TWO COTTON BEGOMOVIRUSES FOUND IN BRAZIL

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Abstract

Begomoviruses are important in many cotton-growing countries. In Brazil, begomovirus epidemics were not reported. This study aimed at surveying and identifying the begomoviruses present in two major cotton producing areas of Brazil: the Cerrado and the North-East region. Plants showing chlorotic spot, mosaic and/or interveinal chlorosis symptoms were collected from these two regions and two viruses were isolated. The first, a begomovirus, was preliminary classified as isolate of *Sida micrantha mosaic virus* (SiMMV) and was found in 22 samples collected in the Cerrado region (central Brazil). The identification was based on the direct sequencing of PCR products using primers directed to part of the coat protein, the entire intergenic region, and part of the Rep protein (94-97% nucleotide identity with SiMMV, accession KC706535.1 and HM357459.3). In the North-East region, another begomovirus was isolated in Paraiba State. PCR confirmed the begomovirus infection. Then, the complete genome (DNA-A and DNA-B) was cloned after rolling circle amplification and digestion with a single site cutting restriction enzyme. From all samples, the isolates were over 99% identical to each other indicating that they were infected with the same virus with low variability. The genome size and the genome organization were typical of New World bipartite begomoviruses. The maximum nucleotide identity with known begomoviruses was 77.8% for DNA-A of *Tomato common mosaic virus* (ToCmMV, accession NC_018350); and 67.8% for DNA-B of *Tomato yellow vein streak virus* (NC_010950). Thus, this virus can be considered as a new species based on the species demarcation criteria of the International Committee on Taxonomy of Viruses, and the name Cotton chlorotic spot virus is proposed.

Introduction

Members of the family Geminiviridae are important worldwide, causing devastating diseases. This family is divided into seven genera, and the genus *Begomovirus* is the largest with many destructive viruses, all transmitted by whiteflies.

Begomoviruses have a genome of ssDNA and are informally divided in two groups: New World and Old World. Those of the New World are typically bipartite (DNA-A and DNA-B), and of the Old World monopartite. Most of the monopartite begomoviruses are associated with beta or alphasatellite molecules. Among those infecting cotton plants, some are bipartite, such as *Cotton leaf crumple virus* (CLCrV) present in South United States (Arizona, California), Mexico, and Guatemala (Idris and Brown, 2004); and some are monopartite like those causing the cotton leaf curl disease, a very important cotton disease occurring in India, Pakistan, and in Africa (Satar et al. 2013). In Brazil, begomoviruses are not reported as causing serious epidemics. This study was carried out to survey for begomoviruses in cotton plants.

Materials and Methods

Plants showing mosaic and interveinal chlorosis symptoms were collected in central Brazil, Cerrado area (State of Goias). The chlorosis was sometimes restricted to parts of the leaf or the plant. A partial genome was amplified from

DNA extracts from symptomatic leaves using the begomovirus universal primers $PAL1_V1978$ and pARV1c715 (Rojas et al. 1993) from 22 samples. The amplified DNA fragments (1320 bp, including part of the coat protein, Rep protein coding regions and the entire intergenic region) were directly sequenced and compared with other begomovirus sequences using the blastN algorithm.

In the North-East region, cotton plants with chlorotic spots, interveinal chlorosis and leaf distortion were collected from Paraiba State, in 2009. First, partial sequences were obtained which confirmed the presence of a begomovirus. Then, the whole genome, including DNA-A and DNA-B, was amplified using bacteriophage *phi*-29 DNA polymerase in a rolling circle amplification mechanism (Inoue-Nagata et al, 2004), followed by digestion with the single cutting enzyme *Xba*I. These monomeric units were cloned in pBlueScript (Stratagene), sequences were obtained and the genome assembled (Almeida et al. 2013).

Results and Discussion

Plants showing mosaic and interveinal chlorosis were collected in the State of Goias (Figures 1 and 2). They were tested by PCR using begomovirus universal primers. At least 22 samples tested positive and the PCR products were directly sequenced using the amplification primers.

Figure 1: *Gossypium hirsutum* showing leaf spots in growing areas of central Brazil.

Figure 2: Symptomatic *G. barbadense* plants in germplasm multiplication in central Brazil.

The partial sequences shared 94 to 97% nucleotide identity with *Sida micrantha mosaic virus* (SiMMV). The virus isolated from cotton genotypes LA RN 910 and 'Plains' shared 95% nucleotide identity with SiMMV isolated from commom bean GO60 (GenBank: KC706535.1 and HM357459.3).This virus was originally found in *Sida micrantha* plants (Jovel et al. 2004), soybean (Fernandes et al. 2009) and okra (Aranha et al. 2011) in Brazil. Plants with begomovirus-like symptoms were compared to healthy ones in the field to estimate the losses caused by infection. Infected plants showed a reduction on plant height and number of bowls, although symptoms frequently did not persist during the plant development.

Plants with symptoms found in Paraiba, North-East Brazil, are shown in Figure 3. The complete genome sequence was determined from viruses isolated from plants with chlorotic spots. The genome organization exhibited the typical characteristics of New World bipartite begomoviruses. The maximum nucleotide identity with known begomoviruses was 77.8% for DNA-A of *Tomato common mosaic virus* (ToCmMV); and 67.8% for DNA-B of *Tomato yellow vein streak virus*. Thus, this virus can be considered as a new species based on the species demarcation criteria of International Committee on Taxonomy of Viruses, and the name Cotton chlorotic spot virus is proposed (Almeida et al. 2013).

Figure 3. Cotton plants in Paraiba, 2009.

Summary

Two begomoviruses species have been demonstrated to infect cotton plants in Brazil.

Abutilon mosaic virus (AbMV) has been attributed as the causal agent of a disease known as common cotton mosaic and variegated chlorosis in malvaceous plants. This is a bipartite virus, collected in West Indies and one of the first begomoviruses described, early in 1906. Later, it was shown that the yellow mosaic symptom in *Sida micrantha* (Synonym of *Sidastrum micranthum*) was caused by an isolate of *Sida micrantha mosaic virus* (SiMMV; Jovel et al. 2004). It is not known if AbMV is present in Brazil.

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