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Different classes of resistance to turnip mosaic virus in *Brassica rapa*

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Abstract

Pathotype-specific and broad-spectrum resistance to turnip mosaic virus (TuMV) have been identified in the diploid A genome brassica species *Brassica rapa*. The pathotype-specific resistance is effective against pathotype 1 isolates of TuMV, which are the most common in Europe. It is almost identical in its specificity to that of a mapped resistance gene (*TuRB01*) present in the A genome of the amphidiploid species *Brassica napus*. A mutant of a pathotype 1 isolate of TuMV (UK 1M) that is able to overcome *TuRB01* also overcame the *B. rapa* resistance. This, combined with the fact that a single-nucleotide mutation in the cylindrical inclusion gene of TuMV that has been shown to induce a change from avirulence to virulence against *TuRB01*, had an identical effect on the *B. rapa* resistance, suggest that the two resistances are conditioned by the same gene. A second source of resistance in *B. rapa* prevented systemic spread of all TuMV isolates tested. A third source of resistance that appears to provide immunity to, or severely restrict replication of most isolates of TuMV has been characterised. This resistance source also prevented systemic spread of all TuMV isolates tested. Prior to this study, no resistance to pathotype 4 or pathotype 12 isolates of TuMV had ever been identified. For each of these three resistance sources, plant lines that are not segregating for some of the resistance phenotypes and that are presumably homozygous for the genes controlling these phenotypes have been generated. Strategies for further characterising and deploying these resistances in different *Brassica* species are described.

Introduction

Turnip mosaic virus (TuMV) is a member of the *Potyviridae*, the largest family of plant viruses. It is an important pathogen of *Brassica* species and other crops worldwide. In certain parts of Asia where large amounts of Chinese cabbage (*Brassica rapa*) are consumed, TuMV is a major constraint on production. In China, following detailed national surveys, it has been shown to be the most important pathogen of *B. rapa*. In other parts of the world, for example Europe where *B. oleracea* vegetable types (cabbage, cauliflower, broccoli, Brussels sprout) are widely grown, again TuMV is a major problem (Tomlinson, 1987). There are a number of reasons why TuMV is such a widespread and important pathogen. It has a very wide host range, the widest in terms of plant genera of any potyvirus. It infects 318 plant species, including weed plants belonging to 14 different families (Shattuck, 1992). Also in many parts of the world, horticultural and arable brassicas are grown in close proximity and often there is year-round production which results in reservoirs of TuMV-infected plants providing constant inoculum sources for aphid vectors (Walsh, 1986).

Due to the ineffectiveness of insecticides in controlling TuMV (Evans and MacNeil, 1983; Niu et al., 1983), deployment of natural plant resistance is likely to be the most effective means of control. Extreme

forms of resistance to TuMV have been described in *B. napus* (Tomlinson and Ward, 1978; Shattuck and Stobbs, 1987; Walsh and Tomlinson, 1985) and *B. rapa* (Provvidenti, 1980; Niu et al., 1983; Suh et al., 1995). The only gene conferring resistance that has been mapped to date (*TuRB01*) is in the A genome of *B. napus* (Walsh et al., 1999). None of the resistances described to date have been found to be effective against all isolates of TuMV. No extreme forms of resistance to TuMV have been reported in *B. oleracea* (C genome) types. There is a need to find sources of broad-spectrum resistance to all pathotypes of TuMV in brassicas, or to combine different resistance sources that, together, will provide broad-spectrum resistance. In the longer term it is desirable to deploy these genes in the different *Brassica* species. In order to achieve this, it will be necessary to fully characterise the resistances, determine their mode of inheritance and map the genes involved. This will facilitate the rapid introgression of these genes into desired genetic backgrounds by marker-assisted selection.

Variation in TuMV in terms of interactions with *Brassica* plants has been studied, is reminiscent of a gene-for-gene relationship and twelve different pathotypes have been described (Jenner and Walsh, 1996). The characterisation and genetic mapping of the virus resistance gene in *Brassica*, *TuRB01* (Walsh et al., 1999) and the identification of the TuMV gene encoding the pathogenic determinant to this gene (Jenner et al., 2000) has established the first gene-for-gene interaction between TuMV and *Brassica*. The diversity of TuMV identified earlier (Jenner and Walsh, 1996) has been utilised in the work described here to characterise new sources of resistance.

As a first and necessary step in the genomic mapping and deployment of resistances to TuMV, this paper describes the characterisation of a pathotype-specific and two different pathotype non-specific resistances to TuMV in *B. rapa*. We also discuss how these resistances could be deployed in different *Brassica* crop species.

Materials and methods

Virus isolates and Brassica *lines*

The TuMV isolates UK 1, CZE 1, CDN 1, JPN 1, DEU 7, GK 1 and UK 4, were used as type members of pathotypes 1, 3, 4, 7, 8, 9 and 12 respectively (Jenner and Walsh, 1996). Additionally, CHN 5 which is a particularly important resistance-breaking isolate representing the strain C5 (Green and Deng, 1985) and belonging to pathotype 3 (Jenner and Walsh, 1996) was used. The isolate UK 1M is a resistance-breaking mutant of UK 1 (Lehmann et al., 1997). The infectious clone of TuMV, p35Tunos (Sánchez et al., 1998) derived from UK 1 was also used. A single-nucleotide change was introduced into this clone at position +5570 (Jenner et al., 2000). Virus derived from p35Tunos (v35Tunos) cannot infect plants possessing the resistance gene *TuRB01*, whereas virus derived from the altered clone (v35Tunos +5570 A > G) is capable of overcoming *TuRB01* (Jenner et al., 2000).

The Chinese cabbage lines studied were the F_1 hybrid cultivar Tropical Delight (T. Sakata and Company) with some resistance to TuMV (Provvidenti, 1980) and lines BP079 and BP058 found to be among the five most resistant lines of 3000 tested (Liu et al., 1996). Selfs were produced by bud pollination and then covering pollinated flowers with bags. *B. napus* line N-o-1 possesses the resistance gene *TuRB01* (Walsh et al., 1999).

Disease assays

The virus-plant interactions were determined as described by Jenner and Walsh (1996). Indirect plate-trapped antigen (PTA) ELISA tests were carried out as described by Walsh et al. (1999). Triple antibody sandwich (TAS) ELISA using a polyclonal antiserum to TuMV to coat the plates, the monoclonal antibody EMA 67 as the secondary antibody (Jenner et al., 1999) and goat anti-mouse alkaline phosphatase conjugate (Sigma) as the tertiary antibody was carried out in some instances. In addition to ELISA testing, leaves from test plants were ground up in inoculation buffer (Jenner and Walsh, 1996) and inoculated to plants of a highly susceptible mustard, *B. juncea* cultivar Tendergreen in some cases, to further check for the presence or absence of TuMV infection. Reverse transcriptase polymerase chain reaction (RT-PCR) was performed on extracts of Tropical Delight leaves systemically infected with v35Tunos +5570 A > G, using the oligonucleotide primers 5′-GGTGGGACGTCCTTTGGTAAC-3′ and 5′-CAGGTTTTGGTCGGCTTTCA-3′ to amplify the region of the cytoplasmic inclusion gene encompassing position +5570. The product was sequenced (Jenner et al., 2000).

In all experiments, all virus isolates were inoculated to the *B. napus* differentials that define their

pathotype (Jenner and Walsh, 1996), in order to check their authenticity and stability. All isolates behaved as described previously on these differentials (Jenner and Walsh, 1996).

Results

Tropical Delight resistance

The cultivar Tropical Delight had extreme resistance (0) to UK 1, was resistant to systemic infection but susceptible to local necrotic infection (R_N) by GK 1 and was susceptible to both CZE 1 ($+_N$) and CDN 1 (+) (Table 1). One plant showing the R_N phenotype to GK 1 was selfed (S_1) and then a single S_1 plant was selfed to give a larger bulk of S_2 seed for further investigation. This S_2 family ($S_2$34.1) was segregating for plants with systemic necrotic infection ($+_N$) and extreme resistance to UK 1, necrotic and non-necrotic systemic infection to CZE 1, CHN 5 and CDN 1, and non-necrotic (R) and necrotic resistance to systemic spread of GK 1 (Table 1). It showed extreme resistance to JPN 1, resistance to systemic spread of DEU 7 and was susceptible to UK 4 (Table 1). The $S_2$34.1 plants from this experiment with extreme resistance to UK 1 were bud self-pollinated to produce S_3 generations that were inoculated with UK 1. All plants of one of the S_3 families (S_3.RL2-43) had extreme resistance (no virus detected in all 10 plants tested) to UK 1. Plants of the same S_3 family inoculated with UK 1M and CDN 1 were all fully susceptible to these isolates (Table 1).

All plants of the S_3.RL2-43 family and the control plant line N-o-1 showed extreme resistance to v35Tunos (and UK 1 inoculated on the same occasion as a control). In contrast, v35Tunos +5570 A > G spread systemically in both S_3.RL2-43 and N-o-1, inducing a severe necrosis, including necrotic spots on leaves and sequencing verified the presence of the mutation at position +5570.

BP079 resistance

In initial tests on BP079 plants derived from the small amount of seed available, all plants were resistant to systemic spread (R) of UK 1, CZE 1, CDN 1 and GK 1 (Table 1). In other words, virus was detected in inoculated leaves following mechanical inoculation but not in uninoculated leaves. Consequently, plants were selfed to produce a S_1 generation and then a S_1 plant was selfed to provide S_2 seed for further evaluation. All

Table 1. The interaction of the Chinese cabbage (*B. rapa*) F_1 hybrid cultivar Tropical Delight, lines BP079 and BP058 and selfs with a range of turnip mosaic virus (TuMV) isolates

Plant line	TuMV isolate (pathotype)								
	UK 1 (1)	UK 1M (3)	CZE 1 (3)	CHN 5 (3)	CDN 1 (4)	JPN 1 (7)	DEU 7 (8)	GK 1 (9)	UK 4 (12)
Tropical Delight	0	n.t.	$+_N$	n.t.	+	n.t.	n.t.	R_N	n.t.
S_2.34.1[1]	$0/+_N$	n.t.	$+/+_N$	$+/+_N$	$+/+_N$	0	R_N	R/R_N	$+_N$
S_3.RL2-43[2]	0	$+_N$	n.t.	n.t.	$+_N$	n.t.	n.t.	n.t.	n.t.
BP079	R[6]	n.t.	R[6]	n.t.	R[6]	n.t.	n.t.	R[6]	n.t.
S_2.22.3[3]	R[6]	n.t.	R[6]	R[6]	R[6]	0	R[6]	R[6]	R[6]
S_1BP058[4]	0	n.t.	0	0/R	0/R	n.t.	n.t.	n.t.	n.t.
S_2BP058[5]	0/R[7]	n.t.	0/R[7]	R[7]	0/R[7]	0	0/R[7]	0	0/R[7]

[1]S_2 family derived from single S_1 plant derived from a Tropical Delight plant.
[2]S_3 family derived from a single UK 1 immune S_2 plant.
[3]S_2 family derived from single S_1 plant derived from a BP079 plant.
[4]S_1 family derived from a single BP058 plant.
[5]S_2 family derived from a single CDN 1 apparently immune S_1 plant.
[6]distinct chlorotic spots and virus detected in inoculated leaves, but no symptoms and no virus detected by ELISA in uninoculated leaves.
[7]PTA ELISA only detected TuMV in leaves inoculated with UK 4, TuMV was detected in inoculated leaves of plants inoculated with all these isolates following back inoculation to *B. juncea*. TAS ELISA on inoculated leaves from plants inoculated with UK 1 and CHN 5 detected TuMV in some, but not all plants.
0 = no symptoms and no virus detected in leaves by ELISA; R = infection of inoculated leaves but no symptoms and no virus detected by ELISA in uninoculated leaves; R_N = as for R but inoculated leaves showed necrotic lesions; + = systemic mosaic symptoms throughout plants and virus detected in uninoculated leaves; $+_N$ = systemic necrotic symptoms in plants and virus detected in uninoculated leaves; n.t. = not tested.

individuals of the S_2 generation were resistant to systemic spread (R) of all isolates of TuMV tested with the exception of JPN 1, where no infection of inoculated leaves was detected (Table 1). Most isolates caused diffuse chlorotic lesions in varying numbers on inoculated leaves of the BP079 and S_2 plants. UK 1 gave very weak symptoms on inoculated leaves of the S_2 plants and it was difficult to detect infection by ELISA in inoculated leaves of all plants. However, inoculations from inoculated leaves to *B. juncea* cultivar Tendergreen confirmed the presence of TuMV in the inoculated leaves of all plants tested.

BP058 resistance

Only one seed of the line BP058 germinated, so this was immediately selfed to provide S_1 seed for further evaluation. PTA ELISA did not detect TuMV in any plants of the S_1 generation that had been inoculated with UK 1 and CZE 1. This generation appeared to segregate for no virus detected (0) and resistance (R) to CHN 5 and CDN 1 (Table 1). A S_1 plant with extreme resistance to CDN 1 was selfed to produce S_2 seed. PTA ELISA tests on inoculated leaves of S_2 plants detected UK 4 but failed to detect any of the other isolates (Table 1). However, back inoculations from inoculated leaves of the BP058 S_2 generation to *B. juncea* detected the presence of TuMV in some, but not all plants that had been inoculated with UK 1, CZE 1, CHN 5, CDN 1, DEU 7 and UK 4. TAS ELISA of leaves inoculated with the UK 1 and CHN 5 isolates of TuMV detected TuMV in leaves of some of the plants inoculated with UK 1 (3/10) and most of the plants inoculated with CHN 5 (9/10). No clear symptoms were seen in leaves inoculated with any of the isolates, occasionally what appeared to be very faint chlorotic spots were seen in the inoculated leaves of some plants. No virus was detected in uninoculated leaves of any of the plants tested by PTA ELISA, TAS ELISA or back inoculation to *B. juncea*.

Discussion

The three *B. rapa* lines described in this study illustrate two very different types of disease resistance. The Tropical Delight resistance was strain-specific; it was only fully effective against two of the seven pathotypes tested and partially effective against another two. It is not surprising that the S_2 generation segregated for some of these resistances, as Tropical Delight is an F_1 hybrid cultivar of Chinese cabbage and hence will be heterozygous at many loci. The S_3 family (S_3RL2-43) derived from a resistant S_2 plant was uniformly extremely resistant to UK 1 TuMV, indicating that it and the S_2 plant were homozygous for at least one major gene controlling extreme resistance (possibly immunity) to UK 1. The specificity of this resistance was virtually identical to that of the R4 differential of the *B. napus* series used to pathotype TuMV isolates (Walsh, 1989; Jenner and Walsh, 1996) and that conferred by the gene *TuRB01* (Walsh et al., 1999). This, along with the fact that the characterised mutant of UK 1 (UK 1M) that overcomes R4 (Lehmann et al., 1997) and *TuRB01* (Walsh et al., 1999), also overcame the Tropical Delight resistance and that *TuRB01* has been mapped to the A genome of *B. napus* (derived from *B. rapa*), suggested that the resistance from Tropical Delight was conferred by *TuRB01*. Results from tests with viruses derived from the infectious clone demonstrated that the single-nucleotide change in the cylindrical inclusion gene of TuMV (demonstrated to be a pathogenic determinant for *TuRB01*) was a pathogenic determinant for the resistance in Tropical Delight. This suggests very strongly that the resistance in Tropical Delight must act by the same mechanism as *TuRB01* and provides further evidence that it may be derived from *TuRB01*. The apparent propensity of pathotype 1 isolates to mutate and overcome *TuRB01* (Jenner and Walsh, 1996; Walsh et al., 1999) suggests that deployment of the Tropical Delight source of resistance is unlikely to provide durable resistance to TuMV in the absence of other sources of resistance. Tropical Delight is one of the differentials described by Provvidenti (1980) and used by others (Green and Deng, 1985) to identify the C1-5 strains of TuMV. C1 strains of TuMV are defined by an inability to infect Tropical Delight (Provvidenti, 1980) and pathotype 1 isolates described by Jenner and Walsh (1996) are defined by an inability to infect the *B. napus* differential R4. This suggests that strain C1 and pathotype 1 are genetically identical in terms of their interactions with these *B. rapa* and *B. napus* differential lines.

The BP079 and BP058 sources of resistance were effective against all TuMV pathotypes tested. This is the first example of resistance to CDN 1 (pathotype 4 isolate of TuMV) and UK 4 (pathotype 12 isolate). In these respects and as they also have resistance to CHN 5, these lines may be especially valuable sources of resistance. The R phenotype exhibited by BP079 and BP058 indicated resistance to long-distance movement of the virus. The detection of virus in inoculated leaves of BP079 plants and the presence of chlorotic spots

in inoculated leaves suggest that TuMV is capable of limited cell-to-cell movement in these plants. The fact that all individuals in the S_2 generation of BP079 and BP058 were resistant to systemic spread of all TuMV isolates tested indicated the S_1 and S_2 individuals were homozygous for gene(s) controlling resistance to the TuMV isolates.

The BP058 line appears to possess a more extreme form of resistance than BP079, in that no clear symptoms were seen in inoculated leaves and no virus was detected by PTA ELISA for most TuMV isolates tested. The inability to detect TuMV by PTA ELISA and the fact that some TuMV isolates were detected in some, but not all inoculated leaves by back inoculation, demonstrates that for all isolates except UK 4, the amounts of virus present must have been very low and at the limits of detection for these techniques. TAS ELISA detected TuMV in BP058 S_2 plants where PTA had failed to detect TuMV, demonstrating the increased sensitivity of the former. The inability to detect CHN 5 in the inoculated leaves of one BP058 S_2 plant that was subsequently shown to be infected following inoculation to mustard plants, again suggests that the amount of virus in inoculated leaves was small or that there is leaf-to-leaf variation for individual plants.

Having produced lines of Tropical Delight, BP079 and BP058 that are not segregating for at least one of their resistance phenotypes, it will now be possible to proceed and genetically characterise these resistances and map genes controlling resistance to TuMV in *B. rapa*. This will then facilitate the rapid introgression of the resistances into other genetic backgrounds by marker assisted selection. As well as deploying the resistances in Chinese cabbage (*B. rapa*) cultivars, it will be possible through the production of resynthesised *B. napus* lines, to deploy the genes in oilseed rape and swede. The markers will facilitate attempts to move the genes into the C genome through intergenomic recombination in resynthesised *B. napus* and ultimately the transfer of genes to *B. oleracea*. The movement of resistance to clubroot (*Plasmodiophora brassicae*) from *B. rapa* into *B. oleracea* has already been achieved (Chiang et al., 1980). We are also investigating other sources of resistance to TuMV and studying the viral determinants of virulence/avirulence in TuMV for the different plant resistance genes (Jenner et al., 2000). The long-term goal is to use this information to identify combinations of resistance genes that not only will give resistance, or preferably immunity, to all isolates of TuMV, but will also provide durable resistance/immunity.

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