The products of the broken Tm-2 and the durable Tm-2² resistance genes from tomato differ in four amino acids

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Abstract

To gain an insight into the processes underlying disease resistance and its durability, the durable Tm-2² resistance gene was compared with the broken Tm-2 resistance gene. The Tm-2 gene of tomato could be isolated via PCR with primers based on the Tm-2² sequence. The Tm-2 gene, like the Tm-2² gene, encodes an 861 amino acid polypeptide, which belongs to the coiled coil/nucleotide binding site/leucine-rich repeat class of resistance proteins. The functionality and the nature of the isolated Tm-2 gene were confirmed by introducing the gene under the control of the 35S promoter into tomato mosaic virus-susceptible tobacco. This transgenic tobacco was crossed with transgenic tobacco plants producing the movement protein (MP)-authenticated MP as the Avr protein of the Tm-2 resistance. The Tm-2² and Tm-2 open reading frames only differ in seven nucleotides, which on a protein level results in four amino acid differences, of which two are located in the nucleotide binding site and two are located in the leucine-rich repeat domain. The small difference between the two proteins suggests a highly similar interaction of these proteins with the MP, which has major implications for the concept of durability. Comparison of the two resistance-conferring alleles (Tm-2 and Tm-2²) with two susceptible alleles (tm-2 and lptm-2) allowed discussion of the structure–function relationship in the Tm-2 proteins. It is proposed that the Tm-2 proteins display a partitioning of the leucine-rich repeat domain, in which the N-terminal and C-terminal parts function in signal transduction and MP recognition, respectively.

Key words: Lycopersicon esculentum, Lycopersicon peruvianum, Tm-2, Tm-2², tomato mosaic virus, plant disease resistance gene, durability, structure–function relationships.

Introduction

Due to breeding and selection for economically valuable traits, crops usually have little variation in their gene pool. Consequently, their resistance to harmful changes or events, like the appearance of a new pathogen, and their adaptability to changing demands are limited. This limited genetic diversity can lead to devastating disasters, which is exemplified by the Irish Potato Famine of the 1840s. Resistance against pathogens requires the presence of resistance (R) genes, whose polypeptide products recognize products of the pathogen and, subsequently, are able to trigger a defense response. These R genes could have been lost from the gene pool due to breeding or could have been absent in the original founder material, which presents a problem to breeders. A commonly used method to increase the genetic flexibility of commercial crops is to make use of the gene pool of closely related wild relatives of the crops. In this way, valuable new genes can be introduced into crops by introgression. Well-studied and successful examples of this procedure for R genes are the introductions of the N-gene of Nicotiana glutinosa, conferring resistance against tobacco mosaic virus (TMV), into N. tabacum (Dinesh-Kumar et al., 1995; Marathe et al.,...
2002), and the Cf-genes of Lycopersicon pimpinellifolium, conferring resistance against the fungus Cladosporium fulvum, into L. esculentum (Boukema, 1980). Another example of this procedure is the introgression of tomato mosaic virus (ToMV) resistances into the cultivated L. esculentum. ToMV infections in modern commercial L. esculentum varieties are controlled by the Tm-1, Tm-2, and Tm-2\textsuperscript{2} R genes (Pelham, 1966; Hall, 1980), which were introgressed from the wild tomato species L. hirsutum (Tm-1) and L. peruvianum (Tm-2 and Tm-2\textsuperscript{2}).

Genetic analysis of ToMV strains capable of overcoming the resistances has shown that for Tm-1 the RNA-dependent RNA polymerase gene of ToMV is the matching Avirulence (Avr) gene (Meshi et al., 1988). The Tm-2 and the Tm-2\textsuperscript{2} resistances are considered to be allelic (Khush et al., 1964; Pelham, 1966; Schroeder et al., 1967; Hall, 1980; Tanksley et al., 1992) and share the movement protein (MP) of ToMV as the matching Avr protein. The Tm-2\textsuperscript{2} gene was recently isolated from tomato and demonstrated to be functional in both tomato and tobacco (Lanfermeijer et al., 2003, 2004). The Tm-2\textsuperscript{2} protein displays all the characteristics of the coiled coil/nucleotide binding site/leucine-rich repeat (CNL) type of R proteins and differs considerably from the polypeptide encoded by the allele which was isolated from susceptible L. esculentum lines (tm-2). The differences are concentrated in the C-terminal half of the leucine-rich repeat (LRR) domain.

The Tm-2 and Tm-2\textsuperscript{2} resistances have characteristics which make the study of the Tm-2\textsuperscript{2} locus meaningful for the study of structure–function relationships of the CNL-type of R proteins. Firstly, the two resistances share the viral MP as the Avr but, in order to break the two resistances, mutations at different locations in the MP are necessary (Meshi et al., 1989; Calder and Palukaitis, 1992; Weber et al., 1993; Weber and Pfitzner, 1998). Secondly, the Tm-2\textsuperscript{2} resistance has been more durable than the Tm-2 resistance (Fraser et al., 1989). Consequently, the Tm-2\textsuperscript{2} resistance is still used in tomato breeding and, therefore, is of ongoing practical and economical importance. In particular, the combination of the three aspects—the sharing of MP as the Avr protein, the different locations of the mutations necessary for circumventing the resistances, and the different durabilities—is intriguing.

Here, the isolation and characterization of the resistance-conferring allele Tm-2 of the L. esculentum variety GCR236 (originally introgressed from L. peruvianum) and the lptm-2 allele of a ToMV-susceptible L. peruvianum are reported. The genes were obtained by PCR using primers developed on the basis of the sequence of the Tm-2\textsuperscript{2} gene. With the differences between the two resistance-conferring alleles as a starting point, possible reasons for the differences between the nature of the Tm-2\textsuperscript{2} and Tm-2 genes is discussed.

### Materials and methods

**Isolation of the Tm-2 allele from L. esculentum, accession Craigella GCR236 and the lptm-2 allele from L. peruvianum (CGN14355)**

The Tm-2\textsuperscript{2} locus contains only a single gene (Lanfermeijer et al., 2003), which simplifies the cloning of the homologous Tm-2 and lptm-2 alleles using the Tm-2\textsuperscript{2}-specific primer set PrRuG84/PrRuG86 (Table 1). PCR was performed on genomic DNA using Platinum Taq (Life Technologies) or ExTaq (TaKaRa Bio Inc). Three independent PCR products for each primer set were cloned into pGEM-T-Easy Vector (Promega) and their nucleotide sequences determined.

**The introduction of the Tm-2 open reading frame (ORF) in Nicotiana tabacum SR1**

The binary vector pTM90 was constructed with the Tm-2 ORF under the control of the cauliflower mosaic virus (CaMV) 35S promoter and the NOS-polyadenylation signal for the transformation of the Nicotiana tabacum SR1 line, which is susceptible to ToMV and TMV infections. Using primer PrRuG97 and PrRuG102 (Table 2), a PCR product containing the complete ORF of Tm-2 with an introduced NcoI site at the ATG and an introduced NcoI site 11 bp downstream of the TGA, was amplified from genomic DNA of tomato line Craigella GCR236. The PCR product was digested with NcoI and this fragment was introduced into the NcoI site of pTM6 (Lanfermeijer et al., 2003). The orientation of the ORF relative to the promoter and polyadenylation signal was assessed by digestion and the plasmid was named pMP1039. The pMP1039 vector was digested with AscI and PacI and the chimeric Tm-2 gene was cloned into the binary vector pVictorHiNK, resulting in plasmid pTM90.

### Table 1. PCR-primers used in this study and their target sequences

<table>
<thead>
<tr>
<th>Target</th>
<th>Name</th>
<th>Primer</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tm-2, lptm-2</td>
<td>PrRuG084</td>
<td>CTGGCAAAAGACTGCAAGCGATGATTGTC</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>PrRuG086</td>
<td>CTACACTACTACGTGTATGCATGAC</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>PrRuG087</td>
<td>TTATCCATGCGTAAATTTCTCTACATCAAAATCTCG</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>PrRuG102</td>
<td>CCTGCATGCATGTATACATCTACATCAAAATCTCG</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>PrRuG151</td>
<td>GATGCTTTCTTTAAACATCTAAAGCTGGAAG</td>
<td>R</td>
</tr>
<tr>
<td>SCAR\textsuperscript{a}</td>
<td>PrRuG248</td>
<td>AGCGTCACTTCTACATGGG</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>PrRuG249</td>
<td>AGCGTCACTTCTACATGGG</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>PrRuG531</td>
<td>ACAAGTCGTGCTACCTCCAA</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>PrRuG532</td>
<td>GCTGGTCAACATAAGATCG</td>
<td>R</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Primer sequences derived from Sobir et al. (2000).
The plasmid pTM90 was introduced into Agrobacterium tumefaciens strain LBA4404 by electroporation. Transformants were selected on L-Broth plates. Subsequently, the transformants were checked for unaltered gene constructs and used to transform Nicotiana tabacum SR1 leaf explants as described (Horsch et al., 1985; Lanfermeijer et al., 2004). After culturing the explants on the appropriate media in order to stimulate shoot and root development, kanamycin-resistant plantlets were transferred to soil and grown in the greenhouse under standard greenhouse conditions.

Seeds, obtained from self-pollination or crosses, were sown and grown on Murashige–Skoog plates, supplemented with 1% w/v sucrose and, if necessary, 100 μg mL−1 kanamycin and, subsequently, transferred to soil and grown in the greenhouse under standard greenhouse conditions.

Transgenic plants with the Tm-2 gene were selected on the basis of two criteria: the ability to grow in the presence of kanamycin, and the presence of the T-DNA. The presence of the T-DNA was assessed using PCR with the T-DNA-specific primers, PrRuG531 and PrRuG532 (Table 1). DNA that served as a template for these assays was isolated from the tobacco plants according to the alkali treatment (Klimyuk et al., 1993).

**Virus resistance assays**

Lycopersicon species and N. tabacum plants to be tested were infected with leaf homogenates of N. tabacum plants infected with Dutch greenhouse isolates of ToMV (Lanfermeijer et al., 2003). In experiments with transgenic plants, untransformed plants were used as controls for virus inoculations. The plants were all inoculated twice with a 4 d interval to rule out random escape of inoculation. Virus symptoms were visually monitored on a daily basis for the duration of the experiment (21 d). After 21 d a leaf homogenate of the infected plants was inoculated onto the ToMV-indicator plant N. glutinosa, and lesions were scored after 3–4 d. Infection of N. glutinosa with TMV and ToMV-containing solutions resulted in the development of local lesions on the infected leaves.

**CAPS and SCAR markers for discrimination of the tM-2, Tm-2, and Tm-2 alleles**

PCR was performed on template-material obtained through the alkali treatment (Klimyuk et al., 1993). Approximately 1 mm² of treated leaf-tissue was transferred to 25 μL of PCR solution. This solution consisted of 0.625 U of Taq DNA polymerase (Roche Diagnostics) in its prescribed reaction buffer with either 5 μM of the primers PrRuG086 and PrRuG151 [CAPS (cleaved amplified polymorphic sequence) markers] or 5 μM of the primers PrRuG248 and PrRuG249 [SCAR (sequence characterized amplified region) markers] (Sobir et al., 2000) (Table 1). The PCR protocol used for both marker-types was: 5 min at 94 °C, followed by 30 cycles that consisted of 15 s at 94 °C, 45 s at 55 °C, and 90 s at 72 °C. The protocol was concluded with 5 min at 72 °C. Fifteen microlitres of the PCR products were subsequently digested with HpaI, BfrI, or AccI.

**Analysis software**

Sequences were analysed using the ClustalW (Thompson et al., 1994), the Clone Manager Software (Scientific and Educational Software), and Blast (Altschul et al., 1990) software.

**Accession numbers**

The accession numbers for the Lycopersicon esculentum Tm-2, Tm-2, and tm-2 genes and the Lycopersicon peruvianum lptm-2 gene are AF536201, AF536200, AF536199, and AY765395, respectively.

**Results**

**Molecular cloning of the Tm-2 gene from L. esculentum**

Isolation of the Tm-2 allele necessitates assessment of the genotypes and phenotypes of the tomato accessions used from which it was derived. The Craigella accessions, which contain either the Tm-2 (Craigella GCR236) or the Tm-2 allele (Craigella GCR267), could only be infected with their breaking virus isolates, ToMV-2 or ToMV-2A, respectively. The susceptible Craigella (Craigella GCR26), which contains the tm-2 allele (Lanfermeijer et al., 2003), and the L. peruvianum plants could be infected with all the isolates used (Table 2). Additionally, the genotype of the Craigella accessions was confirmed using SCAR markers (Sobir et al., 2000) and new CAPS markers developed from the known sequences of tm-2 and Tm-2 and the sequence of Tm-2 presented here. These CAPS markers were based on the absence of an HpaI site in tm-2, which is present in both Tm-2 and Tm-2, and the sole presence of a BfrI site in Tm-2. The PCR product, obtained with the use of the primers PrRuG086 and PrRuG151, was either treated with

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**Table 2. Virus specificity of tomato and transgenic tobacco lines assessed by inoculation with various tobamovirus isolates**

<table>
<thead>
<tr>
<th>Tomato accession</th>
<th>Genotype or introduced T-DNA</th>
<th>Tobamovirus isolates&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>GCR26 (Craigella)</td>
<td>tm-2, tm-2</td>
<td>+&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>GCR236 (Craigella)</td>
<td>Tm-2, Tm-2</td>
<td>−</td>
</tr>
<tr>
<td>GCR267 (Craigella)</td>
<td>Tm-2&lt;sup&gt;2&lt;/sup&gt;, Tm-2</td>
<td>−</td>
</tr>
<tr>
<td>L. peruvianum (CGN14355)</td>
<td>lptm-2, lptm-2</td>
<td>n.d.&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tobacco SR1</td>
<td>None</td>
<td>+</td>
</tr>
<tr>
<td>Tobacco SR1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>pTM90 (Tm-2)</td>
<td>−</td>
</tr>
</tbody>
</table>

<sup>a</sup> Virus isolates: TMV, tobacco mosaic virus-U1 isolate; Cg, TMV-Cg isolate (an Arabidopsis- and tobacco-infecting tobamovirus); 0, ToMV-GdK (wild-type tomato mosaic virus); 1, ToMV-SPS (Tm-1 breaking isolate); 2, ToMV-GeRo (Tm-2 breaking isolate); 2A, ToMV-GM65 (Tm-2<sup>2</sup> breaking isolate). Virus isolates were obtained from Plant Research International, Wageningen, The Netherlands, except TMV-Cg which was obtained from Dr Masayuki of Hokkaido University, Japan.

<sup>b</sup> + indicates infection, − indicates no infection.

<sup>c</sup> Not determined.

<sup>d</sup> Five independent lines (F0071, F0076, F0078, F0080, and F0085) were tested and all displayed the same virus specificity.
By the combination of the results of the two CAPS markers the genotype of a plant can unambiguously be determined (Fig. 1). Tomato accessions (Craigella GCR26 and ATV840) which contain the tm-2 allele (Lanfermeijer et al., 2003) did not display digestion of the PCR product by both HpaI and BfrI, whereas for the Tm-2-carrying accession (Craigella GCR236) only HpaI digested the PCR product. The PCR product obtained from Tm-22-containing accessions (Craigella GCR267 and ATV847; Lanfermeijer et al., 2003) was digested by both enzymes.

*Lycopersicon esculentum* contains a single Tm-22-like gene (Lanfermeijer et al., 2003), which allows the cloning of the homologous Tm-2 allele using the Tm-22-specific primer set PrRuG84/PrRuG86 (Table 1). A 2875 bp PCR product was obtained, which was identical in size to similar PCR products previously obtained from the tm-2 and Tm-22 alleles (Lanfermeijer et al., 2003). Analysis and comparison of the sequence of the PCR product of Tm-2 which was obtained revealed that it contained an intact ORF of a size identical to that of Tm-22. This ORF could be translated into a polypeptide 861 amino acids long, which resembled the Tm-22 protein and, consequently, contained all the features of the CNL class of R proteins (Hammond-Kosack and Jones, 1997; Jones and Jones, 1997; van der Biezen and Jones, 1998; Lanfermeijer et al., 2003). Alignment of the Tm-2 and Tm-22 ORFs and their polypeptides revealed that the difference between the Tm-2 allele and Tm-22 was unexpectedly small; on the DNA level, seven differences (0.3%) were observed which result, on the protein level, in four differences (0.5%) (Fig. 2).

**Introduction of the Tm-2 ORF in Nicotiana tabacum SR1**

Final confirmation of the isolation of the tobamovirus R gene came from the transformation of the *Nicotiana tabacum* SR1 line, which is susceptible to ToMV and TMV infections, with the Tm-2 gene under the control of the CaMV 35S promoter and the NOS-polyadenylation signal. Primary kanamycin-resistant transformants were grown in the greenhouse and three cuttings were taken from each individual plant. The cuttings were inoculated with a Dutch wt-ToMV isolate (ToMV-GdK; Table 2). All cuttings of all primary transformants were resistant towards infection with ToMV. Also, as with the Tm-22 gene in tomato (Lanfermeijer et al., 2003) and in transgenic tobacco (Lanfermeijer et al., 2004), no macroscopically visible symptoms, like local lesions, were observed in tobacco plants containing the Tm-2 R gene. Control tobacco plants displayed the characteristic mosaic symptoms of infection. Inoculation of leaves of the ToMV-indicator species, *N. glutinosa*, with leaf sap from the mosaic-displaying plants resulted in the development of local lesions, whereas these leaves, when inoculated with leaf sap from the symptom-free transgenic plants, developed no local lesions. All five transformants analysed contained the T-DNA as detected by PCR with primers pRUG531 and pRUG532 (Table 1; data not shown). In the progeny of the primary transformants similar results were obtained: all kanamycin-resistant plants contained...
for their virus specificity. For these experiments six tobamoviruses were used: TMV; ToMV-GdK (wild-type tomato mosaic virus); Isolate 1, ToMV-SPS (Tm-1 breaking isolate); Isolate 2, ToMV-GeRo (Tm-2 breaking isolate); Isolate 2A, ToMV-GM65 (Tm-2^2 breaking isolate); and TMV-Cg (an Arabidopsis- and tobacco-infecting tobamovirus). The virus specificity of the transgenic Tm-2 gene in the tobacco background was similar to the virus specificity of the tomato-accession Craigella GCR236, which was the source of the gene. Both the GCR236 and the transgenic tobacco lines (F0071, F0076, F0080, and F0085) were resistant against TMV-U1, ToMV-GdK, ToMV-SPS, ToMV-GM65, and TMV-Cg, but could be infected by ToMV-GeRo, the Tm-2-breaking ToMV-isolate (Table 2). These observations demonstrate that next to the preservation of the ability of conferring resistance, the characteristics of the Tm-2 R gene are also conserved after transformation of the Tm-2 gene into a susceptible tobacco background, which confirms the isolation of the Tm-2 gene. Moreover, as for the Tm-2^2 gene, the use of the CaMV 35S promoter did not influence the characteristics of the Tm-2 gene (Lanfermeijer et al., 2004).

**Crosses between tobacco plants, which express the ToMV-MP gene, and tobacco plants with the Tm-2 transgene**

The Tm-2^2 gene was isolated from tomato through transposon tagging and making use of the lethal combination of the presence of MP transgene and the Tm-2^2 being expressed in the same plant (Weber and Pfitzner, 1998; Lanfermeijer et al., 2003). The same combination was tested to assess the functioning of the Tm-2 gene in tobacco. Homozygous MP-containing tobacco plants (Lanfermeijer et al., 2004) were crossed with homozygous ToMV-resistant tobacco plants containing the Tm-2 transgene. However, contrary to the observations made on the cross between MP-containing tobacco and Tm-2^2-containing tobacco (Lanfermeijer et al., 2004), the progenies of plants containing the MP or the Tm-2 transgenes obtained by self-pollination or by crossing the two genotypes, displayed all germination frequencies in the order of 80% (Table 3). However, seedlings from the cross between plants containing the MP and the Tm-2 gene displayed a severe growth arrest. Roots and shoots from these seedlings did not develop properly. The cotyledons were present and the first true leaves were initiated but neither expanded even after 28 d (Fig. 3).

**The tm-2 allele**

The tm-2 allele is considered to have originated in L. esculentum, whereas the Tm-2 and Tm-2^2 alleles originated in L. peruvianum (Lanfermeijer et al., 2003). In order to study the relationship between tm-2, Tm-2, and Tm-2^2, and to determine what role the origin of the alleles
might play in their difference, a fourth tm-2-like gene was isolated from *L. peruvianum* var. *dentatum* accession CGN14335 by PCR using the *Tm-2*-specific primers PrRuG97 and 102 (Table 1). The 2875 bp PCR product obtained contained one single ORF that encoded a protein identical in size and make-up to the *Tm-2*- protein. Because the accession, from which this allele was obtained, was susceptible to all ToMV strains tested (Table 2), this allele was named lptm-2. The difference between *lptm-2* and *Tm-2*- was less than the difference between *Tm-2*- and *tm-2* (Lanfermeijer et al., 2003). Twenty-six nucleotide differences (1.0%) were observed between *lptm-2* and *Tm-2*- that resulted in 15 amino acid differences (1.7%). Calculating the phylogenetic relationship between the four alleles resulted in an unrooted tree as presented in Fig. 2C. A clear grouping of the alleles *Tm-2*, *Tm-2*, and *lptm-2* can be observed, which is in agreement with the fact that the *Tm-2* and *Tm-2* *R* genes were introgressed into *L. esculentum* from *L. peruvianum* (Pelham, 1966) and that *tm-2* is probably an original allele of *L. esculentum*.

**Discussion**

In this paper, the isolation of the *Tm-2* gene from *L. esculentum* is described. The *Tm-2* gene is the second allele of the *Tm-2* locus that confers resistance to tobamoviruses. Its ability to confer resistance to tobamoviruses was confirmed by the introduction of this gene into *N. tabacum* SR1. The *Tm-2* and *Tm-2*- alleles have always been considered to be allelic (Khush et al., 1964; Pelham, 1966; Schroeder et al., 1967; Hall, 1980; Tanksley et al., 1992), which is now confirmed by the sequence of the *Tm-2* allele. However, it had already been observed that the two resistance-conferring alleles, *Tm-2* and *Tm-2*-, have different levels of durability (Hall, 1980; Fraser et al., 1989). Whereas the *Tm-2*- allele has been used for four decades, the *Tm-2*-conferring resistance was broken by ToMV soon after its introduction in commercial *L. esculentum* varieties. The present isolation of the *Tm-2* allele, together with the recent isolation of the *Tm-2*- allele (Lanfermeijer et al., 2003) allows comparison of these two alleles and the possibility of gaining insight in the reasons for their different characteristics (Table 4).

### The molecular differences between the proteins encoded by the alleles *tm-2*, *lptm-2*, *Tm-2*, and *Tm-2*-2

The four proteins, encoded by the four alleles of the *Tm-2* locus, have an identical structure, with all the typical elements of the CNL type of R proteins (Hammond-Kosack and Jones, 1997; Jones and Jones, 1997; van der Biezen and Jones, 1998; Lanfermeijer et al., 2003). The different characteristics of the *Tm-2* and *Tm-2*-2 resistances should reside in their amino acid composition, but, unexpectedly, the difference between the *Tm-2*- and the *Tm-2* protein is surprisingly small. Only four amino acid differences are present between the proteins of these two alleles (Fig. 2A). Of these four amino acid differences, two are located in the NB-ARC (nucleotide binding site–apoptosis, R gene products, and CED-4 domain) domain [Ile257Phe and Met286Ile (NB-ARC domain)] whereas the other two are in the LRR domain (Tyr268Ala and Ser270Thr) (Fig. 2A). The differences at positions 257 and 286 are in the motifs III and IV of the NB-ARC domain (Pan et al., 2000) but at these two positions both amino acids are allowed based on the functionality of the *Tm-2* and *Tm-2*-2 proteins and the alignments of *R* genes from *Arabidopsis* and tomato (Pan et al., 2000; Meyers et al., 2003). Whether these two differences are involved in the difference between the virus specificity of two alleles has to be studied, because it has been observed that regions outside the LRR domain can affect the specificity of R proteins (Ellis et al., 1999; Luck et al., 2000). However, based on the predominant view that the LRR domain is the major determinant in R-protein specificity and that the NB-ARC domain functions in signal transduction (Jones and Jones, 1997; Bittner-Eddy et al., 2000; Ellis et al., 2000; Halterman et al., 2001; Moffett...
Table 4. Differences between the Tm-2 and Tm-2^2 resistances

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Tm-2</th>
<th>Tm-2^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics of the breaker virus</td>
<td>Phe257; Ile286;</td>
<td>Ile257; Met286;</td>
</tr>
<tr>
<td>Location of the mutations of the breaker virus</td>
<td>Virulent</td>
<td>Tyr767; Ser769</td>
</tr>
<tr>
<td>Phenotype of cross with MP-containing plants</td>
<td>N-terminal and central regions of MP</td>
<td>C-terminus of MP</td>
</tr>
<tr>
<td></td>
<td>Arrested growth</td>
<td>No germination</td>
</tr>
</tbody>
</table>

The interaction between the two R proteins and MP

On the interaction between the Tm-2 and Tm-2^2 R proteins and MP, several, and at first sight contradictory, observations have been made. Firstly, the amino acid changes in MP that are necessary to overcome the Tm-2 and Tm-2^2 resistances are located in different parts of MP. For the Tm-2 resistance those changes are located in the N-terminal half (Meshi et al., 1989; Calder and Palukaitis, 1992), whereas those necessary to overcome the Tm-2^2 resistance are located in the C-terminal half of MP (Weber and Pfitzner, 1998; Calder and Palukaitis, 1992; Weber et al., 1993). This suggests that the two R proteins interact with different domains of MP. Secondly, the experiments performed by Weber et al. (2004) indicate that the interactions of the Tm-2 and Tm-2^2 proteins with MP are not that different. They showed that transgenic expression of a gene, which encodes a protein consisting of the first two-thirds of MP, in tomato plants with either the Tm-2 or Tm-2^2 resistance results in a hypersensitive response, while expression of a gene encoding the last third of MP (amino acids 187–264) in both types of plants did not. This suggests a major role for the N-terminal two-thirds of MP in the interaction with both the Tm-2 and Tm-2^2 proteins (Weber et al., 2004). However, both with Tm-2 and Tm-2^2, resistance observations were made that imply a role of the C-terminal third of MP in the interaction. In Tm-2-containing plants the induction of the necrotic response by MP with a deleted C-terminus was significantly delayed in comparison with the response elicited by the full-length protein. In addition, in Tm-2^2-containing plants, fusion of β-glucuronidase to the C-terminus of MP resulted in an absence of the necrotic response (Weber et al., 2004).

Now, the observation can be added that the difference between the Tm-2 and Tm-2^2 R proteins is only four amino acids. This suggests that the interaction between the Tm-2 and Tm-2^2 proteins and MP or the MP/virulence target complex could be highly similar.

These observations can be reconciled with each other if one considers the proposed topological model of the MP of TMV, which suggests this protein is an integral membrane protein (Brill et al., 2000). If one adapts the model of Brill et al. (2000) for the MP of ToMV it becomes clear that all changes in the Tm-2- and Tm-2^2-overcoming strains, except one, are located in the putative cytoplasmic domain of the MP. Moreover, it is very well possible that, due to the folding of MP, the domains in which the respective mutations are necessary to overcome either Tm-2 or the Tm-2^2 resistance interact or are close together. It is, therefore, possible that the two positionally differing sets of mutations are able to affect two highly similar interactions between the two R proteins and MP (or the complex involving MP). Close proximity or interaction of the two domains could also explain the absence of virus strains able to overcome both the Tm-2 and Tm-2^2 resistance by
harbouring both sets of mutations (Fraser et al., 1989). If both the set of changes for breaking Tm-2 and the set for breaking Tm-2² affect the same area of MP, the combined presence of both sets could then have a fatal impact on the function of MP.

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