Asymmetrical Distribution of Barley Yellow Dwarf Virus PAV Variants Between Host Plant Species

Jamila Mastari, Hervé Lapierre, and Johannes T. Dessens

First and second authors: Unité de Pathologie Végétale, Institut National de la Recherche Agronomique, Route de Saint Cyr, 78026 Versailles, France; and third author: Infection and Immunity Section, Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB, United Kingdom.
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ABSTRACT

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A large epidemiological study of the genetic variation of barley yellow dwarf virus (BYDV) serotype PAV involving different host plant species was conducted. French BYDV PAV isolates were collected from barley and ryegrass, and their capsid protein gene sequences characterized using restriction fragment length polymorphism, single-strand conformation polymorphism, and sequence analyses. The data show that BYDV PAV

isolates from five different continents are separated into two distinct groups named cpA and cpB, which are distributed irrespective of geographical location. Amino acid identity of the capsid proteins ranged from 93 to 99.5% in group cpA and from 95 to 99.5% in group cpB, while this value was only from 82 to 88% between the groups. Moreover, isolates from each group were found preferentially (up to 98%) in one of the two plant species examined. These results show that host plant species play a role in isolate selection and maintenance and that they contribute to the genetic diversity of BYDV PAV.

Barley yellow dwarf is an agronomically important disease of cereal crops worldwide and is caused by a group of luteoviruses known collectively as barley yellow dwarf virus (BYDV) (4). These plant RNA viruses are naturally transmitted by aphids in a circulative, non-propagative manner, and efficient transmission is often limited to one or two specific aphid species. Apart from infecting seasonal crops such as barley, wheat, maize, and oats, some grasses also serve as hosts and may function as important virus reservoirs. BYDV is divided into five different serotypes (PAV, MAV, RPV, RMV, and SGV) based on their specific aphid vectors. Differences in genome organization have led to a further division of the serotypes into two subgroups, I (PAV, MAV, and SGV) and II (RPV and RMV) (4).

Serological as well as capsid protein sequence analyses are techniques used to assess genetic diversity within BYDV serotypes. Although such diversity often appears to be correlated with the geographical origin of the isolates (5), distinct variants have also been isolated from single locations (2), suggesting that other factors such as host plant species may contribute to virus diversity. To test this hypothesis, we assessed capsid protein sequence variation of a large number of BYDV PAV isolates collected from a small area in France, as well as the distribution of variants between two different host plant species, ryegrass (*Lolium multiflorum*) and barley (*Hordeum vulgare*). The results obtained clearly show that host plant species play a role in isolate selection and maintenance, and that they contribute to the genetic diversity of BYDV PAV.

MATERIALS AND METHODS

Isolate collection and serotyping. Many French virus isolates were collected from winter barley fields and fallow lands located

within a 30-km radius around Versailles. Two isolates were collected from barley in Morocco. Sampling was carried out randomly during mid-February and late June from 1994 to 1997. PAV serotypes were identified by reaction with anti-PAV poly- and monoclonal antibodies and by failure to react with anti-MAV and anti-RPV monoclonal antibodies in triple-antibody sandwich enzyme-linked immunosorbent assay (ELISA) (3,8).

Reverse transcription and polymerase chain reaction (PCR) amplification. Virions were recovered from homogenized leaf tissue by immunocapture in anti-PAV antibody-coated microtiter plates and subjected to two freeze-thaw cycles to expose the viral RNA (14). The RNA was reverse transcribed for 1 h at 42°C with avian myeloblastosis virus reverse transcriptase (Boehringer GmbH, Mannheim, Germany) in the presence of primer Lu-4 (GTCTAC-CTATTTGG), corresponding to nucleotides 3,455 to 3,468 of isolate Vic-PAV (11). Subsequently, 3'-truncated capsid protein gene sequences (approximately 530 bp) were PCR-amplified with primers Lu-1 (CCAGTGGTTATGGTC), corresponding to nucleotides 2,938 to 2,952 of isolate Vic-PAV (11), and Lu-4 for use in restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) analyses. Alternatively, primers P3 (GGTGAAATGAATTCAGTAGG), corresponding to nucleotides 2,858 to 2,871 of isolate Vic-PAV (2), and Lu-4 were used to PCR amplify the entire capsid protein genes (approximately 600 bp) for nucleotide sequence analysis. PCR amplification consisted of an initial denaturation step of 5 min at 95°C followed by 35 cycles of 95°C for 15 s, 50°C for 30 s, and 72°C for 1.5 min in a Perkin-Elmer thermal cycler (Perkin-Elmer Corp, Norwalk, CT).

RFLP, SSCP, and sequence analyses. For RFLP analysis, PCR products were digested with the restriction endonuclease *Sau3AI* or *HaeIII* (Boehringer GmbH), fractionated in 5% polyacrylamide gels, and visualized by ethidium bromide staining (2). For SSCP analysis, PCR products were heated to 95°C for 5 min in the presence of 48% formamide, transferred to ice, fractionated in 6% polyacrylamide gels, and visualized by silver staining (1). For sequence analysis, PCR products were purified with JetSorb

Corresponding author: J. T. Dessens; E-mail address: j.dessens@ic.ac.uk

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(GenoMed, Beverly Hills, CA) and directly sequenced in a Perkin-Elmer automated sequencer (Perkin-Elmer Corp.).

Computation. Multiple alignment and determination of sequence homology was carried out using the programs PILEUP and BEST-FIT, respectively (Genetics Computer Group, Madison, WI). Bootstrap tree analysis was conducted using CLUSTAL W (7).

RESULTS

RFLP analysis. Endonuclease digestion of capsid protein-specific PCR products with *Sau3AI* or *HaeIII* gave rise to two distinct, but parallel, RFLP patterns among the isolates tested. Typical RFLP patterns are shown in Figure 1A (*Sau3AI*) and B (*HaeIII*). Of 112 isolates tested, 61 gave pattern B (Fig. 1, lanes 1 and 2), 49 gave pattern A (Fig. 1, lanes 3 and 4), and 2 gave a combination of patterns A and B (Fig. 1, lanes 5 and 6). The latter two samples are the result of mixed infections.

SSCP analysis. SSCP analysis also resulted in two distinct patterns, discriminating in a way similar to that in the RFLP analyses noted above (Fig. 1C). The lower bands on the gel display distinct electrophoretic mobilities for the RFLP pattern B (Fig. 1C, lanes 1 and 2) and pattern A (Fig. 1C, lanes 3 and 4) isolates, pointing to considerable differences in their respective nucleotide sequences. Again, the two samples containing mixed infections gave rise to a combination of both SSCP patterns (Fig. 1C, lanes 5 and 6).

Sequence analysis. Thirteen isolates were analyzed by sequence analysis. This separated the isolates, including previously reported isolates from Australia (Vic-PAV) (9), Japan (Jpn-PAV) (12), and the United States (PAV-129, NY-PAV, and P-PAV) (2,10,13), into two distinct groups of variants named cpA and cpB (Figs. 2 and 3). Amino acid identity of the capsid proteins ranged from 93 to 99.5% in group cpA and from 95 to 99.5% in group cpB, while this value was only from 82 to 88% between the groups. The main amino acid differences were located at amino acid positions 51 to 58 and to a lesser extent in the C-terminal region of the capsid proteins (Fig. 2). The division based on sequence analysis fully correlated to the distinct RFLP and SSCP patterns described above. Indeed, sufficient nucleotide sequence variation existed between isolates from groups cpA and cpB to account for the different electrophoretic mobilities observed in the SSCP analysis (Fig. 1C). Moreover, the *Sau3AI* and *HaeIII* recognition sequences responsible for the distinct RFLP patterns were located within the region of the capsid protein gene encoding the cpA- and cpB-specific amino acid cluster at positions 51 to 58 (Fig. 2). Hence, the absence or presence of these restriction sites (and thus RFLP patterns A and B) directly correlated with the cpA and cpB grouping, respectively.

Distribution of variants between barley and ryegrass. The most striking observation of this study was the distribution of the two virus groups between the two host plant species examined. Although both plant species are hosts for viruses from either group (e.g., isolates FL1, FL2, FH1, and FH3) (Table 1), 49 of 50 French isolates (98%) found in ryegrass belonged to group cpA, whereas 50 of 60 French isolates (83%) present in barley contained the group cpB virus (Table 1). Moreover, group cpB isolates in barley were considerably more dominant (96%) later in the season (data not shown). Hence, variants belonging to group cpA are found preferentially in ryegrass, whereas those belonging to group cpB are present preferentially in barley.

DISCUSSION

In this paper different techniques were used to assess genetic diversity of the capsid protein within French BYDV PAV isolates. RFLP analysis using *Sau3AI* and *HaeIII* identified two distinct patterns, A and B, that have in fact been described previously for BYDV PAV isolates from New York (NY-PAV and PAV-129) (2). These patterns were shown to be the result of *Sau3AI* and *HaeIII* restriction sites at positions 166 and 151 of the capsid protein gene,

Fig. 1. Typical restriction fragment length polymorphism (RFLP) and single-strand conformation polymorphism (SSCP) patterns of different barley yellow dwarf virus serotype PAV isolates. **A**, RFLP analysis with *Sau3AI*. **B**, RFLP analysis with *HaeIII*. **C**, SSCP analysis. Samples constitute isolates FH1 (lane 1), FH2 (lane 2), FH3 (lane 3), FH4 (lane 4), FH5 (lane 5), and FH6 (lane 6). Table 1 contains isolate details. Lane M contains non-denatured DNA size markers (1-kb DNA ladder; Life Technologies, Gaithersburg, MD).

Fig. 3. Phylogenetic tree of different barley yellow dwarf virus serotype PAV isolates. Bar represents branch length.

changes that can eliminate essential restriction sites. Overall, the RFLP and SSCP analyses described here, or a combination of both, appear to be reliable ways to distinguish between the two groups of BYDV PAV variants.

For BYDV isolates of the RMV serotype, considerable diversity in the capsid protein has been reported among isolates from different geographical areas; for example, sequences of isolates from New York and Illinois were only 80% identical, whereas those of isolates from Illinois and nearby Minnesota shared 97% identity (5). A fourth isolate from Montana shared 81% identity with the other isolates (6). Our results show that geographical origin alone cannot explain the genetic diversity found in BYDV PAV, because group cpA and cpB viruses were even found in the same plant. Instead, our findings show a strong correlation between virus genotype and host plant species. In doing so, this study provides the first clear evidence that host plants play a role in isolate selection and maintenance and, consequently, in the genetic diversity of the virus. Two groups of BYDV PAV variants among isolates from five different continents have been identified to date (Table 1). It is not clear why the genetic diversity in the capsid protein appears to be restricted to these two groups, but the observed host preference associated with these virus groups seems unlikely to be based on coincidence. The viruses must benefit (i.e., have greater fitness) in their preferred hosts. Possibly, this observation reflects an early stage in an evolutionary process that will ultimately lead to distinct virus species with a distinct host range. In any event, this study has provided important new information on the genetic diversity and distribution of BYDV PAV isolates in different host plant species, underlining the importance of such epidemiological studies in understanding the complexity of barley yellow dwarf disease in the field. To complete the picture, similar studies must be carried out for the other important BYDV hosts and serotypes.

TABLE 1. Grouping of barley yellow dwarf virus PAV isolates worldwide

Isolate name	Country of origin	Host plant	Isolate group
Vic-PAV	Australia	Barley	cpA
Jpn-PAV	Japan	Barley	cpA
NY-PAV	United States	Oat	cpA
P-PAV	United States	Oat	cpA
PAV-129	United States	Oat	cpB
M1	Morocco	Barley	cpB
M2	Morocco	Barley	cpA
FL1	France	Ryegrass	cpB
FL2 – FL50	France	Ryegrass	cpA
FH1 – FH2	France	Barley	cpB
FH3 – FH4	France	Barley	cpA
FH5 – FH6	France	Barley	cpA, B ^a
FH7 – FH52	France	Barley	cpB
FH53 – FH60	France	Barley	cpA

^a Mixed infections.

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