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Molecular characterization of the SCAR markers tightly linked to the *Tm-2* locus of the genus *Lycopersicon*

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Abstract The *Tm-2* gene and its alleles conferring tomato mosaic virus resistance in tomato originate from *Lycopersicon peruvianum*, a wild relative of tomato. DNA fragments of several RAPD markers tightly linked with the *Tm-2* locus in tomato were successfully cloned and sequenced. Subsequently, the 24-mer oligonucleotide primer pairs of the SCAR markers corresponding to the RAPD markers were designed based on the 5'-endmost sequences. A fragment of the same size as that of a SCAR marker was amplified in the ToMV-susceptible tomato line with no *Tm-2*, but the digests of the PCR fragments by *Acc*I exhibited polymorphism in fragment length between the two lines. We chose three SCAR markers and three RAPD markers tightly linked with the *Tm-2* locus, and examined whether the same-sized fragments corresponding to these markers were also present in three other lines carrying *Tm-2a* or one of the other *Tm-2* alleles. The fragments corresponding to the three SCAR markers were present in all of the three lines, but the other markers (three RAPDs) were absent in one or two lines, suggesting that the three SCAR markers are closer to *Tm-2* than the other markers. Comparison of the nucleotide sequences of these fragments revealed that they are all homologous to the corresponding SCAR markers.

Key words Tomato · Random amplified polymorphic DNA (RAPD) · Sequence characterized amplified region (SCAR) · *Tm-2* gene · Tomato mosaic virus (ToMV) resistance

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Introduction

Tm-2 and *Tm-2a* are tomato mosaic virus(ToMV) resistance genes that were introgressed from two different lines of *Lycopersicon peruvianum* L. into tomato [*L. esculentum* (L.) Mill.] in two independently conducted tomato breeding programs in Hawaii (Kikuta and Frazier 1947) and in Florida (Alexander 1963), respectively. They are allelic, both dominant to an allele (+) for ToMV susceptibility in *L. esculentum* and are located in the heterochromatic region close to the centromere of the long arm of chromosome 9 (Khush et al. 1964; Pecaut 1965; Tanksley et al. 1992). The phenotypes of ToMV resistance expressed by the *Tm-2* and *Tm-2a* genes are similar but can be distinguished by differential responses to certain ToMV strains (Pelham 1972). It has been suggested that ToMV resistance is expressed as a result of an inhibition of the virus-encoded function for the cell-to-cell movement of the virus (Nishiguchi and Motoyoshi 1987; Meshi et al. 1989). On the other hand, plants carrying *Tm-2* or *Tm-2a* have been observed to show a hypersensitive response to infection with ToMV (Cirulli and Alexander 1969; Hall 1980). It remains unknown whether both the inhibition of the cell-to-cell movement of the virus and the induction of the hypersensitive response to ToMV infection are controlled by these genes or whether there are any other genes that are linked with the *Tm-2* locus that control either of the functions.

Previously (Ohmori et al. 1995), we reported that 13 random amplified polymorphic DNA (RAPD) fragments were linked with the *Tm-2* locus and one other locus in a region spanning about 5 cM. Of the 13 markers, 10 were in coupling with *Tm-2*, and 3 were in coupling with *Tm-2a*. The markers in coupling with *Tm-2* were further characterized (Ohmori et al. 1995), and some of them were cloned, sequenced and converted into sequence characterized amplified region (SCAR) markers (Motoyoshi et al. 1996). Of these 10 markers 6 are linked with the *Tm-2* locus more tightly than the other markers, since they all co-segregated with one another in

133 F_2 progeny derived from the crosses between a +/+ line and a *Tm-2*/*Tm-2* line (Ohmori et al. 1995).

For the practical use of these markers, it is desirable to determine which marker is the closest to the *Tm-2* locus. However, it is difficult to predict how many F_2 plants should be analyzed to find recombinants between these tightly linked markers. As an alternative approach, we considered that an examination of whether the homologues of these markers are present or not in different tomato lines carrying one of the ToMV resistance genes allelic to *Tm-2* may permit estimation of the rough order of the markers with respect to distance to the *Tm-2* locus. In the previous study (Ohmori et al. 1995), we estimated that 4 RAPD markers are closer to the *Tm-2* locus than the other 2, since the same-sized polymerase chain reaction (PCR) products corresponding to the former markers were generated in the *Tm-2a* line, whereas no products corresponding to the latter markers were detected in this line.

There are two other *Tm-2*-like genes allelic to *Tm-2*. These genes were introduced from *L. peruvianum* into tomato in France and in Japan (Laterrot and Pecaut 1969; Yamakawa and Nagata 1975). The RAPD or SCAR markers, whose homologues are present in both tomato lines carrying one of these *Tm-2* alleles, would be candidates for the markers nearest the *Tm-2* locus.

In the study reported here, fragments of the same size corresponding to three SCAR markers were found in these lines. We then sequenced these fragments and subsequently compared their sequences with those of the SCAR markers. Fragments corresponding to the three SCAR markers were also cloned from a *L. peruvianum* line, from which the *Tm-2a* allele is derived, and sequenced. The results revealed that the sequences corresponding to the SCAR markers in the ToMV-resistant tomato lines examined are real homologues that originated from *L. peruvianum*.

Materials and methods

Plant materials

GCR26 is a tomato var. Craigella, which is susceptible to ToMV. GCR236 and GCR267 are homozygous for *Tm-2* and *Tm-2a*, respectively, and have a common genetic background with Craigella (Smith and Ritchie 1983). Perou 2 is a line bred in France and carries a *Tm-2*-like gene that is derived from a line of *L. peruvianum* and is allelic to *Tm-2* (Laterrot and Pecaut 1969; Provvidenti and Schroeder 1969). TMJ54–28, a line bred in Japan, also has a *Tm-2*-like gene allelic to *Tm-2* (Yamakawa et al. 1987) that originated from a line of *L. peruvianum* (Yamakawa and Nagata 1975). The line P.I. 128650 of *L. peruvianum* is the source of *Tm-2*a that has been introgressed into tomato (Alexander 1963).

Conversion of RAPD markers into SCAR markers

PCR-amplification and separation of the PCR products to detect RAPD markers, and the cloning of the RAPD fragments were carried out as described previously (Ohmori et al. 1996). The RAPD fragments of $OPB12_{1200}$, $OPI18_{900}$, $OPG10_{800}$, $OPN13_{1000}$, $OPE16_{900}$ and $OPG09_{700}$ were successfully cloned with blunted ends into the SmaI site of a pBluescript II KS vector (Stratagene). The identity of these clones with respect to the corresponding RAPD markers was examined by hybridization with them to Southern blots of PCR products amplified from the genomic DNAs of GCR26 and GCR236. The dideoxy nucleotide chain termination was carried out using oligonucleotide primers and fluorescent dye terminators (Applied Biosystems, Foster City, Calif.). The sequences were analyzed by an automated DNA sequencer (377–18, Applied Biosystems).

A pair of 24-mer oligonucleotide primers was designated based on the sequences of the 5'-endomost 24 bases. PCR and resolution of the products in agarose gels were carried out as described by Ohmori et al. (1996).

Comparative analysis of SCAR fragments in tomato and *L. peruvianum* lines

The SCAR fragments amplified from the DNA samples of the tomato lines and *L. peruvianum* P.I. 128650 were cloned and sequenced as described above. Nucleotide sequences were analyzed using the GENETYX software system (Software Development). Updated versions of the GenBank sequence database were searched using the BLAST algorithm of Altschul et al. (1990).

Results

Conversion of RAPD markers into SCAR markers

We briefly noted previously (Motoyoshi et al. 1996) that we were successful in converting *Tm-2*-linked RAPD markers $OPB12_{1200}$, $OPI18_{900}$, $OPE16_{900}$ and $OPG09_{700}$ into SCAR markers $SCB12_{1200}$, $SCI18_{900}$, $SCE16_{900}$ and $SCG09_{700}$, respectively. These SCAR marker fragments were amplified from the genomic DNA of GCR236 with each pair of 24-mer oligonucleotide primers designated based on 5′-endmost sequences of the cloned RAPD fragments. A pair of the oligonucleotide primers amplifying a SCAR fragment, SCG10800, corresponding to OPG10800, however, amplified a band of the same size from the DNA sample of GCR26(+/+). The nucleotide sequences of the primer pairs used for amplifying the SCAR fragments are shown in Table 1.

A 24-mer oligonucleotide primer pair newly designed based on the sequence of a cloned $OPN13_{1000}$ fragment (Table 1) also amplified a fragment of the same size from the GCR26(+/+) DNA sample (Fig. 1A). However, polymorphism was found in the band patterns between these tomato lines when the PCR products in both lines were previously digested with *Acc*I (Fig. 1A). The SCAR marker, $SCN13_{1000}$, is thus useful as a co-dominant marker for differentiating *Tm-2* and + alleles.

Putative linear order relationship of certain markers tightly linked with the *Tm-2* locus

In the previous study (Ohmori et al. 1995), the primers for $OPD20_{1800}$ and $OPG10_{800}$ in GCR236 were found to amplify none of the fragments of the same size in GCR267, while those for $OPE16_{900}$, $OPN13_{1000}$, $OPG09_{700}$ and $OPH09_{1000}$ in GCR236 amplified the fragments of the

Table 1 Nucleotide sequences of primers (24-mers) used for the SCAR markers converted from the RAPD markers linked to the *Tm-2* locus

SCAR marker	Primer	Sequence[a]	Phenotype
$SCB12_{1200}$	$OPB12U_{1200}$	ccttgacgcaGATAATGTGATGTC	Dominant
	$OPB12T_{1200}$	ccttgacgcaTTGAAAGAAATCAA	
$SCE16_{900}$	$OPE16U_{900}$	ggtgactgtgTAATTATGTTGTGC	Dominant
	$OPE16T_{900}$	ggtgactgtgGTGGAAAATGCAAA	
$SCG09_{700}$	$OPG09T_{700}$	ctgacgtcacCTTGTTGGTCTTTA	Dominant
	$OPG09T_{700}$	ctgacgtcacTTAGCTTTGTAAAC	
$SCG10_{800}$	$OPG10U_{800}$	agggccgtctACGTGTGTTTGAAT	No polymorphism
	$OPG10T_{800}$	agggccgtctATTGGATCAGAGAA	
$SCI18_{900}$	$OPI18U_{900}$	tgcccagcctAATTGTCCCTAGGC	Dominant
	$OPI18T_{900}$	tgcccagcctTGGAAAAAAAAGAT	
$SCN13_{1000}$	$OPN13U_{1000}$	agcgtcactcCATACTTGGAATAA	Co-dominant after *Acc*I digestion
	$OPN13T_{1000}$	agcgtcactcAAAATGTACCCAAA	

[a] The sequences of corresponding RAPD marker are represented in *lowercase*

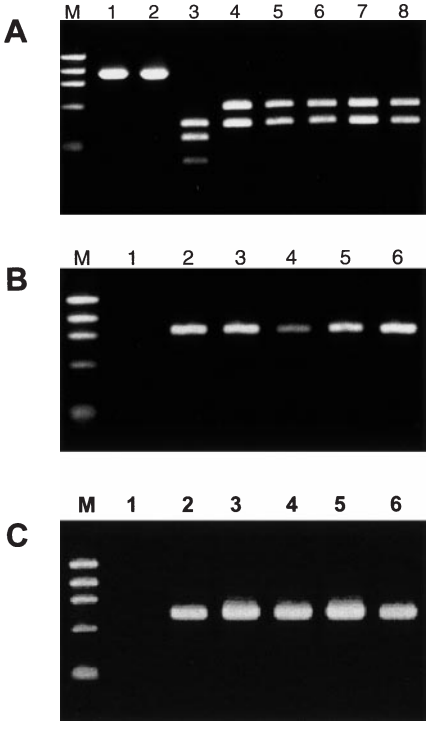

Fig. 1A–C Electrophoretic patterns of the fragments amplified by PCR from genomic DNAs of GCR26 (+/+) and the ToMV-resistant tomato or *L. peruvianum* lines using the primer pairs for SCAR markers. **A** PCR products amplified using the primer pair for $SCN13_{1000}$. The products were electrophoresed before and after digestion with *Acc*I. *Lanes 1* and *2* PCR products from DNAs of GCR26 and GCR236 before *Acc*I digestion, *lanes 3–8* the *Acc*I-digested PCR products from DNAs of GCR26, GCR236, GCR267, TMJ54–28, Perou 2 and *L. peruvianum* P.I.128650, respectively. **B** PCR products amplified using the primer pair for $SCE16_{900}$. *Lanes 1–6* PCR products from DNAs of GCR26, GCR236, GCR267, TMJ54–28, Perou 2 and *L. peruvianum* P.I.128650, respectively. **C** PCR products amplified using the primer pair for $SCG09_{700}$. *Lanes 1–6* PCR products from DNAs of GCR26, GCR236, GCR267, TMJ54–28, Perou 2 and *L. peruvianum* P.I.128650, respectively. *M* (**A–C**) DNA size markers (ϕX174/*Hae*III digest)

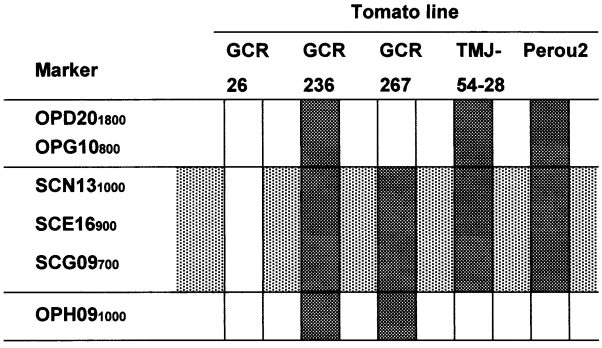

Fig. 2 Putative order of the tightly linked SCAR or RAPD markers in the region surrounding the *Tm-2* locus. The *columns* represent the region surrounding the *Tm-2* locus. The *black areas* are the putative parts originating from *L. peruvianum*, and the *white areas* are those from *L. esculentum*. The *Tm-2* locus is putatively located within the parts shown by the *hatched background*

same size also in GCR267. This suggests that $OPE16_{900}$, $OPN13_{1000}$, $OPG09_{700}$ and $OPH09_{1000}$ are closer to the *Tm-2* locus than either $OPD20_{1800}$ or $OPG10_{800}$. In further analytical studies, we used SCAR markers, $SCN13_{1000}$, $SCE16_{900}$ and $SCG09_{700}$ (Fig 1 A–C) instead of the corresponding RAPD markers. RAPD markers, $OPD20_{1800}$, $OPG10_{800}$ and $OPH09_{1000}$ were continuously used, since we could not convert them into SCAR markers.

To classify these markers with respect to distance to the *Tm-2* locus, we examined whether the fragments of the same size as those of the RAPD or SCAR markers were present or not in each of the ToMV-resistant tomato lines. Fragments of the same size corresponding to $OPD20_{1800}$ and OPG10800 were detected in TMJ54–28 and Perou 2, but not in GCR267. Conversely, $OPH09_{1000}$ was detected in GCR267, but not in TMJ54–28 and Perou 2. Fragments corresponding to $SCN13_{1000}$, $SCE16_{900}$ and $SCG09_{700}$ could be clearly demonstrated in GCR267, TMJ54–28 and Perou 2, suggesting that these three SCAR markers are closer to the *Tm-2* locus than the other markers examined. The putative linear order relationship between these markers is illustrated in Fig. 2.

Table 2 Percentage homologies of the nucleotide sequences of $SCN13_{1000}$ and its corresponding fragments in tomato lines and *L. peruvianum* P.I. 128650

Line[a]	GCR236	GCR267	TMJ54-28	Perou2	P.I. 128650	GCR26
GCR236 (1001 bp)	–	99.20	99.80	99.50	99.20	92.98
GCR267 (1004 bp)		–	99.40	99.30	100.00	92.89
TMJ54-28 (1002 bp)			–	99.70	99.40	93.08
Perou2 (1004 bp)				–	99.30	92.99
P.I.128650 (1004 bp)					–	92.89
GCR26 (1007 bp)						–

[a] Number of the nucleotide residues of the sequences in the lines of tomato or *L. peruvianum* are shown in parenthesis

Table 3 Percentage homologies of the nucleotide sequences of $SCE16_{900}$ and its corresponding fragments in tomato lines and *L. peruvianum* P.I. 128650

Line[a]	GCR236	GCR267	TMJ54-28	Perou2	P.I. 128650
GCR236 (919 bp)	–	98.80	98.69	99.35	98.80
GCR267 (918 bp)		–	99.24	99.24	100.00
TMJ54-28 (918 bp)			–	99.35	99.24
Perou2 (918 bp)				–	99.24
P.I.128650 (918 bp)					–

[a] Number of the nucleotide residues of the sequences in the lines of tomato or *L. peruvianum* are shown in parenthesis

Table 4 Percentage homologies of the nucleotide sequences of $SCG09_{700}$ and its corresponding fragments in tomato lines and *L. peruvianum* P.I. 128650

Line[a]	GCR236	GCR267	TMJ54-28	Perou2	P.I. 128650
GCR236 (716 bp)	–	98.88	97.50	98.61	98.88
GCR267 (716 bp)		–	97.49	98.61	99.72
TMJ54-28 (716 bp)			–	97.360	97.49
Perou2 (715 bp)				–	98.61
P.I.128650 (716 bp)					–

[a] Number of the nucleotide residues of the sequences in the lines of tomato or *L. peruvianum* are shown in parenthesis

Sequence analysis of the SCAR markers tightly linked with the *Tm-2* locus

To determine whether the fragments of the same-size found in different lines are real homologues of the markers linked to *Tm-2*, we cloned and sequenced the $SCN13_{1000}$, $SCE16_{900}$ and $SCG09_{700}$ fragments in GCR236 and the same-sized fragments in GCR267, TMJ54–28 and Perou 2. Fragments corresponding to these markers in *L. peruvianum* P.I. 128650 were also sequenced to estimate whether these fragments had originated from *L. peruvianum.* The fragment in GCR26 generated with the primer pair for $SCN13_{1000}$ was also cloned and sequenced to compare its sequence to those of $SCN13_{1000}$ and the corresponding fragments in the other lines. No open reading frame-like sequences were found in these fragments. The numbers of the nucleotide residues of these SCAR markers and percentage homologies between their nucleotide sequences are shown in Tables 2, 3 and 4.

$SCN13_{1000}$ and its corresponding fragments (Table 2)

$SCN13_{1000}$ in GCR236 consisted of 1001 nucleotides, while the corresponding sequences in GCR267, Perou 2, TMJ54–28 and *L. peruvianum* P.I. 128650 had 1004, 1002, 1002 and 1004 nucleotides, respectively. The sequence of the fragment in GCR26 was 1007 nucleotides long. $SCN13_{1000}$ had high homologies ranging from 99.20% to 99.80% with the corresponding sequences in GCR267, Perou 2, TMJ54–28 and *L. peruvianum* P.I. 128650. The sequence of the 1004-bp fragment in GCR267 was identical to that of *L. peruvianum* P.I. 128650, suggesting that this fragment in GCR267 had been introgressed together with *Tm-2a* into tomato. The 1007-bp fragment in GCR26 became distinguishable from $SCN13_{1000}$ in GCR236 after the PCR products are digested with *Acc*I; in addition to a common 420-kb fragment, the former exhibited a 360-kb and a 220-kb fragment and the latter a 580-kb fragment (Figs. 1A and

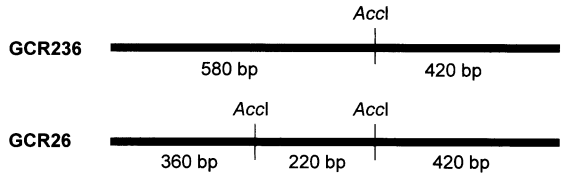

Fig. 3 The *Acc*I sites identified in the nucleotide sequences of $SCN13_{1000}$ and of its homologue in GCR26 (+/+)

3). The GCR26 fragment still had considerably high homologies up to 93.08% with $SCN13_{1000}$ and the corresponding sequences in the other ToMV resistant lines. This result suggests that the sequence in GCR26 is derived from a common ancestral species of *L. esculentum* and *L. peruvianum*.

$SCE16_{900}$ and its corresponding fragments (Table 3)

$SCE16_{900}$ consisted of 919 nucleotide residues, and was one nucleotide longer than its corresponding fragments in the other three ToMV resistant lines. The sequence of $SCE16_{900}$ had 98.80% homology with both of the fragments in GCR267 and *L. peruvianum* P.I.128650, and 98.69% and 99.35% homology with those in TMJ54–28 and Perou2, respectively. The sequences of the fragments in TMJ54–28 and Perou 2 were completely identical. A complete identity was also found between the sequences of the fragments in GCR267 and *L. peruvianum* P.I. 128650, suggesting that the sequence in the *L. peruvianum* line had been introgressed in tomato with no nucleotide substitutions.

$SCG09_{700}$ and its corresponding fragments (Table 4)

$SCG09_{700}$ and its corresponding fragments in GCR267, TMJ54–28 and *L. peruvianum* P.I. 128650 consisted of 716 nucleotides, 1 nucleotide longer than that in Perou 2. The percentage homologies of the sequence of $SCG09_{700}$ were 98.88% with both sequences in GCR267 and *L. peruvianum* P.I. 128650 and 97.50% and 98.61% with those in TMJ54–28 and Perou 2, respectively. The sequences of the fragments in GCR267 and *L. peruvianum* P.I. 128650 were not completely identical, but they were highly homologous.

Discussion

No fragments of the same size as those corresponding to the four SCAR markers, $SCB12_{1000}$, $SCI18_{900}$, $SCE16_{900}$ and $SCG09_{700}$, linked to the *Tm-2* locus in GCR236 (*Tm-2*/*Tm-2*) were found in GCR26 (+/+), indicating that these makers can be used as dominant markers. The SCAR primers for $SCG10_{800}$ and $SCN13_{1000}$ were different in that they amplified fragments of the same size in GCR26. This implies that sequences of the same size as that of to the RAPD markers $OPG10_{800}$ and $OPN13_{1000}$ are present in GCR26 but are not amplified or are too poorly amplified to be detected, and that they become exclusively amplified using the 24-mer primer pairs with increased specificity. When the $SCN13_{1000}$ and the corresponding PCR product from GCR26 were initially digested with *Acc*I, polymorphic bands became discernible in an agarose gel between the two tomato lines, indicating that $SCN13_{1000}$ is useful as a co-dominant marker tightly linked to the *Tm-2* allele.

The main use of these SCAR markers may be to mark the *Tm-2* locus for facilitating the breeding of ToMV resistant tomatoes. In this respect, $SCN13_{1000}$, $SCE16_{900}$ and $SCG09_{700}$ are most specific and convenient markers known so far to be the most closely linked to the *Tm-2* locus. In general, SCAR markers are more easily detected than restriction fragment length polymorphism (RFLP) markers and are more distinct than RAPD markers (Paran and Michelmore 1993). The sequences amplified in the other tomato lines with the primers for $SCN13_{1000}$, $SCE16_{900}$ or $SCG09_{700}$ are all highly homologous to one another. These SCAR primers can be used to mark not only the original *Tm-2* gene, but also the *Tm-2*a and the other two *Tm-2* alleles, all of which are being widely used in the breeding of various tomato varieties.

These markers may be especially useful for selecting an individual whose chromosomal segment surrounding the *Tm-2* locus that is derived from the *L. peruvianum* chromosome has been mostly replaced with that from *L. esculentum* by a recombination event. Such a recombinant may be a useful source of genetic material for breeding varieties homozygous for a limited region surrounding a *Tm-2* allele, since most of the unfavorable genes introgressed from this wild species may be excluded together with the *L. peruvianum* chromosome region to be replaced. In addition, it is also advantageous that the homozygous *Tm-2* (*Tm-2*/*Tm-2*) confers stronger resistance than the heterozygous *Tm-2* (*Tm-2*/+).

In the early stage of infection of tomato leaf tissues with a wild type of ToMV, resistance conferred by *Tm-2* and *Tm-2a* is expressed as an inhibition of virus movement from a primary infected cell to neighboring cells (Nishiguchi and Motoyoshi 1987). On the other hand, *Tm-2* and *Tm-2a* are known as the genes involved in expressing a hypersensitive reaction in response to ToMV infection (Cirulli and Alexander 1969; Hall 1980). In addition, we observed that plants whose genotype is *Tm-2a*/*Tm-2* were severely infected with Ltb1, a mutant ToMV which overcomes the resistance conferred by *Tm-2* but not the resistance conferred by *Tm-2a* (Motoyoshi et al. 1996). This finding suggests the possibility that there is another gene that is linked with *Tm-2* and *Tm-2a* and interacts with them for the resistance to be expressed. In order to elucidate the mechanism of the resistance in which *Tm-2* and *Tm-2a* are involved, we need to select various recombinants in the limited chromosomal region using DNA markers most closely linked with the *Tm-2* locus and to characterize the phenotypes in detail.

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References

Alexander LJ (1963) Host-pathogen dynamics of tobacco mosaic virus on tomato. Phytopathology 61:611–617

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Cirulli M, Alexander LJ (1969) Influence of temperature and strain of tobacco mosaic virus on resistance in a tomato breeding line derived from *Lycopersicon peruvianum*. Phytopathology 59:1287–1297

Hall TJ (1980) Resistance at the Tm-2 locas in the tomato to tomato mosaic virus. Euphytica 29:189–197

Khush GS, Rick CM, Robinson RW (1964) Genetic activity in a heterochromatic chromosome segment of the tomato. Science 145:1432–1434

Kikuta K, Frazier WA (1947) Preliminary report on breeding tomatoes for resistance to tobacco mosaic virus. Proc Am Soc Hortic Sci 49:256–262

Laterrot H, Pecaut P (1969) Gene *Tm2*=new source. TGC Rep 19:13–14

Meshi T, Motoyoshi F, Maeda T, Yoshikawa S, Watanabe H, Okada Y (1989) Mutations in the tobacco mosaic virus 30-kDa protein gene overcome *Tm-2* resistance in tomato. Plant Cell 1:515–522

Motoyoshi F, Ohmori T, Murata M (1996) Molecular characterization of heterochromatic regions around the *Tm-2* locus in chromosome 9 of tomato. Symp Soc Exp Biol 50:65–70

Nishiguchi M, Motoyoshi F (1987) Resistance mechanisms of tobacco mosaic virus strains in tomato and tobacco. In: Evered D, Harnett S (eds) Plant resistance to viruses. John Wiley & Sons, Chichester, pp 38–46

Ohmori T, Murata M, Motoyoshi F (1995) Identification of RAPD markers linked to the *Tm-2* locus in tomato. Theor Appl Genet 90:307–311

Ohmori T, Murata M, Motoyoshi F (1996) Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato. Theor Appl Genet 92:151–156

Paran I, Michelmore RW (1993) Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. Theor Appl Genet 85:985–993

Pecaut P (1965) Tomate. II. Résistance au virus de la mosaïque du tabac (TMV). Rep Station d'Amel des Plantes Maraîchêres (INRA) 1964:50–53

Pelham J (1972) Strain-genotype interaction of tobacco mosaic virus in tomato. Ann Appl Biol 71:219–228

Provvidenti R, Schroeder WT (1969) Further evidence that 'Perou 2' is a new source of *Tm-2* not linked with *nv*. TGC Rep 19:23

Smith JWM, Ritchie DB (1983) A collection of near-isogenic lines of tomato: research tool of the future? Plant Mol Biol Rep 1:41–45

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Roder MS, Wing RA, Wu W, Young ND (1992) High-density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Yamakawa K, Nagata T (1975) Three tomato lines obtained by the use of chronic gamma radiation with combined resistance to TMV and *Fusarium* race J-3 (in Japanese). Technical News 16, Inst Rad Breed

Yamakawa K, Yasui H, Mochizuki T, Hida K, Komochi S (1987) Incorporation of disease resistance from *Lycopersicon peruvianum* L. to cultivated tomatoes (in Japanese with English summary). Bull Natl Res Inst Veg, Ornamental Plants Tea A2:1–37