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Molecular characterization and population structure of blackberry vein banding associated virus, new ampelovirus associated with yellow vein disease

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ABSTRACT

Blackberry yellow vein disease is the most important viral disease of blackberry in the United States. Experiments were conducted to characterize a new virus identified in symptomatic plants. Molecular analysis revealed a genome organization resembling *Grapevine leafroll-associated virus 3*, the type species of the genus *Ampelovirus* in the family *Closteroviridae*. The genome of the virus, provisionally named blackberry vein banding associated virus (BVBaV), consists of 18,643 nucleotides and contains 10 open reading frames (ORFs). These ORFs encode closterovirid signature replication-associated and quintuple gene block proteins, as well as four additional proteins of unknown function. Phylogenetic analyses of taxonomically relevant products consistently placed BVBaV in the same cluster with GLRaV-3 and other members of the subgroup I of the genus *Ampelovirus*. The virus population structure in the U.S. was studied using the replication associated polyprotein 1a, heat shock 70 homolog and minor coat proteins of 25 isolates. This study revealed significant intra-species variation without any clustering among isolates based on their geographic origin. Further analyses indicated that these proteins are under stringent purifying selections. High genetic variability and incongruent clustering of isolates suggested the possible involvement of recombination in the evolution of BVBaV.

1. Introduction

Blackberry yellow vein disease (BYVD) is the most serious and complex viral disease of blackberry in the United States. The disease occurs in both cultivated and wild blackberries, and has been reported in the Southeastern United States and California (Martin et al., 2013). Symptoms include oak-leaf patterns, irregular chlorosis, line patterns, ringspots, yellowing of veins, and malformed leaves (Susaimuthu et al., 2006, 2007) and results in a rapid decline in productivity and longevity of plants (Tzanetakis, 2012). Several viruses; blackberry yellow vein-associated virus, blackberry virus Y, beet pseudo-yellows virus, blackberry chlorotic ringspot virus, blackberry virus E, blackberry virus S, impatiens necrotic spot virus, and tobacco ringspot virus, have often been found associated with the BYVD complexes (Martin et al., 2013). Symptoms appear only in mixed infections of two or more of these viruses. Plants infected with multiple viruses often exhibit identical symptoms with severity directly associated with the number of viruses in plants (Martin et al., 2013). A new ampelovirus (family *Closteroviridae*) provisionally named blackberry vein banding associated virus (BVBaV) was first identified in symptomatic plants in Mississippi (Fig. 1; Sabanadzovic et al., 2011) and is the subject of this communication.

Members of *Closteroviridae* are important pathogens in several horticultural crops. The family comprises viruses with relatively large, single-stranded, positive-sense, RNA genomes that range from 13 to 20 kb in size and are either monopartite or segmented. Viruses of this family belong to four genera; *Closterovirus*, *Ampelovirus*, *Velarivirus* and *Crinivirus*, based on the differences in genome size, type, organization, biological and epidemiological properties (Martelli and Candresse, 2010; Martelli et al., 2012a,b). Although the genome organization varies among the members of the family, all closterovirids encode replication associated proteins in open reading frames (ORFs) 1a and 1b and have a conserved quintuple gene block involved in movement and encapsidation.

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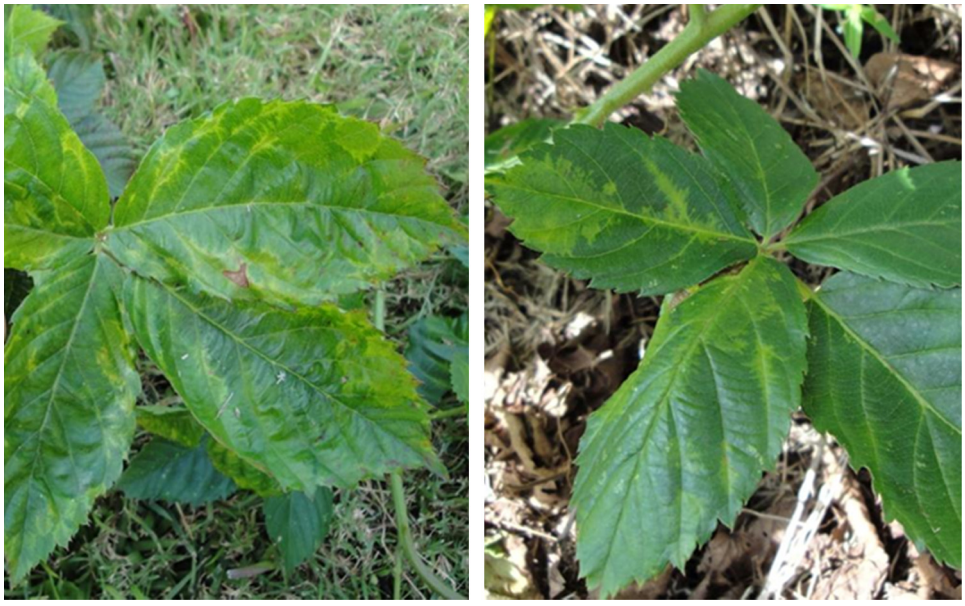

Fig. 1. Symptoms observed on the original source of blackberry vein banding associated virus. The plant was also infected with at least two other viruses.

In the genus *Ampelovirus* there are two types of genome organizations: subgroup I, represented by grapevine leafroll-associated virus 3 (GLRaV-3), includes members with larger genomes that contain 12–13 ORFs whereas subgroup II, represented by pineapple mealybug associated virus 1, comprises viruses with smaller genomes of 7 ORFs, lacking the diverged copy of the coat protein. Closteroviruses, ampeloviruses and velariviruses have monopartite genomes. Closteroviruses are transmitted by aphids, ampeloviruses with mealybugs or scale insects whereas there are not known vectors for velariviruses. Most of the criniviruses have bipartite genomes and are transmitted by whiteflies.

Closteroviruses are reported to have highly diverse genetic population structures (Dolja et al., 2006; Karasev, 2000). Replication of RNA genomes is an error prone process due to the lack of a proof reading mechanism in the RNA-dependent RNA polymerase (RdRp; Domingo, 1997). This and other factors, such as recombination and selection pressure, play significant roles in evolution and shaping of the population structure of RNA viruses. Knowledge on population structure and the extent of intra-species variation are essential to better understand virus dynamics and develop appropriate detection and control tools. Also, the evolutionary potential of a virus is an important factor to be considered when employing control methods through development of resistant cultivars. Few studies have been reported regarding the genetic variation of ampeloviruses and those were concentrated on the RdRp, major coat protein (CP) and heat shock protein 70 homolog (HSP70h) (Fajardo et al., 2001; Habili et al., 1995; Saldarelli et al., 1998; Turturo et al., 2005; Wang et al., 2011). The replication-associated polyprotein contains conserved domains of papain-like protease (PRO), methyltransferase (MTR) and helicase (HEL) whereas the intervening region is highly variable (Dolja et al., 2006). Also, the diverged copy of the coat protein (minor coat protein, CPm) of GLRaV-1, was reported to be highly variable compared to the CP (Alabi et al., 2011; Little et al., 2001). Thus, a diversity analysis on BVBaV polyprotein and CPm sequences in addition to HSP70h, the hallmark gene of *Closteroviridae*, was conducted and the results are reported here. The information obtained in this study contributes to the existing knowledge on blackberry viruses and ampeloviruses in general and gives insight into the forces driving evolution of this diverse group of plant viruses.

2. Materials and methods

2.1. Molecular characterization

Double-stranded RNA from infected plants was extracted using a protocol involving selective chromatography (Valverde, 1990), and used as a template for virus characterization. Initial virus sequences were obtained by cloning randomly amplified cDNA fragments combined with Illumina next-generation sequencing as previously described (Laney et al., 2011; Sabanadzovic and Valverde, 2011). Gaps between contigs were filled by RT-PCR. The 5′ end of the genome was obtained using 5′ RACE (Life Sciences) according to manufacturer's instructions. Terminal sequences at the 3′end of the virus genome were determined by RT-PCR, performed on cDNAs generated on artificially polyadenylated dsRNAs as previously described (Abou-Ghanem et al., 1998). Nucleotide sequences of the same region were further verified with the protocol involving ligation of 5′phosphorylated/3′ amino-blocked oligonucleotide to target dsRNAs (Lambden et al., 1992).

Genome sequences were analyzed with ORF finder (Wheeler et al., 2006) and FGENESV0 software (Anonymous, 2007) to identify the ORFs. Conserved domains of the encoded proteins were identified with the conserved domain database (CDD; Marchler-Bauer et al., 2013). The genome sequence was deposited in GenBank under accession no. KC904540.

Phylogenetic analyses were performed on deduced amino acid sequences of the RdRp and HSP70h proteins of BVBaV and 26 recognized closterovirids. Both datasets, initially aligned by MUSCLE (Edgar, 2004), were submitted to phylogenetic estimations with Maximum Likelihood (ML) and Neighbor Joining (NJ) methods. ML-based phylogenies were inferred with PhyML (Guindon and Gascuel, 2003) implemented in SeaView version 4.4 (Gouy et al., 2010) using the LG substitution model (Le and Gascuel, 2008), whereas MEGA v.5 (Tamura et al., 2011) was used to infer phylogenies applying the Neighbor-Joining method with 1000 bootstrap replicates. Amino acid identities of the conserved proteins of the BVBaV was obtained by pairwise comparisons of the polyprotein, RdRp, HSP70h, HSP90h, CP, CPm and 21 kDa proteins with those of the subgroup I orthologs.