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

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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

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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.

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

Plant gene	TuMV protein	Amino acid mutation
TuRB01	CI	N459D
TuRB01	CI	H630R
TuRB04	P3	F312L
TuRB05	CI	M589T

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 (SÁENZ *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.

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