Theor Appl Genet (1994) 89:583–589

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Genetic mapping of turnip mosaic virus resistance in *Lactuca sativa*

Received: 31 August 1993 / Accepted: 2 February 1994

Abstract Presence of the dominant *Tu* gene in *Lactuca sativa* is sufficient to confer resistance to infection by turnip mosaic virus (TuMV). In order to obtain an immunological assay for the presence of TuMV in inoculated plants, the TuMV coat protein (CP) gene was cloned by amplification of a cDNA corresponding to the viral genome using degenerate primers designed from conserved potyvirus CP sequences. The TuMV CP was overexpressed in *Escherichia coli*, and polyclonal antibodies were produced. To locate *Tu* on the *L. sativa* genetic map, F_3 families from a cross between cvs 'Cobbham Green' (resistant to TuMV) and 'Calmar' (susceptible) were genotyped for *Tu*. Families known to be recombinant in the region containing *Tu* were infected with TuMV and tested by the indirect enzyme-linked immunosorbent assay (ELISA) using the anti-CP serum. This assay placed *Tu* between two random amplified polymorphic DNA (RAPD) markers and 3.2 cM from *Dm5/8* (which confers resistance to *Bremia lactucae*). Also, bulked segregant analysis was used to screen for additional RAPD markers tightly linked to the *Tu* locus. Five new markers linked to *Tu* were identified in this region, and their location on the genetic map was determined using informative recombinants in the region. Six markers were identified as being linked within 2.5 cM of *Tu*.

Key words Coat protein · Disease resistance · Molecular markers

Communicated by G. E. Hart

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Introduction

While plant responses to viral infection are determined in many cultivated crops by single resistance genes (Fraser 1990; Ellis et al. 1988), the cellular mechanisms of viral resistance in plants remain unknown. No plant viral resistance gene has yet been cloned and no gene product characterized. A cloned viral resistance gene would allow examination of the cellular and molecular events involved in resistance. Several proteins, enzymes and cDNAs are induced or repressed upon viral infection (Legrand et al. 1987; Kombrink et al. 1988); however, as many of these factors are also induced by abiotic stresses, their role in resistance is difficult to establish (Bowles 1990). Most differentially expressed genes are thought to be involved in the general stress defense reaction mounted by the plant cell in response to infection and are not thought to be the primary determinants of resistance. Plant viral resistance genes, however, are specific for one or a few strains of a virus (Fraser 1987) and are the key genetic factor that determines whether a plant will be resistant or susceptible. Several models have been proposed to account for the mechanism of inhibition of virus multiplication by resistance gene products. The model for which there is the most evidence was suggested by Bruening et al. (1987) and involves the inhibition of viral polyprotein processing by a plant inhibitor of the viral protease. In the absence of a cloned resistance gene, however, the causal relationship to the resistance phenotype has been difficult to confirm. The use of molecular markers to determine map location followed by map-based cloning appears to be one of the most promising approaches for the isolation of resistance genes from plants (Michelmore et al. 1992).

We are using the interaction between *Lactuca sativa*, turnip mosaic virus (TuMV) and other viruses to study viral resistance genes in plants. TuMV is a member of the potyvirus family of plant viruses, containing a monopartite positive-sense RNA 9,830 nucleotides in

length, that has recently been fully sequenced (Nicolas and Laliberté 1992). A single dominant gene, *Tu*, seems to confer complete resistance to TuMV in *L. sativa* (Duffus and Zink 1969).

A detailed genetic linkage map for *L. sativa* (Kesseli et al. 1993) has been developed using restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) markers. We are now mapping disease resistance genes and saturating the adjacent genomic regions with molecular markers as a prerequisite for map-based cloning. *Tu* and *Dm5/8*, which encodes resistance to lettuce downy mildew caused by *Bremia lactucae*, were reported to be linked in *trans* at approximately 13 cM (Zink and Duffus 1970). However, the exact location of *Tu* relative to *Dm5/8* and other markers on the genetic map was not known. In this report we describe determination of the precise genetic position of *Tu* using individuals previously shown to be recombinant in the region surrounding *Dm5/8*. The phenotypes of resistance and susceptibility were determined using both visual symptoms and antibodies produced to cloned TuMV coat protein (CP) expressed in *E. coli* in an enzyme-linked immunosorbent assay (ELISA). In order to saturate the region surrounding *Tu* with molecular markers, we used bulked segregant analysis (BSA) with pools of DNA from resistant and susceptible homozygous individuals for *Dm5/8* (Michelmore et al. 1991). Linkage of several new markers to *Tu* was confirmed by segregation analysis of individuals that were recombinant in the *Dm5/8* region.

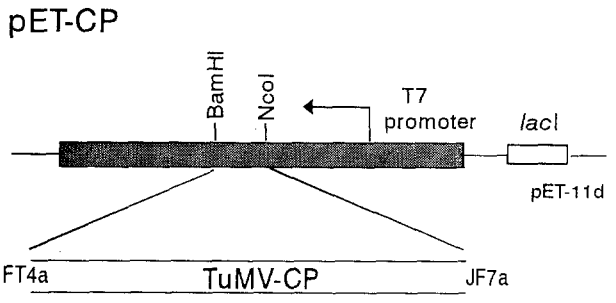
Materials and methods

Turnip mosaic virus purification and propagation

The TuMV isolate described by Tremblay et al. (1990) was propagated on *Brassica perviridis* under greenhouse conditions. Crude extracts were prepared by grinding infected leaf material (0.25 g/ml) in 100 m*M* KH_2PO_4 pH 7.5, and these were used for inoculations. Plants were inoculated by first dusting the leaf surface with Carborundum (180 grit) and then rubbing it with a cotton swab saturated with crude extract from infected leaf tissue. Nine indicator plants [*Chenopodium quinoa*, *C. amaranticolor*, *B. perviridis*, *B. napus*, *B. pekinensis*, *Nicotiana glutinosa* and *L. sativa* cvs. 'Calmar' (TuMV susceptible), 'Cobbham Green' (TuMV resistant) and 'Kordaat' (TuMV resistant)] were used to verify the identity and the purity of the TuMV isolate used. TuMV was purified from *B. perviridis* according to Choi et al. (1977) and stored at −70 °C in 10 m*M* KH_2PO_4 pH 7.5.

Overexpression of the TuMV CP in *E. coli*

The TuMV coat protein gene was cloned from viral RNA by reverse transcriptase-polymerase chain reaction (PCR) into pET-11d, to form pET-CP. The CP sequence was amplified using two oligonucleotides synthesized with the Gene Assembler (Pharmacia LKB). Primer JF7a (Fig. 1) was complementary to the cDNA copy of TuMV RNA between nucleotides 8728 and 8766, which encode the last 9 amino acids of the NIb protein and the first 4 residues of the CP (Nicolas and Laliberté 1992). The primer contained *Bam*HI and *Nco*I restriction sites (lowercase in Fig. 1). The ATG of the *Nco*I site is in phase with the reading frame of the CP gene. Primer FT4a (Fig. 1) was complementary to viral RNA nucleotides 9604–9643, which encode the last 5 amino acids of the CP and 25 nucleotides of the 3′ non-coding region of the viral RNA. The primer introduced a *Bam*HI restriction site (lowercase in Figure 1) after the stop codon. Amplification was carried out as described previously (Nicolas and Laliberté 1992) using *Taq* DNA polymerase (Perkin-Elmer Cetus). The amplification product was gel purified and recovered using GenecleanII (Bio101). The fragment was digested with *Bam*HI and *Nco*I and inserted into pET-11d cleaved with the same enzymes (Studier et al. 1990). *E. coli* strain BL21 (DE3) harbouring pET-CP (MR1 strain) was grown in Luria broth with ampicillin (100 µg/ml) at 37 °C. At $A_{600} = 0.5$, 0.4 m*M* IPTG was added to promote expression of the T7 RNA polymerase and transcription of the CP gene. The cells were incubated for an additional 2 h after which they were harvested by centrifugation.



FT4a 5′-TATAGTCTACCAggatccACTTCATAACCCCTGAAGGCC

JF7a 5′-GGTGTTGAGGCTTggatccGAAccatggCAGGTGAAACG

Fig. 1 Plasmid pET-CP used for the overexpression of cloned TuMV-CP. A reverse-transcribed copy of the viral RNA was amplified using primers JF7a (complementary to the cDNA of the sense virus) and FT7a (complementary to the 3′ end of the TuMV-CP mRNA). The *Nco*I and *Bam*HI sites (*lowercase* in the primer sequence) within the primers were used to generate compatible ends with the pET-11d expression vector. No additional codons were incorporated into the coding region of the CP

Production of antibodies and immunodetection

TuMV CP was isolated from the overexpressing MR1 strain of *E. coli* by cell disruption with lysozyme and differential contrifugation of the inclusion bodies according to Harlow and Lane (1988). Proteins from the inclusion bodies were separated on a 12.5% (w/v) polyacrylamide gel under denaturing conditions (1 *M* urea and 0.4% SDS; Adams 1989). Side strips from the gel were stained, and the protein at approximately 38 kDa was cut from the unstained gel. The polyacrylamide slice was pulverized by several passages in a 26-G needle, emulsified in Freund's incomplete adjuvant (Difco) and injected intramuscularly into 8-week-old female rabbits according to Vaitukaitis (1981). Three injections were performed at 10-day intervals. Ten days after the third injection, serum was collected and stored at −70 °C.

An indirect ELISA assay based on the protocol of Clark et al. (1986) was used for the detection of TuMV CP. All incubations were at room temperature. Wells were washed 6 times between each step using phosphate buffered saline (PBS; 137 m*M* NaCl, 2.7 m*M* KCl, 4.3 m*M* Na_2HPO_4, 1.4 m*M* KH_2PO_4, pH 7.0) containing 0.05% Tween-20 (PBST). Leaf samples to be analysed were ground in extraction buffer (15 m*M* Na_2CO_3, 35 m*M* $NaHCO_3$ and 2% polyvinylpyrrolidone (PVP) 44,000) at a ratio of 0.05 g/ml of leaf in buffer. Samples were centrifuged for 1 min at 14,000 g. Microtiter plate wells (Falcon) were blocked with PBST containing 2% bovine serum albumin (BSA). Anti-TuMV CP serum, diluted 1:1000 in PBST buffer containing BSA (0.2%) and PVP (2%), was added to the wells, and antigen-antibody complexes were detected with goat anti-rabbit IgG

alkaline phosphatase conjugate (Bio-Rad) diluted 1:5000 in the same buffer. Substrate solution containing para-nitrophenyl phosphate (1 mg/ml; Sigma) and diethanolamine (9.7%), pH 9.8, was added and colour intensity was measured at 405 nm.

Disease assays

To determine the disease-resistance phenotype, young lettuce plants were inoculated with TuMV once at the three-leaf stage and again at the five-leaf stage with a crude extract of TuMV-infected *B. perviridis* (0.25 g leaf/ml 100 m*M* KH_2PO_4, pH 7.5). When this procedure was used, 95% of the susceptible plants became infected in control experiments (data not shown). Sixteen F_3 individuals from each family were inoculated and scored individually. ELISA was used to determine whether the inoculated plants were resistant or susceptible, as was the scoring of visual symptoms. The F_3 families were scored as all susceptible (progenitor F_2 plant was *tutu*), all resistant (progenitor was *TuTu*) or segregating for resistance (progenitor was *TuTu*).

Immunoblotting

Extracts from infected and non-infected leaves were prepared by grinding 0.25 g of leaf in 1 ml of buffer (100 m*M* TRIS-Cl, pH 8.0, 5 m*M* EDTA, 0.1% Triton X-100, 0.1% mercaptoethanol). Protein concentration was measured using a Comassie Blue dye-binding assay (Bio-Rad). Samples were diluted in electrophoresis sample buffer (63 m*M* TRIS-Cl pH 6.8, 10% glycerol, 2% SDS, 1 *M* urea and 0.05% bromophenol blue), and run in a 15% (w/v) polyacrylamide gel under denaturing conditions as described by Gallagher et al. (1989). Protein was transferred electrophoretically to nitrocellulose. Anti-TuMV serum (1:800) was diluted in PBS containing 1% BSA. Goat anti-rabbit IgG linked to alkaline phosphatase (Bio-Rad) and a nitroblue tetrazolium-based assay (Bio-Rad) were used for antigen-antibody complex detection.

DNA purification and RAPD analysis

DNA extractions were performed by the CTAB method of Doyle and Doyle (1987). Bulked samples of DNA, from plants characterized as homozygous resistant or susceptible to downy mildew (due to *Dm5/8*), were created as described by Michelmore et al. (1991) using purified DNA of F_2 individuals from a cross between cvs 'Calmar' and 'Kordaat'. These bulks were used for RAPD analysis as described by Williams et al. (1990) using arbitrary decamer oligonucleotides (R. J. Carlson, University of British Columbia). Each amplification reaction contained approximately 20 ng of genomic DNA, 240 n*M* primer, 100 µ*M* of each dNTP, reaction buffer (100 m*M* TRIS-Cl, pH 8.8, 500 m*M* KCl, 15 m*M* $MgCl_2$, 1% Triton) and 1.25 units of *Taq* polymerase. Reactions were performed in a Hybaid thermal cycler placed at 4 °C and programmed for 45 cycles of 1 min at 35 °C, 2 min at 72 °C, 1 min at 92 °C, preceded by an initial denaturation step of 94 °C for 5 min. Amplified DNA fragments were separated by agarose gel electrophoresis (1.2% agarose gels) and stained with ethidium bromide.

Results and discussion

Expression of the TuMV CP in *E. coli* and antibody production

The ability to map a gene relies on the unambiguous determination of the phenotype of individual plants in a segregating population. Visual inspection of symptoms following TuMV infection of lettuce has not been sufficiently reliable for phenotyping purposes. An antibody to the virus was therefore required in order to perform ELISA assays. To obtain sufficient CP to generate antibodies, we have chosen to express the CP in *E. coli* instead of purifying the virus due to the fact that *E. coli*-produced CP does not contain any plant proteins that normally co-purify with the virus, therefore producing a virus-specific antiserum. The coding region of the TuMV CP was introduced into the pET-11d vector (Studier et al. 1990), forming pET-CP, to provide high levels of CP expression in *E. coli*. pET-CP (Fig. 1) was constructed to express a protein with a predicted molecular weight (MW) of 34000 Da that comprised amino acid residues 2876–3863 of the TuMV polyprotein (Nicolas and Laliberté 1992). All the codons between the ATG and the TAA belonged to the TuMV CP. Upon induction of the *E. coli* strain BL21 containing pET-CP with IPTG, almost all of the protein found in inclusion bodies consisted of TuMV CP (data not shown). Protein preparations of cells induced with IPTG and harbouring pET-CP displayed a major 38-kDa protein that was not present in cells containing the pET-11d vector without the CP insert. The over-expressed TuMV CP co-migrated with the CP from a preparation of TuMV on SDS-PAGE. The observed molecular weight of the *E. coli*-produced protein (as determined by SDS-PAGE) was slightly larger than that predicted from the nucleotide sequence (Tremblay et al. 1990). Anomalous behaviour of purified potyviruses CP in SDS-PAGE has been reported by others (Abo El-Nil et al. 1977; Choi and Wakimoto 1979; Hiebert and McDonald 1976).

The TuMV CP protein was excised from the gel and injected into rabbits to obtain a polyclonal antiserum that detected a protein of 38 kDa on Western blots of SDS-PAGE separated proteins from cells harboring pET-CP induced by IPTG. An additional protein was detected, but it was also found in untransformed BL21 strain or in transformed but uninduced bacteria, suggesting that an *E. coli* protein of slightly lower molecular weight than the TuMV CP was also recognized by the anti-TuMV CP serum (Fig. 2). The 38-kDa protein corresponds to TuMV CP since a protein of identical molecular weight is detected in crude extracts of TuMV-infected *B. perviridis* that is absent from healthy plants (Fig. 2). When the viral preparation used for this Western blot was inoculated on diagnostic species (*L. sativa* cvs 'Calmar', 'Kordaat' and 'Cobbham Green', *Nicotiana glutinosa*, *Chenopodium quinoa*, *C. amaranticolor*, *Brassica perviridis*, *B. napus* or *B. pekinensis*), symptoms consistent with TuMV infected were produced. The same virus preparation was used to generate the cDNA clones. This antiserum was used to determine whether inoculated plants were resistant or susceptible to TuMV.

Location of *Tu*

The location of *Tu* in relation to previously mapped markers (Kesseli et al. 1993) was determined by estab-

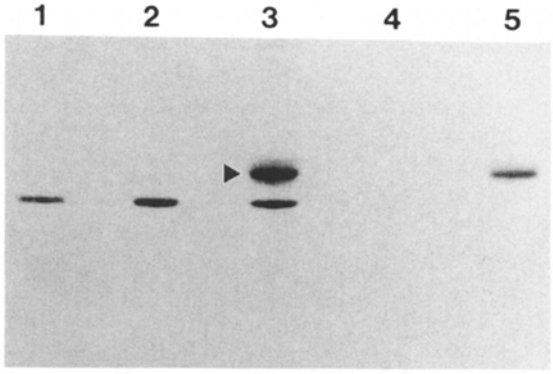


Fig. 2 Immunodetection of TuMV CP in *E. coli* extracts and virus-infected plant sap using antiserum raised against the CP expressed in *E. coli*. Bacterial protein extracts from strain BL21 untransformed (*lane 1*), transformed with pET-CP but uninduced with IPTG (*lane 2*) or transformed with pET-CP and induced to express TuMV CP by addition of IPTG (*lane 3*), were separated by SDS-PAGE and transferred to nitrocellulose. Crude plant extracts from healthy (*lane 4*) and TuMV-infected plant material (*lane 5*) were also run in same gel. The *arrow* indicates the 38-kDa CP

lishing to genotype at the *Tu* locus for each F_2 individual identified as a recombinant in the genomic region surrounding *Dm5/8*. Zink and Duffus (1970) reported linkage at approximately 13 map units in *trans* between the *Dm5/8* and the *Tu* loci in a cross involving *L. sativa* cvs 'Calmar' and 'Kordaat'. We analysed 23 F_3 families of a cross between cvs 'Calmar' (*tutu*) and 'Cobbham Green' (*TuTu*) from F_2 plants that had been identified previously as recombinant in a region of approximately 20 cM (see Table 1). Only recombinant families in the *Dm5/8* region were genotyped since they are the only informative families for mapping purposes. Sixteen F_3 individuals from each family were inoculated with TuMV, and an ELISA protocol, in addition to the scoring of visual symptoms, was used to determine whether the inoculated plants were resistant or susceptible. ELISA readings greater than 4 standard deviations above the mean for 8 uninoculated control plants were considered to be positive for the presence of virus. This high threshold minimized the possibility of classifying *TuTu* plants as *Tutu*. Using ELISA we found that all plants that displayed symptoms contained virus. In approximately 20% of the infected plants, symptoms could not be observed or they were too faint to allow an unequivocal determination of whether the plant was resistant or susceptible. Analysis for segregation of resistance within F_3 families allowed the genotype of progenitor resistant F_2 plants to be determined. The *Tu* gene was found to be located between markers OPm18 and OPy13 (the last three characters refer to the supplier's primer number, the preceding characters refer to the supplier), an interval of 2.5 cM (Table 1).

Detection of RAPD markers linked to *Tu*

Simultaneously, bulked segregant analysis (Michelmore et al. 1991) was used to screen for additional RAPD markers linked to *Tu* (Fig. 3). Pools of DNA from plants homozygous-resistant or susceptible at the *Dm5/8* locus were combined to form bulks of DNA for each of the two phenotypes. Allelic variation at loci unlinked to *Dm5/8* should be randomly distributed and therefore present in

Table 1 Genotypes in the region of the *Tu* locus for F_2 recombinant individuals from a cross between cvs 'Calmar' (TuMV susceptible) and 'Cobbham Green' (TuMV resistant)

F_2[a] individual	Markers							
	$OPf12_{1340}$[b]	$OPm18_{836}$	$OPl08_{634}$	*Tu*	$UBC346_{1067}$	$UBC517_{1397}$	$OPy13_{800}$	$UBC599_{2482}$
8	B[c]	B	B	B	C	D	D	D
11	D	D	D	H	C	D	D	D
12	B	B	B	B	C	B	B	B
15	D	D	D	H	C	D	D	D
27	D	D	D	A	A	D	D	D
28	D	D	D	A	A	D	D	D
34	D	D	D	B	C	B	B	B
35	D	D	D	H	C	B	B	B
37	D	D	D	H	C	D	D	D
38	B	B	B	B	C	B	B	B
40	D	B	B	B	C	B	B	B
62	B	B	B	B	C	B	B	B
85	D	D	D	A	C	D	D	D
87	D	D	D	H	C	D	D	D
95	D	D	D	H	C	D	D	D
106	D	D	D	H	C	B	B	B
108	D	D	D	A	A	D	D	D
132	D	D	D	H	C	D	D	D
134	D	B	B	B	C	B	B	B
140	D	B	B	B	C	B	B	B
144	B	B	B	B	C	B	B	B
145	B	B	B	B	C	B	B	B
149	D	D	D	A	A	D	D	D

[a] Numbers in the left column represent F_2 individuals
[b] Marker subscripts indicate the size (in bp) of the amplified polymorphic band
[c] Letters in the table represent genotypes: A, homozygous 'Calmar'; B, homozygous 'Cobbham Green'; C, homozygous 'Cobbham Green' or heterozygote; D, homozygous 'Calmar' or heterozygote; H, heterozygote

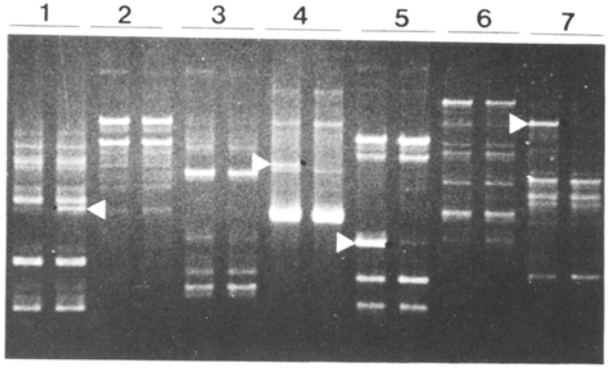


Fig. 3 Identification of RAPD polymorphisms by bulk segregant analysis (BSA). The polymorphisms revealed by BSA were all markers linked to the *Tu* locus within an interval of approximately 20 cM. Primers 10 nucleotides in length were used to amplify DNA from homozygous-resistant or susceptible pools of F_2 individuals. The pools were formed on the basis of the genotype at the *Dm5/8* locus that is linked to *Tu*. The F_2 individuals were from a cross between *L. sativa* cvs 'Calmar' and 'Kordaat'. Each pair of lanes (1–7) contains amplification products from one 10-nucleotide primer, using the two pools of DNA as template. Amplification products were separated by agarose (1.2%) gel electrophoresis. *Arrows* in *lanes 1, 4, 5* and 7 points to polymorphic DNA fragments that were amplified using primers UBC346, UBC517, UBC566 and UBC599, respectively. These polymorphisms were used in segregation analysis

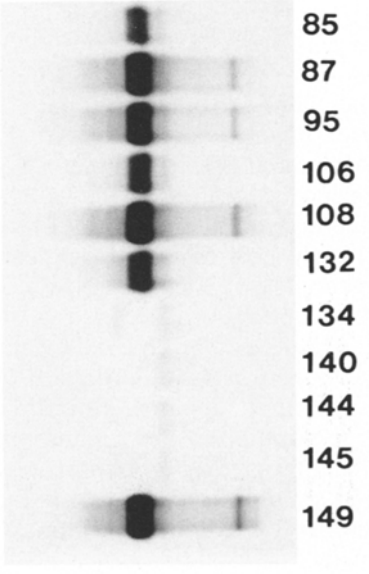
both pools. Only loci linked to *Dm5/8* will be detected as polymorphic between the two pools. Pools were made on the basis of the *Dm5/8* phenotype rather than *Tu*, since the two loci are linked and an F_2 population characterized at the *Dm5/8* locus was already available from previous studies (Kesseli et al. 1993). Homozygosity of the F_2 individuals at the *Dm5/8* locus had been determined by testing F_3 families for downy mildew resistance. The F_2 population used was from a cross between cvs 'Kordaat' (*TuTu*) and 'Calmar' (*tutu*). DNA extracted from 12 F_3 plants of each family was used to create each pool.

Over 250 arbitrary oligonucleotide primers were screened using the two pools, and seven polymorphic markers were identified. Five markers detecting a DNA polymorphism between the resistant and the susceptible bulks were confirmed as being linked to the *Tu* locus. The other two markers did not segregate in the mapping population used (from a cross between cvs 'Calmar' and 'Cobbham Green'), indicating that the DNA polymorphism identified in the bulks (the F_3 individuals were from a 'Calmar' × 'Kordaat' cross) was absent in the parents used to generate the mapping population. With some primers, the polymorphic band was faint, making it difficult to determine whether the band was absent or simply low in intensity. Since the genetic distances examined were small, one mis-scored individual could alter the distances significantly. The RAPD marker data for F_2 individuals recombining in the vicinity of the *Tu* locus (Table 1) were verified by Southern blotting to confirm the RAPD phenotypes obtained. The polymorphic DNA fragment obtained from amplification from each of five primers (UBC346, UBC599, UBC517, UBC675, OPm18) was excised from the gel, labelled and used as a probe on a Southern blot of amplification products from the same primer (Fig. 4). This additional scoring allowed the correction of data obtained from primer OPm18 and UBC675.

RAPD marker data and the genotypic information for *Tu* for all 23 recombinant F_2 individuals in the region were analysed for linkage using Mapmaker 3.0 (Lander et al. 1987). In addition to locating *Tu* relative to markers previously mapped in the *Dm5/8* region, this analysis determined that five markers were linked to the *Tu* locus (Fig. 5). The order of markers $OPy13_{800}$, $UBC517_{1397}$ and $UBC599_{2482}$ could not be resolved using the 23 recombinants derived from a population of 142 F_2 individuals.

Although the markers identified by bulked segregant analysis were not distributed randomly, all of the polymorphic markers identified mapped to a region spanning approximately 20 cM around *Dm5/8*, and three mapped in a cluster of approximately 1.4 cM close to *Tu*. This is in contrast to our experience screening near-isogenic lines, generated by six backcrosses to the recurrent parent (Ryder 1979), to map resistance to lettuce mosaic virus (confered by the *mo* locus). Of 16 primers detecting polymorphism (from 300 screened), only 3 were found to be linked to *mo* (Fortin and Ubalijoro,

Fig. 4 Southern blot of amplification products using primer OPm18 and template DNA from a sample of the F_2 population segregating for *Tu*. The presence of weak polymorphic bands (in *lanes 87* and *106* for example) was verified on these blots by using a labelled probe prepared by excising and labelling the polymorphic band observed in BSA. Each lane contains amplification products from an F_2 individual. The F_2 population was from a cross between *L. sativa* cvs 'Calmar' and 'Cobbham Green', and the individuals used here had previously been identified as recombinants in the *Dm5/8* region using RAPD markers. The genotype for the *Tu* locus was determined by inoculation with TuMV of F_3 individuals resulting from the selfing of the F_2 parent. The genotypes corresponding to each F_2 individual are shown in Table 1



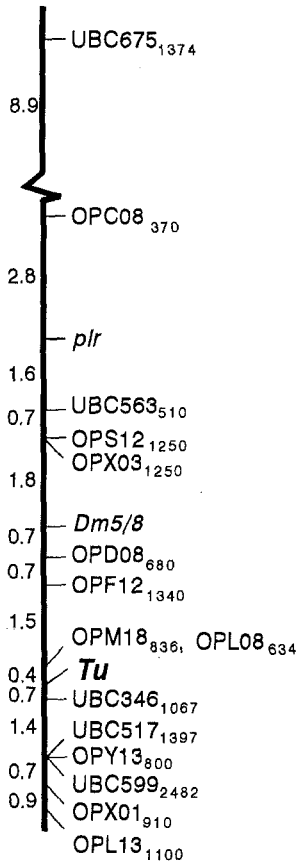


Fig. 5 Genetic map of *L. sativa* surrounding the *Tu* locus. The mapping population consisted of 142 F_2 individuals from a cross between cvs 'Calmar' and 'Cobbham Green'; 23 individuals recombinant in the *Dm5/8* region were analysed to construct this map. Genetic distances between loci are shown in cM. All the markers are RAPD markers, except for *Dm5/8* (resistance to *Bremia lactucae*), *plr* (resistance to *Plasmopara lactucae radicis*) and *Tu*. New markers added by BSA were $UBC675_{1374}$, $UBC563_{710}$, $UBC346_{1067}$, $UBC517_{1397}$, and $UBC599_{2482}$

unpublished observations). Other markers segregated independently of the resistance gene and appeared to be linked to additional genomic fragments from the donor parent that became fixed during the backcross programme.

We have identified three RAPD markers in a region of 1.1 cM surrounding *Tu*. It is likely that more markers will be required before map-based cloning becomes feasible. However, there is a wide variation in the relationship between genetic and physical distances, from approximately 14 kbp/cM for the *Bz* locus in corn (Dooner 1986) to over 4 Mbp/cM around the *Tm*-2a viral resistance gene in tomato (Ganal et al. 1989). In the latter case, proximity of the gene to a centromeric region probably reduces recombination frequency. The linkages detected between the *Tu* resistance gene and molecular markers in sufficiently close to warrant examination of the physical distance separating the latter from the *Tu* gene. The physical distance between the two closest markers and *Tu* will represent the maximum distance that will need to be covered by clones in order to recover a cloned copy of the gene. We are also currently saturating the region with more markers and screening large populations for recombinants close to *Tu*. These will allow us to develop a fine structure map of the region and should provide markers that will minimize the number of overlapping clones required to obtain a cloned copy of the *Tu* gene.

Acknowledgements This research was supported by grants from the Natural Sciences and Engineering Research Council of Canada (MGF, JFL), the Louis G. Johnson Foundation (MGF), the Conseil de Recherches en Pêches et en Agroalimentaire du Québec (MGF), the Netherlands Organization for Scientific Research (HW), and the USDA/NRI #91-37300-6364 (RWM).

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