

The *cyy-2* resistance to *Clover yellow vein virus* in pea is controlled by the eukaryotic initiation factor 4E

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Abstract The same mutant allele of eukaryotic initiation factor 4E (*eIF4E*) that confers resistance to *Pea seed-borne mosaic virus* (*sbm-1*) and the white lupine strain of *Bean yellow mosaic virus* (*wlv*) also confers resistance to *Clover yellow vein virus* (CIYVV) in pea. The *eIF4E* genes from several pea lines were isolated and sequenced. Analysis of the *eIF4E* amino acid sequences from several resistant lines revealed that some lines, including PI 378159, have the same sequence as reported for *sbm-1* and *wlv*. When *eIF4E* from a susceptible pea line was expressed from a CIYVV vector after mechanical inoculation of resistant PI 378159, the virus caused systemic infection, similar to its effects in susceptible line PI 250438. The resistance to CIYVV in line PI 378159 was characterized through a cross with PI 193835, which reportedly carries *cyy-2*. Mechanical inoculation of the F1 progeny with CIYVV resulted in no infection, indicating that the resistance gene in PI 378159 is identical to *cyy-2* in PI 193835. Furthermore, particle bombardment of pea line PI 193835 with infectious cDNA of CIYVV (pCIYVV/C3-S65T) resulted in the same resistance mode as that described for PI 378159. These results demonstrate that the resistance to CIYVV conferred by *cyy-2* is mediated by *eIF4E* and that *cyy-2* is identical to *sbm-1* and *wlv*.

Keywords *Clover yellow vein virus* · *cyy-2* · *sbm-1* · *wlv* · Eukaryotic initiation factor 4E · Pea resistance

Introduction

Previous genetic studies have shown that pea contains two clusters of recessive resistant genes to a number of potyviruses (Provvidenti and Hampton 1991). The first cluster (linkage group II), which includes *bcm*, *cyy-1*, *mo*, *pmv*, and *sbm-2*, confers resistance to strain NL-8 of *Bean common mosaic virus* (BCMV), *Clover yellow vein virus* (CIYVV), *Bean yellow mosaic virus* (BYMV), *Pea mosaic virus* (PMV), and the L1 pathotype of *Pea seed-borne mosaic virus* (PSbMV), respectively. The second cluster (linkage group VI), which consists of *sbm-1*, *cyy-2*, and *wlv*, confers resistance to the P1 pathotype of PSbMV, CIYVV, and the white lupin strain of BYMV (BYMV-W), respectively.

Gao et al. (2004a) reported that *eIF4E* is a marker that is tightly linked to *sbm-1* in linkage group VI. Gao et al. (2004b) further identified a number of amino acid differences between the *eIF4E* genes in lines susceptible (JI2009 and 744) and resistant (JI1405 and PI 193835) to pathotype P1 of PSbMV. Co-bombardment with a vector carrying *eIF4E* from a susceptible line and an infectious cDNA of PSbMV complemented replication and cell-to-cell movement of the virus. Thus, resistance gene *sbm-1* in pea lines JI1405 and PI 193835 is controlled by *eIF4E*. In addition, sequence analyses by Bruun-Rasmussen et al. (2007) demonstrated that the resistance gene *wlv* corresponds to the *sbm-1* allele of *eIF4E*.

The involvement of *eIF4E* in the multiplication of CIYVV in *Arabidopsis thaliana* has also been reported (Sato et al. 2005). Sato et al. (2005) demonstrated that a null mutant of *A. thaliana* lacking *eIF4E* was unable to support CIYVV multiplication; however, the transformation of that mutant with intact *eIF4E* restored viral multiplication. It was also shown that a null mutant of

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A. thaliana lacking *eIF4G*, but not one lacking *eIF(iso)4G*, was resistant to CIYVV infection (Nicaise et al. 2007). On the basis of their data and on studies of other potyviruses, Nicaise et al. (2007) hypothesized that either eIF4F, which is composed of eIF4E and eIF4G, or eIF(iso)4F, which is composed of eIF(iso)4E and eIF(iso)4G, is required for potyviral infections in *A. thaliana*, including CIYVV infection.

In addition, we previously described two distinct modes of resistance in pea, represented by lines PI 378159 and PI 347295, to isolate 30 of CIYVV (Andrade et al. 2007). Resistance was inherited in a recessive fashion, and the lines did not become infected after mechanical inoculation with CIYVV using crude extracts of infected tissue. However, both lines were locally infected after particle bombardment with infectious cDNAs.

The two modes of resistance described by Andrade et al. (2007) can be discriminated based on the cell-to-cell movement of the virus after particle bombardment. The virus moved slowly to neighboring cells in line PI 378159, but it scarcely moved in PI 347295. Several other resistant pea lines with a recessive character were identified by infection with cDNAs via particle bombardment; lines PI 356986 and PI 378158 had the same resistance mode as PI 378159, whereas PI 162908 and PI 429853 had the same resistance mode as PI 347295. However, the correspondence or relationships between the resistance genes and resistance modes described by Andrade et al. (2007) were not analyzed in the previous study. Here we show that PI 378159 carries *cyv-2* and that *eIF4E* is involved in the resistance conferred by *cyv-2*.

Materials and methods

Viral culture and plant material

Infectious cDNA constructed from full-length cDNA corresponding to the genomic RNA of CIYVV isolate 30 (Takahashi et al. 1997; Uyeda et al. 1975) was used as the viral source. Resistant pea lines PI 162909, PI 347295, PI 429853, PI 356986, PI 378158, and PI 378159; susceptible line PI 250438 (Andrade et al. 2007), and lines carrying the *cyv-2* resistance gene to CIYVV (PI 193586, PI 193835, PI 347465, PI 347466, PI 347467, and PI 347492; Provvidenti 1987) were provided by Dr. C. Coyne (Western Regional Plant Introduction Station, Washington State University, Pullman, WA, USA). The lines were selected based on the screening of 202 pea lines for resistance (Andrade et al. 2007; Ravelo et al. 2007).

Particle bombardment and detection of virus after inoculation

Particle bombardment and GFP monitoring were performed as previously described (Andrade et al. 2007). ELISAs were conducted 21 days after inoculation (dai) to confirm infection (Andrade et al. 2007).

Isolation and sequencing of *eIF4E*

Total RNA was extracted from 0.1 g of pea leaves using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. Reverse transcription-polymerase chain reaction (RT-PCR) was performed using an RNA PCR kit (Takara Bio, Otsu, Shiga, Japan). The primers used to isolate the ORF of *eIF4E* [5'-GATTCCA TGGTTGTAGAAGAAACCCCAAATC-3' (sense) and 5'-ACGCGAATTCACACAACATATTTGTTTTAGCA T-3' (antisense)] were designed based on published sequences (Gao et al. 2004b). First-strand cDNA was synthesized at 58°C for 30 min in a 5- μ l mixture containing 500 ng of total RNA and the antisense primer. Each cDNA encoding the full-length *eIF4E* ORF was in turn amplified using 2.5 μ l of cDNA in a 12.5- μ l reaction mixture containing the sense and antisense primers with the following program: 94°C for 5 min; followed by 28 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s; with a final hold at 72°C for 5 min. The products were fractionated on a 0.8% agarose gel and purified using a QIAquick Gel Extraction Kit (Qiagen, Valencia, CA, USA). Each PCR fragment was then inserted into the pGEM-T Easy vector system (Promega, Madison, WI, USA). For determining the *eIF4E* cDNA sequence, three positive clones per line were sequenced using 4000LS Long Read IR (Li-Cor, Lincoln, NE, USA) and ABI PRISM 310 Genetic Analyzer (Foster City, CA, USA) sequencing systems. Because the sense primer included the first eight amino acids of the published sequence, the N-terminal amino acid sequences were determined in a separate experiment based on the nucleotide sequences of the PCR products generated using the primers 5'-CAGGTTTGGCGGAGAAAGAAAC CGAGAGAG-3' (sense) and 5'-AATTTTATGCTTGAAA CAATAGAAATCTGC-3' (antisense).

Viral plasmid construction

pCIYVV/C3-S65T, which carries *GFP*, was described by Sato et al. (2003). pCIYVV-SeIF4E, pCIYVV-ReIF4E, and pCIYVV-SeIF4E-GFP (Fig. 1) were constructed as described for pCIYVV-GFP by Masuta et al. (2000). The *eIF4E* cDNAs derived from the susceptible pea line PI

250438 (*SeIF4E*) and the resistant pea line PI 378159 (*ReIF4E*) were inserted between P1 and HC-Pro in pCIYVV to produce pCIYVV-*SeIF4E* and pCIYVV-*ReIF4E*, respectively. For pCIYVV-*SeIF4E*-GFP, the *SeIF4E* was inserted into pCIYVV/GFP-*Cl*/CP/W, which is identical to pCIYVV/GFP/CP/W (Wang et al. 2003) except that it lacks the *Cl*I site in *GFP*. All plasmids were used to particle-bombard broad bean (*Vicia faba*) plants (Andrade et al. 2007; Gal-On et al. 1997). The virus particles were subsequently recovered from systemically infected leaves and used as inoculum.

Detection of *eIF4E* in CIYVV by RT-PCR

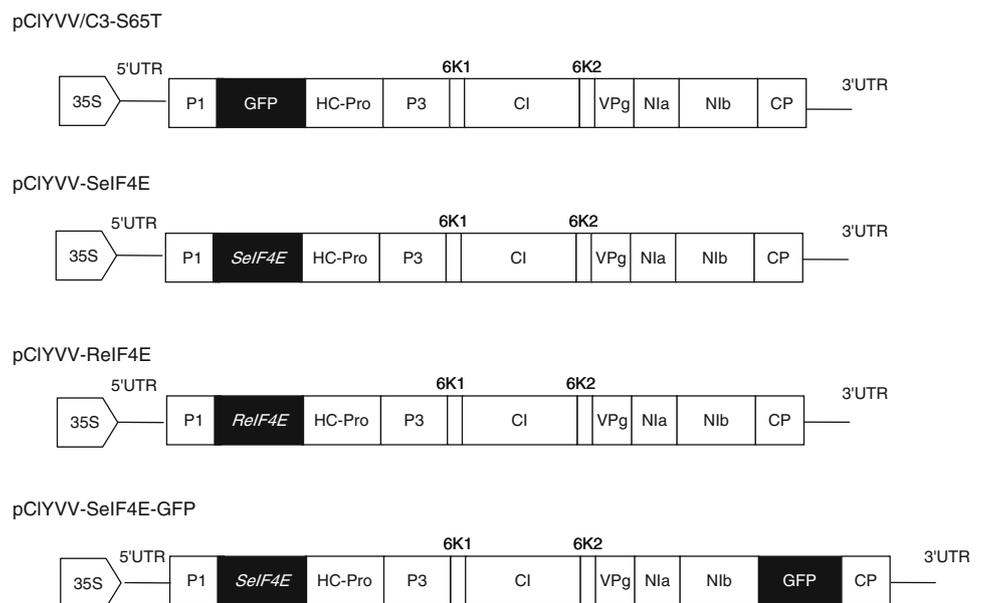
RT-PCR was used to detect viral infection and the integrity of the insert in plants inoculated with CIYVV-*SeIF4E* and CIYVV-*ReIF4E*. Approximately 0.1 g of the youngest and most apical leaves were macerated in extraction buffer (0.1 M Tris, pH 7.5, 0.05 M EDTA, and 1% mercaptoethanol). The extract was then mixed with 20% chloroform (v/v) and centrifuged. The aqueous phase was collected, and 20% PEG + 2.5 M NaCl was added to a final concentration of 4% PEG. This solution was then centrifuged, and the precipitate was suspended in 100 µl of TE (pH 8.0). Next, 500 µl of TRIzol reagent (Invitrogen) was added, and the solution was centrifuged again; the supernatant was then treated with chloroform/isoamyl alcohol. Total RNA was precipitated in ethanol and suspended in distilled water. The cDNA was then synthesized using cloned avian myeloblastosis virus (AMV) reverse

transcriptase (Invitrogen) with an oligo-dT primer (Takara Bio) as per the manufacturer’s specifications. For detecting the insertion of *eIF4E*, the cDNA was used as a template for PCR to amplify a 1,000-bp fragment with the primers 5’CATGCACATAATTGTTAACC-3’ and 5’-GTGTGTC CAATGTCTTTTGG-3’ and the following program: 94°C for 5 min; followed by 28 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s; with a final hold at 72°C for 5 min. This primer pair was designed to hybridize to the end of P1 and the start of HC-Pro to detect *eIF4E*, which was inserted between the P1 and HC-Pro regions. An RT-PCR-based assay was carried out at 21 dai. To detect a systemic infection, we used the cDNA as a template for PCR to amplify a 600-bp fragment within the coat protein-coding region using the primers 5’-AATGTTGGTGAGCAAC AA-3’ and 5’-CATACCCGACGTCTCTTTAG-3’ under the following conditions: 94°C for 5 min; followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 1 min; with a final hold at 72°C for 5 min.

Allelism test

Pea line PI 378159 (father) was crossed with line PI 193835 (mother). To confirm successful crossing between the susceptible and resistant lines, we isolated genomic DNA from the parental lines and progeny using DNAzol reagent (Invitrogen). The primers 5’CCTGAGTCATC-ACATAGGAGAT-3’ and 5’-GCAGAAGTATTTGACTT GATGGAA-3’ (Loridon et al. 2005) were then used to amplify a polymorphic band of approximately 300 bp.

Fig. 1 pCIYVV derivatives carrying *eIF4E* from the susceptible and resistant lines PI 250438 (pCIYVV-*SeIF4E*) and PI 378159 (pCIYVV-*ReIF4E*), respectively. pCIYVV-*SeIF4E*-GFP carries both *GFP* and *SeIF4E*



Results

Association of resistance with differences in the amino acid sequence of *eIF4E*

We previously described two different modes of recessive resistance in pea, represented by PI 378159 and PI 347295 (Andrade et al. 2007). A separate study showed that CIYVV requires *eIF4E* to establish an infection in *A. thaliana* (Sato et al. 2005). We therefore analyzed whether *eIF4E* is involved in recessive resistance in pea by searching for differences in the amino acid sequence of *eIF4E* between susceptible and resistant lines. Total RNA was extracted from pea leaves, and cDNAs to *eIF4E* were generated by RT-PCR. Five substitutions were detected in the *eIF4E* amino acid sequences from susceptible line PI 250438 and the resistant line PI 378159 (Fig. 2). In contrast, the sequence from PI 347295, another resistant line, was the same as that from PI 250438, suggesting that *eIF4E* is involved in resistance in pea line PI 378159 but not in PI 347295. The *eIF4E* from pea line PI 378159 was thus identified as a resistant allele of *eIF4E* (*ReIF4E*), and that from susceptible line PI 250438 was identified as a susceptible allele of *eIF4E* (*SeIF4E*).

The amino acid sequence of *ReIF4E* was the same as that from PI 193835, which reportedly carries *sbm-1* (Gao et al. 2004b) and *wlv* (Bruun-Rasmussen et al. 2007). As shown in Fig. 2, five amino acid differences have been reported to be correlated with the resistance of *sbm-1*, four of which are also said to be correlated with the resistance of *wlv* to BYMV-W. Because *sbm-1* and *wlv* are tightly linked to *cyv-2*, we examined whether the same five amino acid differences were correlated with *cyv-2* resistance in pea. The cDNAs encoding the ORF in *eIF4E* were generated from the pea lines PI 193586, PI 347465, PI 347466, PI 347467, and PI 347492, which carry *cyv-2*, and sequenced. Each of the lines that carried *cyv-2* was found to have the same five mutations reported in *sbm-1* by Gao et al. (2004b).

Involvement of *eIF4E* in the resistance of pea lines carrying *ReIF4E*

To test whether *eIF4E* is responsible for the resistance of line PI 378159, *SeIF4E* and *ReIF4E* were inserted into pCIYVV to create pCIYVV-*SeIF4E* and pCIYVV-*ReIF4E*, respectively (Fig. 1). Viruses carrying the *eIF4Es* were recovered from infected broad bean leaves that had been particle-bombarded with the plasmids. Gene *eIF4E* was stably expressed after inoculation and maintained in the CIYVV genome (data not shown). Systemic mosaic and vein clearing, which is typical of CIYVV infections in non-

necrotic lines (Ravelo et al. 2007), appeared in line PI 378159 after inoculation with the virus recovered from pCIYVV-*SeIF4E* (Fig. 3a). Systemic infection by CIYVV carrying *eIF4E* was confirmed by RT-PCR analysis of the CIYVV genome using RNA extracted from symptomatic pea leaves (Fig. 3b). CIYVV carrying *eIF4E* could not infect PI 347295 plants carrying *SeIF4E*, based on symptom observation and RT-PCR analysis of the CIYVV genome (Table 1). As a negative control, PI 378159 was inoculated with the virus recovered from pCIYVV-*ReIF4E*; the plants were not infected. A systemic GFP signal was also detected when PI 378159 was inoculated with CIYVV-*SeIF4E*-GFP (Fig. 3c).

Complementation testing involving mechanical inoculation of the virus from CIYVV-*SeIF4E* into several pea lines was also conducted. As expected, PI 162909 and PI 429853 plants carrying *SeIF4E* were not infected (Table 1). Resistance in these lines obviously is controlled by a different resistance mode other than involvement of a mutant *eIF4E* (*ReIF4E*). A gene involved in resistance of these lines has not been identified. On the other hands, PI 356986 and PI 378158 carrying *ReIF4E* were infected (Table 1). The presence of two complementation groups among resistant pea lines correlates well with the two modes of resistance described by Andrade et al. (2007).

The resistance gene in PI 378159 is allelic to *cyv-2*

Pea line PI 378159 carries the same *eIF4E* allele and resistance mode as pea lines carrying *cyv-2*. Therefore, we next examined whether the resistance gene present in PI 378159 is *cyv-2*. Because the pea line PI 193835 carries *sbm-1* (Hagedorn and Gritton 1973; Provvidenti and Hampton 1993), *wlv* (Provvidenti and Hampton 1993), and *cyv-2* (Provvidenti 1987), we crossed it with PI 378159. Molecular analysis with a single marker indicated that all eight F1 plants obtained were successfully crossed. Each F1 plant was then mechanically inoculated with CIYVV/C3-S65T. Three PI 250438 plants, which were simultaneously inoculated as a susceptible control, developed mosaic symptoms and GFP fluorescence on the upper noninoculated leaves at 7 dai. RT-PCR analysis at 21 dai revealed that none of the F1 plants were infected, indicating that the resistance gene in PI 378159 is *cyv-2*.

Resistance conferred by *cyv-2* is mediated by *eIF4E*

Andrade et al. (2007) previously reported that resistance in PI 378159 differs depending on the method of inoculation. No infection occurs after mechanical inoculation with viral

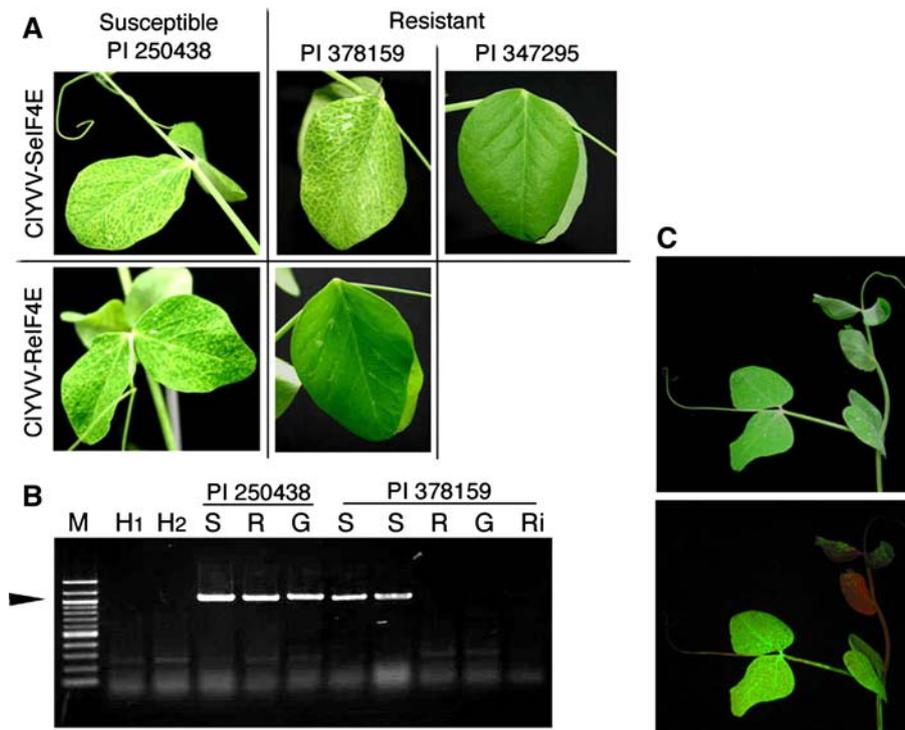


Fig. 3 Resistant pea line PI 378159 inoculated with either CIYVV-SeIF4E or CIYVV-SeIF4E-GFP. **a** Symptom expression in systemically infected leaves of PI 378159 at 11 days after inoculation. Vein clearing was observed on a systemically infected leaf of pea line PI 378159 inoculated with CIYVV-SeIF4E but not with CIYVV-ReIF4E. No symptoms were observed on an upper non-inoculated leaf of pea line PI 347295, even in the presence of CIYVV-SeIF4E. **b** Agarose gel (1.5%) electrophoresis of RT-PCR products. Total RNA was extracted from susceptible line PI 250438 and the resistant line PI 378159 11 days after inoculation and subjected to RT-PCR to amplify a 1,000-bp fragment (arrowhead marks expected position).

Tissues were collected from non-inoculated upper (lanes S, R, and G) or inoculated (lane Ri) leaves of plants inoculated with either CIYVV-SeIF4E (lane S), CIYVV-ReIF4E (lanes R and Ri), or CIYVV/C3-S65T (lane G). A primer pair complementary to the end of P1 and the start of HC-Pro was used to detect the *eIF4E* insertion, located between the P1 and HC-Pro regions. M molecular size marker. RNA was prepared from healthy PI 250438 (H1), PI 378159 (H2), inoculated PI 250348, and PI 378159 plants. **c** A GFP signal (lower panel) was systemically detected in PI 378159 (upper panel) inoculated with CIYVV-SeIF4E-GFP, confirming a loss of resistance in PI 378159 due to CIYVV expressing *SeIF4E*

Table 1 Complementation of *Clover yellow vein virus* (CIYVV) infection through the expression of susceptible *eIF4E* in pea lines having two different modes of resistance

Pea line	<i>eIF4E</i> Genotype	Infection ^a		
		CIYVV-SeIF4E	CIYVV-ReIF4E	CIYVV/C3-S65T
PI 250438	Susceptible	+	+	+
PI 162909	Susceptible	–	NT	–
PI 347295	Susceptible	–	NT	–
PI 429853	Susceptible	–	NT	–
PI 356986	Resistant	+	NT	–
PI 378158	Resistant	+	NT	–
PI 378159	Resistant	+	–	–

+ : Infection positive, – : infection negative, NT: not tested

^a Three to four pea plants were mechanically inoculated with sap from broad bean plant infected with one of the viruses. CIYVV-SeIF4E has *eIF4E* from susceptible pea PI 250438, CIYVV-ReIF4E has *eIF4E* from resistant pea PI 378159; infection was examined by RT-PCR of the *eIF4E* insert. CIYVV/C3-S65T with green fluorescent protein (GFP) (Sato et al. 2003); infection was determined by GFP monitoring and ELISA

particles CIYVV/C3-S65T. But when plants are inoculated with infectious cDNA from pCIYVV/C3-S65T via particle bombardment, the plants do become infected. The virus then spreads slowly to neighboring cells from the site of infection.

Given that pea lines known to carry *cyv-2* have been shown to carry *ReIF4E*, we next investigated whether *ReIF4E* controls the resistance conferred by *cyv-2*. Pea lines known to carry *cyv-2* were mechanically inoculated with CIYVV/C3-S65T to assess their resistance to isolate 30 of CIYVV. No GFP fluorescence was observed in either the inoculated or upper non-inoculated leaves; moreover, the virus was not detected by ELISA at 21 dai, indicating that these lines were indeed resistant to CIYVV (Table 2). We next inoculated the same lines with CIYVV-SeIF4E-GFP (Fig. 1). The virus was able to systemically infect the plants, as demonstrated by GFP expression analysis and ELISA (Table 2). These data indicate that resistance in pea lines carrying *cyv-2* is also controlled by *eIF4E*.

We next examined the resistance modes of pea lines carrying *cyv-2* by monitoring GFP fluorescence after particle bombardment with pCIYVV/C3-S65T (Fig. 4). GFP fluorescence was first detected in a single cell at 1 dai, and then spread to neighboring cells at 3–5 dai. At 7–9 dai, fluorescence was detected along the veins. Viral spread as indicated by GFP fluorescence was essentially the same as that described for PI 378159 (Andrade et al. 2007).

Table 2 Complementation of *Clover yellow vein virus* (CIYVV) infection through the expression of susceptible *eIF4E* in pea lines carrying *cyv-2* resistant gene

Pea line	Infection ^a	
	CIYVV-C3/S65T ^b	CIYVV-SeIF4E-GFP ^c
PI 250438	+	+
PI 193586	–	+
PI 193835	–	+
PI 347465	–	+
PI 347466	–	+
PI 347467	–	+
PI 347492	–	+

+: Infection positive, –: infection negative

^a Six pea plants were mechanically inoculated with sap from infected broad bean plants. Infection was determined by green fluorescent protein (GFP) monitoring and ELISA

^b CIYVV carrying GFP (Sato et al. 2003)

^c CIYVV carrying both GFP and *eIF4E* from susceptible PI 250438

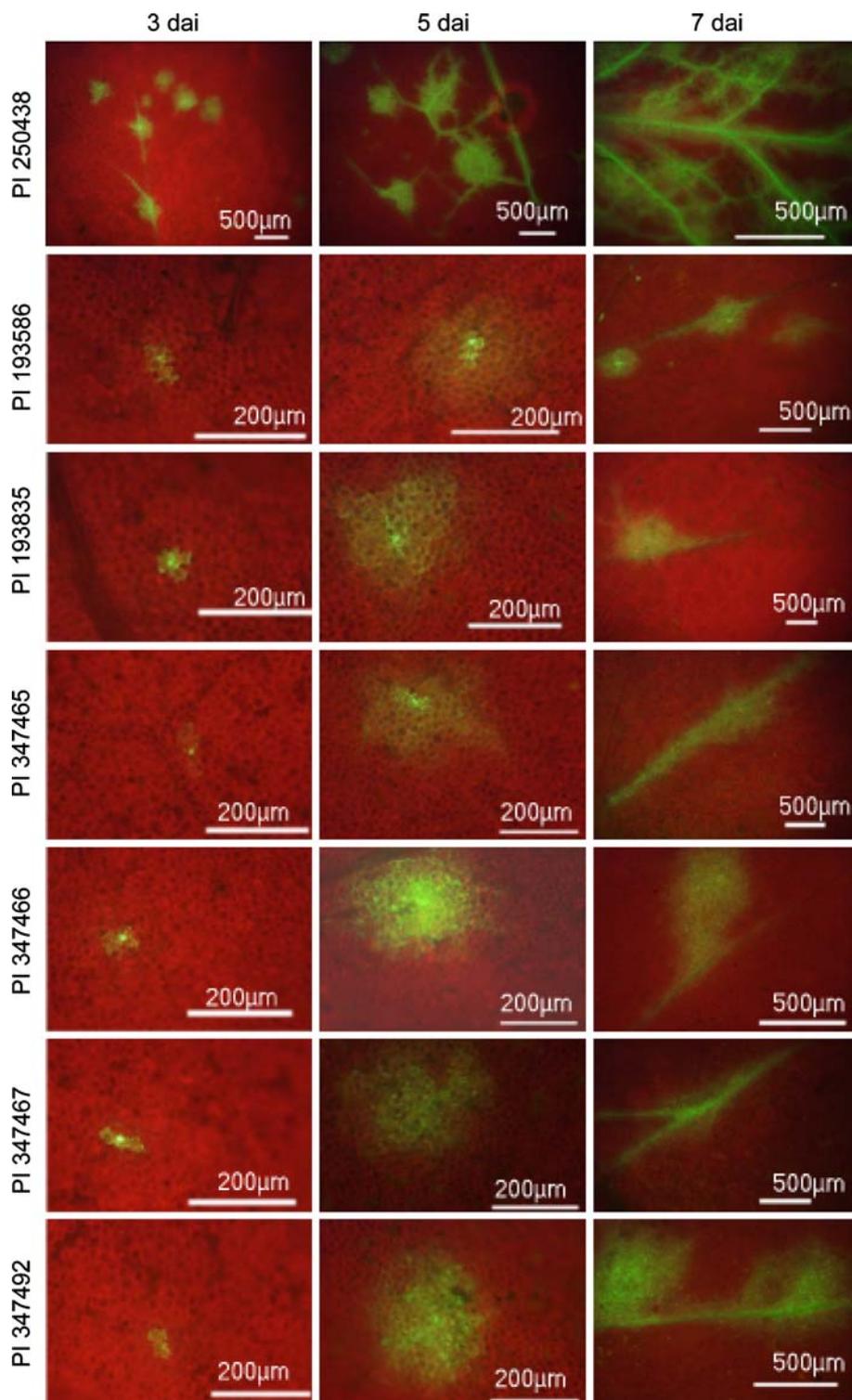
Discussion

Provvidenti and Hampton (1991) previously reported that *cyv-2*, *sbm-1*, and *wlv* are part of the same linkage group and are closely linked. Here, we found that PI 378159 carries a *cyv-2* resistance gene that is identical to a mutant allele of *eIF4E* that confers resistance to PSbMV (*sbm-1*) (Gao et al. 2004b) and BYMV-W (*wlv*) (Bruun-Rasmussen et al. 2007).

It is interesting that the same mutant allele of *eIF4E* confers resistance to several distinct viral species within the genus *Potyvirus*. However, the mode of resistance mediated by *eIF4E* is virus-dependent. PSbMV was restricted to a single cell when infectious cDNA was used in the particle bombardment of resistant leaves of JI1405, whereas CIYVV was able to spread to neighboring cells (Andrade et al. 2007; Gao et al. 2004b). In contrast, the interactions of CIYVV with PI 378159 are similar to those of BYMV with pea lines carrying *wlv*. When resistant pea line PI 193835 was agro-inoculated with BYMV-W using full-length viral cDNA, a variant virus capable of systemically infecting PI 193835 was generated at a low frequency. When infectious cDNA of CIYVV was used in the particle bombardment of the resistant pea line PI 378159, resistance-breaking variants were frequently detected (Andrade et al. 2007).

That the same allele confers resistance to three different viruses indicates that the components required for infection by diverse potyviruses in pea are conserved. It is well known that *eIF4E* or *eIF(iso)4E* is involved in the infection of several potyviruses, including CIYVV; however, their exact role in the infection process is unclear. At the very least, the early translation of viral genes occurs in resistant pea lines. Mechanical inoculation with viral particles did not result in infection, but viral particles carrying *SeIF4E* were fully infectious after mechanical inoculation of PI 378159. Thus, the first round of viral gene translation in resistant pea lines appears to proceed without complementation by *SeIF4E* from susceptible pea upon the initial event of viral multiplication. Robaglia and Caranta (2006) suggested three roles for *eIF4E* or *eIF(iso)4E* during viral multiplication. One possibility is that *eIF4E* functions in the initiation of minus-strand synthesis. This idea is a tempting hypothesis because *Vpg*, a key gene in viral replication, is known to bind *eIF4E* in the case of *Turnip mosaic virus* or *Potato virus Y* (Charron et al. 2008; Wittman et al. 1997). Furthermore, a resistance-breaking gene of BYMV in pea carrying *wlv* was mapped to *Vpg* (Bruun-Rasmussen et al. 2007). We have described several resistance-breaking mutant viral isolates

Fig. 4 Infection by and spread of *Clover yellow vein virus* (CIYVV) in six resistant pea lines and one susceptible line as monitored by fluorescence of green fluorescent protein. Resistant lines carrying *cyv-2* (PI 193586, PI 193835, PI 347465, PI 347466, PI 347467, and PI 347492) and susceptible pea line PI 250438 were particle-bombarded with pCIYVV/C3-S65T. The infection was monitored at 3, 5, and 7 days after inoculation (dai)



(Andrade et al. 2007); it would be interesting to test whether a mutation in *Vpg* makes resistant pea lines susceptible.

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