mosaic virus* (TuMV) (Lellis *et al.*, 2002). Similarly, a transposon-disrupted eIF(iso)4E mutant in Arabidopsis conferred broad-spectrum resistance to TuMV and *Lettuce mosaic virus* (LMV) (Duprat *et al.*, 2002). In *Capsicum* and lettuce, naturally occurring mutations in eIF4E are responsible for strain-specific *Potato virus* Y (PVY) resistance conferred by the resistance genes, $pvr2^1$ and $pvr2^2$ (Ruffel *et al.*, 2002) and for resistance to LMV conferred by *mo1* (Nicaise *et al.*, 2003). More recently, another potyvirus recessive resistance gene, *sbm1*, in pea was also identified as *eIF4E* (Gao *et al.*, 2004), and *Hv-eIF4E* has been proposed as a candidate for the bymovirus resistance locus, *rym4/5*, in barley (Graner *et al.*, 1999; Pellio *et al.*, 2005; Wicker *et al.*, 2005).

The recessive resistance gene, *pvr1*, from *Capsicum chinense* confers broad-spectrum resistance to all known strains of PVY, *Pepper mottle virus* (PepMoV), and most known TEV strains (Kyle and Palloix, 1997). Originally described as alleles of the *et* locus for resistance to TEV (Cook, 1961), *pvr1* and $pvr2^2$, which also control a few TEV isolates, were subsequently assigned to distinct loci (Greenleaf, 1986). These genes have a common mechanism of resistance (blocked replication) to the highly aphid transmissible (HAT) strain of TEV (Deom *et al.*, 1997; Murphy *et al.*, 1998). In addition to a shared mechanism of resistance to at least one viral isolate, comparative mapping data place both genes near a common RFLP marker TG135, which in tomato is closely linked to *pot-1*, a recessive resistance gene in tomato for TEV and PVY resistance (Murphy *et al.*, 1998; Parella *et al.*, 2002). Another allele at the *pvr2* locus, $pvr2^1$, provides narrow-spectrum resistance to only pathotype 0 of PVY, apparently by blocking cell-to-cell movement (Arroyo *et al.*, 1996).

The central region of the potyviral protein, VPg, is crucial in race-specific replication, cell-to-cell and long-distance movement in relation to several recessive potyvirus resistance genes in diverse host species (Keller *et al.*, 1998; Masuta *et al.*, 1999; Moury *et al.*, 2004; Nicolas *et al.*, 1997a; Rajamaki and Valkonen, 2002; Schaad *et al.*, 1997). This protein is translated as a polyprotein known as NIa or VPg-Pro, composed of N-terminal VPg and C-terminal protease domains and participates in replicative and proteolytic functions during potyvirus infection (Revers *et al.*, 1999). Strong interaction was observed in yeast two-hybrid assays between TEV NIa and eIF4E isolated from tomato and tobacco (Schaad *et al.*, 2000). Interactions were also observed between *Arabidopsis thaliana* eIF4E or eIF(iso)4E and TuMV VPg-Pro both in yeast two-hybrid and enzyme-linked immunosorbent assay (ELISA)-based *in vitro* binding assays (Wittmann *et al.*, 1997). Furthermore, the interaction of Arabidopsis eIF(iso)4E and TuMV VPg-Pro was correlated with viral infectivity (Leonard *et al.*, 2000).

These studies provide the foundation for the hypothesis that a physical interaction between eIF4E and/or eIF(iso)4E and viral VPg is necessary for viral infection. The objective of the present study was to test this hypothesis in the *Capsicum* system. First, it was necessary to critically assess the relationship between strain-specific potyvirus resistance conferred by the *pvr2* resistance genes known to affect an *eIF4E* locus in pepper and broad-spectrum resistance conferred by the *pvr1* gene. Upon confirmation of allelism, a systematic study of the physical interaction between *eIF4E* alleles and viral strains either controlled or not controlled by respective resistance alleles was performed, together with careful studies of viral infectivity in various host genotypes. Our results shed light on the similarities and differences between TEV resistance conferred by various *eIF4E* alleles at the molecular and whole plant levels.

Results

Cosegregation of eIF4E *with* pvr1

Two tomato *eIF4E* ORF primers based on a 696-bp tomato eIF4E coding sequence were used to amplify pepper *eIF4E* cDNA fragments via RT-PCR. These primers amplified a 687-bp fragment in *Capsicum* showing 87% nucleotide sequence identity to tomato and 62% nucleotide sequence identity to Arabidopsis *eIF4E* (EMBL accession Y10548). A 537-bp fragment was also amplified from pepper which showed 96 and 71% nucleotide identity to tomato and Arabidopsis *eIF4E*, respectively. The *eIF4E* fragment was used as a hybridization probe for DNA blot analysis, revealing three homologs in the pepper genome. One homolog showed

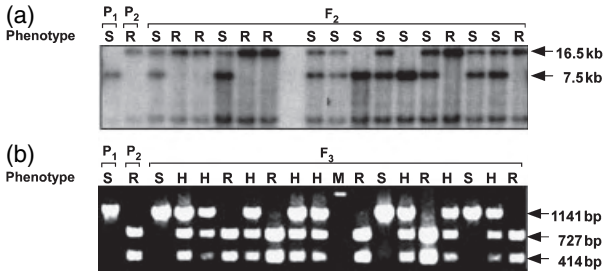


Figure 1. Cosegregation of *eIF4E* with *pvr1*.
(a) Polymorphism detected with eIF4E ORF probe co-segregated with susceptible (S) or resistant (R) phenotypes in an inter-specific F_2 population of 75 individuals screened with PepMoV. Susceptible parent *Capsicum annuum* RN (P_1) and resistant parent *C. chinense* 234 (P_2) are indicated.
(b) The *Ssp*I CAPS marker P56 co-segregated with response to TEV-HAT and PepMoV in 114 *C. chinense* F_3 families scored resistant (R), segregating (H), or uniformly susceptible (S) to virus infection in two independent screens. Susceptible parent HAB (P_1) and resistant parent 234 (P_2) are indicated. M, marker lane.

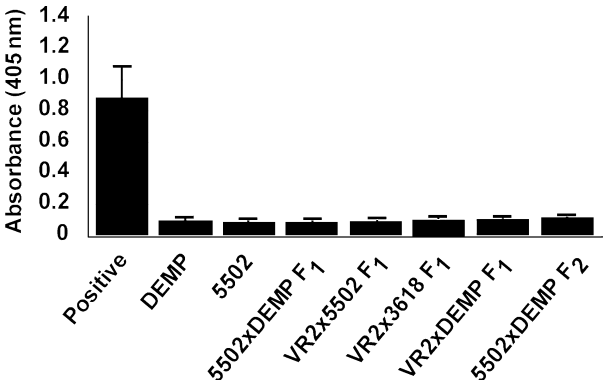


Figure 2. Genetic complementation analysis of *pvr1* and *pvr2*^{2}. For genetic complementation test, crosses were made between *Capsicum annuum* 5502 or 3618 (both *pvr1/pvr1*) with DEMP or VR2 (both *pvr2*^{2}/*pvr2*^{2}). Parents and F_1 progeny were inoculated with TEV-HAT and assayed for presence of viral coat protein by indirect ELISA at 21 dpi. The parents and F_1 populations showed lower ELISA absorbance than the threshold indicating that they are all resistant. These results show that *pvr1* and *pvr2*^{2} fail to show genetic complementation for resistance to TEV-HAT. The susceptible check was *C. annuum* JP (*pvr1*$^+$/*pvr1*$^+$). The 5502 × DEMP F_2 population was also resistant to TEV-HAT. Error bars indicate standard error.

polymorphism between two pepper genotypes, which was in marked contrast to potyvirus resistance controlled by the resistance gene, *pvr1*. *Capsicum annuum* RN (*pvr1*$^+$/*pvr1*$^+$) is susceptible to all potyviruses used in this study; *C. chinense* PI159234 (*pvr1/pvr1*) is resistant to PVY, PepMoV and TEV (Murphy *et al.*, 1998). When we mapped the polymorphic copy of *eIF4E* relative to potyvirus resistance in the inter-specific AC mapping population derived from a cross between these two parents (Figure 1a) (Murphy *et al.*, 1998), one *eIF4E* polymorphism cosegregated precisely with *pvr1*. This result was confirmed in a second intra-specific population derived from a cross between resistant and susceptible *C. chinense* genotypes, *C. chinense* Habanero (fully susceptible) × PI 159234. An *Ssp*I CAPS marker based in the 5′ upstream region of the gene was used to genotype 114 *C. chinense* F_3 families that had been inoculated with TEV, again revealing precise co-segregation (Figure 1b). The second *eIF4E* homolog corresponding to the 537 bp fragment did not map near known resistance genes and lacked any characteristic polymorphism between resistant and susceptible genotypes.

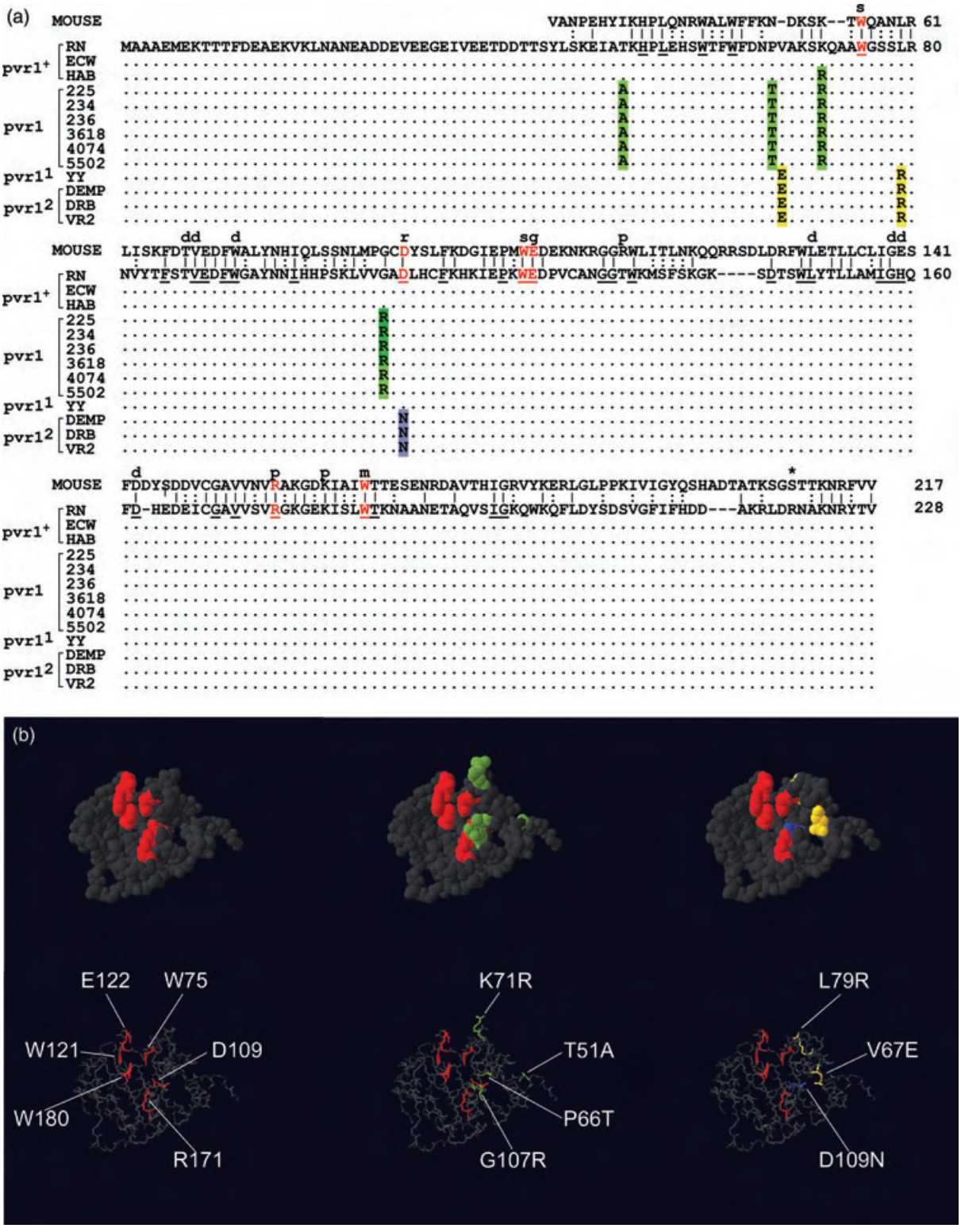
Similar experiments were undertaken to clone pepper *eIF(iso)4E*. Primers based on a 603-bp tomato *eIF(iso)4E* sequence were used to amplify pepper *eIF(iso)4E* cDNA sequence which was cloned and sequenced, revealing a 609-bp *eIF(iso)4E* homolog with 89% nucleotide sequence identity to tomato and 63% nucleotide sequence identity to Arabidopsis *eIF(iso)4E* (EMBL accession Y10547). DNA blot analysis of pepper *eIF(iso)4E* on genomic DNA of pepper showed at least two copies of *eIF(iso)4E* in the pepper genome. When *eIF(iso)4E* was mapped in the AC population, it was unlinked to *pvr1*, localizing instead to a chromosomal interval containing the recessive resistance gene *pvr6* (data not shown), previously reported to confer resistance to the potyvirus, *Pepper veinal mottle virus* (PVMV), when combined with *pvr2*^{2} (Caranta *et al.*, 1996).

Genetic complementation analysis of pvr1 *and* pvr2^2 *demonstrates allelism*

The formal genetic relationship of the resistance genes, *pvr1* and *pvr2*^{2}, has not been previously addressed. Both *pvr1* and *pvr2*^{2} confer resistance to TEV-HAT infection at the cellular level (Deom *et al.*, 1997; Murphy *et al.*, 1998), therefore this viral strain can be used to evaluate genetic complementation, also known as allelism, of these two resistance genes. Resistant × resistant and resistant × susceptible crosses were made between *C. annuum* genotypes known to carry *pvr1* (5502 or 3618) and *pvr2*^{2} (Dempsey and VR2). Evaluation of parental genotypes and F_1 progeny for response to inoculation with TEV-HAT confirmed that each of these genes demonstrates fully recessive inheritance for resistance to TEV-HAT (Figure 2). When the F_1 progeny from crosses between resistant parents carrying *pvr1* and *pvr2*^{2} were assayed for response to inoculation with TEV-HAT, uniform resistance was observed (Figure 2). These results unequivocally establish that *pvr1* and *pvr2*^{2} fail to complement genetically, and by definition map to the same genetic locus. Because the *pvr1* allele was described first (Greenleaf, 1956), the designation *pvr1* takes priority for this *eIF4E* locus on chromosome 3. Based upon our genetic complementation results and potyviral resistance gene nomenclature in *Capsicum* (Kyle and Palloix, 1997), we propose that the *pvr2*^{1} allele should be re-designated *pvr1*^{1}, while the *pvr2*^{2} allele should be redesignated *pvr1*^{2}. We will use this nomenclature throughout the paper.

Resistance alleles have distinct amino acid substitutions

Full-length coding sequences for *eIF4E* from 13 *Capsicum* genotypes known to be homozygous for *pvr1*$^+$, *pvr1*, *pvr1*^{1} or *pvr1*^{2} genotypes were cloned and sequenced. Deduced amino acid sequence alignment revealed that the two susceptible (*pvr1*$^+$/*pvr1*$^+$) genotypes, *C. annuum* RN and ECW, showed 100% identical amino acid sequence. Another



susceptible ($pvr1^+/pvr1^+$) genotype, *C. chinense* HAB, also showed sequence identical to the susceptible *C. annuum* genotypes, except for one amino acid substitution at position 71 (K to R substitution, K71R) (Figure 3a). This mutation appeared to be specific to *C. chinense* because all eIF4E amino acid sequences obtained from *C. chinense* showed the K71R substitution relative to the *C. annuum* sequence, regardless of viral resistance or susceptibility (Figure 3a). Three *C. chinense* accessions (225, 234, and 236) allelic for *pvr1* (Murphy *et al.*, 1998) and three *C. annuum* bell pepper breeding lines known, based on breeding pedigrees, to carry *pvr1* introgressed from *C. chinense* (3618, 4074, 5502) all carried identical sequence at the *pvr1* locus (Figure 3a). The *pvr1* allele contains three amino acid substitutions relative to susceptible *C. annuum* $pvr1^+$ genotypes: T55A, P66T, and G107R (Figure 3a).

Capsicum annuum bell pepper varieties 'Dempsey' and 'Florida VR2,' are known to be homozygous for $pvr1^2$ (Deom *et al.*, 1997; Greenleaf, 1986). Their identical sequence at the eIF4E locus is also consistent with a commonly introgressed resistance gene. The $pvr1^2$ allele carries three point mutations relative to $pvr1^+$, V67E, L79R, and D109N, all within the same region of the protein as observed in *pvr1*, but at different residues (Figure 3a). This suggests that *pvr1* and $pvr1^2$ arose after speciation via different point mutations in the two lineages. The amino acid substitution L79R was not previously reported in the VR2 eIF4E sequence (Ruffel *et al.*, 2002), however, our results confirmed this substitution is present in three independent germplasm sources. The eIF4E sequence for $pvr1^1$ revealed that this allele, which controls a narrower range of isolates than $pvr1^2$, shared two of the amino acid substitutions also observed in the $pvr1^2$ allele. The reported pedigrees do not identify a common ancestor for these two alleles (Cook, 1961).

Three point mutations in *pvr1* and all of the mutations in $pvr1^1$ and $pvr1^2$ result in non-conservative amino acid substitutions in the respective predicted proteins. To understand how mutations in resistance allele *eIF4E* affect protein structure, 3-D models of pepper eIF4E proteins were predicted based on known 3-D structures of mouse eIF4E (Figure 3b). The predicted pepper eIF4E protein structure was very similar to mouse, therefore we assessed the predicted conformational differences in the proteins as a result of the combination of point mutations observed in each allele. It is evident that in all three resistance alleles, substituted residues occurred in similar regions of the protein (Figure 3b). Substitutions in the proteins pvr1, $pvr1^1$, and $pvr1^2$, however, are generally not located at highly conserved residues involved in cap binding or binding of eIF4G, except in the case of $pvr1^2$ D109R mutation. In contrast to an earlier interpretation (Ruffel *et al.*, 2002), this mutation does affect a highly conserved residue involved in stabilization of cap binding. In general, however, the mutations do occur near residues known to be important in protein function, e.g., the G107R substitution in pvr1 occurs at two residues away from this position. Taken together, our results define four alleles at the *pvr1* locus in *Capsicum* that are precisely consistent with specific differences in resistance spectra and known breeding pedigrees.

Cap-binding activity of pepper eIF4E is not essential for TEV resistance phenotype

To determine the functional consequences of these point mutations at the *pvr1* locus, we undertook a systematic assessment of cap-binding activity to determine the effect, if any, on the cellular role of the mRNA cap-binding proteins encoded by $pvr1^+$, *pvr1*, $pvr1^1$, and $pvr1^2$. Recombinant proteins from each allele were expressed in *Escherichia coli* and assayed for binding-activity on m^7-GTP cap-analog columns. All of the pepper eIF4E proteins were stably expressed (Figure 4a). The eIF4E encoded by the susceptible host RN ($pvr1^+/pvr1^+$) showed strong cap-binding activity, as did recombinant proteins from $pvr1^1$ and $pvr1^2$ (Figure 4b) indicating that the D109R mutation specific to $pvr1^2$ did not abolish cap binding. In contrast, the eIF4E protein from *pvr1* failed to bind to the column, either as a result of the G107R mutation or some other disruption in the protein. Plants homozygous for *pvr1* are phenotypically normal in every respect, suggesting that if the cap-binding activity of

Figure 3. Alignment of amino acid sequences and predicted structures of pepper eIF4E proteins.
(a) Amino acid alignment of mouse eIF4E with eIF4E sequences from *Capsicum*. *Capsicum* sequences were aligned with the reference $pvr1^+$ sequence *C. annuum* RN using the Clustal algorithm of DNASTAR. The other genotypes and alleles are depicted on the left column. The pepper eIF4E sequences were translated from $pvr1^+$, *pvr1*, $pvr1^1$ and $pvr1^2$ and were designated $pvr1^+$, pvr1, $pvr1^1$, and $pvr1^2$, respectively. Amino acids of $pvr1^+$ identical to mouse are denoted by vertical bars; substitutions resulting in a similar amino acid are indicated by colons. Amino acids widely conserved across kingdoms (Marcotrigiano *et al.*, 1997) are underlined. Highly conserved cap-binding residues are colored red. Amino acid substitutions specific to pvr1 are colored green. Amino acid substitutions shared by $pvr1^1$ and $pvr1^2$ are in yellow. The substitution specific to $pvr1^2$ is colored blue. Upper and lower rulers denote mouse and pepper amino acid position, respectively. Functional classifications are: s, stacking tryptophan; d, conserved dorsal residue; g, hydrogen bonding to guanine; r, stabilizing Arg-157; p, interaction with phosphate groups of m^7GDP; m, van der Waals interaction with m^7GDP; *, site of phosphorylation. *Capsicum* amino acids identical to $pvr1^+$ are denoted by a period; substituted residues are listed by symbol.
(b) Comparison of eIF4E variants from potyvirus-susceptible and resistant *Capsicum* genotypes using models derived from the crystal structure of mouse eIF4E. Space-filling (upper) and chain (lower) models depict backbones of the proteins in gray with side chains shown only for colored residues. Highly conserved cap-binding residues are shown in red labeled with their position in the pepper protein. Left panel: homology model of $pvr1^+$ from $pvr1^+$ genotype *C. annuum* RN; center panel: homology model for pvr1 from *pvr1* genotypes with amino acid substitutions (green) relative to $pvr1^+$ labeled by position; right panel: a composite homology model for $pvr1^+$ showing substitutions shared by $pvr1^1$ and $pvr1^2$ (yellow) and the unique mutation in $pvr1^2$ (blue).

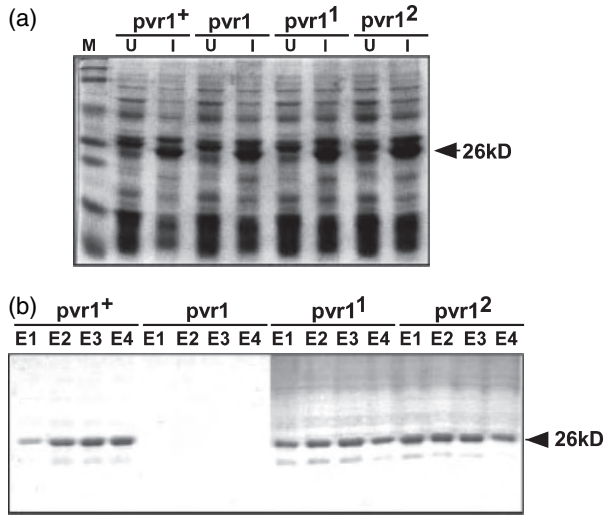


Figure 4. Cap-binding assay of recombinant eIF4E proteins encoded by the *pvr* alleles.

(a) Expression of eIF4E proteins in *Escherichia coli* (DE3). Coomassie blue-stained 12% SDS-polyacrylamide gel of whole cell lysates of *E. coli* before (U) and after (I) induction by the addition of 20 μM IPTG. Arrows indicate the position of recombinant protein at approximately 26 kDa. The recombinant protein was induced in all *E. coli* cells containing various eIF4E genes. M, molecular size marker; U, un-induced; I, induced.

(b) SDS-PAGE analysis of recombinant eIF4E proteins after m^7-GTP-Sepharose affinity chromatography. The soluble fraction of bacterial cell lysates containing recombinant eIF4E protein was applied to the m^7-GTP-Sepharose column followed by a wash step as described in Experimental procedures. After washing, the bound eIF4E protein was eluted from the column by m^7-GTP. The recombinant proteins pvr1$^+$, pvr1^1, and pvr1^2 were retained on the column upon washing; however, no binding to m^7-GTP-Sepharose was observed for pvr1. E1–E4, eluted fractions using m^7-GTP.

eIF4E is defective *in planta*, this activity is not essential for host viability or normal growth.

All three resistance alleles at pvr locus control two strains of TEV

pvr1 and *pvr1*^{2} in the homozygous condition block TEV-HAT replication at a single cell level (Deom *et al.*, 1997; Murphy *et al.*, 1998); however, the response of *pvr1*^{1}/*pvr1*^{1} plants to this TEV isolate has not been reported. To definitively establish TEV resistance phenotypes on plants homozygous for these four *pvr1* alleles, we undertook a systematic study to characterize infectivity of two TEV strains, TEV-HAT and TEV-NW, observed to differ with respect to virulence (Chu *et al.*, 1997).

After mechanical inoculation of the susceptible genotypes, JP and RN, symptoms induced by TEV-HAT and TEV-NW were first observed as mild vein-clearing on un-inoculated leaves between 5 and 7 days post-inoculation (dpi). During this time, no visually detectable symptoms were observed on 234, DEMP, or YY plants. As new leaves emerged on TEV-NW-infected JP and RN plants, obvious mosaic and puckering symptoms due to large dark green islands developed. TEV-HAT-infected JP and RN plants also expressed mosaic symptoms that were milder than those observed for TEV-NW, confirming a difference in symptom intensity on susceptible hosts between these two TEV isolates. When inoculated and un-inoculated tissue from JP and RN plants were tested for the presence of virus by ELISA, 100% of all plants were shown to be infected (Figure 5a,b).

In contrast to observations of fully susceptible hosts, emerging un-inoculated leaves on YY plants (*pvr1*^{1}/*pvr1*^{1}), positive for virus by ELISA developed only a very mild mosaic symptom that was not clearly distinct from mock-inoculated plants. Although YY plants did not express obvious symptoms, both TEV-HAT and TEV-NW infection eventually did occur in most plants. Again, in contrast to fully susceptible genotypes where we observed uniform infection, TEV-HAT was detected in the inoculated leaves in 4 of 10 YY plants and in un-inoculated leaves from six of nine plants at 10 dpi.

The average ELISA value for inoculated leaves representing all YY plants in the TEV-HAT treatment was below the threshold for a positive infection; this was due to the fact that only four plants contained detectable amounts of TEV-HAT in inoculated leaves with the ELISA values for the infected samples being fairly low (data not shown). More YY plants were infected with TEV-NW when compared with TEV-HAT; TEV-NW was detected in the inoculated leaf from 8 of 10 YY plants and in un-inoculated leaves from 9 of 10 plants. In each case, the average ELISA value was positive for the presence of TEV-NW (Figure 5a,b). In summary, this genotype does not display a response to TEV-HAT and TEV-NW that is entirely similar to the susceptible response when assessed via visual symptoms or ELISA; however, systemic infection of this genotype occurs with regular frequency, and infection of inoculated leaves was detected quite reliably with the more severe isolate, TEV-NW.

To shed further light on the relationship between the susceptible response and the response observed in YY plants, we performed a time course experiment shown in Figure 5(b). These results confirmed the observations reported in Figure 5(a), but revealed one clear difference between YY plants and the fully susceptible genotypes, JP and RN. At 3 dpi, YY plants show significantly less accumulation of virus in un-inoculated tissue than observed in JP or RN plants. Although YY plants eventually go on to accumulate similar levels of virus that are not statistically different from that of susceptible plants, a clear difference in virus accumulation is observed early in the course of infection.

None of the 234 (*pvr1*/*pvr1*) or DEMP (*pvr1*^{2}/*pvr1*^{2}) plants inoculated with either TEV-HAT or TEV-NW developed any apparent symptoms. TEV-HAT and TEV-NW were not detected in any tissue from 234 and DEMP plants.

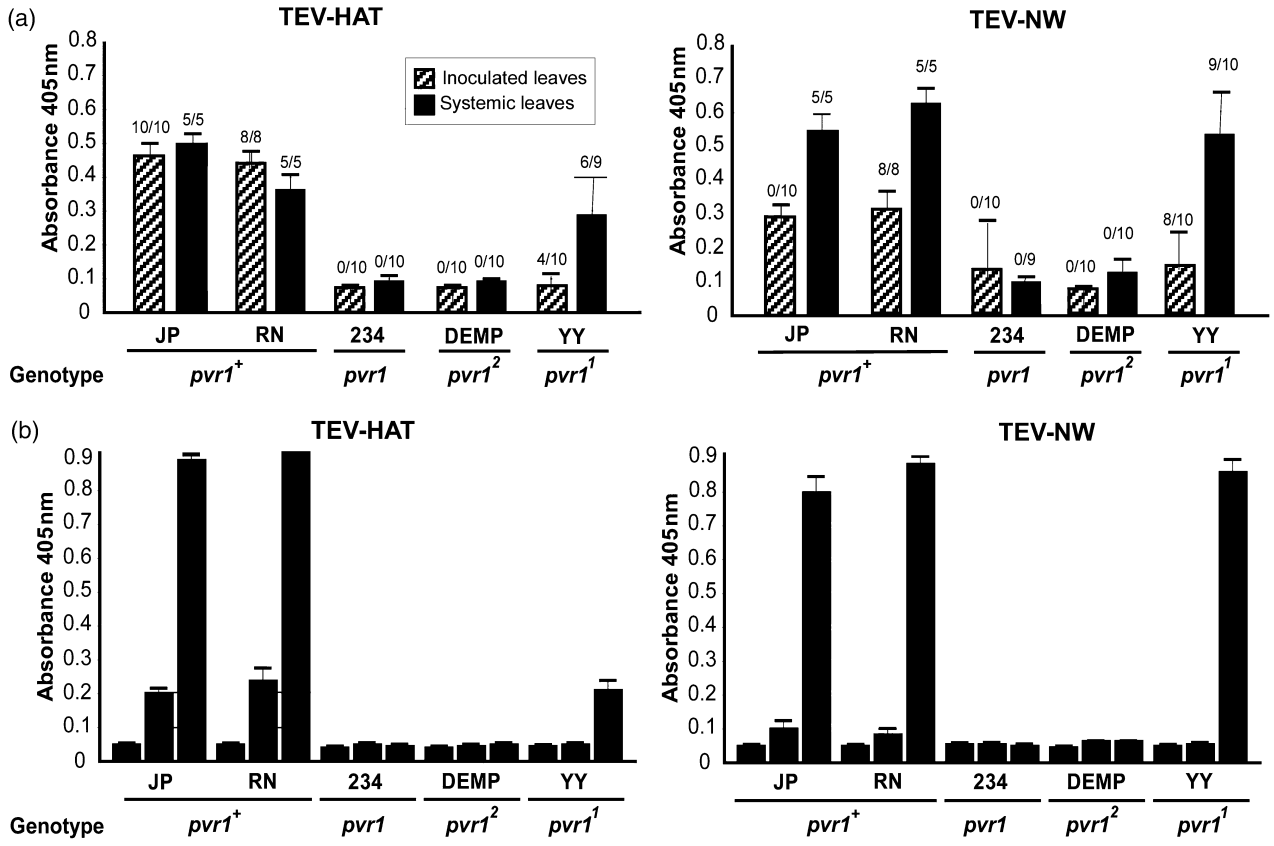


Figure 5. Infectivity test of pepper plants having different *pvr* alleles.
(a) Accumulation of TEV coat protein in inoculated and un-inoculated leaves of pepper plants. Representative responses were determined by indirect ELISA of various *pvr1* genotypes to TEV-HAT (left) or TEV-NW (right) inoculation. Virus accumulation in inoculated leaf 2 was tested at 10 dpi (hatch bar) while two un-inoculated leaves were tested at 21 dpi (closed bar). A sample was considered positive for virus when the ELISA absorbance value was greater than the threshold. Numbers above each bar represent susceptible plants over total number of plants tested.
(b) Time course showing virus accumulation in un-inoculated leaves after inoculation of various genotypes with TEV-HAT and TEV-NW. The bars for each genotype represent mean values of the following samples: the first bar, mock-inoculated healthy plants; the second, virus inoculated at 3 dpi; the third, virus inoculated at 6 dpi. Standard deviation is indicated on the top of bars. The total number of the plants used for ELISA analysis was six for each time and treatment.

In summary, TEV infectivity studies demonstrated that *pvr1* (234) and *pvr1*^{2} (DEMP) genotypes were completely resistant to both TEV-HAT and TEV-NW. The *pvr1*^{1} (YY) genotype developed considerably milder symptoms, and systemic infection was not consistently established in all plants, in contrast to positive control treatments. On average, however, TEV-HAT and TEV-NW eventually accumulated in systemically infected YY tissue to levels indistinguishable from the susceptible control. These results indicate that while YY would be considered generally susceptible to TEV-HAT and TEV-NW, this response is not exactly equivalent to the fully susceptible response.

VPg interaction with eIF4E aligns with TEV resistance phenotype

To test the hypothesis that it is the physical interaction or absence of interaction between eIF4E and the avirulence determinant in this system, VPg, that determines the outcome of infection at the organismal level, we undertook a systematic analysis of the physical interaction between proteins encoded by resistance alleles at *pvr1* and TEV VPg. If this hypothesis is correct, we predict that conformational changes in eIF4E proteins encoded by the potyvirus resistance alleles should abolish or impair binding of TEV-HAT or TEV-NW VPg. We therefore examined the interaction of VPg from the two TEV strains with pepper eIF4E proteins from resistant and susceptible genotypes used for virus infectivity test above. Five amino acid substitutions are evident in the VPg region of TEV-HAT and TEV-NW (Figure 6a). Two of these mutations at amino acid 127 and 129 occur near amino acids which are known to be specificity determinants in other potyvirus–host interactions (Ayme *et al.*, 2004; Rajamaki and Valkonen, 2002).

Figure 6(b) shows an immunoblot analysis of yeast cells harboring the four *eIF4E* fusion constructs and empty vector, confirming that these proteins were stably expressed in yeast at similar levels. Activity values from the interaction

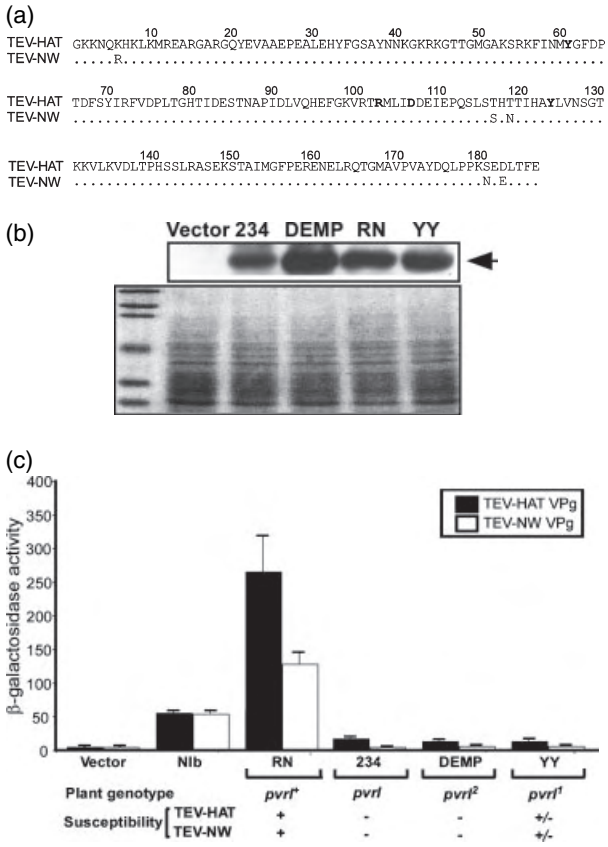


Figure 6. Protein–protein interaction assay using the yeast two-hybrid system.

(a) Amino acid sequence alignment of VPg region of different TEV virus strains. The amino acids conserved among VPg of different potyviruses are in bold. Amino acids identical to TEV-HAT VPg are denoted by a period; substituted residues are listed by symbol.

(b) Expression of wild type and mutant eIF4E proteins in the yeast two-hybrid system. Yeast cells containing empty vector (lane 1) or eIF4E fusion gene in pJG4-5 vector (lanes 2–5) were grown on selection medium lacking leucine and tryptophan. Proteins were extracted and fractionated on 10% SDS-polyacrylamide gels (lower panel), and immunoblotted with eIF4E antibody (upper panel). The eIF4E fusion proteins about 48 kDa was detected in all yeast cells containing various *eIF4E* genes as indicated by arrow but not in the cells containing empty vector (lane 1).

(c) β-Galactosidase assay of yeast two-hybrid interaction between TEV VPg proteins and eIF4E from RN (*pvr1*$^+$), 234 (*pvr1*), DEMP (*pvr1*^{2}) and YY (*pvr1*^{1}). Bait plasmid pEG202 was used to express the fusion protein TEV-HAT VPg (black bar), and TEV-NW VPg (open bar), while the prey plasmid pJG4-5 was used to express pepper eIF4E. The empty vector pJG4-5 served as a negative interaction control. Yeast containing known interactors pEG202: TEV-HAT NIa and pJG4-5: TEV-HAT NIb served as a positive interaction control. β-galactosidase activity was detected only in the susceptible eIF4E and TEV VPg-HAT or TEV VPg-HAT, but not in other resistant eIF4E and TEV VPg combinations.

between NIa and NIb were used as positive control and was consistent with those reported in previous studies using TEV NIa (Schaad *et al.*, 2000). The eIF4E proteins from TEV-susceptible genotypes RN interacted in *yeast* with VPg cloned from both viral isolates (Figure 6c). In contrast, fusion proteins pvr1, pvr1^1, and pvr1^2 translated from resistance alleles cloned from *Capsicum* genotypes 234 (*pvr1*), DEMP (*pvr1*^{2}), and YY (*pvr1*^{1}) failed to interact with VPg from either virus.

To confirm the yeast two-hybrid data, pvr1$^+$, pvr1, pvr1^1, and pvr1^2 were translated *in vitro* and incubated with glutathione *S*-transferase (GST) alone or GST-tagged VPg proteins (VPg-HAT and VPg-NW) immobilized on glutathione-agarose beads (Figure 7a). Upon washing the beads, no eIF4E proteins were retained on the glutathione beads with GST alone (Figure 7b–e, lane 1). As expected, pvr1$^+$ protein was retained by the beads in combination with VPg-HAT and VPg-NW (Figure 7b, lanes 2 and 3). None of the proteins (pvr1, pvr1^1, or pvr1^2) encoded by resistance alleles were retained in combination with either VPg-HAT or Vpg-NW (Figure 7c–e). These results confirmed that interactions were only observed between susceptible eIF4E and TEV-HAT and TEV-NW VPg. These data uniformly supported the prediction that the translational products of *pvr1*, *pvr1*^{1}, and *pvr1*^{2} alleles would show altered function with respect to VPg binding.

Discussion

These studies confirm that eIF4E is the host factor responsible for recessive potyvirus resistance attributed to *pvr1* and the alleles at this locus formerly known as *pvr2*^{1} and *pvr2*^{2}. Hybridization and sequence-based markers for the *eIF4E* gene co-segregated completely with the *pvr1* resistance phenotype in two independent populations. Genetic complementation data confirmed that all three alleles occur at the same locus, necessitating revision of the locus designation. Each resistance allele carried signature point mutations localized to similar regions of the gene that could be tracked through inter-specific introgression breeding programs. These data, together with published evidence that transient expression of wild-type eIF4E in *pvr1*^{1} and *pvr1*^{2} genotypes restored susceptibility to PVY (Ruffel *et al.*, 2002), clearly indicate that mutations in this gene can result in broad-spectrum potyvirus resistance, in addition to the strain-specific resistance previously known.

VPg is known to be the avirulence determinant for several potyvirus–host interactions, including PVY, PSbMV, TEV, and TuMV (Borgstrom and Johansen, 2001; Johansen *et al.*, 2001; Keller *et al.*, 1998; Masuta *et al.*, 1999; Moury *et al.*, 2004; Nicolas *et al.*, 1997b; Rajamaki and Valkonen, 1999; Schaad *et al.*, 1997). Furthermore, it has been proposed that the outcome of the interaction of eIF4E [or eIF(iso)4E] and VPg may determine viral infectivity, at least in part. In some cases, data from protein–protein interaction experiments align with infectivity (Leonard *et al.*, 2004; Schaad *et al.*, 2000; Wittmann *et al.*, 1997). In other studies, however, either the eIF4E/VPg interaction was not consistent with infectivity, or the interaction was not detected. For example, TEV-HAT VPg is the avirulence determinant for recessive

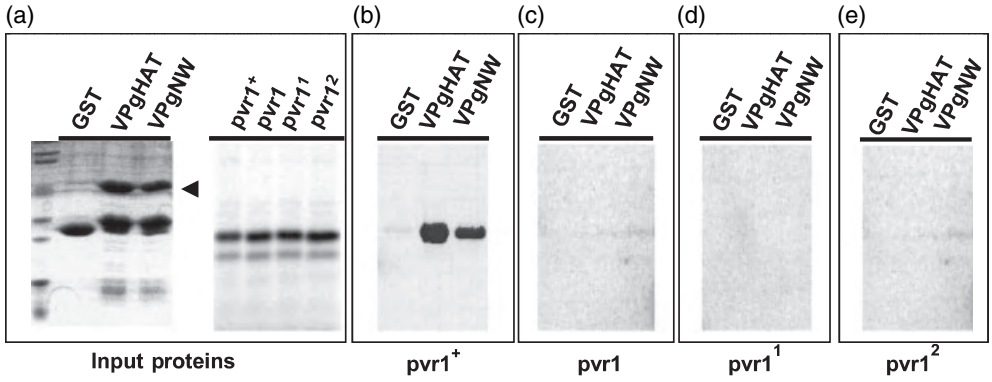


Figure 7. GST pull-down assay demonstrating eIF4E and VPg interaction *in vitro*. VPg pull-down assays were conducted with GST, GST-VPgHAT (VPg-HAT), and GST-VPgNW (VPg-NW) immobilized on glutathione-Sepharose 4B beads and ^{35}S-labeled *in vitro*-translated eIF4E alleles, pvr1^{+}, pvr1, pvr1^{1}, and pvr1^{2}. Panel (a) represents equivalent amounts of GST, VPgHAT, and VPgNW used in the pull-down assay and 20% of *in vitro*-translated eIF4Es. An arrow indicates GST fusion proteins. *In vitro*-translated eIF4E proteins were mixed with the immobilized GST-VPg proteins or GST alone. The mixture was incubated and washed as described in Experimental procedures. Pull-down eluates were separated by 12% SDS-PAGE and exposed to a phosphorimager screen. The eIF4E proteins not retained in the beads with GST alone (panel b lane 1 and panel c–e). The eIF4E protein from pvr1^{+} retained by both VPg-HAT and VPg-NW beads (panel b, lane 2 and 3), but the other three eIF4E proteins translated from *pvr1*, *pvr1*^{1}, and *pvr1*^{2} were not retained by either of the beads (panel c–e, lanes 2 and 3) confirming yeast two-hybrid results.

resistance to systemic movement in tobacco. The eIF4E protein from the susceptible genotype failed to interact with VPg of a virulent TEV strain, Oxnard, and no difference was observed between eIF4E sequences from resistant and susceptible tobacco genotypes (Schaad *et al.*, 2000). Further experiments using chimeric NIa proteins suggested that the interaction determinants were genetically distinct from the avirulence determinant. In another system where *eIF4E* is known to be the resistance gene, no interaction between the viral avirulent determinant VPg of PSbMV and eIF4E from a susceptible pea variety was demonstrated (Gao *et al.*, 2004).

Our data support the importance of the eIF4E interaction with respect to the outcome of viral infection. We observed strong interactions of eIF4E proteins from susceptible genotypes of *C. annuum* and *C. chinense* with VPg from TEV-HAT and TEV-NW, both of which cause severe infection. Absence of an interaction was observed between the VPg from the two TEV stains and eIF4E proteins translated from resistant genotypes (*pvr1* and *pvr1*^{2}). To the extent that interaction assays *in vitro* and in yeast are indicative of interactions *in planta*, our data support the hypothesis that physical interaction between eIF4E and TEV-VPg may be required for full susceptibility.

The case of the *pvr1*^{1} allele is of special note. Although the response of the YY genotype to inoculation with TEV-HAT and TEV-NW is not equivalent to the susceptible response, plants can sustain infection. This observation indicates that disruption of the eIF4E interaction with VPg may be necessary for complete resistance, but it is not sufficient. Slower symptom development and delayed viral antigen accumulation could be a consequence of impaired eIF4E/VPg interaction that may affect viral replication and/or movement; however, in most (*pvr1*^{1}/*pvr1*^{1}) plants, infection was established that eventually became indistinguishable from the susceptible interaction (Figure 5). The nature of the compensatory shift(s) on the viral side that promote systemic infection despite the interruption of the eIF4E/VPg interaction is not clear. Within plant cells, interaction of eIF4E and VPg would likely be influenced by conformational changes brought on by interaction with other host and viral proteins and RNA. There may be alternative ways to stabilize the association of eIF4E with VPg or to substitute for the function the interaction provides. The difference in degree of susceptibility may explain the failure to observe interactions in other eIF4E-mediated potyvirus systems that apparently show a clear difference in infectivity.

One important observation from the present study is that the ability to bind capped mRNA is interrupted in only one of the three resistance alleles. This clearly demonstrates that in our system impaired cap-binding ability is not essential for potyvirus resistance. Gao *et al.* (2004) showed that eIF4E protein encoded by *sbm1* in pea also abolished cap-binding activity and suggested a possible relationship between loss of this activity and resistance. However, our cap binding and VPg-binding assays support the observations made by Leonard *et al.* (2000) that the domains of eIF4E involved in cap binding are distinct. The eIF4E proteins encoded by *pvr1*^{1} and *pvr1*^{2} alleles lack VPg-binding affinity *in vitro* but retain cap-binding affinity, confirming that the amino acid sequence essential for VPg binding in these assays is different from those for cap binding. A second implication of this result is that impaired cap binding of this eIF4E paralog has no apparent phenotypic consequences in *pvr1* plants, consistent with functional redundancy in *Capsicum*. Specifically, it is possible that the 537 bp *eIF4E* homolog, eIF(iso)4E, or other molecules may function in place of the mutant eIF4E in *pvr1* genotypes. Likewise, no phenotypic defects were found in potyvirus-resistant Arabidopsis

Table 1 Comparison of amino acid mutations in eIF4E proteins encoded by natural recessive genes cloned in three plant species

	Mutations[a] and amino acid positions[b]													
Plants	50	62	67	68	73	74	76	79	106	107	108	109	175	183
Pepper	T/G	–	P/T	V/E	–	–	–	L/R	–	G/R	–	D/N	–	–
Lettuce	–	–	A/P	–	–	–	–	–	Δ	G/H	Δ	–	–	–
Pea	–	W/L	–	–	A/D or A/P	A/D	Δ	–	–	G/R	–	–	N/K	A/S

[a]Amino acid substitutions are represented as wild type/mutation and Δ represents deletion.
[b]Amino acid positions are based on the pepper eIF4E sequence.

eIF(iso)4E mutants, suggesting that redundancy for this function may be universal in plants. Although it is possible that eIF4E has cellular functions other than cap binding that are as yet unknown, our results imply that the eIF4E of this study is a homolog whose function may be dispensable for the host, but essential for the virus.

One of the interesting features of recessive potyvirus resistances in *Capsicum* is that the *pvr1* alleles show various specificities against several distinct potyviruses or viral strains. In light of previous work, sequence analysis of these *pvr1* alleles indicates that point mutations resulting in single amino acid substitutions result in significantly altered resistance specificity. For example, one amino acid difference between $pvr1^1$ and $pvr1^2$ resulted in a shift in resistance spectrum and also an apparent shift in resistance phenotype (cellular resistance to resistance to cell–cell movement) (Arroyo *et al.*, 1996). The allele that is the focus of this study, *pvr1*, has the broadest resistance spectrum among the three resistance *pvr1* alleles and shows unique amino acid substitutions that resulted in defects of both VPg binding and cap binding. When eIF4E proteins encoded by natural recessive genes cloned from three plant species were aligned, the only position showing a consistent change occurred at amino acid 107 (Table 1). Among the three *pvr* alleles reported here, only *pvr1* shares this mutation. Assuming eIF4E is involved in multiple steps in the viral infection cycle, the distinctive features of pvr1 protein may be related to the increased resistance spectrum. Similarly, premature stop codons and insertions into Arabidopsis eIF(iso)4E almost certainly interrupted both VPg and cap binding and led to broad-spectrum potyvirus resistance in Arabidopsis (Duprat *et al.*, 2002; Lellis *et al.*, 2002).

Interaction of VPg with eIF4E has been proposed to confer a translational advantage to the virus (Lellis *et al.*, 2002); however, there is evidence from both potyvirus and poliovirus that both eIF4E and VPg are dispensable for cap-independent translation (Gallie, 2001; Nomoto *et al.*, 1977; Sachs *et al.*, 1997). In many RNA virus-host systems, interaction between cellular translation machinery, viral proteins and RNA appears to be important in bringing 5′ and 3′ regulatory elements together for RNA replication (Herold and Andino, 2001; Lai, 1998). Interaction of these regulatory regions favors viral replication for a number of reasons, including efficient recruiting of the RNA polymerase to the replication start site, ensuring that only intact viral templates are replicated, and coordinating RNA replication with translation (Herold and Andino, 2001). TEV has RNA secondary structures at the 3′ end of its genome that are required in *cis* for RNA replication (Haldeman-Cahill *et al.*, 1998; Mahajan *et al.*, 1996). With VPg attached to the 5′ end of the potyviral genome, interaction with eIF4E could create a bridge to the 3′ poly-A tail through interaction with eIF4G and poly-A binding protein, thus allowing regulation of RNA synthesis (Gallie *et al.*, 1995; Revers *et al.*, 1999). Mutations in eIF4E that interrupt this association could explain how *pvr1* and $pvr1^2$ genotypes block replication of TEV-HAT. Second site compensatory shifts that restore this bridging role, at least to some extent, may account for partial recovery of susceptibility in $pvr1^1/pvr1^1$ genotypes.

Even though the precise mechanisms through which cap-binding factors promote potyvirus infection have yet to be resolved, it is now clear that both *eIF4E* and *eIF(iso)4E* are strong candidates for a number of naturally occurring recessive potyvirus resistance genes (Diaz-Pendon *et al.*, 2004). Based on mapping data in tomato and pepper, respectively, *pot-1* and *pvr5* are likely to be *eIF4E* homologs (Parella *et al.*, 2002). Results presented here indicate that *pvr6*, which confers resistance to PVMV when combined with $pvr1^2$ (Caranta *et al.*, 1996), is likely to be *eIF(iso)4E*. The combined effects of mutations in two cap-binding isomers may also explain the epistatic effect of the recessive *bc-u* gene on recessive *Bean common mosaic virus* (BCMV) resistance genes *bc-1*, *bc-2*, and *bc-3* (Drijfhout, 1978). In other recessive potyvirus-host systems, candidate gene analysis will reveal the universality of *eIF4E* and *eIF(iso)4E* mutants (Keller *et al.*, 1998; Nicolas *et al.*, 1997a).

Our study confirms that eIF4E may be an important point of interaction between the virus and its host. Furthermore, our results suggest the possibility that cellular-level resistance may be engineered by site-directed mutagenesis to create dominant negative mutations or targeted silencing of this host sequence in transgenic plants (Azevedo *et al.*, 2002). Because of the well-characterized genetic variability on both host and pathogen sides of this interaction, the *pvr1* system offers a unique opportunity to extend our understanding of the role host gene products play in viral pathogenesis, and the identity and mechanisms of the molecular determinants of host-pathogen specificity.

Further, this study confirms *eIF4E* and *eIF(iso)4E* as viable candidates for a plethora of naturally occurring recessive virus resistance genes for which molecular markers may be very useful in agriculture.

Experimental procedures

Plants, viruses, and populations for genetic studies

Capsicum annuum 'NuMex RNaky' (RN), 'Jupiter' (JP), 'Early Cal Wonder' (ECW), 'Florida VR2' (VR2), 'Yolo Y' (YY), 'Del Ray Bell' (DRB), and breeding lines '3618', '5502', and '4074' were obtained from Asgrow Seed Co. (San Juan Bautista, CA, USA). *Capsicum annuum* 'Dempsey' (DEMP) was provided by M. Deom, University of Georgia, Athens, GA, USA and *C. annuum* 'Jupiter' was provided by Syngenta Seeds (Naples, FL, USA); *C. chinense* PI 152225 (225), PI 159234 (234), and PI 159236 (236) were obtained from the USDA Southern Regional Plant Introduction Station (Griffin, GA, USA); *C. chinense* 'Habanero' (HAB) was obtained from Tomato Growers Supply Co. (Fort Myers, FL, USA). TEV-HAT and TEV-NW (non-wilting on *C. frutescens* Tabasco) cultures were obtained from T. Pirone, University of Kentucky (Lexington, KY, USA). PepMoV-FL (Florida) was obtained from T. Zitter, Cornell University (Ithaca, NY, USA). All potyvirus strains were maintained on TMV-resistant *Nicotiana tabacum* 'Kentucky 14' and were transferred every 4–8 weeks.

For genetic complementation experiments, the set of resistant parents (DEMP, VR2, 3618, 5502) were intercrossed in all combinations and crossed with the susceptible parents JP and ECW. Parents and F_1 progeny were inoculated with TEV-HAT and assayed for symptoms and presence of virus by indirect ELISA.

DNA samples from F_2 individuals of an inter-specific mapping population (AC population) used in a previous study to map the *pvr1* locus were used to assess genetic markers (Livingstone *et al.*, 1999). A second population of 114 F_3 families was generated for this study from an intra-specific cross between *C. chinense* HAB and *C. chinense* 234 (CC population). This population was scored for resistance to TEV-HAT and PepMoV in two separate screens. DNA samples were extracted as described previously for genotypic analysis (Livingstone *et al.*, 1999; Murphy *et al.*, 1998).

Virus screening procedure

Seed were sown in Styrofoam trays in Pro-Mix soilless potting medium (Premier Peat; Riviére-du-Loup, Québec, Canada) or Cornell Mix. Plants were inoculated when at the five to six leaf stage of development. Virus was applied to the two oldest leaves (leaves 1 and 2) by mechanical inoculation after being lightly dusted with Carborunum. Inoculum consisted of TEV-HAT, TEV-NW, or PepMoV systemically infected tobacco tissue ground in 50 mM potassium phosphate buffer, pH 7.5 (1 g tissue: 20 ml buffer). Each virus treatment was inoculated onto 8–10 plants for each genotype with the addition of four plants serving as a mock inoculation treatment (leaves inoculated with buffer alone).

Plants were monitored daily for the timing of appearance and severity of symptoms. Leaf tissue was tested for the presence of virus using antigen plate-coating indirect ELISA as described (Murphy *et al.*, 1998). Anti-viral immunoglobulins were obtained from Agdia, Inc. (Elkhart, IN, USA) and used according to the manufacturer's instructions. Virus accumulation was tested either in inoculated at 10 dpi or un-inoculated leaves at 21 dpi. For each test, ELISA substrate-enzyme reactions were read every 30 min using a Tecan Sunrise microtiter plate reader at 405 nm (Tecan Sunrise, Sunnyville, CA, USA). A sample was considered positive for virus when the ELISA absorbance value was greater than the threshold determined from the mean absorbance value of healthy control samples of each genotype plus three standard deviations. In addition, another set of ELISA using the same pepper genotypes was performed in order to detect differential viral accumulation in systemic leaves at earlier time points: 3 and 6 dpi.

RNA isolation, RNA blots, and RT-PCR

Total RNA was isolated from young pepper leaves using Trizol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA blots were performed according to standard methods (Sambrook and Russell, 2001). First strand cDNA was synthesized in 25 μl containing 2 μg total RNA and 500 ng oligo dT using M-MLV reverse transcriptase (Promega, Madison, WI, USA) according to the manufacturer's instructions. For RT-PCR, 2 μl of cDNA was added to a reaction volume of 25 μl containing 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 2 mM $MgSO_4$, 0.1% Triton X-100, 0.2 mM each dNTP, 0.4 uM each forward and reverse primer, and 1 unit *Taq* polymerase (New England Biolabs, Beverly, MA, USA). PCR cycling conditions were 95°C 3 min, (95°C 30 sec, 55°C 30 sec, 72°C 5 min) × 1, (95°C 30 sec, 55°C 30 sec, 72°C 90 sec) × 29, 72°C 10 min. Full-length *eIF4E* ORF primers (forward 5′-ATGGCAACAGCTGAAATGG-3′; reverse 5′-TATACGGTGTAACG-ATTCTTGGCA-3′) were based on tomato eIF4E sequence (GenBank accession AF259801). Full-length *eIF(iso)4E* ORF primers (forward 5′-AACAATGGCCACCGAAGC-3′; reverse 5′-ATTTCACAGTATATC-GGCTCT-3′) were based on published tomato sequence (TIGR accession TC103222) (http://www.tigr.org). Full-length 537 bp *eIF4E* ORF primers (forward 5′-TTAGGCAAACCAATCACAATG-3′; reverse 5′-CCTGTTGTAACGATAGAACTA-3′) were based on published tomato sequence (TIGR accession TC96888). PCR products were run on 1.5% agarose. PCR products were gel purified using the Qiaquick gel purification kit (Qiagen, Valencia, CA, USA) and cloned using the pGEM-T Easy kit (Promega).

cDNA sequence alignment and protein modeling

At least two positive clones were sequenced from both ends for each PCR product and analyzed using Seqman software (DNASTAR Inc., Madison, WI, USA). Amino acid sequence alignments were produced using the Clustal algorithm within Megalign software (DNASTAR). For protein homology models, pepper sequence was submitted to the SwissProt database via the application DeepView (http://www.expasy.ch/spdbv/text/server.htm). The model was generated using the murine crystal structure for eIF4E (sequence 1EJ1.B).

DNA blots

DNA blotting and hybridization were carried out as reported previously (Livingstone *et al.*, 1999). Probes were amplified via PCR from cloned DNA fragments, purified from agarose using the Qiaquick gel extraction kit and labeled with ^{32}P using the Prime-It Random Primer Labeling Kit (Stratagene, La Jolla, CA, USA) according to the manufacturer's instructions. Labeled probe was purified through a Sephadex G50 column, incubated with blots overnight at 65°C, then washed once with 2X SSC, once with 1X SSC and twice with 0.5X SSC. All washes included 0.1% SDS. Filters were placed on Kodak XAR-5 film. Genetic maps were assembled using MapMaker/EXP v3.0b (Livingstone *et al.*, 1999).

CAPS markers

The upstream primer used to amplify the promoter region containing the *Ssp*I CAPS differential cleavage site was based on sequence obtained by genome-walking using a Genome Walker kit (Clontech, Palo Alto, CA, USA). The reverse primer lies 56 bases within the eIF4E open reading frame. The reaction volume of 50 μl contained 50 ng genomic DNA, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 20 mM Tris-HCl, 2 mM $MgSO_4$, 0.1% Triton X-100, 0.2 mM each dNTP, 0.4 μM each forward primer 5′-TTACACGCGCCGATACACTTG-3′ and reverse primer 5′-CATCATCTGCCTTCATTAGCATTCAAT-3′, and 2 units *Taq* polymerase (New England Biolabs). Cycling conditions were 95°C 3 min, (95°C 30 sec, 60°C 30 sec, 72°C 5 min) × 1 cycle, (95°C 30 sec, 60°C 30 sec, 72°C 90 sec) × 29 cycles, 72°C 10 min. A 20-μl aliquot of PCR product was digested with 2.5 units of *Ssp*I (New England Biolabs) in a 30 μl reaction volume.

Yeast two-hybrid analysis

Yeast two-hybrid analysis was performed according to published methods (Golemis *et al.*, 1996). Yeast strains and plasmid vectors were provided by G. Martin (Boyce Thompson Institute, Ithaca, NY, USA). A bait plasmid, pEG202, was used for the fusion of NIa and VPg to the DNA binding domain of LexA; a prey plasmid, pJG4-5, was used to express *Capsicum eIF4E* genes and NIb. The DNA sequences encoding NIa from TEV-HAT and TEV-NW were amplified by PCR from full-length clones using forward primer 5′-GCCGAATTCATGGGGAAGAAGAATCAGA-3′ (*Eco*RI site underlined) and reverse primer 5′-CCCTCGAGCTATTGCGTGTACACCAATTC-3′ (*Xho*I site underlined). The DNA sequences encoding VPg was amplified by PCR from full-length clones using forward primer 5′-GCCGAATTCATGGGGAAGAAGAATCAGA-3′ (*Eco*RI site underlined) and reverse primer 5′-CCCTCGAGCTATTCAAACGTCAAGTCCT-3′ (*Xho*I site underlined; TEV-HAT) or reverse primer 5′-CCCTCGAGCTATTCAAACGTCAACTCCT-3′ (*Xho*I site underlined; TEV-NW). The eIF4E sequences from different pepper genotypes known to be homozygous for each resistance allele were amplified using forward primer 5′-GAATTCATGGCAACAGCTGAA-3′ (*Eco*RI site underlined) and 5′-CTCGAGCTATACGGTGTAACG-3′ (*Xho*1I site underlined). TEV-HAT NIb was amplified by PCR using forward primer 5′-CCGAATTCATGGGAGAGAAGAGGAAATG-3′ (*Eco*RI site underlined) and reverse primer 5′-CCCTCGAGCTACTGAAAATAAAGATTCTC-3′ (*Xho*I site underlined) and cloned into pJG4-5 as a positive control for interaction for yeast two hybrid assay assay. The amplified fragments were digested with *Eco*RI and *Xho*I and cloned into corresponding restriction sites of pEG202 and pJG4-5, respectively. In addition, all constructs were confirmed by sequencing. Bait recombinant plasmids were transformed into yeast strain EGY48 containing the *lacZ* reporter plasmid pSH18-34 using the lithium acetate method. Yeast cells containing pSH18-34 and bait plasmids were subsequently transformed with the prey plasmid pJG4-5:NIa. For two-hybrid assays, transformants were grown in complete minimal medium lacking histidine, tryptophan, and uracil, supplemented with 2% galactose/1% raffinose and X-gal. The degree of interaction was determined by the β-galactosidase liquid assay (Victoria, 1996) and protein expression was assayed by immmunoblots probed with anti-eIF4E (New England Biolabs) to assure equivalent expression and stability in all treatments.

GST in vitro *pull-down assay*

The eIF4Es from each of the three resistant alleles: *pvr1*, *pvr2*^{1}, and *pvr2*^{2}, and the susceptible allele *pvr1*$^+$, were cloned into the pET16b vector and translated *in vitro* using TnT coupled reticulocyte lysate (Promega) according to the manufacturer's instructions using T7 RNA polymerase and ^{35}S-methionine (Amersham Biosciences, Piscataway, NJ, USA). GST, GST-VPgHAT, and GST-VPgNW proteins were expressed in *E. coli* and bound to 10 μl GST-Sepharose beads and re-suspended in 195 μl IPAB-gelatin buffer (20 mM HEPES, pH 7.8, 150 mM KCl, 0.1% gelatin, 0.1% Triton X-100, 0.1% NP-40, 5 mM $MgCl_2$, 2 mM DTT). TnT translation products (5 μl) were added to prepared GST fusion Sepharose, mixed by end-over rotation at 4°C for overnight, and washed three times with 1X PBS buffer containing 2 mM DTT. The Sepharose beads were re-suspended in 20 μl SDS-PAGE loading buffer and incubated for 2 min at 100°C. Released proteins were separated by SDS-PAGE. Following electrophoresis, gels were fixed, dried, and exposed to a phosphorimaging screen.

Cap-binding assays

The coding region of pepper eIF4E was amplified by PCR with forward primer 5′-CCATATGGCAACAGCTGA-3′ (*Nde*I site underlined) and reverse primer 5′-CCCTCGAGCTATACGGTGTAACGA-3′ (*Xho*I site underlined). The amplified fragments were then digested by *Nde*I and *Xho*I and cloned into pET16b (Novagen, Madison, WI, USA) in frame with the 6X histidine tag. The constructs were confirmed by sequencing before transformation into *E. coli* BL21(DE3)pLysS (Novagen). Expression of the recombinant proteins and purification of the proteins by m^{7}GTP-Sepharose 4.B (Amersham Bioscience) were carried out as described previously with minor modification (Hagedorn *et al.*, 1997). Expression of recombinant proteins was induced at 20°C for 20 h by addition of 20 μM IPTG. The bacterial cells from 500 ml culture were re-suspended in 5 ml of extraction buffer [20 mM Tris-HCl, pH 7.5 (4°C), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 1 mM PMSF] and sonicated. After cell lysis, Triton X-100 was added to a final concentration of 0.1% and stirred for 15 min at 4°C. The samples were centrifuged at 22 000 ***g*** for 20 min; the supernatant was recovered for the cap-binding assay and applied to a 0.5-ml affinity column saturated with extraction buffer. The column was washed extensively with extraction buffer and eIF4E protein was eluted using extraction buffer containing 100 μM m^{7}GTP. SDS-PAGE and immunoblot analysis were carried out as described previously (Murphy and Kyle, 1994), using human anti-eIF4E antibody (New England Biolabs) for detection of recombinant eIF4E.

Acknowledgements

We thank R.C. Grube, M. Deom, R.F. Heisey, G.B. Martin, and T.A. Thorup for experimental materials and L.G. Landry, G. Moriarty, F.T. Lao, P. McCord, and K. Liu for technical assistance. We thank S.G. Lazarowitz, R.C. Grube, Carole Caranta, and Alain Palloix for useful discussions and Helen Griffiths for critical review of this manuscript. This work was supported in part by USDA NRICGP Plant Genome Award No. 94-37300-0333, USDA IFAFS Award No. 2001-52100-113347 and NSF Plant Genome Award No. 0218166. J.D.F. was supported in part by a USDA National Needs Fellowship in Plant Biotechnology. I.Y. was supported in part by a fellowship from the Kwanjeong Educational Foundation, Korea.

References

Arroyo, R., Soto, M.J., Martinez, Z.J.M. and Ponz, F. (1996) Impaired cell-to-cell movement of potato virus Y in pepper plants carrying

the *y-a* (*pr2-1*) resistance gene. *Mol. Plant Microbe Interact.* **9**, 314–318.

Ayme, V., Pierrugues, O., Souche, S., Nemouchi, G., Caranta, C., Jacquemond, M., Chadoeuf, J., Palloix, A. and Moury, B. (2004) PVY evolution toward virulence to the resistance conferred by *pvr2*[3] in pepper and consequences for its durability. In *XIIth Meeting on Genetics and Breeding of Capsicum & Eggplant*, Netherlands, 17–19 May 2004, 139–146.

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K. and Schulze-Lefert, P. (2002) The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. *Science*, **295**, 2073–2076.

Borgstrom, B. and Johansen, I.E. (2001) Mutations in pea seedborne mosaic virus genome-linked protein VPg after pathotype-specific virulence in *Pisum sativum*. *Mol. Plant Microbe Interact.* **14**, 707–714.

Caranta, C., Palloix, A., Gebre, S.K., Lefebvre, V., Moury, B. and Daubeze, A.M. (1996) A complementation of two genes originating from susceptible *Capsicum annuum* lines confers a new and complete resistance to pepper veinal mottle virus. *Phytopathology*, **86**, 739–743.

Carrington, J.C., Kasschau, K.D., Mahajan, S.K. and Schaad, M.C. (1996) Cell-to-cell and long-distance transport of viruses in plants. *Plant Cell*, **8**, 1669–1681.

Chu, M., Lopez-Moya, J.J., Llave-Correas, C. and Pirone, T.P. (1997) Two separate regions in the genome of the tobacco etch virus contain determinants of the wilting response of Tabasco pepper. *Mol. Plant Microbe Interact.* **10**, 472–480.

Cook, A.A. (1961) A mutation for resistance to potato virus Y in pepper. *Phytopathology*, **51**, 550–552.

Deom, C.M., Murphy, J.F. and Paguio, O.R. (1997) Resistance to tobacco etch virus in *Capsicum annuum*: inhibition of virus RNA accumulation. *Mol. Plant Microbe Interact.* **10**, 917–921.

Diaz-Pendon, J.A., Truniger, V., Nieto, C., Garcia-Mas, J., Bendahmane, A. and Aranda, M.A. (2004) Advances in understanding recessive resistance to plant viruses. *Mol. Plant Pathol.* **5**, 223–233.

Drijfhout, E. (1978) Genetic interaction between *Phaseolus vulgaris* and bean common mosaic virus with implications for strain identification and breeding for resistance. *Agric. Res. Rep.* **872**, 1–89.

Duprat, A., Caranta, C., Revers, F., Menand, B., Browning, K.S. and Robaglia, C. (2002) The *Arabidopsis* eukaryotic initiation factor (iso)4E is dispensable for plant growth but required for susceptibility to potyviruses. *Plant J.* **32**, 927–934.

Fraser, R.S.S. (1992) The genetics of plant-virus interactions: implications for plant breeding. *Euphytica*, **63**, 175–185.

Gallie, D.R. (2001) Cap-independent translation conferred by the 5′ leader of tobacco etch virus is eukaryotic initiation factor 4G dependent. *J. Virol.* **75**, 12141–12152.

Gallie, D.R. and Browning, K.S. (2001) eIF4G functionally differs from eIFiso4G in promoting internal initiation, cap-independent translation, and translation of structured mRNAs. *J. Biol. Chem.* **276**, 36951–36960.

Gallie, D.R., Tanguay, R.L. and Leathers, V. (1995) The tobacco etch viral 5′ leader and poly(A) tail are functionally synergistic regulators of translation. *Gene*, **165**, 233–238.

Gao, Z., Johansen, E., Eyers, S., Thomas, C.L., Noel Ellis, T.H. and Maule, A.J. (2004) The potyvirus recessive resistance gene, *sbm1*, identifies a novel role for translation initiation factor eIF4E in cell-to-cell trafficking. *Plant J.* **40**, 376–385.

Goldbach, R., Eggen, R., de Jager, C. and van Kammen, A. (1990) Genetic organization, evolution and expression of plant viral RNA genomes. In *Recognition and Response in Plant–Virus Interactions* (Fraser, R.S.S., ed.). Heidelberg: Springer-Verlag, pp. 147–162.

Golemis, E.A., Gyuris, H. and Brent, R. (1996) Interaction trap/two-hybrid system to identify interacting proteins. In *Current Protocols in Molecular Biology* (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Stuhl, K., eds). New York: Wiley, pp. 20.21.21–20.21.28.

Graner, A., Streng, S., Kellermann, A., Schiemann, A., Bauer, E., Waugh, R., Pellio, B. and Ordon, F. (1999) Molecular mapping and genetic fine-structure of the rym5 locus encoding resistance to different strains of the Barley Yellow Mosaic Virus Complex. *Theor. Appl. Genet.* **98**, 285–290.

Greenleaf, W.H. (1956) Inheritance of resistance to tobacco etch virus in *Capsicum frutescens* and in *Capsicum annuum*. *Phytopathology*, **46**, 371–375.

Greenleaf, W.H. (1986) Pepper breeding. In *Breeding Vegetable Crops* (Basset, M.J., ed.). Westport, CT: AVI Pub., pp. 67–134.

Hagedorn, C.H., Spivak-Kroizman, T., Friedland, D.E., Goss, D.J. and Xie, Y. (1997) Expression of functional eIF-4E human: purification, detailed characterization, and its use in isolating eIF-4E binding proteins. *Protein Expr. Purif.* **9**, 53–60.

Haldeman-Cahill, R., Daros, J.A. and Carrington, J.C. (1998) Secondary structures in the capsid protein coding sequence and 3′ nontranslated region involved in amplification of the tobacco etch virus genome. *J. Virol.* **72**, 4072–4079.

Herold, J. and Andino, R. (2001) Poliovirus RNA replication requires genome circularization through a protein–protein bridge. *Mol. Cell*, **7**, 581–591.

Johansen, I.E., Lund, O.S., Hjulsager, C.K. and Laursen, J. (2001) Recessive resistance in *Pisum sativum* and potyvirus pathotype resolved in a gene-for-cistron correspondence between host and virus. *J. Virol.* **75**, 6609–6614.

Keller, K.E., Johansen, I.E., Martin, R.R. and Hampton, R.O. (1998) Potyvirus genome-linked protein (VPg) determines *Pea seedborne mosaic virus* pathotype-specific virulence in *Pisum sativum*. *Mol. Plant Microbe Interact.* **11**, 124–130.

Kyle, M.M. and Palloix, A. (1997) Proposed revision of nomenclature for potyvirus resistance genes in *Capsicum*. *Euphytica*, **97**, 183–188.

Lai, M.M. (1998) Cellular factors in the transcription and replication of viral RNA genomes: a parallel to DNA-dependent RNA transcription. *Virology*, **244**, 1–12.

Lellis, A.D., Kasschau, K.D., Whitham, S.A. and Carrington, J.C. (2002) Loss-of-susceptibility mutants of *Arabidopsis thaliana* reveal an essential role for eIF(iso)4E during potyvirus infection. *Curr. Biol.* **12**, 1046–1051.

Leonard, S., Plante, D., Wittmann, S., Daigneault, N., Fortin, M.G. and Laliberte, J.F. (2000) Complex formation between potyvirus VPg and translation eukaryotic initiation factor 4E correlates with virus infectivity. *J. Virol.* **74**, 7730–7737.

Leonard, S., Viel, C., Beauchemin, C., Daigneault, N., Fortin, M.G. and Laliberte, J.F. (2004) Interaction of VPg-Pro of turnip mosaic virus with the translation initiation factor 4E and the poly(A)-binding protein in planta. *J. Gen. Virol.* **85**, 1055–1063.

Livingstone, K.D., Lackney, V.K., Blauth, J.R., van Wijk, R. and Jahn, M.M. (1999) Genome mapping in *Capsicum* and the evolution of genome structure in the *Solanaceae*. *Genetics*, **152**, 1183–1202.

Mahajan, S., Dolja, V.V. and Carrington, J.C. (1996) Roles of the sequence encoding tobacco etch virus capsid protein in genome amplification: requirements for the translation process and a *cis*-active element. *J. Virol.* **70**, 4370–4379.

Marcotrigiano, J., Gingras, A.C., Sonenberg, N. and Burley, S.K. (1997) Cocrystal structure of the messenger RNA 5′ cap-binding protein (eIF4E) bound to 7-methyl-GDP. *Cell*, **89**, 951–961.

Masuta, C., Nishimura, M., Morishita, H. and Hataya, T. (1999) A single amino acid change in viral genome-associated protein of *Potato virus Y* correlates with resistance breaking in 'Virgin A Mutant' tobacco. *Phytopathology*, **89**, 118–123.

Moury, B., Morel, C., Johansen, E., Guilbaud, L., Souche, S., Ayme, V., Caranta, C., Palloix, A. and Jacquemond, M. (2004) Mutations in potato virus Y genome linked protein determine virulence towards recessive resistances in *Capsicum annum* and *Lycopersicum hirsutum*. *Mol. Plant Microbe Interact* **17**, 322–329.

Murphy, J.F. and Kyle, M.M. (1994) Isolation and viral infection of *Capsicum* leaf protoplasts. *Plant Cell Rep.* **13**, 397–400.

Murphy, J.F., Blauth, J.R., Livingstone, K.D., Lackney, V.K. and Jahn, M.M. (1998) Genetic mapping of the *pvr1* locus in *Capsicum* spp. and evidence that distinct potyvirus resistance loci control responses that differ at the whole plant and cellular levels. *Mol. Plant Microbe Interact.* **11**, 943–951.

Nicaise, V., German-Retana, S., Sanjuan, R., Dubrana, M.P., Mazier, M., Maisonneuve, B., Candresse, T., Caranta, C. and LeGall, O. (2003) The eukaryotic translation initiation factor 4E controls lettuce susceptibility to the potyvirus *Lettuce mosaic virus*. *Plant Physiol.* **132**, 1272–1282.

Nicolas, O., Dunnington, S.W., Gotow, L.F., Pirone, T.P. and Hellmann, G.M. (1997) Variations in the VPg protein allow a potyvirus to overcome *va* gene resistance in tobacco. *Virology*, **237**, 452–459.

Nomoto, A., Kitamura, N., Golini, F. and Wimmer, E. (1977) The 5′-terminal structures of poliovirion RNA and poliovirus mRNA differ only in the genome-linked protein VPg. *Proc. Natl Acad. Sci. USA*, **74**, 5345–5349.

Parella, G., Ruffel, S., Moretti, A., Morel, C., Palloix, A. and Caranta, C. (2002) Recessive resistance genes against potyviruses are localized in colinear genomic regions of the tomato (*Lysopersicon* spp.) and pepper (*Capsicum* spp.) genomes. *Theor. Appl. Genet*, **105**, 855–861.

Pellio, B., Streng, S., Bauer, E., Stein, N., Perovic, D., Schiemann, A., Friedt, W., Ordon, F. and Graner, A. (2005) High-resolution mapping of the *Rym4/Rym5* locus conferring resistance to the barley yellow mosaic virus complex (BaMMV, BaYMV, BaYMV-2) in barley (*Hordeum vulgare* ssp. *vulgare* L.). *Theor. Appl. Genet.* **110**, 283–293.

Provvidenti, R. and Hampton, R.O. (1992) Sources of resistance to viruses in the *Potyviridae*. *Arch. Virol.* **5**, 189–211.

Rajamaki, M.L. and Valkonen, J.P. (1999) The 6K2 protein and the VPg of potato virus A are determinants of systemic infection in *Nicandra physaloides*. *Mol. Plant Microbe Interact.* **12**, 1074–1081.

Rajamaki, M.L. and Valkonen, J.P. (2002) Viral genome-linked protein (VPg) controls accumulation and phloem-loading of a potyvirus in inoculated potato leaves. *Mol. Plant Microbe Interact.* **15**, 138–149.

Revers, F., Le, G.O., Candresse, T. and Maule, A.J. (1999) New advances in understanding the molecular biology of plant/potyvirus interactions. *Mol. Plant Microbe Interact.* **12**, 367–376.

Riechmann, J.L., Lain, S. and Garcia, J.A. (1992) Highlights and prospects of potyvirus molecular biology. *J. Gen. Virol.* **73**, 1–16.

Rodriguez, C.M., Freire, M.A., Camilleri, C. and Robaglia, C. (1998) The *Arabidopsis thaliana* cDNAs coding for eIF4E and eIF(iso)4E are not functionally equivalent for yeast complementation and are differentially expressed during plant development. *Plant J.* **13**, 465–473.

Ruffel, S., Dussault, M.H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C. and Caranta, C. (2002) A natural recessive resistance gene against potato virus Y in pepper corresponds to the eukaryotic initiation factor 4E (eIF4E). *Plant J.* **32**, 1067–1075.

Sachs, A.B., Sarnow, P. and Hentze, M.W. (1997) Starting at the beginning, middle, and end: translation initiation in eukaryotes. *Cell*, **89**, 831–838.

Sambrook, J. and Russell, D.W. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Schaad, M.C., Lellis, A.D. and Carrington, J.C. (1997) VPg of tobacco etch potyvirus is a host genotype-specific determinant for long-distance movement. *J. Virol.* **71**, 8624–8631.

Schaad, M.C., Anderberg, R.J. and Carrington, J.C. (2000) Strain-specific interaction of the tobacco etch virus NIa protein with the translation initiation factor eIF4E in the yeast two-hybrid system. *Virology*, **273**, 300–306.

Urcuqui-Inchima, S., Haenni, A.L. and Bernardi, F. (2001) Potyvirus proteins: a wealth of functions. *Virus Res.* **74**, 157–175.

Victoria, L. (1996) Yeast vectors and assays for expression of cloned genes. In *Current Protocols in Molecular Biology* (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Stuhl, K., eds). New York: Wiley, pp. 13.16.11–13.16.14.

Ward, C.Q. and Shukla, D.D. (1991) Taxonomy of potyviruses: current problems and some solutions. *Intervirology*, **32**, 269–296.

Wicker, T., Zimmermann, W., Perovic, D., Paterson, A.H., Ganal, M., Graner, A. and Stein, N. (2005) A detailed look at 7 million years of genome evolution in a 439 kb contiguous sequence at the barley Hv-eIF4E locus: recombination, rearrangements and repeats. *Plant J.* **41**, 184–194.

Wittmann, S., Chatel, H., Fortin, M.G. and Laliberte, J.F. (1997) Interaction of the viral protein genome linked of turnip mosaic potyvirus with the translational eukaryotic initiation factor (iso) 4E of *Arabidopsis thaliana* using the yeast two-hybrid system. *Virology*, **234**, 84–92.

Data deposition: The sequences reported in this paper have been submitted to the GenBank database and have been assigned the following accession numbers: AY485127, AY485128, AY485129, AY485130, and AY485131.