Characterising Resistance to *Turnip mosaic virus* (TuMV) in *Turnip* (*Brassica rapa rapa*).

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**Abstract**


A *Brassica rapa* *rapa* L. line has been identified with high resistance to seven isolates of *Turnip mosaic virus* (TuMV) (including UK 1, CHN 5, CZE 1, CDN 1, GBR 6, POL 1 and UK 4) representing the major pathotypes of the virus. Resistant plants showed no symptoms following mechanical inoculation with TuMV and no virus was detected in the plants by ELISA. A cross was made between the rapid-cycling *Brassica rapa* line R-o-18 (which has been found to be susceptible to all the TuMV isolates) and a plant from the resistant *B. rapa rapa* line. The small amount of the F₁ generation seed available from this cross has been grown and inoculated with the seven TuMV isolates. F₂ plants were uniformly resistant to the UK 1 isolate of TuMV, uniformly susceptible to the CHN 5 isolate (only 2 plants inoculated) and segregated for resistance and susceptibility to the other five TuMV isolates. This suggested that the parent *B. rapa rapa* plant used in the cross was probably homozygous for one, or more dominant resistance genes to the UK 1 isolate of TuMV and heterozygous for one, or more dominant resistance genes to the other TuMV isolates. When self seed (S₁) from the parent plant from the resistant line was inoculated with the TuMV isolates GBR 6 and UK 4, the segregation for the former isolate was not significantly different from 3 resistant to 9 susceptible, suggesting resistance to GBR 6 is controlled by a single dominant gene, whereas resistance to UK 4 is controlled by two or more dominant resistance genes. The putative resistance genes appear to confer hitherto unknown dominant TuMV resistance specificities, and in combination have the exciting potential of providing durable resistance to TuMV.

**Keywords:** *Brassica; TuMV isolates, Plant lines resistance.*

**Introduction**

*Turnip mosaic virus* (TuMV), a member of the *Potyviridae* family, has the widest host range of the *Potyvirus* genus (16). It is among the most damaging viruses of *Brassica* species and other crops worldwide. In certain parts of Asia including North China and Taiwan, where large amounts of Chinese cabbage (*Brassica rapa* L.) are consumed, TuMV causes serious economic losses in many important vegetables. It also infects many plant species in temperate and tropical regions of the world and is the second most important virus to infect field-grown vegetables in the world (19). It is particularly important in *B. oleracea* vegetable types (cabbage, cauliflower, broccoli and kale) and other crops including oilseed rape, radish, horseradish, lettuce, chicory, peas, rhubarb and ornamentals (*Abutilon*, stocks and wallflowers) (15).

TuMV has a very wide host range infecting at least 318 plant species of 43 families (2), including weed species belonging to 14 different families (15). The distribution of TuMV in infected plants influences acquisition and spread of the virus by aphids (1). TuMV is transmitted, in the field, in the non-persistent stylet-borne manner by eighty-nine aphid species (2). The most important vectors are thought to be *Myzus persicae*, *Brevicoryne brassicae* and *Aphis gossypii* (9, 16). Some *Brassica* cultivars develop progressive necrosis of leaves, petioles and stem with some TuMV isolates, leading to plant death, particularly in *B. napus* (22). Cabbage growers in the UK have experienced complete loss of stored material (up to 1200 tons in one store), with others suffering substantial losses in the range of 15-20%. In 2005, one grower alone recorded losses of 200,000 GB pounds (Walsh, unpublished).

Strategies for the management of viral diseases normally include control of vector populations using insecticides, use of virus-free propagating material, appropriate cultural practices and use of resistant cultivars. However, each of the above methods has its own drawbacks.

Natural plant resistance is likely to be the most effective and environmentally friendly method of controlling TuMV. Recently identified resistances in *Brassica rapa* appear to be effective against a broad range of TuMV isolates (14, 18, 24). Several different modes of inheritance of TuMV resistance in *B. rapa* have been described (18, 25).

A number of systems for discriminating strains/pathotypes of TuMV have been described. That of Provvidenti (11) using Chinese cabbage (*B. rapa*) differentials discriminated strains C1–C4 and subsequently (3) described C5. Stobbs and Shattuck (17) described C6, and Liu et al. (10) C7 and C8. Walsh (20) distinguished four TuMV groups depending on the interaction of isolates with three *B. napus* differentials. Using these differentials plus one further *B. napus* line, Jenner & Walsh (5) characterised 124 isolates from around the world, revealing 12 distinct pathotypes. They concluded that the most common pathotypes in Europe were 1, 3 and 4. Variation in TuMV in terms of interactions with *Brassica* plants has been studied further and a gene-for-gene relationship has...
been described (21). The characterisation and genetic
classification of the first virus resistance gene in *

\textit{Brassica},

\textit{TuRB01} (23) and the identification of the TuMV gene
coding the pathogenic determinant to this gene (7) established this gene-for-gene interaction between TuMV and *

\textit{Brassica}. Other genes for resistance to TuMV have been
mapped in lettuce (Tu, 12), \textit{B. napus} (TuRB02, 23; TuRB03, 4; TuRB04 and TuRB05, J. A. Walsh and D. J. Lydiate, unpublished) and in *

\textit{Brassica rapa} (TuRB01h, 13). All are dominant resistance genes (R genes) that control resistance to narrow spectra of TuMV isolates.

There is a need to find sources of broad-spectrum resistance to all pathotypes of TuMV in brassicas, or to combine different resistance sources which, together, will provide broad-spectrum resistance.

In this paper we describe the characterisation of resistance to TuMV in a \textit{B. rapa} line which had previously been shown to be resistant to TuMV (Walsh & Bambridge, unpublished), with a range of TuMV isolates representing the three most common pathotypes in Europe (1, 3, and 4) and a particularly aggressive pathotype 12 isolate.

\section*{Materials and Methods}

\textbf{Virus isolation and inoculation procedure}

\textit{Turnip mosaic virus} isolates UK 1, CHN 5, CZE 1, CDN 1, GBR 6, POL 1 and UK 4, were used in this study. UK 1 was isolated from \textit{B. napus} in Warwickshire, UK (20), the Chinese isolate CHN 5 (5) was isolated from \textit{Brassica} sp. in 1994 and was used because it is representative of the aggressive C5 TuMV strain from Taiwan and China (3, 10); CZE 1 (5) was isolated from \textit{Brassica oleracea} in the Czech Republic; CDN 1 (20) was isolated from \textit{Brassica napus} by Dr V.I. Shattuck, Guelph, Canada; GBR 6 was isolated from winter cauliflower in Lincolnshire, UK in 1991 (5); POL 1 (5) was isolated from \textit{B. napus} in Poland by K. Ostrowka and UK 4 was isolated from \textit{Brassica oleracea} in Lincolnshire UK in 1991 (5). The isolates were selected as representatives of pathotypes 1, 3, 4 and 12 (Table 1). Most of the virus isolates were maintained in infected mustard plants (*\textit{Brassica juncea}, cultivar Tendergreen) in the glasshouse, those that weren’t, were revived from liquid nitrogen storage by inoculating to

healthy mustard plants, or test plants at the two to three true-leaf stage (≈ 4 weeks after planting) growing in 9 cm pots, as described in Jenner & Walsh (5). Inoculated plants were maintained in an insect-proof greenhouse at 18-20°C.

\textbf{Plant material}

The \textit{B. rapa rapa} line used in this study was obtained from Warwick HRI Genetic Resources Unit (GRU). It was sent to the GRU as an accession of *\textit{Brassica oleracea}, however, it was identified as turnip by the GRU and subsequent cytometry testing of plant tissue confirmed that it was *\textit{B. rapa rapa} (turnip). R-o-18 is an inbred line of *\textit{B. rapa rapa}, \textit{trilocularis} that is susceptible to all the TuMV isolates it has been inoculated with (Rusholme and Walsh, pers. comm.).

The TuMV-susceptible line, R-o-18 was bud-pollinated with pollen from a single \textit{B. rapa rapa} plant to produce the F1 generation (BR05 085). A number of buds were pollinated per inflorescence and all the remaining buds were removed. The inflorescences were enclosed in cellophane bread bags to prevent cross-pollination and allowed to mature. Once the seed pods had ripened (≈ 8 weeks after the last bud was pollinated) they were dried, harvested, and the seed was collected. The S1 generation (BR05 058) was produced by selfing the parent *\textit{B. rapa rapa} plant. Inflorescences of the parent plant were covered with a cellophane bag at the unopened bud stage. As buds opened, plants were shaken to encourage transfer of pollen from stamen to stigma. Seed was collected when mature. The breeding strategy used to characterise the resistance is shown in Figure 1.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{breeding_strategy.png}
\caption{The breeding strategy used to characterise the resistance in *\textit{B. rapa rapa} and the genetic model for the resistance to *\textit{Turnip mosaic virus} isolate GBR 6.}
\end{figure}

\textbf{Testing plants for resistance to TuMV}

Seed was sown in half seed trays (22 x 17 x 5cm) of Levington F2+S (Seed and Modular Compost). One hundred seeds of the original *\textit{B. rapa rapa} line, 30 seeds of line R-o-18, 40 seeds of the F1 (BR05 085) generation and 85 seeds of S1 generation (BR05 058) were sown in rows. Trays were maintained in an insect-proof isolation glasshouse at 18-20°C.

Seven days later, seedlings at the cotyledon stage, were transplanted into FP7 pots of compost (Levington M2 Pot and Bedding Compost). Four TGM were transplanted as controls. When the majority of the seedlings had reached the 2-3 true leaf stage (≈ 4 weeks), each was labelled with a unique number. Each plant was marked using a pipette tip to make a small hole in the youngest leaf to be inoculated. All plants were dusted with carborundum and then the upper surface of all leaves including the cotyledons were inoculated with one virus isolate per plant by gently rubbing them with a piece of muslin soaked in either healthy mustard leaf sap (as a control) or sap from TuMV-infected mustard leaves ground with a pestle and mortar in inoculation buffer (1% K2HPO4 + 0-1% Na2SO4 in distilled water). The different plant lines and isolates of TuMV used are shown in Table 1.

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
\textbf{Plant line} & \textbf{TuMV isolate} \\
\hline
R-o-18 & UK 1 \\
F1 (BR05 085) & CZE 1 \\
S1 (BR05 058) & CDN 1 \\
\hline
\end{tabular}
\caption{Plant lines and TuMV isolates used in the study.}
\end{table}
Table 1. The reaction of lines derived from Brassica rapa rapa to seven different isolates of Turnip mosaic virus (TuMV).

<table>
<thead>
<tr>
<th>Plant line</th>
<th>Number of plants infected with each TuMV isolate (pathotype)/total number of inoculated plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UK 1 (4)</td>
</tr>
<tr>
<td>B. rapa</td>
<td>0/22</td>
</tr>
<tr>
<td>F1 (BR05 085)</td>
<td>0/5</td>
</tr>
<tr>
<td>S1 (BR05 058)</td>
<td>0/15</td>
</tr>
</tbody>
</table>

+ = systemic mosaic infection (susceptible); ++N = systemic necrotic infection (susceptible); n.t. = not tested.

Infection assessment

Plants were visually assessed for the presence of virus symptoms at weekly intervals. Resistance and susceptibility were verified by results from ELISA tests on inoculated and uninoculated leaves of a sample of plants from those showing resistance and susceptibility. Where ELISA results showed values were borderline, further tests were carried out by inoculating leaves of susceptible mustard plants with extracts of leaves of test plants.

Observed segregation ratios of resistant and susceptible phenotypes were analysed by chi-square tests for goodness of fit to expected Mendelian models.

Serological tests

Indirect plate-trapped antigen ELISA as described by Walsh et al. (23) was used to test for the presence of TuMV in inoculated plants at the four weeks post inoculation period to determine whether or not there was any infection in those plants where no symptoms were observed. Fresh extracts of leaves of test plants were diluted 1:1 in 0.05M sodium carbonate buffer. The first antibody (diluted 1/2500) was a mouse monoclonal antibody (EMA 67) produced against TuMV isolate CZE 1 and shown to be capable of recognising all isolates of this virus (6). The second antibody was goat anti-mouse IgG conjugated to alkaline phosphatase (Sigma Chemical co., A-3562; Poole, UK) (diluted 1/5000) which was incubated for 3 hours at lab temperature. The substrate was made up in 10% diethanolamine (20 ml substrate buffer + 4 alkaline phosphatase tablets, Sigma S0942 ; 1/5 ml).

Results

Broad-spectrum high resistance to a diverse range of TuMV isolates (including UK 1, CHN 5, CZE 1, CDN 1, GBR 6, POL 1 and UK 4) has been identified in the B. rapa rapa line. Resistant plants showed no detectable local or systemic symptoms following mechanical inoculation with the isolates and no virus was detected in those resistant plants tested by ELISA. The parental B. rapa rapa line was segregating for resistance to the GBR 6 isolate (three of the 22 plants inoculated were susceptible showing systemic mosaic and necrotic symptoms) and was uniformly resistant to UK 1, CHN 5 and UK 4 TuMV isolates (Table 1). The inbred rapid-cycling B. rapa line R-o-18 was susceptible to all of the TuMV isolates it was inoculated with (UK 1, CHN 5, CZE 1, CDN 1, GBR 6, POL 1 and UK 4; Table 1). ELISA tests confirmed the presence of TuMV in tested plants showing symptoms and failed to detect any TuMV in tested plants that had no symptoms.

Due to the small amount of F1 generation seed available from the cross between R-o-18 and B. rapa rapa, only a small number of plants could be inoculated with the different TuMV isolates. Plants of the F1 generation were uniformly resistant to the UK 1 isolate of TuMV, uniformly susceptible (only two plants tested) to CHN 5 (necrotic symptoms) and segregated for resistance and susceptibility to isolates CZE 1, CDN 1, GBR 6, POL 1 and UK 4 (susceptible plants segregated for mosaic and necrotic symptoms).

When self seed (S1) from the resistant parent plant was inoculated with the TuMV isolate UK 1, all S1 plants were resistant, whereas with isolates GBR 6 (13 out of 48 plants inoculated were susceptible) and UK 4 (9 out of 13 plants inoculated were susceptible) there was segregation for resistance and susceptibility (Table 1).

χ² tests on the segregation ratios of the number of resistant to susceptible plants in the F1 generation showed that those for TuMV isolates CZE 1, CDN 1, GBR 6, POL 1 and UK 4 were not significantly different from 1:1 or 1:3. χ² tests on the segregation ratio of the number of resistant to susceptible plants for the S1 generation inoculated with TuMV isolate GBR 6 was not significantly different from 3:1 and that for the UK 4 isolate was not significantly different from 1:3, or 9:7. All comparisons were at P= 0.05.

Discussion

Resistance to a range of TuMV isolates representing the major pathotypes of the virus has been found and characterised in a line of B. rapa rapa (turnip). There appear to be a number of different genes for resistance to the different isolates of TuMV which in combination seem capable of conferring broad-spectrum resistance to all of the isolates.

The fact that the F1 generation of the cross between the resistant B. rapa rapa line and the uniformly susceptible inbred B. rapa line R-o-18 was completely resistant to TuMV isolate UK 1 and there was no segregation suggests that the resistant parent plant was homozygous for one or more dominant resistance genes to UK 1 conferring extreme resistance. As all the parental plants tested were resistant, they must all have possessed at least one copy of the resistance gene(s). The lack of observed segregation in the S1 generation further supports the assumption that the resistant parent plant was homozygous for the resistance gene(s). The dominant resistance gene TuRB01b has been mapped in the B. rapa A genome (13, 21) and confers resistance to the UK 1 isolate of TuMV (24). What appears to the same gene (TuRB01)
It is possible that the resistance gene we have identified in *B. rapa rapa* is TuRB01. It might be possible to investigate this using chimeric versions of the infectious clone of TuMV isolate UK 1 that overcome TuRB01b (7, 24), however, this approach might be confounded by the presence of the other resistance gene(s) in the *B. rapa rapa* line.

As the F1 generation of the cross between the plant from the resistant *B. rapa rapa* line and the uniformly susceptible inbred *B. rapa line R-o-18* segregated for resistance to all the other TuMV isolates, the resistance to these isolates must be controlled by different gene(s) to that/those responsible for the UK 1 resistance. As there was some resistance to these isolates in the F1 generation, it suggests that the resistant parent plant was heterozygous for one or more dominant resistance genes, or that the resistance was incompletely dominant. As the first self (S1) generation segregated for resistance to the two isolates it was tested with (GBR 6 and UK 4), this further supports the notion that the parent plant was heterozygous for one or more dominant resistance genes. For the resistance to GBR 6, the segregation ratio of the F1 generation wasn’t significantly different from 1 resistant : 1 susceptible and that of the S1 wasn’t significantly different to 3 resistant : 1 susceptible (Figure 1). This is consistent with the resistance being controlled by a single dominant gene where the resistant parent plant used in the cross was heterozygous for the resistance gene. As the segregation ratio of the S1 generation following inoculation with UK 4 was quite different (not significantly different from 1 resistant: 3 susceptible, or 9 resistant: 7 susceptible), it suggests that the genetic basis of this resistance is different to that for GBR 6. The most likely basis of the resistance to UK 4 is the requirement of two (or possibly more) dominant resistance genes, where the resistant parent plant used in the cross was heterozygous for both of the resistance genes. Such a scenario would result in a ratio of 9 resistant: 7 susceptible plants in the S1 generation and 1 resistant: 3 susceptible in the F2 generation, assuming balanced segregation. The χ2 tests showed that the observed ratios in these two generations were not significantly different from the 9 resistant: 7 susceptible for the S1 generation and 1 resistant: 3 susceptible in the F2 generation.

A gene-for-gene relationship between TuMV and resistance genes in the *Brassica* A genome has already been established, with five plant *R* genes and four TuMV avirulence determinants (8, 21). The resistance in the *B. rapa rapa line R-o-18* adds to this pathosystem with the discovery of the new resistance specificities involving what appears to be dominant resistance genes to the pathotype 4 TuMV isolate GBR 6 and the pathotype 12 TuMV isolate UK 4. Further dissection, characterisation and mapping of these genes will be needed to confirm the number of resistance genes present and their individual, as well as collective resistance specificities for different TuMV isolates. Recessive broad-spectrum resistance to TuMV has been described and characterised (14), however, the dominant and apparent broad-spectrum nature of the *B. rapa rapa* resistance provides exciting opportunities for breeding *B. rapa* lines with durable resistance to TuMV.
References


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