Genetic control of broad-spectrum resistance to turnip mosaic virus in *Brassica rapa* (Chinese cabbage)

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The *Brassica rapa* line RLR22 was resistant to eight diverse turnip mosaic virus (TuMV) isolates. A *B. rapa* genetic map based on 213 marker loci segregating in 120 first back-cross (B$_1$) individuals was established and aligned with the *B. rapa* genome reference map using some of the RFLP probes. B$_1$ individuals were self-pollinated to produce B$_2$S$_1$ families. The existence of two loci controlling resistance to TuMV isolate CDN 1 was established from contrasting patterns of segregation for resistance and susceptibility in the B$_2$S$_1$ families. The first gene, recessive TuMV resistance 01 (retr01), had a recessive allele for resistance, was located on the upper portion of chromosome R4 and was epistatic to the second gene. The second gene, Conditional TuMV resistance 01 (ConTR01), possessed a dominant allele for resistance and was located on the upper portion of chromosome R8. These genes also controlled resistance to TuMV isolate CZE 1 and might be sufficient to explain the broad-spectrum resistance of RLR22. The dominant resistance gene, ConTR01, was coincident with one of the three eukaryotic initiation factor 4E (eIF(iso)4E) loci of *B. rapa* and possibly one of the loci of eIF4E. The recessive resistance gene retr01 was apparently coincident with one of the three loci of eIF(iso)4E in the A genome of *Brassica napus* and therefore, by inference, in the *B. rapa* genome. This suggested a mode of action for the resistance that is based on denying the viral RNA access to the translation initiation complex of the plant host. The gene retr01 is the first reported example of a recessive resistance gene mapped in a *Brassica* species.

INTRODUCTION

Turnip mosaic virus (TuMV; genus *Potyvirus*) infects a wide range of cultivated plant species (Edwardson & Christie, 1991) and causes significant economic losses in *Brassica* crops (Shattuck, 1992). TuMV is a positive-strand RNA virus with a genome comprising 9830–9835 nt (Nicolas & Laliberté, 1992; Ohshima, 1996; Jenner et al., 2000, 2003). The viral avirulence determinant is the P3 protein (Jenner et al., 1994), and *Brassica napus* (TuRB01, Walsh et al., 1999; TuRB03, Hughes et al., 2003; TuRB04 and TuRB05, J. A. Walsh & D. J. Lydiate, unpublished) and *Brassica rapa* (TuRB01b, Rusholme et al., 2007). All are dominant resistance genes (R genes) that control resistance to narrow spectra of TuMV isolates. In the case of TuRB01, TuRB01b and TuRB04, the cytoplasmic inclusion protein has been identified as the viral avirulence determinant (Jenner et al., 2000, 2002; Walsh et al., 2002). For TuRB03 and TuRB05, the viral avirulence determinant is the P3 protein (Jenner et al., 2002, 2003).

Plants possess a range of defence responses against viral pathogens. The best-characterized defence responses lead to a hypersensitive reaction with localized cell death and are often associated with activation of systemic acquired resistance and restriction of the virus to the initial site of infection (Staskawicz et al., 1995; Yang et al., 1997). This type of response is strain-specific and is controlled by R genes that play a role in virus recognition. The N gene, controlling resistance to tobacco mosaic virus (Erickson et al., 1999), and Rx, which controls resistance to potato virus X (Köhln et al., 1993), are well-characterized examples of dominant R genes. Both genes have a nucleotide binding site and a leucine-rich repeat motif (Erickson et al., 1999; Bendahmane et al., 1999) that are...
common to many R genes active not only against viruses, but also against a wide range of other pathogens (Dangl & Jones, 2001).

An additional general mechanism by which plants have developed resistance to viruses is by the modification of host functions that are involved in the virus life cycle. It has been shown that the pvr2 locus in pepper, which confers recessive resistance against the potyviruses potato virus Y and tobacco etch virus (TEV), corresponds to the eukaryotic initiation factor 4E gene (eIF4E) (Ruffel et al., 2002). Similarly, in Arabidopsis thaliana, disrupting the function of elf(iso)4E produces plants that are resistant to TEV (Lellis et al., 2002), TuMV (Duprat et al., 2002; Lellis et al., 2002) and lettuce mosaic virus (LMV) (Duprat et al., 2002). The elf(iso)4E protein of Arabidopsis has also been shown to interact with the genome-linked protein (VPg), which is attached to the 5' end of the viral genome and is assumed to be responsible for recruiting the viral RNA to the translation initiation complex (of TuMV in the yeast two-hybrid system (Wittmann et al., 1997). Since these original findings, a number of recessive resistances to other members of the Potyviridae have been shown to correspond to elf4E or elf(iso)4E (Robaglia & Caranta, 2006). The natural role of elf4E and elf(iso)4E is in the initiation of translation of capped mRNAs (Browning, 1996). elf4E binds elf4G, which is a scaffold for other components of the translation initiation complex.

Several plant genes with recessive alleles that restrict the cell-to-cell movement (Nicolas et al., 1997) or the systemic spread (Schaad & Carrington, 1996; Murphy et al., 1998; Hamalainen et al., 2000) of viruses have been identified. One example is a mutant gene of A. thaliana that is thought to disrupt the interaction of turnip vein clearing virus with plasmodesmata at the boundary between vascular and non-vascular tissue (Lartey et al., 1998). Genes with dominant (wild-type) alleles that restrict virus movement have also been identified in A. thaliana (RTM1, RTM2 and RTM3; Chisholm et al., 2001). All of the above plant defence mechanisms are distinct from virus-induced gene silencing, which involves the recognition and sequence-specific degradation of viral RNA by plants (Vance & Vaucheret, 2001).

B. rapa is grown worldwide as vegetable, oilseed and fodder crops. In addition, it has contributed the Brassica A genome to the amphidiploid crop species B. napus and Brassica juncea (U, 1935). Several accessions of B. rapa exhibiting broad-spectrum resistance to TuMV have been identified (Anonymous, 1996; Liu et al., 1996) but the genetic control of resistance in even the best-characterized accessions, 0-2 and BP058, is unclear (Yoon et al., 1993; Suh et al., 1995, 1996; Hughes et al., 2002). This paper describes the application of established Brassica genetic marker technology (Parkin et al., 1995; Sharpe et al., 1995) and the European TuMV pathotyping system (Jenner & Walsh, 1996) to identify the genes controlling broad-spectrum resistance to TuMV in the Chinese cabbage line RLR22 and to position these genes on the B. rapa genome. It establishes a solid foundation for understanding a mechanism of broad-spectrum resistance to potyviruses based on modifying the host eIF4E and eIF(iso)4E genes.

METHODS

Parental plant material. The Chinese cabbage accession BP079 exhibits resistance to a wide variety of TuMV isolates (Anonymous, 1996; Liu et al., 1996; Walsh et al., 2002). It was obtained from Dr Sylvia Green (Asian Vegetable Research and Development Centre, Shanhua, Tainan, Taiwan). BP079-8 was an inbred line derived from BP079, and RLR22 was a plant selfed from BP079-8 (Walsh et al., 2002). R-o-18 was an inbred line of B. rapa ssp. trilocularis (D. J. Lydiate).

TuMV isolates and disease assays. The origins and propagation of the eight TuMV isolates used in this study (UK 1, CHN 5, CZE 1, CDN 1, JPN 1, DEU 7, GK 1 and UK 4, representative of TuMV pathotypes 1, 3, 4, 7, 8, 9 and 12, respectively) and their phenotypes on the B. napus differentials of the European pathotyping system have been described by Jenner & Walsh (1996). In disease assays, the cotyledons and the first and second leaves of plants were mechanically inoculated at the two true-leaf stage (Jenner & Walsh 1996) and resistance (the absence of systemic spread) was established by negative results from ELISA on the uninoculated third and fourth leaves, 4 weeks post-inoculation (Jenner et al., 1999; Walsh et al., 1999). The eight TuMV isolates were inoculated separately to batches of six RLR22S1 plants and batches of three R-o-18 plants. TuMV isolates UK 1, CZE 1 and CDN 1 were inoculated separately to batches of seven F1 plants from the cross R-o-18 × RLR22 (Fig. 1).

DNA extraction and genetic marker analysis. DNA extraction and Southern blot hybridization were carried out as described by Sharpe et al. (1995) except that filters were washed only at low stringency. Genetic marker analysis of the first back-cross (B1) mapping population (Fig. 1) employed a range of Brassica genomic and cDNA clones as Southern blot hybridization probes in RFLP analysis including those described by Thormann et al. (1994) (pW)

![Fig. 1. Breeding strategy used to develop the first back-cross (B1) population segregating for resistance to TuMV, the selfed progeny of the B1 individuals (B1S1 families) used to assess resistance/susceptibility to different TuMV isolates and an inbred line with broad-spectrum resistance to TuMV (RLR22S1), ⊙, Self-pollination; ♂, male parent; ♀, female parent. RLR22 is resistant to TuMV isolate CDN 1 and R-o-18 is susceptible to TuMV isolate CDN 1.](image-url)
and Sharpe et al. (1995) (pN, pO and pR), new sets of Brassica genomic clones (pL and pM; A. Sharpe & D. J. Lydiate, unpublished) and Z18443 (Sillito et al., 2000). In addition, Brassica microsatellite markers (A. Sharpe & D. J. Lydiate, unpublished) were used as described by Naom et al. (1995). The B. rapa/Brassica A genome loci representing elF4E and elF(iso)4E were mapped using B. napus cDNA clones selected on the basis of homology to the corresponding A. thaliana genes (A. Sharpe, unpublished). The clone LL61 represented B. napus domesticated the SG population was derived from a resynthesized B. napus genome (Parkin et al., 1995) on the basis of well-characterized RFLP loci (Parkin et al., 2005) (I. Parkin, personal communication, and D. J. Lydiate, unpublished).

**Statistical analysis of genetic linkage data.** Genetic linkage analysis was performed using MAPMAKER version 3.0 (Lander et al., 1987). A logarithm of odds (LOD) score of 4.0 was used to associate genetic loci into linkage groups, and three-point and multipoint analyses were performed to determine the most probable order of loci. The final locus order for each linkage group was established by minimizing double crossovers after proofreading. Recombination frequencies were converted to map distances using Kosambi’s mapping function (Kosambi, 1944).

**RESULTS**

**Resistance spectra of RLR22S₁₁, R-o-18 and F₁ progeny**

Self progeny of RLR22 (RLR22S₁₁), plants of the line R-o-18 and F₁ progeny from the R-o-18 × RLR22 cross were inoculated with eight diverse isolates of TuMV to establish the resistance spectra of the three B. rapa genotypes. Individual plants were inoculated with only one virus isolate. RLR22S₁₁ exhibited broad-spectrum resistance; all plants inoculated were immune to JPN 1 and resistant to systemic spread of the other seven TuMV isolates. All R-o-18 plants and all F₁ plants from the R-o-18 × RLR22 cross were susceptible to the TuMV isolates tested, indicating that at least one gene controlling resistance to TuMV in RLR22 was recessive. A single F₁ plant was used to pollinate an RLR22S₁₁ plant to produce a back-cross population (B₁) (Fig. 1) that was expected to segregate for resistance to TuMV. Each individual of the back-cross population was self-pollinated to produce a separate B₁S₁ family (Fig. 1) family that could be tested to infer the resistance of the parental B₁ genotype to a range of TuMV isolates.

**Genetic linkage map of the B₁ population**

One hundred and twenty B₁ individuals were assayed at 213 genetic marker loci and the segregation data for these loci were assembled into ten substantial linkage groups with no unlinked loci. The linkage groups were unambiguously aligned with those of established maps of the Brassica A genome (Parkin et al., 1995; Sharpe et al., 1995) using loci that shared identical or indistinguishable alleles, and the resulting genetic linkage map along with the framework loci are presented in Fig. 2.

The distribution of interval sizes in the new map was compared with the distribution expected from an equal-expected theoretical population with both markers and crossovers randomly distributed across the genome (Fig. 3) and the observed and expected distributions were essentially identical (Kolmogorov–Smirnov statistic = 0.051, P = 0.65), suggesting that neither crossovers nor marker loci were clustered. The frequency distribution of segregation ratios at marker loci showed that segregation distortion was not a problem in the back-cross population (Fig. 4), although a slight but significant (χ² = 21.1, P = 4.42 × 10⁻⁶) bias against R-o-18 alleles was evident. The above parameters suggested that the B₁ population was near-ideal for genetic analyses.

**Identifying a recessive gene controlling resistance to TuMV**

Eight individuals from each of 61 B₁S₁ families (Fig. 1) were tested for resistance/susceptibility to TuMV isolate CDN 1 and the results are summarized in Fig. 5. Nineteen of the B₁S₁ families were uniformly resistant (no susceptible individuals among the eight plants tested) and these families were expected to be derived from B₁ individuals homozygous for a recessive resistance gene. Marker data from the 19 B₁ plants from which the uniformly resistant B₁S₁ families were derived were surveyed to identify loci exhibiting an excess of homozygotes. Four loci at the top of R4 (pN220e3, pW205e1, pN202e1 and pW103e2) were homozygous in all 19 B₁ individuals and this degree of linkage was highly significant (χ² = 19, P = 1.31 × 10⁻⁵). However, ten B₁ individuals also homozygous in the region associated with control of resistance to TuMV did not produce uniformly resistant B₁S₁ families (Fig. 5), suggesting that at least one more locus was involved in controlling the trait.

**A two-gene model for control of resistance to TuMV**

The expected values for the experimental design described above were calculated based on a hypothesis invoking a resistance locus with a recessive allele for resistance on the upper half of linkage group R4 and epistatic to a second locus with a dominant allele for resistance. In this model, RLR22 would be homozygous for the recessive resistance allele at the first locus (aa) and homozygous for the dominant resistance allele at the second locus (RR), whilst R-o-18 would be homozygous for the dominant allele for susceptibility at the first locus (A⁺A⁺) and homozygous for the recessive allele for susceptibility at the second locus (rr). The A⁺ locus would be epistatic to the R locus. The F₁ would be heterozygous at both loci (A⁺a⁺R⁻r⁻), assuming balanced segregation ratios and that A and R are unlinked, and the B₁ population would contain equal proportions of four genotypes: aaR⁺R⁺, aaR⁻r⁻, A⁺R⁺r⁻ and A⁺R⁻r⁺. The first B₁
Fig. 2. Genetic map of *B. rapa*. The vertical lines, R1 to R10, represent the ten established linkage groups of the *B. rapa* genome. Marker loci are to the left of the linkage groups and interval sizes in cM are to the right of the linkage groups. Locus nomenclatures with prefixes ‘p’ or ‘s’ refer to RFLP markers and microsatellite markers, respectively. *, Loci used to align and orientate the new linkage groups with those of the established maps; *retr01*, locus with recessive allele for resistance to TuMV; *ConTR01*, locus with dominant allele for resistance to TuMV.
genotype \((aaRR)\) would be true-breeding and yield \(B_1S_1\) families uniformly resistant to TuMV \((aaRR)\), whereas the other three genotypes (heterozygous at one, the other or both loci) would yield \(B_1S_1\) families segregating for resistant and susceptible individuals. The \(B_1\) genotype \(aaRr\) would yield \(B_1S_1\) families segregating into three resistant \((aaR-)\) to one susceptible \((aarr)\). The \(B_1\) genotype \(AaRR\) would yield \(B_1S_1\) families segregating into one resistant \((aaRR)\) to three susceptible \((A-RR)\). The \(B_1\) genotype \(AaRr\) would yield \(B_1S_1\) families segregating into three resistant \((aaR-)\) to 13 susceptible \((A—\text{and} aarr)\).

With the above model, the limited sample size of eight individuals employed to assess the phenotype of each \(B_1S_1\) family imposed a degree of uncertainty in deducing the genotypes of \(B_1\) individuals. Based on the model, the majority of those \(B_1S_1\) families where all eight individuals were resistant to TuMV would be derived from \(B_1\) plants of the \(aaRR\) genotype, although almost 10% of such families would be predicted to be derived from \(B_1\) plants of the \(aaRr\) genotype. Similarly, the vast majority of \(B_1S_1\) families exhibiting one, two or three susceptible plants amongst the eight tested would be derived from \(B_1\) plants of the \(aaRr\) genotype, whilst those families exhibiting five, six, seven or eight susceptible individuals would be derived from \(B_1\) plants of the \(AaRR\) and \(AaRr\) genotypes.

**Mapping retr01 and ConTR01**

Based on the predictions of the above model, almost all of the \(B_1\) plants that gave rise to \(B_1S_1\) families exhibiting three or fewer susceptible individuals (amongst the eight tested) would be homozygous for the recessive resistance allele at the first locus; 27 of the 61 families tested fell into this class. Similarly, almost all of the \(B_1\) plants that gave rise to \(B_1S_1\) families exhibiting five or more susceptible individuals would be heterozygous at the first locus, and 27 of the 61 families fell into this second class. Comparing the segregation pattern for the predicted genotypes for the first resistance locus (the hypothetical \(A\) locus) with the segregation at marker loci indicated that the \(A\) locus was closely linked to \(pN202e1\) on chromosome R4 (Fig. 2). This association was highly significant \((\chi^2=46.3, P=4.9\times10^{-10}; \text{Table 1})\) and the resistance locus thus identified was named \(retr01\) (recessive TuMV resistance locus 01). The two \(B_1S_1\) families with phenotypes different from those anticipated based on the \(B_1\) genotype at the \(pN202e1\) locus were those derived from \(B_1\) plants 101 and 102. Plant 101 was homozygous at the \(pN202e1\) locus but the \(B_1S_1\) progeny exhibited five out of eight susceptible individuals, whilst plant 102 was heterozygous at \(pN202e1\) but the \(B_1S_1\) progeny exhibited three out of eight susceptible individuals. The phenotypes exhibited by \(B_1S_1\) families 101 and 102 were both particularly prone to
misclassification. On testing a further nine individuals of family 102 for resistance to TuMV isolate CDN 1, eight of the nine individuals were susceptible, suggesting that reclassification of the $B_1$ plant 102 to heterozygous at retr01 was appropriate. Limited quantities of $B_1S_1$ seed precluded further testing of 101. These results do not preclude the possibility that retr01 is coincident with $pN202e1$.

Again following the predictions of the above model, those $B_1$ plants that were heterozygous at retr01 would give rise to $B_1S_1$ families for which the influence of variation at the second resistance locus was unresolvable using the current experimental system. In contrast, those $B_1$ plants homoygous for the RLR22 allele at retr01 would give rise to $B_1S_1$ families that were uniformly resistant when the second locus was homozygous for the dominant resistance allele, but would exhibit at least one in eight susceptible individuals (in 90% of tests) when the second locus was heterozygous. Twenty-nine of the 61 $B_1$ plants for which $B_1S_1$ TuMV resistance/susceptibility scoring data were available were homozygous at $pN202e1$ (Fig. 5). Of these 29 $B_1$ plants, 19 individuals gave rise to uniformly resistant $B_1S_1$ families (based on the limited sample size employed) and were presumed to be homozygous for the dominant resistance allele at the second locus, whilst ten individuals gave rise to $B_1S_1$ families segregating for resistance/susceptibility and were predicted to be heterozygous at the second locus. Comparing the predicted genotypes for the putative second resistance locus (the $R$ locus) with marker data identified a pair of coincident marker loci on linkage group R8 ($pO52e2$ and $pO85e1$; Fig. 2) with an identical segregation pattern in all but two of the 29 informative families. This association was highly significant ($x^2 = 21.8, P = 6.7 \times 10^{-5}$; Table 1) and the resistance locus thus identified was named ConTR01 (Conditional TuMV Resistance 01). The two $B_1S_1$ families with phenotypes different from those anticipated based on the $B_1$ genotype at the $pO85e1$ locus were those derived from $B_1$ plants 43 and 109. Both plants were homozygous at the $pO85e1$ locus but the $B_1S_1$ progeny exhibited no susceptible individuals out of eight progeny. This type of discrepancy was precisely that expected from the inevitable misclassification of a proportion of $B_1S_1$ families derived from $B_1$ plants homozygous for the RLR22 allele at retr01 and heterozygous at ConTR01. On testing a further 12 individuals of family 109 for resistance to TuMV isolate CDN 1, two of the 12 individuals were susceptible, indicating that reclassification of the $B_1$ plant 109 to heterozygous at ConTR01 was appropriate. Limited quantities of $B_1S_1$ seed prevented further testing of family 43. These results do not preclude the possibility that ConTR01 is coincident with $pO85e1$.

The genetic markers tightly linked to retr01 ($pN202e1$) and ConTR01 ($pO85e1$) were used to test for segregation distortion in the regions of the $B. rapa$ genome carrying the resistance genes. Of the 120 $B_1$ plants, 62 were homozygous and 58 heterozygous at $pN202e1$ and again 62 were homozygous and 58 heterozygous at $pO85e1$; these observed ratios were extremely close to 1:1 and demonstrated efficient transmission of the two alleles at each locus to the $B_1$ progeny. $Brassica$ microsatellite markers known to detect loci in the region of R4 containing retr01 or the region of R8 containing ConTR01 were screened to identify those loci polymorphic in the $B_1$ mapping population. The segregation patterns of the informative microsatellite markers in the $B_1$ population were scored, allowing these markers to be positioned on the genetic linkage map (Fig. 2).

### Table 1. $\chi^2$ tests for linkage of marker allele segregation at loci $pN202e1$ and $pO85e1$ to segregation of the resistance phenotype

<table>
<thead>
<tr>
<th>Predicted genotype</th>
<th>Genotype*</th>
<th>$\chi^2$</th>
<th>d.f.†</th>
<th>$P$</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Observed</td>
<td>Expected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$pN202e1$‡</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>$aa$</td>
<td>1</td>
<td>26</td>
<td>13.5</td>
<td>13.5</td>
</tr>
<tr>
<td>$Aa$</td>
<td>26</td>
<td>1</td>
<td>13.5</td>
<td>13.5</td>
</tr>
<tr>
<td>$pO85e1$§</td>
<td>$RR$</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>17</td>
<td>9.5</td>
<td>9.5</td>
</tr>
<tr>
<td>$Rr$</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>5</td>
</tr>
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</table>

* +, Heterozygous for the RLR22 allele; −, homozygous for the RLR22 allele.

†d.f., Degrees of freedom.

‡Predicted $B_1$ genotype at the locus with a recessive allele for resistance ($a$). $B_1S_1$ families with $<4/8$ susceptible individuals ($aa$) and $B_1S_1$ families with $>4/8$ susceptible individuals ($Aa$).

§Predicted $B_1$ genotype at the locus with a dominant allele for resistance ($R$). $B_1S_1$ families with 0 out of 8 susceptible individuals ($RR$) and $B_1S_1$ families with 1, 2 or 3 out of 8 susceptible ($Rr$).
**ConTR01 and retr01 control resistance to multiple TuMV pathotypes**

The initial mapping of retr01 and ConTR01 employed resistance/susceptibility tests based solely on CDN 1, a pathotype 4 TuMV isolate (Jenner & Walsh, 1996). To test whether retr01 and ConTR01 were sufficient to control broad-spectrum resistance, individuals from 17 B1S1 families, tested with CDN 1 in the initial mapping of retr01 and ConTR01, were tested with CZE 1. CZE 1 is a representative of TuMV pathotype 3 (Jenner & Walsh, 1996). The results are summarized in Table 2 and clearly demonstrated that the B1S1 families exhibited the same resistance/susceptibility phenotypes when tested with CZE 1 as when tested with CDN 1. Thus, retr01 and ConTR01 control resistance to two distinct TuMV pathotypes and might explain the genetic basis for the broad-spectrum resistance observed in RLR22.

**Association of eIF4E with ConTR01 and of eIF(iso)4E with retr01**

Southern blot hybridization analysis of members of the B. napus SG mapping population using the LL61 and es2686 clones as probes for the members of the eIF4E and eIF(iso)4E gene families, respectively, indicated that there were probably six members of each gene family in B. napus (three copies of each in the A genome and three copies of each in the C genome). The six members of the eIF4E gene family mapped on A genome chromosomes N1, N3 and N8 and on C genome chromosomes N11, N17 and N18 of B. napus. The eIF4E gene on N8 was closely linked to the p085d locus, with only one recombinant in the 90 doubled haploid (DH) lines of the SG population that were assayed.

The p085d locus on the SG map was the same as the p085e1 locus on the B1 map and was possibly coincident with ConTR01. LL61 was then used as a probe in RFLP mapping in the B1 mapping population and identified a member of the eIF4E gene family that was coincident with the p085e1 and ConTR01 loci. Five members of the eIF(iso)4E gene family mapped on A genome chromosomes N4, N5 and N8 and C genome chromosomes N14 and N16, whilst the remaining gene was provisionally assigned to another C genome chromosome, N18. The eIF(iso)4E gene on N4 was closely linked to the pN202b locus with no recombinants represented in the 90 DH lines. The pN202b locus on the SG map was the same as the pN202e1 locus on the B1 map and was possibly coincident with retr01. Similarly, the eIF(iso)4E gene on N8 was closely linked to the p085d locus with only two recombinants in the population of 90 DH lines. The clone es2686 was then used as an RFLP probe on the B1 mapping population but none of the regions containing members of the eIF(iso)4E gene family was polymorphic in that population.

**DISCUSSION**

Two genes that act together to confer resistance to the TuMV isolates CDN 1 (a pathotype 4 isolate) and CZE 1 (a pathotype 3 isolate) on Chinese cabbage were identified and positioned in the B. rapa genome. The first resistance locus, retr01, mapped to the upper portion of chromosome R4 and was epistatic to the second locus. The second resistance locus, ConTR01, mapped to the upper region of chromosome R8. Plants exhibited resistance to TuMV isolates CDN 1 and CZE 1 when retr01 was homozygous for the recessive allele and at least one copy of the dominant allele was present at the ConTR01 locus.

Plants expressing resistance to mechanical inoculation developed chlorotic blotches in the inoculated leaves, with no detectable systemic spread of the virus. This indicated that the resistance mediated by retr01 and ConTR01 operated by limiting viral replication and/or restricting viral movement to only the few cells surrounding the initial foci of infection. The same resistance genes were even more effective against the low levels of inoculum resulting from aphid-mediated TuMV challenge. This better reflects the conditions experienced by plants in the field; CDN 1 failed to establish any detectable infection in the plants with retr01 and ConTR01 in contrast to the clear infection and systemic spread of the virus in control plants (J. A. Walsh & J. M. Bambridge, unpublished).

One of the three copies of initiation factor 4E (eIF4E) in the B. rapa genome was probably coincident with ConTR01 on chromosome R8. Similarly, one of the three copies of the isoform of eIF4E [eIF(iso)4E], was probably coincident with retr01 on chromosome R4, whilst another copy of eIF(iso)4E was closely linked to (and possibly coincident with) ConTR01 on chromosome R8. It is difficult to estimate precisely the significance of these observations as the phenotypes of B1 plants were deduced from samples of their self progeny and because the positions of the eIF(iso)4E genes relative to retr01 and ConTR01 were determined on the basis of linkage to common marker loci in two different mapping populations. However, if we

<table>
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<th>CZE 1 phenotype*</th>
<th>CDN 1 phenotype†</th>
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<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td>X</td>
<td>6</td>
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<tr>
<td>Y</td>
<td>0</td>
</tr>
<tr>
<td>Z</td>
<td>0</td>
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*Phenotype of B1S1 families tested with TuMV isolate CZE 1: X, 0/8 susceptible; Y, 1/8, 2/8 or 3/8 susceptible; Z, 5/8, 6/8, 7/8 or 8/8 susceptible.
†B1S1 families tested with TuMV isolate CDN 1; data reduced to three classes as above.
assume that retr01 and ConTR01 were each definitively localized within their respective 5 cM segments of the genome, and we know that there are six copies in total of elf4E and elf(iso)4E and we know that the total length of the assayed genome was 801 cM, then the chance of elf4E or elf(iso)4E being fortuitously coincident with retr01 or ConTR01 was $6 \times (5 \text{ cM} + 5 \text{ cM})/801 \text{ cM}$, or 7.5 %. After the first observation that a copy of elf(iso)4E was apparently coincident with retr01 on chromosome R4, then the likelihood of a second copy of elf4E or elf(iso)4E being fortuitously coincident with ConTR01 on chromosome R8 was $5 \times 5 \text{ cM}/796 \text{ cM}$, or 3.1 %. The likelihood of both marker associations occurring by chance was therefore 7.5 % × 3.1 %, or 0.23 %.

It has been shown in a number of plant–potyvirus interactions that the potyvirus VPg is able to bind to the elf4E protein and that mutations in members of the elf4E gene family can confer resistance to potyviruses (Robaglia & Caranta, 2006). The exact biochemical role of elf4E in potyvirus infection has yet to be defined. The VPg that is covalently bound to the 5' end of the viral RNA might mimic the cap of mRNA and recruit the viral RNA to the translation initiation complex to initiate translation. elf4E in pea (Pisum sativum) has been shown to be involved in movement of the potyvirus pea seed-borne mosaic virus (PsbMV) from cell to cell, as well as its probable support of viral translation (Gao et al., 2004). Studies on the binding of the VPg from the potyvirus LMV with lettuce elf4E suggest that this binding increases the strength of interaction between elf4E and the elf4E-binding domain on elf4G (the central component of the complex required for the initiation of protein translation), showing that VPg is an efficient modulator of elf4E biochemical functions (Michon et al., 2006). The potyviral genome is known to have an alternative mechanism for initiation of translation (Carrington & Freed, 1990; Levis & Astier-Manifacier, 1993) but this might be of secondary importance to the VPg–elf4E/elf(iso)4E pathway. It is clear that some potyviruses need at least one member of the elf4E/elf(iso)4E gene family in order to infect plants. TuMV needs elf(iso)4E in order to infect Arabidopsis; it does not appear to be able to use any of the elf4E gene family in this species, whereas for another potyvirus, clover yellow vein virus, the opposite is true (Sato et al., 2005). Where known, most potyviruses are able to use elf4E but not elf(iso)4E, and other genera of plant viruses use elf4E (Robaglia & Caranta, 2006). Our results suggest that TuMV is able to use only specific members of either the elf(iso)4E or both the elf(iso)4E and elf4E gene families in B. rapa. It is likely that the recessive allele of elf(iso)4E at the retr01 locus is non-functional for TuMV replication, whereas the dominant allele at this locus is functional for viral replication. The simplest model to explain the involvement of the second locus (ConTR01) is that the dominant allele of elf(iso)4E or elf4E at this locus is non-functional for TuMV, whereas the recessive allele either is only partially functional for TuMV or is only weakly expressed. As a consequence, when the retr01 locus is homozygous for the virus-incompatible allele, TuMV can only achieve replication above the threshold sufficient for full systemic infection of plants when the virus-compatible allele at the ConTR01 locus is homozygous.

The TuMV protein VPg is covalently linked to the viral genome and a mutation in the VPg that abolished the interaction with elf(iso)4E in vitro prevented viral infection in planta (Léonard et al., 2000). The VPg protein has been implicated in overcoming resistance mediated by these initiation factors in a range of other plant species (Robaglia & Caranta, 2006); however, as we have not been able to identify any TuMV isolates that can overcome the resistance controlled by retr01 and ConTR01 (Walsh et al., 2002), it is not currently possible to investigate differences that would distinguish the VPg proteins from virulent and avirulent TuMV isolates. Other elf4E- and elf(iso)4E-based resistances appear to be strain-specific. The gene sbm-1 with a recessive allele for resistance to the potyvirus PsbMV has been reported in pea (Johansen et al., 2001). sbm-1 behaves in a gene-for-gene manner with the potyvirus pathogen avirulence gene (VPg) (Keller et al., 1998) and sbm-1 has been shown to be elf4E (Gao et al., 2004). This facet of the behaviour of these recessive resistance genes parallels the gene-for-gene interactions of classical R genes. The resistance controlled by retr01 and ConTR01 is effective against two geographically and genetically diverse TuMV isolates (Jenner & Walsh, 1996; Lehmann et al., 1997; Tomimura et al., 2003) and indeed the resistance spectrum of the parental line RLR22 suggests that retr01 and ConTR01 together confer broad-spectrum resistance to TuMV on Chinese cabbage (Walsh et al., 2002).

Previous investigations into the genetic control of broad-spectrum resistance to TuMV in Chinese cabbage have produced variable results that probably reflect the allelic differences between the precise parents employed (Yoon et al., 1993; Suh et al., 1995, 1996). The generation of a new mapping population by crossing RLR22 to a susceptible parent of a different genotype could still uncover the involvement of additional copies of elf4E and/or elf(iso)4E at other loci in the susceptibility and resistance of B. rapa to TuMV. Similarly, testing B1S1 families with TuMV isolates representing additional pathotypes could still identify other resistance genes required for resistance to different isolates of TuMV.

Based on the hypothesis that retr01 and ConTR01 are members of the elf(iso)4E and possibly elf4E gene families, it will now be possible to clone these genes and sequence their allelic variants. This will pave the way for direct investigation of the interaction between the TuMV proteins (most notably VPg) and the different copies of elf4E and elf(iso)4E present in the parental B. rapa lines RLR22 and R-o-18. Such studies will shed light on the precise molecular mechanism of resistance. Molecular investigation of the elf4E and elf(iso)4E loci in B. rapa other than retr01 and ConTR01 will determine whether
TuMV-incompatible alleles for these genes have already become fixed in both RLR22 and R-o-18. The acquisition of TuMV-incompatible alleles at retr01 and ConTR01 could then be seen as the end of a long evolutionary process of establishing resistance to TuMV.

The broad-spectrum resistance identified in RLR22 is as yet undefeated. The ability of pathogens to overcome resistance controlled by simply inherited dominant resistance genes has resulted in a repetitive cycle of developing new cultivars with new resistance specificities and the consequent mutation of the pathogen to overcome these resistance genes (Bergelson et al., 2001). Therefore, this broad-spectrum resistance represents a novel and potentially durable source of resistance to TuMV that can be deployed in susceptible Brassica crops. The PCR-based microsatellite markers that have been identified as flanking the resistance genes retr01 and ConTR01 can be employed in marker-assisted selection for TuMV resistance, assisting in the elimination of linkage drag ( Tanksley et al., 1989 ) and promoting rapid and accurate breeding for these useful genes. In future, single nucleotide polymorphism markers that select directly for the resistance alleles at the retr01 and ConTR01 loci could also be very helpful in this process.

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