Mapping and candidate-gene screening of the novel Turnip mosaic virus resistance gene *retr02* in Chinese cabbage (*Brassica rapa L.)*

Wei Qian[1] · Shujiang Zhang[1] · Shifan Zhang[1] · Fei Li[1] · Hui Zhang[1] · Jian Wu[1] · Xiaowu Wang[1] · John A. Walsh[2] · Rifei Sun[1]

[1]Department of Chinese Cabbage, Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing 100081, China

[2]School of Life Sciences, University of Warwick, Wellesbourne, Warwick, CV35 9EF, UK

Corresponding author:

Dr. Rifei Sun

Tel: +86-10-82109511

Fax: +86-10-62174123

E-mail: rifei.sun@caas.net.cn

Abstract The extreme resistance to Turnip mosaic virus (TuMV) observed in the Chinese cabbage (*Brassica rapa*) line, BP8407, is monogenic and recessive. Bulked segregant analysis was carried out to identify simple sequence repeat (SSR) and Indel markers linked to this recessive resistance gene, termed *recessive Turnip mosaic virus resistance 02* (*retr02*). Mapping of PCR-specific Indel markers on 239 individuals of a BP8407 × Ji Zhao Chun F_2 population, located this resistance gene to a 0.9-cM interval between two Indel markers (BrID10694 and BrID101309) and in scaffold000060 or scaffold000104 on chromosome A04 of the *B. rapa* genome. Eleven eukaryotic initiation factor 4E (*eIF4E*) and 14 eukaryotic initiation factor 4G (*eIF4G*) genes are predicted in the *B. rapa* genome. A candidate gene, Bra035393 on scaffold000104, was predicted within the mapped resistance locus. The gene encodes the eIF(iso)4E protein. Bra035393 was sequenced in BP8407 and Ji Zhao Chun. A polymorphism (A/G) was found in exon 3 between BP8407 and Ji Zhao Chun.

This gene was analysed in four resistant and three susceptible lines. A correlation was observed between the amino acid substitution (Gly/Asp) in the eIF(iso)4E protein and resistance/susceptibility. eIF(iso)4E has been shown previously to interact with the TuMV genome-linked protein, VPg.

Keywords *Brassica rapa*·TuMV resistance·mapping·gene candidates

Introduction

Chinese cabbage (*Brassica rapa L.*) originates in China and is one of its most important vegetable crops in the world, with the largest planting area and yield (Cao et al. 2006), however, outbreaks of Turnip mosaic virus (TuMV) greatly decrease yield and quality.

TuMV is a member of the *Potyvirus* genus (family *Potyviridae*) and is the only *potyvirus* known to infect brassicas (Tomlinson 1987; Walsh and Jenner 2002). It has the widest host range of any member of the *Potyvirus* genus (Shukla et al. 1994), infecting a wide range of cultivated plant species (Edwardson and Christie 1991) and causing significant economic loss in brassica crops (Shattuck 1992). TuMV was first described in *B. rapa* by Gardner and Kendrick (1921) and Schultz (1921) and has since been widely studied. It is difficult to control because of its wide host range and non-persistent stylet-borne mode of transmission by aphids (Walsh and Jenner 2002). Chemical control of the disease is ineffective, so natural plant resistance is likely to be the most effective method of control (Hughes et al. 2002). Resistances have been identified in *B. rapa* that are effective against a broad range of TuMV isolates (Suh et al. 1995; Hughes et al. 2002; Walsh et al. 2002).

A number of different pathotypes of TuMV have been identified. Four strains, C1-4, were described from Chinese cabbage (*Brassica rapa ssp. Pekinensis*) in 1980 (Provvidenti 1980) and a fifth strain in 1985 (Green and Deng 1985). Liu et al. (1990a, b) identified 19 TuMV isolates from 10 areas of China and defined seven strains (Tu1-7) with a new set of differentials. Jenner and Walsh (1996) described 12 different pathotypes based on the interactions of 124 TuMV isolates with *Brassica napus* lines. Plant resistance genes to TuMV have been mapped in lettuce (Robbins et al. 1994)

and brassicas (Walsh and Jenner 2002). Most of the genes mapped in brassicas are specific to certain TuMV isolates, being found in *B. napus* and *B. rapa* (Walsh and Jenner 2002). Most of them are dominant, such as *TuRB01* (Walsh et al. 1999), *TuRB03* (Hughes et al. 2003), *TuRB04-05* (Jenner et al. 2002, 2003), and *TuRB01b* (Rusholme 2000). *TuRB01* is a single dominant resistance gene and was the first TuMV resistance gene described in *Brassica*. Dominant resistance genes (*R* genes) control resistance to narrow spectra of TuMV isolates. Broad-spectrum resistance has also been identified, and appears to be controlled by the combined action of a recessive (*retr01*) and a dominant (*ConTR01*) gene (Rusholme 2000; Walsh and Jenner 2002; Rusholme et al. 2007).

Molecular markers have been used to position TuMV resistance genes on genetic linkage maps (Walsh et al. 1999; Rusholme 2000; Hughes et al. 2003; Rusholme et al. 2007), which provide a powerful tool to facilitate marker-assisted selection for TuMV-resistant *Brassica* varieties. Five TuMV resistance genes and one quantitative trait locus (QTL) have been mapped in *B. rapa* and *B. napus* (Walsh and Jenner 2002). Zhang et al. (2008a) reported four QTLs controlling TuMV-C4 resistance in Chinese cabbage. Zhang et al. (2008b) reported four QTLs controlling TuMV-C3 resistance in Chinese cabbage and Zhang et al. (2009) reported three QTLs controlling TuMV-C4 resistance in Chinese cabbage. Han et al. (2004) found a dominant marker linked to TuMV-C5 resistance in Chinese cabbage. Zhang et al. (2006) noted two EST-PCR-RFLP markers linked to TuMV-C3 resistance in Chinese cabbage.

This paper describes molecular markers linked to a resistance gene effective against TuMV-C4 in *B. rapa* and the genetic and physical mapping of the gene *retr02* in Chinese cabbage, using the bulked segregant analysis (BSA) approach of Michelmore et al. (1991). The candidate TuMV resistance gene is described and sequences from resistant and susceptible plants provided.

Materials and Methods

Plant materials

The Chinese cabbage accessions BP8407 and Ji Zao Chun are homozygous

breeding lines. BP8407 is resistant to a TuMV isolate belonging to the pathotype C4 described by Provvidenti (1980) and Ji Zao Chun is susceptible to this TuMV isolate. F_1 and F_2 populations were produced from a cross between BP8407 and Ji Zao Chun.

TuMV isolate and phenotypic assessments

TuMV isolate (pathotype C4; Institute of Vegetables and Flowers Chinese Academy of Agriculture Sciences, IVF-CAAS) was used to inoculate plants to determine resistance and susceptibility. It was maintained in the susceptible mustard (*Brassica juncea*) cultivar Tender Green. BP8407 (20 individuals), Ji Zao Chun (20 individuals), F_1 (20 individuals) and F_2 (239 individuals) were inoculated with the TuMV C4 isolate. In the disease assays, the second and third true leaves of plants were mechanically inoculated at the three true-leaf stage (Jenner and Walsh 1996). Resistance (the absence of systemic spread) was established by ELISA on the non-inoculated fourth and fifth leaves, 4 weeks post-inoculation (Jenner et al. 1999; Walsh et al. 1999). The ELISA reagents were from Agdia Inc. (Indiana, USA) and absorbances were measured at 405nm on a Microplate Reader (BioTek, ELx800808).

Statistical analysis

Segregation data (susceptibility and resistance) for the F_2 generation was analysed by chi-square for goodness of fit to the expected segregation ratios.

Microsatellite and Indel markers assay

Upon completion of disease assessments, leaves were collected from BP8407, Ji Zao Chun, F_1 and F_2 individuals. Each leaf sample was immediately frozen in liquid nitrogen and freeze-dried for 3 days. Leaf materials were stored in a drying cupboard until required.

Genomic DNA was extracted from dried leaves by the conventional cetyltrimethyl ammonium bromide (CTAB) method and stored at –20°C. One resistant and one susceptible DNA bulk were made (10 resistant, or 10 susceptible F_2 individuals/bulk) after genomic DNA had been extracted from individual plants.

Primers for *B. rapa* microsatellites and Indels were obtained from the Biotechnology Department of IVF-CAAS. The SSR and Indel marker amplification reactions were performed in 15μl reaction mixtures containing 0.3μl forward primer, 0.3μl reverse primer, 50ng DNA, 1.5μl 10×PCR buffer (containing $MgCl_2$), 1.2μl 0.8mM dNTP, and 0.5U Ampli Taq Gold. The SSR conditions were 10 min at 95°C; 36 cycles of 40s at 95°C, 40s at 57°C, 40s at 72°C; and finally, 10 min at 72°C.

Linkage analysis and resistance gene mapping

Linkage between DNA markers and the TuMV resistance locus was established using Joinmap 4.0 software on 239 F_2 individuals. The Kosambi function was applied to convert recombination fractions into map distances. The resistance gene was mapped on the genetic and physical maps of the *B. rapa* genome (Wang et al. 2011;)

Candidate gene prediction and analysis

The *B. rapa* genome has been sequenced and the *Brassica* database (BRAD) includes the predicted genes and associated annotations (InterPro, KEGG2, SwissProt), *B. rapa* genes orthologous to those in *Arabidopsis thaliana* and the genetic markers and maps of *B. rapa*.

Conserved domains and motifs were identified using hmmpfam comparison to pfam (Finn et al. 2008). Protein sequences of candidate eukaryotic initiation factor 4E (*eIF4E*) genes were compared to the Pfam v23 hidden Markov models (HMMs) using HMMER 2.3.2 (Eddy 2003; Mun et al. 2009). To classify the candidate *eIF4E* and eukaryotic initiation factor 4G (*eIF4G*) genes based on domain structure, each sequence was examined for domain regions: the eIF4E and eIF4G domains plus regions using the Multiple Expectation Maximization for Motif Elicitation (MEME) program (Bailey and Elkan 1995; Mun et al. 2009).

In *A. thaliana*, the TuMV resistance gene *lsp* (AT5G35620) has been cloned (Lellis et al. 2002). We used the AT5G35620 sequence in a blastn search of the *B. rapa* genome (http://brassicadb.org/brad/) to identify the physical positions of the orthologues/paralogues.

Sequence analysis of the candidate gene

Generic primers were designed using the candidate gene sequence from the *B. rapa* genome, to encompass the majority of the open reading frame (ORF). PCR was performed on genomic DNA using standard protocols with Taq DNA polymerase. The full-length candidate gene was sequenced in the BP8407 and Ji Zhao Chun lines.

To analyse the allelic variability of the gene, a PCR fragment was amplified from genomic DNA of the TuMV-susceptible line Ji Zao Chun and TuMV-resistant line BP8407. Another two TuMV C4-susceptible lines 80403 (Chun Da Jiang) and 80461 (Qiang Shi) and a further three TuMV C4-resistant lines (80124 (89B), 80186 (Er Qing) and Chiifu) were sequenced. Finally, we compared the allelic variation of the gene in these six lines.

Results

Resistance spectra of BP8407, Ji Zao Chun, and the F_1 progeny

Challenge of Chinese cabbage (BP8407, Ji Zao Chun and F_1) plants with the TuMV C4 isolate caused different symptoms and developmental defects within 20 days post inoculation (d.p.i). BP8407 had no symptoms of virus infection and no TuMV capsid protein (CP) was detected by ELISA. A_{405} values for BP8407 were similar to non-inoculated control plants. Ji Zao Chun and F_1 plants were stunted, had reduced apical dominance and mosaic patterns on the leaves following TuMV inoculation and TuMV CP was detected in their leaves by ELISA. Thus, the BP8407 line was resistant to the TuMV isolate, whereas the Ji Zao Chun and F_1 plants were susceptible (Fig. 1).

Genetic analysis of resistance

BP8407 line plants were all resistant to TuMV C4, but the Ji Zao Chun line and F_1 progeny were all susceptible to the TuMV isolate, indicating that TuMV resistance was recessive. The segregation data from the TuMV inoculation of the F_2 generation (52 resistant and 187 susceptible) fitted the expected segregation for a Mendelian model based on the action of a single recessive allele ($\chi_c^2=1.34 < \chi_{0.05}^2=3.84$) (Table 1).

Identification of SSR markers linked to the gene in the parents and the bulks

According to the polymorphisms between parent 1 (P1, BP8407), parent 2 (P2, Ji Zao Chun) and F_1, 64 polymorphic SSR primer pairs were selected from 200 SSR primers screened. These 64 SSR primer pairs were used to screen a P1 plant, a P2 plant, the resistant bulk, the susceptible bulk and one F_1 plant. Only one primer pair (BC84) provided a clear polymorphism in the P1 plant, the P2 plant and F1 plant. The resistant bulk was coincident with the P1 plant and the susceptible bulk was coincident with the P2 plant. (Fig. 2A).

Development of Indel markers and anchoring on a genetic reference map

To map the TuMV resistance locus in the F_2 population, we developed Indel markers from the BC84 marker identified as linked to the resistance gene. The SSR marker BC84 locus was located on scaffold000048 of *B. rapa* chromosome A04 of the sequenced-based genetic map of *B. rapa* (Wang et al. 2011); scaffold000070 is adjacent to scaffold000048 and scaffold000016 is located at the other end of chromosome A04 (Wang, et al. 2011). To check the location on chromosome A04, we designed 145 Indel markers on chromosome A04 and obtained 17 indel markers (on scaffold000048, scaffold000070, scaffold000060, scaffold000104, scaffold000083, scaffold040552, scaffold040579, scaffold000235, scaffold000177 and scaffold000016, all located on chromosome A04, Wang et al. 2011) linked to the resistance gene.

Indel markers BrID90209 (scaffold000048), BrID90211 (scaffold000048), BrID90143 (scaffold000070), BrID90275 (scaffold000016) were polymorphic (Fig. 2B). These Indel markers were genotyped on the 239 individuals of the F_2 generation. The linkage between the DNA markers and the TuMV resistance locus was established using Joinmap 4.0 software on the 239 F_2 individuals (Fig. 2C). This scoring data positioned the recessive resistance allele on chromosome A04 between Indel markers BrID90211 and BrID90275. The gene was positioned between scaffold000048 and scaffold000016 on chromosome A04.

Fine genetic and physical mapping of the resistance gene

Fifty-five Indel primers were designed for the *B. rapa* genome interval between scaffold000048 and scaffold000016; 17 of them were closely linked with the resistance gene. The gene was mapped to an interval on chromosome A04 between markers BrID10694 (0.3cM) and BrID101309 (0.6cM) on scaffold000060 or scaffold000104 (Fig.3). BrID101309 co-segregated with the resistance gene. However, there was a recombinant (a resistant individual) between BrID10694 and the resistance gene.

Candidate resistance genes

The two closest markers to the resistance gene were on two different scaffolds; therefore, it was not possible to accurately define the interval between these markers nor identify all the genes in the region. In plants, both active and passive resistance mechanisms operate against viruses. Passive resistance mechanisms are always related to the absence of susceptibility factors, such as eIF4E, eIF(iso)4E, eIF4G, and eIF(iso)4G. Therefore, we hypothesized that an *eIF4* gene would be responsible for the observed resistance. We identified all the *eIF4E*, *eIF(iso)4E*, *eIF4G*, *eIF(iso)4G* genes in the *B. rapa* genome, based on the domains and motifs found using hmmpfam. eIF4E and eIF(iso)4E share some similar domains and motifs, as do eIF4G and eIF(iso)4G. A total of 11 *eIF4E* and 14 *eIF4G* gene candidates were identified from the genomic sequences (Table 2).

In *A. thaliana*, a TuMV recessive resistance gene *lsp* (AT5G35620) that encodes *eIF(iso)4E* has been cloned. We used AT5G35620 as a blastn query sequence against the *B. rapa* genome to identify a candidate resistance gene among the 11 *eIF4E* and 14 *eIF4G* genes. We identified three candidate genes similar to AT5G35620 from the 11 *eIF4E* and 14 *eIF4G* genes (Fig. 4A). These were *Bra035393*, *Bra035531* and *Bra039484*, all of which encode the eIF(iso)4E protein. The three candidate genes and AT5G35620 have the same eIF(iso)4E domain and encode eIF(iso)4E proteins. By searching for genes in the sub-genomes of *B. rapa* that are

syntenic for AT5G35620, we identified it as *Bra035393* (http://brassicadb.org/brad/searchSynteny.php).

The TuMV resistance gene was mapped to scaffold000104, or scaffold000060 and *Bra035393* is the syntenic, orthologous gene for AT5G35620 on scaffold000104. Therefore, *Bra035393* is the candidate resistance gene, which encodes eIF(iso)4E, one of two cap binding protein isoforms that are known to interact with the potyviral genome-linked protein, VPg.

Characterisation of the candidate resistance gene *Bra035393*

To characterise the transcribed sequence of the candidate gene *Bra035393*, primers (Table 3) were designed to sequence the full length of the candidate resistance gene *Bra035393* of BP8407 and Ji Zao Chun. The cDNA and protein sequences were then used as queries in blast searches. The cDNA of *Bra035393* includes a 600 nucleotide coding sequence. The corresponding genomic fragment on both BP8407 and Ji Zao Chun is 1319 nucleotides in length and comparison with the cDNA uncovered five exons (200bp, 175bp, 125bp, 67bp, and 32bp) and four introns (63bp, 83bp, 488bp, and 86bp) (Fig. 4B). The coding sequence encodes a protein of 199 amino acid residues.

The coding sequences of *Bra035393* from BP8407 and Ji Zao Chun were compared and only a single nucleotide polymorphism between the parents, (SNP; A/G), in exon3 was identified, 455 nucleotides downstream from the ATG. The SNP results in an amino acid substitution (Gly/Asp, amino acid 152) in eIF(iso)4E (Fig. 5). *Bra035393* was sequenced from the 52 resistant individuals (including the recombinant between BrID10694 and *retr02*) and all had A at position 455, as in BP8407. The gene was also sequenced from thirteen susceptible individuals. Ten homozygotes had G at position 455 and three heterozygotes had A and G at this position.

To investigate this difference, a further two susceptible lines – 80403 (Chun Da Jiang) and 80461(Qiang Shi) - and three further resistant lines – 80124 (89B), 80186 (Er Qing) and Chiifu - were sequenced. The base in 80124, 80186 and Chiifu at the

SNP position was the same as in BP8407 (A). In 80403 and 80461, it was the same as Ji Zao Chun (G). Hence, in the resistant lines, the SNP base in exon 3 is A (amino acid Asp) and in the susceptible lines, the SNP base in exon 3 is G (amino acid Gly). It is possible that these base differences in exon 3 may determine the interaction with the VPg of TuMV.

Discussion

Here we report the genetic and physical mapping of a TuMV resistance gene, *retr02*, in the Chinese cabbage BP8407 line. Eight resistance genes have been mapped in *Brassica*, *TuRB01* and *TuRB02* (Walsh et al. 1999), *TuRB03* (Hughes et al. 2003), *TuRB04* and *TuRB05* (Jenner et al. 2002, 2003), *TuRB01b* (Rusholme 2000), and *retr01* and *ConTR01* (Rusholme 2000; Walsh and Jenner 2002; Rusholme et al. 2007). These genes were mapped with RFLP, AFLP, and SSR markers. The resistance gene *retr02* was mapped with PCR-based insertion/deletion (Indel) markers in our study. These markers were developed from *B. rapa* genomic sequence and a sequence-based genetic linkage map was constructed (Wang et al. 2011). The Indel markers covered the *B. rapa* genome and were used as the basis for fine mapping of the gene.

In *B. rapa*, broad-spectrum resistance appears to be controlled by the combined action of a recessive (*retr01*) and a dominant (*ConTR01*) gene (Rusholme 2000; Walsh and Jenner 2002; Rusholme et al. 2007). The recessive resistance gene, *retr01*, mapped to the upper portion of chromosome A04 and was probably coincident with an *eIF(iso)4E* gene. The resistance gene in the present study could be an allele with *retr01* as they both correspond to the only copy of *eIF(iso)4E* on A04, hence the resistance gene was named *retr02*. *retr02* may be a broad-spectrum resistance gene that could be used in marker-assisted selection for TuMV-resistant *Brassica* varieties. *retr01* was mapped in the BP079 and R-o-18 lines; however, *retr02* was mapped from BP8407 and Ji Zao Chun lines. Ji Zao Chun and R-o-18 are different susceptible lines. The F_2 population (from the BP8407×Ji Zao Chun cross) has three genotypes: RR, Rr, and rr. We took 10 F_3 families derived from each of the three F_2 genotypes at random

and inoculated 10 plants in each F_3 family with the TuMV C4 isolate. The ELISA results showed that the 10 F_3 lines from genotype RR F_2 individuals were uniformly susceptible, the 10 F_3 lines from genotype rr F_2 individuals were uniformly resistant, whereas the 10 F_3 lines from genotype Rr F_2 individuals segregated for resistance and susceptibility, hence resistance is controlled by one recessive gene. *retr02* and *retr01* appear to have different genetic inheritance. The predicted protein sequences (*BraA.eIF(iso)4E*) of R-o-18 have already been obtained (Jenner et al. 2010). Compared to *retr02* (*Bra035393*), the predicted protein sequences of the four resistant lines in our research, *BraA.eIF(iso)4E* from R-o-18 has two more amino acid differences (Asp/His, amino acid 27; Phe/Tyr, amino acid 108) in *eIF(iso)4E* on A04, compared to the other susceptible lines (Fig. 5).

Gómez, et al. (2009) stated that 51% of resistance traits to plant viruses were dominant, 35% were recessive, and the remainder were more complex (incomplete dominance or dose-dependent). Plant resistance mechanisms (active and passive) operate against viruses. The active resistance mechanisms are mediated through *R* genes and/or gene silencing. *R* genes are always dominant and have characteristic domains, such as NBS-LRR, (CC)-NBS-LRR, etc. Examples of passive resistance are mainly attributed to the absence of susceptibility factors, such as eIF4E, eIF(iso)4E, eIF4G, eIF(iso)4G, e.g. *lsp1* (Lellis et al. 2002), *pvr2* (Ayme et al. 2006), *nsv* (Nieto et al. 2006; Truniger et al. 2008), *cum2-1* (Yoshii et al. 1998), *cum1-1* (Yoshii et al. 1998), *sbm1* (Gao et al. 2004), *rymv-1* (Albar et al. 2003; Albar et al. 2006), *rym4/5* (Stein et al 2005). A number of recessive resistances to other members of the *Potyviridae* correspond to eIF4E or eIF(iso)4E (Robaglia and Caranta 2006). The natural role of eIF4E and eIF(iso)4E is the initiation of translation of capped mRNAs (Browning 1996). eIF4E binds eIF4G, which is a scaffold for the other components of the translation initiation complex. In this paper, *retr02* appears to be a recessive gene located on chromosome A04 between Indel markers BrID10694 and BrID101309 and presumably provides passive resistance. *retr02* (*Bra035393*) has one homologous, syntenic and orthologous gene, *lsp* (AT5G35620) in *A. thaliana*. The Arabidopsis gene *lsp* (AT5G35620) encodes *eIF(iso)4E*, one of two cap-binding protein isoforms known

to interact with the genome-linked protein (VPg) of potyviruses and contains a single induced point mutation, resulting in a premature stop codon. In contrast, *retr02* (*Bra035393*) also contains a single point mutation relative to the allele in susceptible plants, but it does not create a premature stop codon. TuMV can use both eIF4E and eIF(iso)4E from *B. rapa* for replication; it is also capable of using eIF4E and eIF(iso)4E from multiple loci of a single host plant when expressed ectopically in Arabidopsis, the first example of this for a plant virus (Jenner et al. 2010). *BraA.eIF(iso)4E.a* from R-o-18 complemented an Arabidopsis line (Col-0::*dSpm*) with a transposon knockout of the *eIF(iso)4E* gene (Jenner et al. 2010). *Bra035393* is the syntenic and orthologous gene to AT5G35620. A future experiment is required to determine whether *Bra035393* in Ji Zao Chun could also complement the Arabidopsis line (Col-0::*dSpm*).

In plants, eIF4E and eIF4G form the eIF4F complex and eIF(iso)4E and eIF(iso)4G form the eIF(iso)4F complex. These complexes are involved in the binding of the mRNA cap and ribosome recruitment in the initial steps of translation (Lellis et al. 2002; Albar et al. 2006). Recent research indicated that some potyviruses require at least one member of the *eIF4E/eIF(iso)4E* or *eIF4G/eIF(iso)4E* gene family to infect plants. Many potyviruses can use eIF(iso)4E, but not eIF4E, and other genera of plant viruses use eIF4E (Robaglia and Caranta 2006). TuMV can use both eIF4E and eIF(iso)4E from *B. rapa* for replication (Jenner et al. 2010). However, TuMV can use specific members of either the *eIF(iso)4E*, or both the *eIF(iso)4E* and *eIF4E* gene families in *B. rapa* (Rusholme et al. 2007). In the *B. rapa* genome, we predicted the presence of the *eIF4E* and *eIF4G* gene families: 11 *eIF4E*, and 14 *eIF4G* (Table 2). This suggests that there are potentially more resistance genes in the *eIF* family in Chinese cabbage and that their protein products may interact with the VPg of TuMV. It will be useful to sequence all these genes for comparison with those whose sequences have already been determined.

Acknowledgements

We thank Dr Binyan Xie for providing the TuMV C4 isolate. This work was supported

by Key Laboratory of Horticultural Crop Biology and Germplasm Innovation, Ministry of Agriculture.

References

Albar L, Ndjiondjop MN, Esshak Z, Berger A, Pinel A, Jones M, Fargette D, Ghesquière A (2003) Fine genetic mapping of a gene required for *Rice yellow mottle virus* cell-to-cell movement. Theor Appl Genet 107:371–378

Albar L, Reyser MB, Hébrard E, Ndjiondjop MN, Jones M, Ghesquière A (2006) Mutations in the eIF(iso)4G translation initiation factor confer high resistance of rice to *Rice yellow mottle virus*. Plant J 47:417-426

Ayme V, Souche S, Caranta C, Jacquemond M, Chadœuf J, Palloix A et al. (2006) Different mutations in the Genome-Linked Protein VPg of *Potato virus Y* confer virulence on the *pvr2*^{3} resistance in pepper. Mol Plant Microbe Interact 9:557–563

Bailey TL, Elkan C (1995) The value of prior knowledge in discovering motifs with MEME. Proc Int Conf Intell Syst Mol Biol 3:21–29

Browning KS (1996) The plant translational apparatus. Plant Mol Biol 32:107–143

Cao JS, Yu XL, Ye WZ, Lu G, Xiang X (2006) Functional analysis of a novel male fertility *CYP86MF* gene in Chinese cabbage (*Brassica campestris L. ssp. chinensis* makino). Plant Cell 24:715-723

Eddy SR (2003) HMMER user's guide. ftp://selab.janelia.org/pub/software/hmmer/CURRENT/Userguide.pdf

Edwardson JR, Christie RG (1991) A monograph on the potyvirus group. Fla Agric Exp Stn Tech Bull Monograph 16 Vol 1-4

Finn RD, Tate J, Mistry J, Coggill PC, Sammut JS, Hotz HR, Ceric G, Forslund K, Eddy SR, Sonnhammer EL, Bateman A (2008) The Pfam protein families database. Nucleic Acid Res 36:D281–D288

Gao Z, Eyers S, Thomas C, Ellis N, Maule A (2004) Identification of markers tightly linked to *sbm* recessive genes for resistance to *Pea seed-borne mosaic virus*. Theor Appl Genet 109:488–494

Gardner MW, Kendrick JB (1921) Turnip Mosaic. J Agric Res 22:123-124

Gómez P, Hernández AMR, Moury B, Aranda MA (2009) Genetic resistance for the sustainable control of plant virus diseases: breeding, mechanisms and durability. Eur J Plant Pathol 125:1–22

Green SK, Deng TC (1985) Turnip mosaic virus strains in cruciferous hosts in Taiwan. Plant Dis 69:28-31

Han HP, Sun RF, Zhang SJ, Li F, Zhang SF, Niu XK (2004) AFLP marker linked to *Turnip mosaic virus* susceptible gene in Chinese cabbage (*Brassica rapa L. ssp. pekinensis*). Agric Sci China 3:292-298

Hughes SL, Green SK, Lydiate DJ, Walsh JA (2002) Resistance to *Turnip mosaic virus* in *Brassica rapa* and *B. napus* and the analysis of genetic inheritance in selected lines. Plant Pathol 51:567-573

Hughes SL, Hunter PJ, Sharpe AG, Kearsey MJ, Lydiate DJ, Walsh JA (2003) Genetic mapping of the novel Turnip mosaic virus resistance gene *TuRB03* in *Brassica napus*. Theor Appl Genet 107:1169-1173

Jenner CE, Keane GJ, Jones JE, Walsh JA (1999) Serotypic variation in turnip mosaic virus. Plant Pathol 48:101–108

Jenner CE, Walsh JA (1996) Pathotypic variation in turnip mosaic virus with special reference to European isolates. Plant Pathol 45:848–856

Jenner CE, Tomimura K, Ohshima K, Hughes SL, Walsh JA (2002) Mutations in *Turnip mosaic virus P3* and cylindrical inclusion proteins are separately required to overcome two *Brassica napus* resistance genes. Virology 300:50-59

Jenner CE, Wang X, Tomimura K, Ohshima K, Ponz F, Walsh JA (2003) The dual role of the potyvirus P3 protein of turnip mosaic virus as a symptom and avirulence determinant in *brassicas*. Mol Plant Microbe Interact 16:777–784

Jenner CE, Nellist CF, Barker GC, Walsh JA (2010) *Turnip mosaic virus* (TuMV) is able to use alleles of both *eIF4E* and *eIF(iso)4E* from multiple loci of the diploid *Brassica rapa*. Mol Plant Microbe Interact 23:1498-505

Lellis AD, Kasschau KD, Whitham SA, Carrington JC (2002) Loss-of-Susceptibility Mutants of *Arabidopsis thaliana* reveal an Essential Role for eIF(iso)4E during Potyvirus Infection. Current Biology 12:1046–1051

Liu XP, Lu WC, Liu BX, Li SY, Li JL, Zhao ZY, Wang HJ, Wang CH (1990a) A study on TuMV strain differentiation on cruciferous vegetables from ten regions of China: Identification results with Green's methods. Virologica Sinica 1:82-87

Liu XP, Lu WC, Liu YK, Li JL (1990b) A study on TuMV strain differentiation of cruciferous vegetables from ten provinces in China: New host differentiator screening and strain classification. Chin. Sci. Bull. 35:1734-1739

Michelmore RW, Paran I, Kesseli RV (1991) Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. Proc Natl Acad Sc USA 88:9828–9832

Mun JH, Yu HJ, Park S, Park BS (2009) Genome-wide identification of NBS-encoding resistance genes in *Brassica rapa*. Mol Genet Genomics 282:617–631

Nieto C, Morales M, Orjeda G, Clepet C, Monfort A, Sturbois B et al. (2006) An *eIF4E* allele confers resistance to an uncapped and non-polyadenylated RNA virus in melon. Plant J 48:452–462

Provvidenti R (1980) Evaluation of Chinese cabbage cultivars from Japan and the People's Republic of China for resistance to turnip mosaic virus and cauliflower mosaic virus. J Am Soc Hortic Sci 105:571-573

Robaglia C, Caranta C (2006) Translation initiation factors: a weak link in plant RNA virus infection. Trends Plant Sci 11:40–45

Robbins MA, Witsenboer H, Michelmore RW, Laliberte JF, Fortin MG (1994) Genetic mapping of turnip mosaic virus resistance in *Lactuca sativa*. Theor Appl Genet 89:583–589

Rusholme RL (2000) The genetic control of resistance to *turnip mosaic virus* (TuMV) in Brassica. PhD Thesis, University of East Anglia, Norwich

Rusholme RL, Higgins EE, Walsh JA, Lydiate DJ (2007) Genetic control of broad-spectrum resistance to turnip mosaic virus in *Brassica rapa* (Chinese cabbage). J General Virol 88:3177-3186

Shattuck VI (1992) The biology, epidemiology and control of turnip mosaic virus. In: Janick J (ed) Plant breeding reviews 14. John Wiley and Sons, New York, pp

199–238

Shukla DD, Ward CW, Brunt AA (1994) The Potyviridae. CAB Int, Wallingford, UK

Schultz ES (1921) A transmissible mosaic disease of Chinese cabbage, mustard and turnip. J Agric Res 22:173–177

Suh SK, Green SK, Park HG (1995) Genetics of resistance to five strains of turnip mosaic virus in Chinese cabbage. Euphytica 81:71–77

Stein, N. et al. (2005) The eukaryotic translation initiation factor 4E confers multiallelic recessive Bymovirus resistance in Hordeum vulgare. Plant J. 42, 912–922

Tomlinson JA (1987) Epidemiology and control of virus diseases of vegetables. Ann Appl Biol 110:661–681

Truniger V, Nieto C, González-Ibeas D, Aranda MA (2008) Mechanism of plant eIF4E-mediated resistance against a Carmovirus (*Tombusviridae*): cap-independent translation of a viral RNA controlled in cis by an(a) virulence determinant. Plant J 56:716–727

Walsh JA, Sharpe AG, Jenner CE, Lydiate DJ (1999) Characterisation of resistance to turnip mosaic virus in oilseed rape (*Brassica napus*) and genetic mapping of *TuRB01*. Theor Appl Genet 99:1149-1154

Walsh JA, Jenner CE (2002) Turnip mosaic virus and the quest for durable resistance. Mol Plant Pathol 3:289-300

Walsh, J.A., Rusholme, R.L., Hughes, S.L., Jenner, C.E., Bambridge, J.M. Lydiate, D.J. & Green, S.K. (2002). Different classes of resistance to turnip mosaic virus in *Brassica rapa*. Eur. J. Plant Path. 108:15-20

Wang yan, Sun SL, Liu B, Wang H, Deng J, Liao YC, Wang Q, Cheng F, Wang XW, Wu J (2011) A sequence-based genetic linkage map as a reference for *Brassica rapa* pseudochromosome assembly. BMC Genomics 12:239

Wittmann S, Chatel H, Fortin MG, Laliberté JF (1997) Interaction of the viral protein genome linked of turnip mosaic potyvirus with the transitional eukaryotic initiation factor (iso) 4E of *Arabidopsis thaliana* using the yeast two-hybrid system. Virology 234:84–92

Yoshii M, Yoshioka N, Ishikawa M, Naito S(1998) Isolation of an *Arabidopsis thaliana*

mutant in which accumulation of cucumber mosaic virus coat protein is delayed. Plant J 13:211–219

Zhang JH, Pan CQ, Zhang YW, Cui CS (2006) EST-PCR-RFLP markers linked to turnip mosaic virus (TuMV) resistance gene in Chinese cabbage (*Brassica rapa* ssp. pekinensis). Acta Phytopathol Sinica 36:523-527

Zhang FL, Wang M, Liu XC, Zhao XY, Yang JP (2008a) Quantitative trait loci analysis for resistance against turnip mosaic virus based on a doubled-haploid population in Chinese cabbage. Plant Breed 127:82-86

Zhang JH, QU SP, CUI CS (2008b) Analysis of QTL for Turnip mosaic virus resistance in Chinese cabbage. Acta Phytopathologica Sinica 38:178-184

Zhang XW, Yuan YX, Wang XW (2009) QTL Mapping for TuMV Resistance in Chinese Cabbage [Brassica campestris L. ssp. pekinensis (Lour.) Olssom]. Acta Horticulturae Sinica 36:731–736

FIGURE CAPTIONS

Fig. 1 A Phenotypes of TuMV (pathotype C4)-challenged parental Chinese cabbage plants BP8407, Ji Zao Chun, and the F_1, non-inoculated plants (BP8407$^-$, Ji Zao Chun$^-$, F_1^-) and inoculated plants (BP8407$^+$, Ji Zao Chun$^+$, F_1^+). There were 20 plants in each treatment. **B** ELISA results for TuMV in leaves of BP8407, Ji Zao Chun, and F_1. Extracts from the leaves of TuMV-inoculated and non-inoculated plants were prepared at 21d.p.i. and tested for TuMV capsid protein (CP) by ELISA.

Fig. 2 The polymorphism in the P1 plant, the P2 plant, the resistant bulk, the susceptible bulk, and the F_1 generation. **A** Marker BC84. **B** Marker BrID90211: lane 1,

BP8407; lane 2, Ji Zao Chun; lane 3, the resistant bulk; lane 4, the susceptible bulk; lane 5, F_1.

C Genetic linkage map of the *retr02* locus on chromosome A04, generated from the F_2 population (239 individuals) derived from a BP8407/Ji Zao Chun cross. Genetic distance in cM was calculated using the Kosambi function.

Fig. 3 Genetic map (left) and physical map (Wang et al. 2011) (right). The resistance gene is located on chromosome A04 and on scaffold000104, or scaffold000060. The genetic distance in cM was calculated using the Kosambi function.

Fig. 4 A The blastp results for *Bra035393*, *Bra035531*, *Bra039484* and AT5G35620

B The gene *Bra035393* is the candidate gene that codes eIF(iso)4E.

Fig. 5 Alignment of sequences corresponding to the coding sequence of the resistance locus in eight lines (80122(BP8407), 80124(89B), 80186(Er Qing), 80425(Ji Zao Chun), 80403(Chun Da Jiang), 80461(Qiang Shi), *BraA.eIF(iso)4E*(R-o-18)). 80122, 80124, 80186 and Chiifu are resistant lines; 80425, 80403, 80461 and R-o-18 are susceptible lines.

Table 1. Responses of the F_2 population derived from a cross between BP8407 and Ji Zao Chun to TuMV isolate C4

Plant line	Number of plants			Expected ratio(R:S)	Goodness of fit	
	Resistant (R)	Susceptible (S)	Total		χ^2	Probability
BP8407	20	0	20			
Ji Zao Chun	0	20	20			
BP8407×Ji Zao Chun F1	0	20	20			
BP8407×Ji Zao Chun F2	52	187	239	1:3	1:34	0.25

Table 2 . **Predicted *eIF4E* and *eIF4G* genes in the *B. rapa* genome**

Gene name	Length	HMMPfam	PFname	Domain	E value
Bra035393	199	HMMPfam	PF01652	IF4E	1.50E-85
Bra035531	200	HMMPfam	PF01652	IF4E	7.60E-91
Bra039484	198	HMMPfam	PF01652	IF4E	1.50E-89
Bra002134	189	HMMPfam	PF01652	IF4E	1.80E-73
Bra006439	218	HMMPfam	PF01652	IF4E	1.30E-108
Bra012622	131	HMMPfam	PF01652	IF4E	7.30E-54
Bra013283	228	HMMPfam	PF01652	IF4E	2.60E-113
Bra021026	234	HMMPfam	PF01652	IF4E	6.40E-118
Bra023664	224	HMMPfam	PF01652	IF4E	3.50E-104
Bra030147	244	HMMPfam	PF01652	IF4E	3.00E-88
Bra032325	248	HMMPfam	PF01652	IF4E	2.40E-84
Bra000126	1175	HMMPfam	PF02854	MIF4G	3.10E-51
Bra003593	399	HMMPfam	PF02854	MIF4G	2.80E-09
Bra008429	822	HMMPfam	PF02854	MIF4G	1.80E-48
Bra010275	445	HMMPfam	PF02854	MIF4G	0.011
Bra011211	749	HMMPfam	PF02854	MIF4G	1.00E-63
Bra011218	547	HMMPfam	PF02854	MIF4G	3.40E-58
Bra013145	845	HMMPfam	PF02854	MIF4G	2.10E-39
Bra014505	1656	HMMPfam	PF02854	MIF4G	3.80E-58
Bra020407	770	HMMPfam	PF02854	MIF4G	1.50E-63
Bra023646	1311	HMMPfam	PF02854	MIF4G	1.90E-13

Bra024051	748	HMMPfam	PF02854	MIF4G	7.70E-65
Bra035142	492	HMMPfam	PF02854	MIF4G	7.50E-12
Bra037092	496	HMMPfam	PF02854	MIF4G	2.80E-12
Bra013145	845	HMMPfam	PF09088	MIF4G_like	2.00E-39

Table 3. Primers used for cloning *Bra035393*

Primer	Sequence(5'-3')	Location	Direction
Bio11001	TAAAACCCAAAACTGACT	5'UTR	Forward
Bio11002	TATCTCCTTCCACTTCTT	Exon4	Reverse
Bio11005	GGGTATTGGGAAGAAGTGG	Exon4	Forward
Bio11006	GGTTTTAGAGCAGTCGGT	3'UTR	Reverse
Bio11007	ACAAGGACCAAATCTAATGA	Exon3	Forward
Bio11008	GTGGGTAATATCCAACAAAT	Exon5	Reverse