Genome Sequence of Cotton Leafroll Dwarf Virus Infecting Cotton in Georgia, USA

Afsha Tabassum, Phillip M. Roberts, Sudeep Bag

ABSTRACT Cotton leafroll dwarf disease (CLRDD), caused by the aphid-borne Cotton leafroll dwarf virus (CLRDV; genus, Polerovirus; family, Luteoviridae), has been recently reported from the major cotton-growing regions of the United States. Here, we present the nearly complete genome sequence of a CLRDV isolate from cotton in Georgia.

Cotton is the second most important agricultural commodity for the state of Georgia, with a farm gate value of $901.5 million (1). Cotton leafroll dwarf virus (CLRDV), a phloem-limited virus, is associated with the emerging cotton leafroll dwarf disease (CLRDD) in the United States. It was first reported from Alabama in 2019 (2) and subsequently from the major cotton-growing regions in the United States, including Florida (3), Georgia (4), Louisiana (5), Mississippi (6), South Carolina (7), and Texas (8). Symptoms of the disease include reddening of the leaves and petioles and drooling, crinkling, and deformation of the leaves (Fig. 1A and B), and it has the potential to cause significant yield and economic losses. The viral genome consists of a single-stranded positive-sense RNA approximately 5.8 kb long encoding seven different proteins (9, 10).

In summer 2018, symptomatic plants (n = 20) showing reddening, drooling of leaves, and reddening of petioles, along with asymptomatic plants (n = 20), were collected from three counties, Early, Seminole, and Tift, in Georgia. CLRDV was detected from symptomatic samples but not from the asymptomatic tissue tested (4). To understand the genomic composition of CLRDV from Georgia, a nearly complete genome of an isolate from Seminole County was sequenced using Sanger’s method and analyzed.

Total RNA was extracted from a pooled sample of symptomatic leaves, petioles, and bark tissues using the modified cetyltrimethylammonium bromide method (11, 12). Complementary DNA (cDNA) was synthesized from 2.5 μg of total RNA using SuperScript III reverse transcriptase (Invitrogen, USA) and specific reverse primers targeting different open reading frames (ORFs) (Table 1) of the virus genome following the manufacturer’s recommended conditions. The cDNA (2 μl) and specific primer combinations (Table 1) were used to amplify different ORFs of the CLRDV genome using Platinum Taq DNA polymerase (Invitrogen, USA). Products of predicted sizes were cloned into the pGEM-T Easy I cloning vector (Promega, USA), and both strands were sequenced using the SP6 to T7 sequencing primers (GenScript, USA). The nearly complete nucleotide sequence was assembled from the consensus sequence of three clones for the target region. The sequence was annotated with the help of BioEdit (13) and MEGA X (14) software and submitted to GenBank (accession number MT633122).

The maximum likelihood phylogenetic tree of nearly full-length nucleotide sequences was constructed using the CLRDV sequences from GenBank and the isolate sequenced in this study with MEGA X (14) software. Pairwise comparisons of the nucleotide sequences were performed with SDT v.2.1 (15) software.

Citation Tabassum A, Roberts PM, Bag S. 2020. Genome sequence of Cotton leafroll dwarf virus infecting cotton in Georgia, USA. Microbiol Resour Announc 9:e00812-20. https://doi.org/10.1128/MRA.00812-20.

Editor Kenneth M. Stedman, Portland State University
Copyright © 2020 Tabassum et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.
Address correspondence to Sudeep Bag, sudeepbag@uga.edu.
Received 14 July 2020
Accepted 24 July 2020
Published 20 August 2020
The CLRDV genome from Georgia characterized in this study was 5,868 bp long and encoded seven ORFs, as reported earlier for isolates from North and South America (8–10). It was 95 to 98% identical to the genome of other CLRDV isolates from the United States (Alabama, GenBank accession number MN071395; Texas, MN872302) and South America (KF359947, KF906261, KF906260, NC_014545, GU167940, and HQ827780) (Fig. 1C). The US isolates formed a clade separate from that of the South American isolates in the phylogenetic analysis based on nearly full-length nucleotide sequences (Fig. 1D).

**Data availability.** The nearly complete genome of CLRDV from Georgia described in this study was deposited in GenBank under accession number MT633122.

**TABLE 1** Sequences of oligonucleotide primer combinations, target regions, and annealing temperatures used to amplify the *Cotton leafroll dwarf virus* genome sequence in this study

| Strain name | Sequence (5’–3’) | Primer position | Amplicon length (bp) | \(T_m^{\text{a}}\) (°C) |
|-------------|------------------|-----------------|----------------------|-------------------|
| SB19F       | ACAAAAGAAGGATGAGGTTTGGT | 1–25             | 1,352                | 60                |
| SB20R       | ACCACCAAGCTGAGCTCCGAGC | 1352–1332        | 1,547                | 58                |
| SB28F       | TCAGGGATTACAAGCTGGGAC | 1286–1306        | 1,075                | 56                |
| SB19R       | GTGGGAAACGGATTCCCGGC | 2813–2833        | 775                  | 60                |
| SB25F       | CGCCCATCTCATGTGTATCCCC | 2609–2630        | 1,307                | 64                |
| SB15F       | CCTACTGCTGTCCCATTTTTGGT | 3683–3659        | 1,065                | 68                |
| SB11F       | AGTGTTTCTGTTGAGTACCAATTAATTCAATGTTA | 3544–3569 | 775 | 60 |
| SB11R       | TATTTTTCTGTTGAGTACCAATTAATTCAATGTTA | 4346–4319 | 1,307 | 64 |
| SB16F       | ACGACGAAGACGGAGGAGTC | 3752–3771        | 1,065                | 68                |
| SB16R       | AAAAGTCTCGGCAGTCTGTCGGTT | 5059–5039        | 5794–5771            | 68                |

\(T_m^{\text{a}}\): annealing temperature.
ACKNOWLEDGMENTS

We acknowledge support received in part from the Georgia Cotton Commission, Cotton, Inc., and the USDA National Institute of Food and Agriculture (Hatch Project 1020319) to accomplish the work done.

We are thankful for the comments and suggestions from two anonymous reviewers.

REFERENCES

1. The University of Georgia Center for Agribusiness and Economic Development. 2018. Georgia farm gate value report 2017. The University of Georgia Center for Agribusiness and Economic Development, Athens, GA.
2. Avelar S, Schrimsher DW, Lawrence K, Brown JK. 2019. First report of Cotton leafroll dwarf virus associated with cotton blue disease symptoms in Alabama. Plant Dis 103:592. https://doi.org/10.1094/PDIS-09-18-1550-PDN.
3. Iriarte FB, Dey KK, Small IM, Conner KN, O’Brien GK, Johnson L, Savery C, Carter E, Sprague D, Nichols RL, Wright DL, Mulvaney MJ, Paret M. 2020. First report of Cotton leafroll dwarf virus in Florida. Plant Dis https://doi.org/10.1094/PDIS-10-19-2150-PDN.
4. Avelar S, Schrimsher DW, Lawrence K, Brown JK. 2019. First report of Cotton leafroll dwarf virus associated with cotton blue disease symptoms in Alabama. Plant Dis 103:592. https://doi.org/10.1094/PDIS-09-18-1550-PDN.
5. Price T, Valverde R, Singh R, Davis J, Brown S, Jones H. 2020. First report of Cotton leafroll dwarf virus in Louisiana. Plant Health Prog 21:142–143. https://doi.org/10.1094/PHP-03-20-0019-BR.
6. Price T, Valverde R, Singh R, Davis J, Brown S, Jones H. 2020. First report of Cotton leafroll dwarf virus in Louisiana. Plant Health Prog 21:142–143. https://doi.org/10.1094/PHP-03-20-0019-BR.
7. Wang H, Greene J, Muellner J, Conner K, Jacobson A. 2020. First report of Cotton leafroll dwarf virus in cotton fields of South Carolina. Plant Dis https://doi.org/10.1094/PDIS-03-20-0635-PDN.