

# The ability of a bymovirus to overcome the *rym4*-mediated resistance in barley correlates with a codon change in the VPg coding region on RNA1

Thomas Kühne,<sup>1</sup> Nongnong Shi,<sup>2,3</sup> Gerhard Proeseler,<sup>1</sup> Michael J. Adams<sup>2</sup> and Konstantin Kanyuka<sup>2</sup>

## Correspondence

Konstantin Kanyuka

kostya.kanyuka@bbsrc.ac.uk

<sup>1</sup>Federal Centre for Breeding Research on Cultivated Plants, Institute of Resistance Research and Pathogen Diagnostics, Theodor-Roemer-Weg 4, Aschersleben D-06449, Germany

<sup>2</sup>Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK

<sup>3</sup>Life Science Department, Hangzhou Teacher's College, Hangzhou 310006, China

The genome difference(s) that enable the European pathotype 2 isolates of *Barley yellow mosaic virus* (BaYMV-2) to infect barley genotypes with the *rym4* resistance gene were investigated. Stable deletions of different sizes occurred in RNA2 of laboratory isolates of the common pathotype (BaYMV-1) and BaYMV-2. After mechanical inoculation of susceptible or *rym4* genotypes with a mixture of both isolates, immunocapture-RT-PCR with RNA2-specific primers flanking stable deletion regions was used to detect and distinguish the two pathotypes. Individual leaves contained RNA2 of either or both isolates, showing that RNA2 of BaYMV-1 can replicate and move systemically in *rym4* plants when co-inoculated with BaYMV-2. In contrast, sequences of RNA1-specific RT-PCR fragments showed that in resistant plants these were always BaYMV-2, suggesting that the pathogenicity determinant was on RNA1. The complete ORFs of RNA1 of three BaYMV-1 and four BaYMV-2 isolates from the UK and Germany were sequenced, and the RNA2 sequences of one BaYMV-1 and two BaYMV-2 isolates from the UK were also determined. All sequences were very similar to one another and to the published German BaYMV-1 isolate. The only consistent amino acid difference between the BaYMV-1 and BaYMV-2 isolates was in the RNA1-encoded polyproteins and this was confirmed by sequencing the relevant region of eight further German isolates. All BaYMV-1 isolates had lysine at aa 1307, whereas BaYMV-2 isolates had asparagine (or, in one isolate, histidine). The polymorphism occurred in the central region of VPg, which has been shown to be required for pathogenicity on genotypes carrying recessive resistance genes in several potyvirus/dicotyledonous plant pathosystems.

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## INTRODUCTION

*Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) are bipartite, fungally transmitted viruses of the genus *Bymovirus* in the family *Potyviridae* (Huth & Adams, 1990). These viruses are widespread throughout Europe (particularly United Kingdom, France and Germany) and east Asia and are major pathogens of winter barley. Yield losses of > 50 % may occur when susceptible barley crops are grown on severely infected soils (Plumb *et al.*, 1986). Like other members of the family *Potyviridae*, bymoviruses have single-stranded positive-sense RNA genomes encapsidated in long, flexuous particles. The other five genera of the family *Potyviridae* (*Ipomovirus*, *Macluravirus*, *Potyvirus*,

*Rymovirus* and *Tritimovirus*) have monopartite genomes but the bymoviruses have two separately encapsidated mRNA species. RNA1 and RNA2 are both translated into large polyproteins, which are then processed by virus-encoded proteases into ten structural and non-structural products. Bymoviruses are also distinguished from other members of the *Potyviridae* by their ability to be transmitted by the root-inhabiting endoparasite, *Polymyxa graminis*. Chemical control of soil-borne virus diseases is neither efficient nor acceptable for economic and ecological reasons. Moreover, *P. graminis* produces resting spores that can lie dormant but viable in soil for several decades, and the viruses are protected from the environment within the spores. This makes crop rotation inadequate as a control measure. Often entire fields infected with bymoviruses can only be used to grow non-cereal crops. Therefore, in recent years considerable scientific effort has been directed mainly towards breeding disease-resistant varieties.

Virus sequences have been deposited at EMBL (accession nos AJ517471–AJ517477). Amino acid sequence alignments for the entire polyproteins of RNA1 and RNA2 of all European BaYMV isolates are available as supplementary data at <http://vir.sgmjournals.org>.

One recessive gene, *rym4*, that confers complete immunity against BaYMV and BaMMV has been extensively used in breeding for resistance against these viruses in Europe, where the majority of resistant commercial barley cultivars carry this gene (Graner & Bauer, 1993). However, in the late 1980s another pathotype of BaYMV, BaYMV-2, which is able to overcome *rym4*-controlled resistance, was detected in Germany and United Kingdom and later in several other European countries (Adams, 1991; Hariri *et al.*, 1990; Huth, 1989; Steyer *et al.*, 1995). Very little is known about the pathogenicity mechanisms deployed by BaYMV and BaMMV to attack barley or about the mechanism(s) for recessively inherited resistance to these viruses. Therefore, knowledge of the molecular interaction between bymoviruses and cereal crops is urgently required to help develop novel options for control, or even to prevent these soil-borne virus diseases before they become widespread.

Despite serious efforts in the past, pathotype 2 of BaYMV can only be distinguished from the common BaYMV-1 isolates by its ability to infect *rym4* genotypes. Several previous attempts to differentiate the pathotypes by serological and molecular techniques have failed (Hariri *et al.*, 1996; Shi *et al.*, 1995, 1996), because there were no consistent differences between the viral coat protein sequences of the two pathotypes and because only selected regions of the genome or limited numbers of virus isolates were examined.

The current study aimed to investigate which of the two genomic RNAs determines virulence towards resistant varieties and which BaYMV-2 cistron(s) are responsible for *rym4* resistance breaking.

## METHODS

**BaYMV isolates.** Virus isolates of the common pathotype were obtained from susceptible barley varieties growing at different locations in Germany (BaYMV-1<sup>QLB</sup>) and the UK (BaYMV-1<sup>EST</sup>, BaYMV-1<sup>ROT</sup>). BaYMV had never been reported from resistant (*rym4*) varieties at these locations. German virus isolates BaYMV-1<sup>WST</sup> and BaYMV-1<sup>HKL</sup> were also obtained from susceptible varieties at two different sites. These failed to infect *rym4* varieties in the laboratory tests and were therefore classified as common pathotype isolates. Resistance-breaking isolates were from *rym4* barley varieties in the UK (BaYMV-2<sup>CRW</sup>, BaYMV-2<sup>HAT</sup>, BaYMV-2<sup>BRZ</sup>) and Germany (BaYMV-2<sup>QLB</sup>). BaYMV-2<sup>VIR</sup> came from an anonymous field location in Germany as dried root powder of barley variety Viresa (*rym4*). Virus material obtained from a susceptible barley variety grown at Schladen, Germany (the main experimental field used to test barley varieties for resistance to BaYMV-2), proved to contain isolates of two virus pathotypes: BaYMV-1<sup>SLA</sup> and BaYMV-2<sup>SLA</sup>.

Isolate BaYMV-1<sup>ASL</sup> and isolates BaYMV-2<sup>ASL</sup> and BaYMV-2<sup>GB</sup> had been maintained in climate chambers by serial mechanical passages for about 10 years on susceptible or resistant (*rym4*) barley plants, respectively.

**Immunocapture (IC)-RT-PCR, cloning and sequencing.** IC-RT-PCR was performed according to Mumford & Seale (1997). To amplify fragments of the BaYMV genome by IC-RT-PCR, sap from

infected leaves was incubated overnight at 4 °C in PCR tubes pre-coated with anti-BaYMV IgG. The RNA was reverse-transcribed and then amplified by PCR using sequence-specific primers. The DNA fragments obtained were separated electrophoretically and when required were extracted from the agarose gel using the Wizard PCR Purification kit (Promega) and then cloned into pGEM-T (Promega) according to the manufacturer's instructions. cDNA clones were subsequently sequenced with universal M13 forward and reverse primers in both orientations on the ALF-Express automated sequencer (Pharmacia) using Cy5-labelled primers and the ThermoSequenase Cycle Sequencing Kit (Amersham Pharmacia Biotech). Sequence data were processed using the software package Vector NTI 6 (InforMax Inc.).

Primers FY (5'-AGGAACCAAGACCTGTTACTCA-3') and RY (5'-AGGAGGAAGAAATGGATAATGT-3') were used for differential detection of two mechanically transmitted BaYMV pathotypes. These primers were designed to amplify the P2 cistron fragment corresponding to nt 1480–2903 of the wild-type RNA2 of BaYMV-1<sup>MPI</sup> (EMBL accession no. D01099; Davidson *et al.*, 1991).

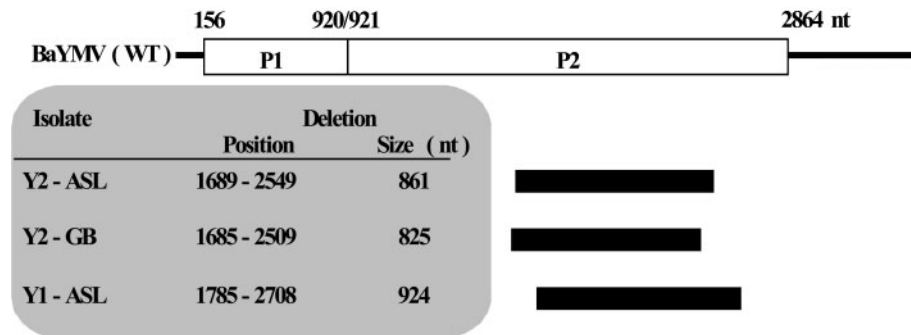
Two pairs of primers (P15, 5'-GCTGTTGAGAGCAAACCTATGTG-3', with P16, 5'-GAACTGTCCTCGGTGTTCT-3', and P17a, 5'-CATCAGCGGAAGCTACTAGAAAGAA-3', with P18, 5'-TGGTTCCTCAATAGCAAAAG-3') were designed for amplification of overlapping DNA fragments in the region of RNA1 polymorphic between BaYMV-2<sup>GB</sup> and BaYMV-1<sup>ASL</sup> by IC-RT-PCR in routine co-inoculation experiments. The RT-PCR products were isolated, cloned and sequenced. For each primer pair and each plant RNA sample, five individual clones were sequenced in both orientations.

**Cloning and sequencing of full-length viral cDNAs.** Double-stranded cDNAs that corresponded to full-length RNA1 and RNA2 of BaYMV were produced by long-range PCR using the Expand Long Template PCR System (Invitrogen) following reverse transcription with the Expand Reverse Transcriptase (Invitrogen) according to the manufacturer's instructions. Reverse transcription used either isolated viral RNA or total plant RNA as a template and the specific primers RESK-20 [5'-(T)<sub>14</sub>GGTCCTCGTAATTGAACTAAGTGAA-CCACCAT-3'] for RNA1, or RESK-21 [5'-(T)<sub>14</sub>GTCACATTTCCTGTGTACAAAGGTTGTTGTTTC-3'] for RNA2. The long-range PCR was performed with primer RESK-19 [5'-(A)<sub>6</sub>TAAACAACCCTAA-ACCAAAACAAACAAAACGA-3'] in combination with either RESK-20 or RESK-21 for RNA1 and RNA2, respectively. The resulting DNA fragments were separated electrophoretically, extracted from the agarose gel and cloned into the pCR-XL-TOPO vector (Invitrogen) as recommended by the manufacturer. Full-length cDNA clones were sequenced with BaYMV-specific primers using the ABI-377 automated DNA sequencer (Amersham) and the ABI PRISM BigDye Terminators Kit (Applied Biosystems). Sequence data were processed using the UNIX-based Staden software package (Medical Research Council, Laboratory of Molecular Biology, Cambridge, UK) or programs from the Wisconsin (GCG) package. Sequences were aligned using CLUSTALW version 1.8 (<http://searchlauncher.bcm.tmc.edu/multi-align/multi-align.html>).

## RESULTS

### Differential detection of two mechanically transmitted BaYMV pathotypes

It is known that BaMMV can undergo spontaneous deletions in the P2 coding region of RNA2 when the virus isolates are maintained by serial mechanical passages under controlled conditions (Jacobi *et al.*, 1995; Timpe & Kühne, 1994, 1995). RNA2 molecules of three German mechanically

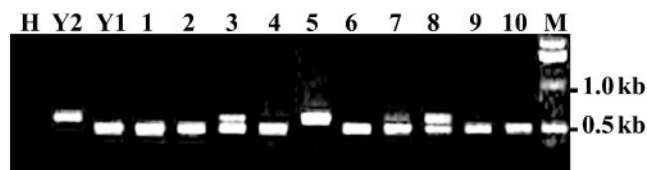


**Fig. 1.** Diagram showing the organization of RNA2 of BaYMV wild-type (WT). Black bars indicate the position and size of deleted regions in the isolates BaYMV-2<sup>ASL</sup>, BaYMV-2<sup>GB</sup> and BaYMV-1<sup>ASL</sup>.

transmitted laboratory isolates of BaYMV (BaYMV-1<sup>ASL</sup>, BaYMV-2<sup>ASL</sup>, BaYMV-2<sup>GB</sup>) were also noticeably shorter than those of field isolates. Cloning and sequencing of the 3' portions of the RNA2 molecules showed, as predicted, that P2 cistrons of these isolates contain large isolate-specific deletions of variable size (Fig. 1 and supplementary data Fig. 1 at JGV Online: <http://vir.sgmjournals.org>; EMBL accession nos AJ517479–AJ51781). IC-RT-PCR using primers FY and RY, which flank the P2 cistron region prone to deletions, and genomic RNA of BaYMV-1<sup>ASL</sup>, BaYMV-2<sup>ASL</sup> and BaYMV-2<sup>GB</sup> as templates consistently resulted in amplification of DNA fragments of the expected sizes of 497, 560 and 596 bp, respectively (data not shown). To investigate whether RNA2 molecules of these pathotypes could occur together in the same leaf, seedlings of the susceptible barley variety Corona were co-inoculated with BaYMV-1<sup>ASL</sup> and BaYMV-2<sup>GB</sup>. IC-RT-PCR with leaves showing typical virus symptoms showed that the two isolates occurred either separately or together in single leaves and that isolate-specific PCR fragments could easily be identified and differentiated by gel electrophoresis (Fig. 2).

### RNA2 molecules of BaYMV pathotypes are interchangeable *in vivo*

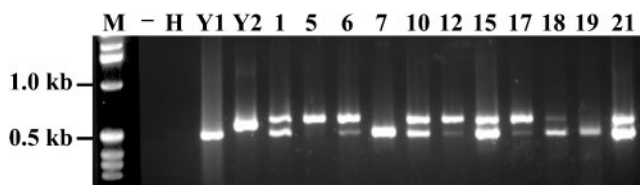
The winter barley variety Express carries the resistance gene *rym4*, which confers resistance to BaYMV-1 but allows infection with BaYMV-2. Twenty-five seedlings of variety



**Fig. 2.** Electrophoresis of IC-RT-PCR-amplified RNA2 fragments from single leaves of individual plants of the susceptible variety Corona inoculated with BaYMV-2<sup>GB</sup> (Y2), BaYMV-1<sup>ASL</sup> (Y1), or simultaneously with both isolates (lanes 1–10). H, mock-inoculated plant; M, DNA size markers.

Express at the three-leaf stage were each inoculated with either BaYMV-1<sup>ASL</sup>, BaYMV-2<sup>GB</sup>, or a mixture of the two isolates. Six weeks after inoculation with BaYMV-1<sup>ASL</sup>, there were no symptoms on any plants and the virus was not detected by ELISA or IC-RT-PCR (data not shown). In contrast, and as expected, approximately 50 % of the plants became infected with BaYMV-2<sup>GB</sup>. It must be noted here that: (i) BaYMV is naturally transmitted only by *P. graminis*; the artificial mechanical transmission is very difficult and the infection rate is usually low because of an inherent instability of the viral RNA or the virus particles in the plant extracts (Kuntze *et al.*, 2000); and (ii) BaYMV-2 is likely to be less virulent than BaYMV-1, as after the mechanical inoculation the proportion of susceptible plants infected with BaYMV-2 (30–50 %) is consistently smaller than that with BaYMV-1 (50–90 %) and as judged by ELISA the BaYMV-2 titre in the infected plants is usually lower than that for BaYMV-1 (data not shown). The latter phenomenon is quite common – the plant pathogen's acquired ability to overcome disease resistance usually comes at a cost of associated reduced fitness (Jones, 2001; Leach *et al.*, 2001).

Eleven plants of variety Express (*rym4*) out of 25 co-inoculated with both virus isolates also developed typical symptoms of virus infection, which were indistinguishable from those seen on susceptible genotypes: yellow streaking, especially of younger leaves, and also small brown necrotic patches. Individual leaves showing symptoms were tested by IC-RT-PCR for the presence of BaYMV RNA2 (Fig. 3). Based on the size of the amplified DNA fragments, the majority of samples (eight) contained the RNA2 of both BaYMV-2<sup>GB</sup> and BaYMV-1<sup>ASL</sup>, one sample contained only the BaYMV-2<sup>GB</sup> RNA2 and, importantly, in two samples only the BaYMV-1<sup>ASL</sup> RNA2-specific amplification product was detected. To confirm that the smaller DNA fragments (see Fig. 3) predicted to be BaYMV-1<sup>ASL</sup> RNA2 were not further deletions of BaYMV-2<sup>GB</sup> RNA2 or recombinant BaYMV-2<sup>GB</sup>/BaYMV-1<sup>ASL</sup> RNA2 molecules, three of these fragments (from sample nos 1, 7 and 18) were recovered from the gel, cloned and sequenced. As expected, all



**Fig. 3.** Electrophoresis of IC-RT-PCR-amplified RNA2 fragments from single leaves of the resistant (*rym4*) variety Express co-inoculated with BaYMV-2<sup>GB</sup> and BaYMV-1<sup>ASL</sup> (plant nos 1–21). M, DNA markers; –, negative control (water in place of a plant sap); H, mock-inoculated plant; Y1, susceptible variety Corona inoculated with BaYMV-1<sup>ASL</sup>; Y2, *rym4* variety Express inoculated with BaYMV-2<sup>GB</sup>.

sequences were identical and characteristic of BaYMV-1<sup>ASL</sup> RNA2 molecules.

RNA2 of BaYMV-2<sup>GB</sup> and BaYMV-1<sup>ASL</sup> was detected in nine and ten leaves, respectively. This suggests that the *rym4* genotype did not impose a selection pressure for RNA2 of a specific BaYMV pathotype. Similar results were obtained in co-inoculation experiments using another *rym4* barley variety, Carola (data not shown). Therefore, these data provide the first experimental proof that RNA2 of BaYMV-2 is not required to overcome the *rym4* resistance and that RNA2 of BaYMV-1 can replicate and move systemically in *rym4* barley genotypes when co-inoculated with the resistance-breaking pathotype BaYMV-2.

### RNA1 of BaYMV is required for pathogenicity

Polymorphism in RNA1 sequences between the two pathotypes was then used to determine whether infection by BaYMV-2 also allows replication and movement of BaYMV-1 RNA1 in the resistant genotypes. Overlapping PCR-derived fragments of RNA1 of both isolates were generated and sequenced, starting at the 5' end of this genome component, to identify polymorphic regions. All primers were designed based on the published sequence of the German isolate BaYMV-1<sup>MPI</sup> (EMBL accession no. X69757; Peerenboom *et al.*, 1992). This molecular analysis showed that there were a number of consistent nucleotide differences in the VPg and the 5' half of the NIa-proteinase cistrons that could be used to distinguish RNA1 of BaYMV-1<sup>ASL</sup> and BaYMV-2<sup>GB</sup>. Several leaf samples of the variety Express (*rym4*) that had been co-inoculated with two BaYMV pathotypes, including those that had tested positive for the presence of RNA2 from both pathotypes (see Fig. 3), were subjected to IC-RT-PCR to amplify overlapping DNA fragments in these polymorphic regions of RNA1, as described in the Methods. The sequences of the RT-PCR products obtained from the infected leaves all corresponded to BaYMV-2<sup>GB</sup> and there were no sequences of BaYMV-1<sup>ASL</sup> (data not shown).

To rule out the possibility that the elimination of BaYMV-1<sup>ASL</sup> RNA1 in the co-inoculation experiment was caused by

factors other than the presence of *rym4*, a control co-inoculation of the susceptible variety Corona was performed. Eleven out of 25 co-inoculated plants showed virus symptoms and tested positive by ELISA. IC-RT-PCR on the individual leaf samples was performed with the RNA1-specific P17a/P18 primer pair. The amplified DNA fragments were cloned and five independent clones from each of the 11 RT-PCR reactions were sequenced. The sequencing results demonstrated that BaYMV-1<sup>ASL</sup> RNA1 was predominant in the samples from the co-inoculated susceptible plants that do not possess the *rym4* gene. This was exactly as expected if BaYMV-2 has a reduced fitness.

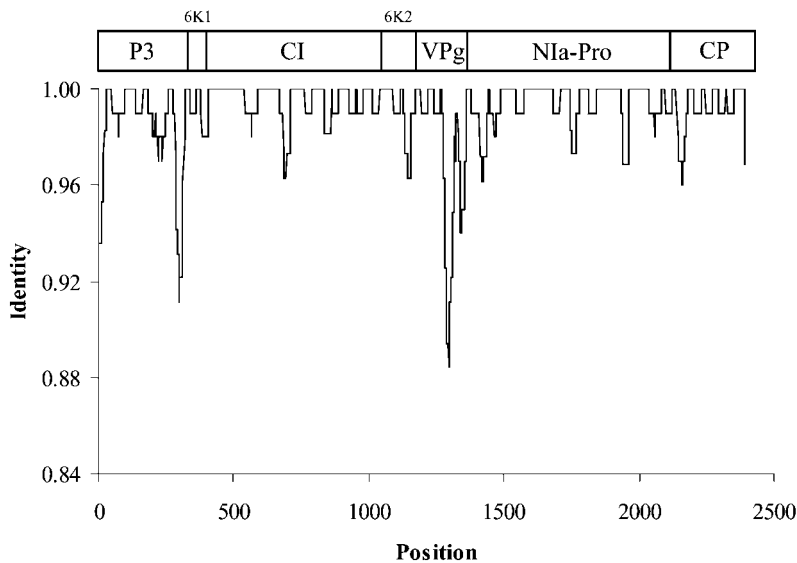
These experiments therefore show that RNA1 of BaYMV-2 determines its ability to overcome the *rym4*-controlled resistance.

### Pathogenicity towards the *rym4* barley genotypes correlates with amino acid changes in the RNA1-encoded VPg protein

To determine whether nucleotide or amino acid substitutions in the BaYMV genome correlate with resistance breaking, the complete, or almost complete, RNA1 molecules were cloned and sequenced from three BaYMV-1 (BaYMV-1<sup>EST</sup>, accession no. AJ515479; BaYMV-1<sup>ROT</sup>, AJ515485; BaYMV-1<sup>ASL</sup>, AJ515484) and five BaYMV-2 isolates (BaYMV-2<sup>CRW</sup>, AJ515480; BaYMV-2<sup>HAT</sup>, AJ515481; BaYMV-2<sup>ASL</sup>, AJ515483; BaYMV-2<sup>BRZ</sup>, AJ515482; BaYMV-2<sup>GB</sup>, AJ517478). All sequences had a single predicted ORF of identical size and were very similar to one another and to the published German BaYMV-1<sup>MPI</sup> isolate (X69757). Nucleotide identity between the different sequences was between 97.9 and 99.4%, with amino acid identities of 98.3–99.7%. The most variable regions were the P3 protein and the VPg (Fig. 4).

In addition to the partial RNA2 sequences of BaYMV-1<sup>ASL</sup>, BaYMV-2<sup>GB</sup> and BaYMV-2<sup>ASL</sup> described above, the complete RNA2 of isolates BaYMV-1<sup>EST</sup> (AJ515486) and BaYMV-2<sup>HAT</sup> (AJ515487) and most of BaYMV-2<sup>BRZ</sup> (AJ515488, including the complete ORF) were also sequenced. These were again very similar to one another and to the published German BaYMV-1<sup>MPI</sup> isolate (D01099). Nucleotide identity between the different sequences was between 94.8 and 99.4%, with amino acid identities of 97.5–99.4%. There was no consistent amino acid difference between the polyproteins of the two pathotypes (supplementary data Fig. 2 at JGV Online: <http://vir.sgmjournals.org>). These sequencing data are in agreement with the results of virus co-inoculation experiments of *rym4* barley varieties described above and strengthen the case against the involvement of RNA2 in resistance-breaking.

However, there was one distinct and consistent amino acid sequence difference between polyproteins encoded by RNA1 of BaYMV-1 and BaYMV-2 isolates. This was at aa 1307, which is in the central region of VPg. To rule out the



**Fig. 4.** Graph showing the proportion of identical amino acids among BaYMV RNA1 isolates generated by a GCG Plotsimilarity plot with a window of 25.

possibility that this difference might be a spurious coincidence, the VPg region only was cloned and sequenced from four more German BaYMV-1 (BaYMV-1<sup>WST</sup>, BaYMV-1<sup>QLB</sup>, BaYMV-1<sup>SLA</sup>, BaYMV-1<sup>HKL</sup>) and BaYMV-2 (BaYMV-2<sup>QLB</sup>, BaYMV-2<sup>VIR</sup>, BaYMV-2<sup>GB</sup>, BaYMV-2<sup>SLA</sup>) field isolates and compared with the corresponding sequence of other isolates (Fig. 5 and supplementary data Fig. 2 at JGV Online: <http://vir.sgmjournals.org>). All BaYMV-1 isolates examined encoded a lysine at aa 1307, whereas BaYMV-2 isolates encoded an asparagine (or, in BaYMV-2<sup>ASL</sup>, a histidine) at this position.

## DISCUSSION

We have demonstrated for the first time that the pathogenicity of BaYMV-2<sup>GB</sup> towards barley varieties harbouring the *rym4* gene is determined solely by RNA1. The RNA1 genome component of BaYMV-2<sup>GB</sup> accepted the RNA2 of BaYMV-1<sup>ASL</sup> as a target and supported its replication and movement in resistant plants. In contrast, there was no evidence for systemic movement and/or replication of RNA1 of BaYMV-1<sup>ASL</sup> in co-inoculated seedlings of the resistant genotypes. Using similar experimentation, it was previously demonstrated that the symptomatology and pathogenicity of a *rym5*-resistance breaking isolate of BaMMV in barley variety Misato Golden (*rym5*) is also determined by RNA1 (Kashiwazaki & Hibino, 1996).

An extensive sequencing analysis of 16 BaYMV isolates was used to identify a region in the VPg protein that is polymorphic between *rym4* resistance-breaking and non resistance-breaking isolates (Fig. 5). A substitution of the basic amino acid lysine (K<sub>1307</sub>) for the uncharged amide asparagine (N<sub>1307</sub>) is likely to affect the biochemical properties of VPg. We believe that the same hypothesis applies to the VPg protein of BaYMV-2<sup>ASL</sup>, which contains the weak basic amino acid histidine at position 1307. The

isoelectric point (pI) of lysine is 9.7, whereas histidine has nearly a neutral pI of 7.6. Also, lysine and histidine have structurally different side chains with very different pK<sub>a</sub> values of 10.79 and 6.06, respectively. Moreover, the VPg of BaYMV-2<sup>ASL</sup> contains two more unique amino acid substitutions (lysine→arginine at position 1216 and valine→isoleucine at position 1297), which may additionally contribute to a conformational change and affect the ability of this protein to interact with other molecules. Interestingly, it was recently reported that a single amino acid substitution in the VPg protein of spontaneous mutants of *Potato virus Y* (PVY) correlates with their ability to overcome tobacco *va* gene-specified resistance (Masuta *et al.*, 1999).

The VPg protein is a multifunctional protein necessary for potyvirus multiplication and movement in host plants (Rajamäki & Valkonen, 2002; Schaad *et al.*, 1997). Both the recessive resistance gene and the corresponding virus component that determines pathogenicity have been characterized for several other potyviruses. Thus, it was shown that variations in the VPg protein allow *Tobacco vein mottling virus* to overcome *va* gene resistance in tobacco (Nicolas *et al.*, 1997) and *Tobacco etch virus* (TEV) to break resistance controlled by two unlinked recessive genes in the tobacco cultivar V20 (Schaad *et al.*, 1997). The ability of *Lettuce mosaic virus* (LMV) to overcome *mol*<sup>1</sup> and *mol*<sup>2</sup> resistance genes of *Lactuca sativa* was also mapped to the 3' half of the LMV genome, which encompasses the VPg cistron (Redondo *et al.*, 2001).

It is becoming clear that in many characterized *Potyvirus*/plant interaction systems modifications of the VPg protein are responsible for resistance breaking. In the current study, we have shown that this appears also to be the case for the bymovirus BaYMV/*rym4* barley interaction. Multifunctionality of potyviral VPg and the recessive nature of resistance suggest it is likely that in susceptible

		1225
Y1-MPI	GKGNKYRPFEDARLMYSTREDATFDWNEKAKERRKVTDKAEPELRRAY	
Y1-EST	.....	
Y1-ROT	.....	
Y1-WST	.....D.....	
Y1-ASL	.....	
Y1-QLB	.....	
Y1-HKL	.....	
Y1-SLA	.....	
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Y2-HAT	.....	
Y2-CRW	.....	
Y2-BRZ	.....	
Y2-QLB	.....S.....	
Y2-ASL	.....R.....	
Y2-VIR	.....L.....	
Y2-GB	.....	
Y2-SLA	.....	
		1275
Y1-MPI	EKRPYFNFYDLQTDNSILEAIFYTTEGDEFFRTADPNRDMNLVADKLRSF	
Y1-EST	.....K.....	
Y1-ROT	.....K.....	
Y1-WST	.....	
Y1-ASL	.....	
Y1-QLB	.....	
Y1-HKL	.....	
Y1-SLA	.....	
-----		
Y2-HAT	.....	
Y2-CRW	.....	
Y2-BRZ	.....	
Y2-QLB	.....	
Y2-ASL	.....	
Y2-VIR	.....K.....	
Y2-GB	.....K.....	
Y2-SLA	.....T.....	
		1325
Y1-MPI	LDTKLVVGHQKLEETANVVIKDTKGTAKMEISQHDPDFLKQNGSGK	
Y1-EST	.....H.V.....K.....ED.....E.....	
Y1-ROT	.....K.....	
Y1-WST	.....E.....K.A.....S.....	
Y1-ASL	.....A.K.....E.....	
Y1-QLB	.....K.....	
Y1-HKL	.....K.....	
Y1-SLA	.....K.....	
-----		
Y2-HAT	.....I.....N.....D.....	
Y2-CRW	.....N.....D.....	
Y2-BRZ	.....N.....	
Y2-QLB	.....I.....N.....	
Y2-ASL	.....I.....H.....	
Y2-VIR	.....A.R.....V.N.....	
Y2-GB	.....A.R.....V.N.....	
Y2-SLA	.....I.....N.....	
		1362
Y1-MPI	VGYPEHRGQFRQEGVAVTGDYDLEAEFGTDTDEISFG	
Y1-EST	.....I.....A.A.....TLE	
Y1-ROT	.....A.A.....TLE	
Y1-WST	.....I.....A.A.....TLE	
Y1-ASL	.....A.A.....TLE	
Y1-QLB	.....A.A.....TLE	
Y1-HKL	.....A.A.....TLE	
Y1-SLA	.....A.A.....RFE	
-----		
Y2-HAT	.....A.A.....TLE	
Y2-CRW	.....A.A.....TLE	
Y2-BRZ	.....A.A.....TLE	
Y2-QLB	.....R.....A.A.....TLE	
Y2-ASL	.....A.A.....TLE	
Y2-VIR	.....I.A.....A.....TLE	
Y2-GB	.....I.A.....A.....TLE	
Y2-SLA	.....R.....A.A.....TLE	

**Fig. 5.** Alignment of the amino acid sequences of the VPg protein of 16 BaYMV isolates. Residues are shown when they differ from the top line (Y1-MPI, X69757) sequence and the position of the pathotype-related polymorphism is highlighted (arrow).

plants there is a functional interaction between this virus protein and plant proteins encoded by the dominant alleles of resistance genes. In resistant plants, this host factor component may be either missing or modified and therefore compromises a particular step of virus infection (Revers *et al.*, 1999). By using the yeast two-hybrid system to test for protein–protein interactions, two laboratories have independently demonstrated that the VPg protein of *Turnip mosaic virus* (TuMV) and TEV interact with the plant cap binding protein eIF4E (Léonard *et al.*, 2000; Schaad *et al.*, 2000). Importantly, the recessive *Arabidopsis* mutants that lack eIF(iso)4E were immune to TuMV and TEV (Lellis *et al.*, 2002). Moreover, it was recently demonstrated that a natural recessive resistance gene, *pvr2*, against PVY corresponds to the eukaryotic translation initiation factor eIF4E (Ruffel *et al.*, 2002). Therefore, eIF4E and eIF(iso)4E play an important role in potyvirus genome expression or replication at the single-cell level. It remains to be seen whether the BaYMV VPg protein interacts with the eIF4E or eIF(iso)4E homologue of barley and whether these genes play an important role in host resistance during bymovirus infection.

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