Molecular characterization of RAPD and SCAR markers linked to the *Tm-1* locus in tomato

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**Abstract** We have cloned and sequenced six RAPD fragments tightly linked to the *Tm-1* gene which confers tomato mosaic virus (ToMV) resistance in tomato. The terminal ten bases in each of these clones exactly matched the sequence of the primer for amplifying the corresponding RAPD marker, except for one in which the 5'-endmost two nucleotides were different from those of the primer. These RAPD clones did not cross-hybridize with each other, suggesting that they were derived from different loci. From Southern-hybridization experiments, five out of the six RAPD clones were estimated to be derived from middle- or high-repetitive sequences, but not from any parts of the ribosomal RNA genes (rDNA), which are known to be tightly linked with the *Tm-1* locus. The remaining clone appeared to be derived from a DNA family consisting of a few copies. These six RAPD fragments were converted to sequence characterized amplified region (SCAR) markers, each of which was detectable using a pair of primers having the same sequence as that at either end of the corresponding RAPD clone. All pairs of SCAR primers amplified distinct single bands whose sizes were the same as those of the RAPD clones. In four cases, the SCAR markers were present in the line with *Tin-1* but absent in the line without it, as were the corresponding RAPD markers. In the two other cases, the products of the same size were amplified in both lines. When these SCAR products were digested with different restriction endonucleases which recognize 4-bp sequences, however, polymorphisms in fragment length were found between the two lines. These co-dominant markers are useful for differentiating heterozygotes from both types of homozygote.

**Key words** Tomato · Random amplified polymorphic DNA (RAPD) · Sequence characterized amplified region (SCAR) · *Tm-1* gene · Tomato mosaic virus (ToMV) resistance

**Introduction**

Mosaic disease caused by tomato mosaic virus (ToMV) is one of the most serious diseases in tomato (*Lycopersicon esculentum*). A ToMV-resistance gene, *Tm-1*, has been used, either independently or in combination with one of the other ToMV-resistance genes, *Tm-2* or *Tm-2a*, to breed resistant varieties. The *Tm-1* gene originated from *L. hirsutum*, a wild relative of *L. esculentum*, by interspecific crossing (Holmes 1957). This gene inhibits synthesis of viral RNA in isolated protoplasts as well as in intact plants (Motoyoshi and Oshima 1977; Watanabe et al. 1987), presumably by interacting with a viral RNA replicase (Meshi et al. 1988).

A number of DNA markers linked to the *Tm-1* locus have so far been identified. Using restriction fragment length polymorphism (RFLP) markers *Tm-1* was mapped to a position close to a ribosomal RNA gene (rDNA) which is located on the short arm of chromosome 2 ( Tanksley et al. 1992). The length of the intergenic spacer sequence in the rDNA differs between tomato lines with and without *Tm-1* (Levesque et al. 1990). Therefore the rDNA itself can be used as an RFLP marker linked to *Tm-1*

In addition to these DNA markers, we have detected eight random amplified polymorphic DNA (RAPD) bands present in a line carrying *Tm-1* but absent in its nearly isogenic line (NIL) without *Tm-1*. Six RAPD markers were arbitrary chosen out of the eight to examine whether they are linked to *Tm-1* (Ohmori et al. 1995 b). In 125 BC1 plants, obtained by backcrossing an *F1* plant to the parent line without *Tm-1*, 63 ToMV-resistant plants carried five RAPD markers and 62 susceptible plants lacked them, suggesting tight linkage between *Tm-1* and each of these five RAPD markers. The remaining marker was also shown to be linked to *Tm-1*, because 63 ToMV-resistant plants carried this
marker, while 56 susceptible plants lacked it. In six ToMV-susceptible plants, however, the presence of the RAPD band could not be confirmed because one PCR product band was very close to the position of the RAPD band. As in the case of these six plants, RAPD primers sometimes amplify multiple non-specific DNA fragments, some of which cause difficulty in identifying specific RAPD bands.

Recently, sequence characterized amplified region (SCAR) markers converted from RAPD markers have proved to be useful (Paran and Michelmore 1993; Adam-Blondon et al. 1994; Masionneuve et al. 1994). A SCAR marker represents a specific genomic region that is amplified by PCR using a pair of specific oligonucleotide primers. SCAR markers are advantageous over RAPD markers because they are identified as distinct single bands in agarose gels, and some of them show co-dominance which differentiates heterozygotes from both types of homozygote.

In this paper, we have characterized fragments cloned from the six RAPD markers linked to the Tm-1 locus. We have also designed SCAR markers based on the sequences of the clones corresponding to the RAPD markers.

**Materials and methods**

**Plant materials**

GCR26 is a tomato variety 'Craigella' which is susceptible to ToMV. GCR237 is homozygous for Tm-1 and has a common genetic background with 'Craigella' (Smith and Ritchie 1983). These NILs, originally bred at the Glasshouse Crops Research Institute (Littlehampton, UK), have been maintained through selfing from those used in previous studies (Motoyoshi and Oshima 1975, 1977).

**DNA extraction**

Total DNA was extracted by the method of Doyle and Doyle (1987) with minor modifications. Fresh leaf tissue (1.5 g) was frozen in liquid nitrogen and ground using a mortar and pestle. The homogenate of the leaf tissue was placed in a test-tube together with 10 ml of DNA extraction buffer [2% (w/v) CTAB (hexadecyltrimethylammonium bromide), 1.4 M NaCl, 0.2% (v/v) 2-mercaptoethanol, 20 mM EDTA, 100 mM Tris-HCl, pH 8.0], and incubated in a water bath at 60 ~C for 30 min with occasional swirling. After incubation, the lysate was resuspended in TE (10 mM Tris-HCl, pH 8.0, 1 mM EDTA). The aqueous phase was mixed with a two-third volume of cold iso-}

**Results**

Cloning and characterization of RAPDs linked to the Tm-1 gene

PCR-amplified products corresponding to the six RAPDs linked to the Tm-1 gene (Ohmori et al. 1995b) were extracted from agarose gels after electrophoresis and cloned into a pBluescript II KS vector. These clones were found to be identical to their corresponding RAPD products by Southern hybridization.

At least 200 nucleotides were sequenced from both 5'-ends of each clone. In both ends of the clones from OPB10_1000, G12_800, N09_1000, and N20_1400, the terminal ten bases exactly matched the sequence of each primer for the corresponding RAPD. The proximal eight bases of the clone from OPA15_1000 also matched the eight bases of the primers, but the terminal two bases at either end of the clone were not identical to those of the primer. This result indicates that the primer annealed with the proximal eight bases of genomic DNA to generate OPA12_1000. These six clones did not cross-hybridize with each other (data not shown), suggesting that the six RAPD markers occupy independent loci close to Tm-1.