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A member of a new genus in the *Potyviridae* infects *Rubus*

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Abstract

Blackberry yellow vein disease causes devastating losses on blackberry in the south and southeastern United States. Blackberry yellow vein associated virus (BYVaV) was identified as the putative causal agent of the disease but the identification of latent infections of BYVaV led to the investigation of additional agents being involved in symptomatology. A potyvirus, designated as Blackberry virus Y (BVY), has been identified in plants with blackberry yellow vein disease symptoms also infected with BYVaV. BVY is the largest potyvirus sequenced to date and the first to encode an AlkB domain. The virus shows minimal sequence similarity with known members of the family and should be considered member of a novel genus in the *Potyviridae*. The relationship of BVY with Bramble yellow mosaic virus, the only other potyvirus known to infect *Rubus* was investigated. The presence of the BVY was verified in several blackberry plants, but it is not the causal agent of blackberry yellow vein disease since several symptomatic plants were not infected with the virus and BVY was also detected in asymptomatic plants.

Keywords: AlkB; Blackberry yellow vein disease; Blackyvirus; Detection; Transmission

1. Introduction

Several virus and virus-like agents infect *Rubus* spp. (Converse, 1987). In recent years, a new disease designated as blackberry yellow vein (BYVD) has emerged in the south and southeastern United States. The disease is characterized by the appearance of yellow vein symptoms on the older leaves of primocanes and vein-yellowing and die-back of floricanes. A new crinivirus, Blackberry yellow vein associated virus (BYVaV) has been associated with symptoms (Martin et al., 2004) but the identification of 'Chickasaw' plants that were asymptomatic suggested that additional agents may be involved in symptom development (Susaimuthu et al., 2006). Using standard techniques we verified the presence of a novel potyvirus, designated as Blackberry virus Y (BVY).

The family *Potyviridae*, the largest plant virus family, is comprised of six genera of positive-sense single-stranded RNA viruses (Hull, 2002). Viruses in the family can be transmitted by aphids (genera *Potyvirus* and *Macluravirus*), eriophyid mites (genera *Rymovirus* and *Tritimovirus*), whiteflies (genus *Ipomovirus*) and plasmodiophorid fungi (genus *Bymovirus*) (Adams et al., 2005a). Potyviruses, with the exception of members of the *Bymovirus* genus, which are bipartite, have monopartite and monocistronic genomes, with a VPg attached to the 5′ end and a poly-adenosine tail at the 3′ end of the genome. The genome is expressed as a single polypeptide which is proteolytically processed to mature virus proteins, in a similar fashion to animal picornaviruses. A major difference between the two groups is that unlike picornaviruses, potyviruses have flexuous filamentous particles.

This communication presents molecular and epidemiological data for BVY, investigates the relationship of the virus with other members of the *Potyviridae* and the possibility BVY is an

Abbreviations: HC-Pro, helper component protease; CI, cylindrical inclusion bodies protein; VPg, genome-linked protein; NI, nuclear inclusion bodies protein; CP, coat protein.

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isolate of Bramble yellow mosaic virus (BrYMV), the only other potyvirus known to infect *Rubus* species (Engelbrecht, 1976).

2. Materials and methods

2.1. Plant material

The nucleotide sequence of BVY presented in this communication was obtained from a blackberry plant ('Chickasaw') from Arkansas (C3ARK) with BYVD symptoms. In order to study the spread of BVY in the field, an experiment was designed in which 10 BVY-free blackberry plants ('Chester') were placed on pedestals next to the symptomatic field plants ('Chickasaw') affected with BYVD. These sentinel plants were replaced at two-week intervals throughout the growing seasons (April–September) of 2004 and 2005 and were tested by reverse transcription-PCR (RT-PCR) and dot-blot hybridization for BVY-infection 6–10 months after field placement. Wild blackberry plants in Arkansas were sampled in 2005 from more than 50 locations throughout the state to determine if these plants serve as a source for the BVY found in cultivated blackberry plantings. Cultivated blackberry plants affected with BYVD from Georgia, Tennessee, Kentucky, South and North Carolina were also tested for the presence of BVY.

2.2. Detection

Double-sandwich enzyme linked immunosorbent assay (DAS-ELISA) (Converse and Martin, 1990) with potyvirus-specific monoclonal antibodies (Agdia, Elkhart, IN) and RT-PCR using a universal potyvirus primer (Chen et al., 2001) were originally evaluated for detection of the virus.

BVY was detected by RT-PCR using BVY-specific primers BVY312F (5′ CTGTGGGGAGATTTGGAGAA 3′) and BVY695R (5′ TCATTCCATGGGTGTGTC 3′) that amplified a 383-base region of the genome. Leaf samples were used for RNA extraction and RT-PCR was performed as described by Susaimuthu et al. (2006) or Tzanetakis et al. (2007).

Hybridization experiments were carried out to detect BVY from symptomatic and asymptomatic blackberry plants. The RNA blots were prepared by dotting RNA on a nylon membrane as described (Susaimuthu et al., 2007). Probe DNA was synthesized by RT-PCR with primers BVY312F and BVY1105R (5′ TATCTCCCCTCCTGCTCTCA 3′) specific for BVY. An established protocol was followed for the hybridization procedure (Sambrook et al., 1989), and the radioactive blots were exposed to BioMax MS X-ray film (Eastman Kodak Company, Rochester, NY) for 4 h.

2.3. Transmission studies

Seedlings of the following species were dusted with carborundum and mechanically inoculated with sap from BVY-infected blackberry leaves and petals: *Chenopodium quinoa* Willd., *C. amaranticolor* Coste and Reyn, *Nicotiana benthamiana* Domin, *N. rustica* L., *N. glutinosa* L., *N. clevelandii* Gray, *N. tabacum* L. 'Kentucky-16', *Phaseolus vulgaris* L. 'Black Valentine', *Pisum sativum* L., *Vigna unguiculata* (L.) Walp. ssp. *unguiculata* 'Georgia 21', *Cucumus sativus* L. 'Boston Pickling', *C. melo* L., *Lycopersicon esculentum* Mill., *Petunia hybrida* Vilm. 'Dream Rose'. Two sets of inoculations were carried out with inoculation buffer (0.05 M phosphate buffer, pH 7.2) containing a 10 mM sodium sulfite or 2% nicotine. Test plants were inspected for symptom development and evaluated for virus infection using RT-PCR. To verify transmission of viruses from blackberry tissue, sap from *Tobacco ringspot virus* (TRSV)-infected raspberry leaves was used to inoculate *C. sativus* L. cv. 'Boston Pickling' as described above.

2.4. Electron microscopy

Leaf tissue from BVY-infected asymptomatic 'Chester' blackberry obtained by placement of sentinel 'Chester' plants in a BYVD affected production field was prepared for thin-section electron microscopy as described previously (Susaimuthu et al., 2006). Sections were double-stained for 15 min in a 2% aqueous solution of uranyl acetate (pH 5.0) and for 5 min in lead citrate (pH 12.0). Specimens were examined using a JEOL 100 CX transmission electron microscope.

2.5. Nucleic acid extractions and cloning

Double-stranded RNA was purified as described by Tzanetakis and Martin (2005) from 20 g of tissue from symptomatic 'Chickasaw' plants from Arkansas. Complementary DNA was prepared using dsRNA as template and cloned as described (Tzanetakis et al., 2005a) without the use of restriction endonucleases. Plasmids were screened by PCR and those with the largest inserts were sequenced at the Macrogen Inc. facilities (Seoul, South Korea) using an ABI3730 XL automatic DNA sequencer.

2.6. Sequence analysis

Sequences obtained were screened against sequences found in Genbank using blastn and blastx (Altschul et al., 1997) and the ones identified as BVY-specific were used for development of oligonucleotide primers for RT-PCR amplification of the genome (Tzanetakis et al., 2005b). Acquisition of the 5′ terminal region of the genome was done as described (Tzanetakis and Martin, 2004b). The consensus of the sequence presented, deposited in Genbank under accession number AY994084, was obtained with CAP3 (Huang and Madan, 1999) and represents an at least a 3× sequence coverage. The putative polyprotein cleavage sites were determined after comparison of orthologous domains of BVY and with those of other potyviruses and the use of the PredictProtein server (Adams et al., 2005b; Rost et al., 2004). Phylogenetic analysis of the helicase and polymerase conserved motifs and the CP of potyviruses was performed with ClustalW (Thompson et al., 1994) using the neighbor-joining algorithm, Kimura's correction and bootstrap consisting of 1000 pseudoreplicates. Phylogenetic trees were visualized with Treeview (Page, 1996).