**Turnip Mosaic Virus Determinants of Virulence for Brassica napus Resistance Genes**

C. E. JENNER1*, F. SÁNCHEZ2, K. TOMIMURA3, K. OHSHIMA3, F. PONZ2 and J. A. WALSH1

1Horticulture Research International, Wellesbourne, Warwick, CV35 9EF, United Kingdom; 2INIA, 28040, Madrid, Spain; 3Laboratory of Plant Virology, Saga University, Saga 840-8502, Japan

*Tel.: +44 1789 470 382, Fax: +44 1789 470 552, E-mail: carol.jenner@hri.ac.uk

**Abstract**

Dominant resistance genes identified in *Brassica napus* lines are effective against some, but not all, *Turnip mosaic virus* (TuMV) isolates. An infectious clone of an isolate (UK 1) was used as the basis of chimeric virus constructions using resistance-breaking mutants and other isolates to identify the virulence determinants for three dominant resistance genes. For the resistance gene *TuRB01*, the presence of either of two mutations affecting the cylindrical inclusion (CI) protein converted the avirulent UK 1 to a virulent isolate. Acquisition of such mutations had a slight cost to viral fitness in plants lacking the resistance gene. A similar strategy is being used to identify the virulence determinants for two more resistance genes present in another *B. napus* line.

**Keywords:** Turnip mosaic virus; Brassica napus; resistance genes; pathogenicity determinants

**INTRODUCTION**

*Turnip mosaic virus* (TuMV) is a potyvirus of considerable economic importance. Its wide host range, widespread occurrence and mode of transmission combine to make control using resistance genes of particular interest. Many sources of resistance have been identified, some of which have been used in a scheme for differentiating (pathotyping) isolates (WALSH 1989; JENNER & WALSH 1996). The gene-for-gene hypothesis is often used to explain the specificity of interaction between plant hosts and pathogens. This paper describes progress towards identifying the pathogen components involved in resistance specificity. It is hoped that some understanding of the basis of the specificity will inform the choice of genes for use as durable field resistance.

**MATERIALS AND METHODS**

An infectious cDNA clone of the TuMV isolate UK 1 was constructed (SÁNCHEZ et al. 1998). *B. napus* lines R4, 165 and N-o-1 have been described previously (WALSH et al. 1999). Mutants of UK 1, able to infect the resistant lines R4 and 165 were isolated from spontaneous events in the glasshouse. TuMV isolates UK 1 and CDN 1 have been described previously (WALSH 1989). Sequences of parental and mutant isolates were obtained using standard techniques. Recombinant plasmids were constructed using restriction enzymes cutting at conserved sites, and standard cloning techniques. 10 µg of recombinant plasmid was rubbed onto plant leaves to initiate viral infection. Subsequent inoculations were performed as described previously (WALSH 1989). Sequences

This work was funded by the BBSRC core strategic Grant to HRI, the EU and DEFRA.
of recombinant viruses in plants were confirmed following reverse transcription PCR. PCR primers were also designed to distinguish between isolates differing by single nucleotides. Viral constructs were tested on families of plant lines derived from crosses between susceptible and resistant plants segregating for the presence of the various resistance genes.

RESULTS

TuRB01: Two mutants of the isolate UK 1 gained the ability to infect lines R4 and N-o-1, in contrast to the parental isolate. The full-length sequence of one mutant differed from UK 1 by a single nucleotide affecting an amino acid in the cytoplasmic inclusion (CI) protein (Table 1). The second mutant possessed a different mutation in the same gene (Table 1). Introduction of either mutation into UK 1 endowed UK 1 with the ability to infect those plant lines of mapping populations that possessed TuRB01.

TuRB04 and TuRB05: A mutant of UK 1 gained the ability to infect line 165, in contrast to the wild-type isolate. The full-length sequence of the mutant differed from UK 1 by 16 nucleotides throughout the genome. Introduction of a single nucleotide change affecting the P3 protein caused UK 1 to induce necrotic lesions on inoculated leaves; introduction of this mutation in combination with a second affecting the CI protein promoted full virulence without necrosis. Analysis of plant crosses indicated that line 165 possesses two resistance genes. TuRB04 confers extreme symptomless resistance and is epistatic to TuRB05, a gene conferring a hypersensitive response. TuRB04 resistance was overcome by a mutation in the P3 gene; TuRB05 resistance was overcome by a mutation in the CI gene (Table 1).

Stability of mutations: Both of the mutations identified that permitted UK 1 to infect line 165 (possessing TuRB04 and TuRB05) were unstable in the propagation host B. juncea (lacks resistance genes) and mutants reverted to wild-type in this host. The wild-type virus rapidly dominated cultures. The mutation in the CI protein causing TuRB01-resistance breaking ability was stable in B. juncea, but co-culturing the wild-type and mutant versions together over five serial passages produced a culture lacking the resistance-breaking mutation.

DISCUSSION

The CI protein has now been identified as a TuMV pathogenic determinant for TuRB01 (Jenner et al. 2000) and TuRB05 (Jenner et al. 2002a). These resistance genes cause different phenotypes and interact with different parts of the viral protein. The P3 protein functions as a pathogenic determinant for TuRB04 (Jenner et al. 2002a). The CI protein is an RNA helicase and has a function in the cell-to-cell movement of the virus (UrCuqui-Inchima et al. 2001). The function of the P3 protein is not understood but may have a role in the symptoms caused by potyviruses (Saenz et al. 2000). The slight cost to viral fitness in acquiring the ability to overcome TuRB01 (Jenner et al. 2002b) is reiterated in the strong tendency of TuRB04- and TuRB05-breaking mutants to revert to the wild-type sequence when cultured in plants lacking known resistance genes. Although each of the three resistance genes examined was overcome by spontaneous viral mutation, there is evidently a fitness penalty to the virus in the absence of selection pressure. Such information will help inform decisions about deployment of resistance genes, singly and in combinations.

Acknowledgements: We thank X. Wang for assistance in differentiating isolates, and A. Sharpe, D. Lydiate and S. Hughes for plant lines from various mapping populations.

Table 1. TuMV UK 1 pathogenicity determinants for Brassica napus resistance genes

<table>
<thead>
<tr>
<th>Plant gene</th>
<th>TuMV protein</th>
<th>Amino acid mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TuRB01</td>
<td>CI</td>
<td>N459D</td>
</tr>
<tr>
<td>TuRB01</td>
<td>CI</td>
<td>H630R</td>
</tr>
<tr>
<td>TuRB04</td>
<td>P3</td>
<td>F312L</td>
</tr>
<tr>
<td>TuRB05</td>
<td>CI</td>
<td>M589T</td>
</tr>
</tbody>
</table>

References


