


Cotton leafroll dwarf disease: An enigmatic viral disease in cotton

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Abstract

Taxonomy: Cotton leafroll dwarf virus (CLR DV) is a member of the genus *Polerovirus*, family *Solemoviridae*.

Geographical Distribution: CLR DV is present in most cotton-producing regions worldwide, prominently in North and South America.

Physical Properties: The virion is a nonenveloped icosahedron with $T=3$ icosahedral lattice symmetry that has a diameter of 26–34 nm and comprises 180 molecules of the capsid protein. The CsCl buoyant density of the virion is 1.39–1.42 g/cm³ and S_{20w} is 115–127S.

Genome: CLR DV shares genomic features with other poleroviruses; its genome consists of monopartite, single-stranded, positive-sense RNA, is approximately 5.7–5.8 kb in length, and is composed of seven open reading frames (ORFs) with an intergenic region between ORF2 and ORF3a.

Transmission: CLR DV is transmitted efficiently by the cotton aphid (*Aphis gossypii* Glover) in a circulative and nonpropagative manner.

Host: CLR DV has a limited host range. Cotton is the primary host, and it has also been detected in different weeds in and around commercial cotton fields in Georgia, USA.

Symptoms: Cotton plants infected early in the growth stage exhibit reddening or bronzing of foliage, maroon stems and petioles, and drooping. Plants infected in later growth stages exhibit intense green foliage with leaf rugosity, moderate to severe stunting, shortened internodes, and increased boll shedding/abortion, resulting in poor boll retention. These symptoms are variable and are probably influenced by the time of infection, plant growth stage, varieties, soil health, and geographical location. CLR DV is also often detected in symptomless plants.

Control: Vector management with the application of chemical insecticides is ineffective. Some host plant varieties grown in South America are resistant, but all varieties grown in the United States are susceptible. Integrated disease management strategies, including weed management and removal of volunteer stalks, could reduce the abundance of virus inoculum in the field.

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KEYWORDS

cotton blue disease, cotton leafroll dwarf disease, cotton leafroll dwarf virus, *Gossypium hirsutum*, *Polerovirus*

1 | INTRODUCTION

Cotton (*Gossypium hirsutum*) is a major cash crop in many parts of the world, including the United States. In 2021, 4.1 million hectares of cotton were harvested in the United States, valued at approximately \$7.4 billion (USDA, 2021). Cotton blue disease (CBD) is a viral disease capable of causing significant losses in the cotton industry. CBD was first described in the Central African Republic in 1949 and has since then been reported from several regions in Africa, Asia, and the Americas (Cauquil, 1977). However, the nature of the causal agent of CBD was not known until 2005, when the entire capsid gene and a partial RNA-dependent RNA polymerase gene revealed its association with a virus belonging to the genus *Polerovirus*, family *Luteoviridae*, and named it cotton leafroll dwarf virus (CLRVD) (Corrêa et al., 2005). Distéfano et al. (2010) sequenced the complete genome of a CLRVD isolate from Argentina. Recently, ICTV reclassified CLRVD as a member of the family *Solemoviridae* (Sömera et al., 2021). In 2006, a less aggressive resistance-breaking genotype of CLRVD was observed in Brazil on cotton varieties known to be resistant against CBD. This new disease was referred to as “atypical” CBD (Agrofoglio et al., 2017; da Silva et al., 2015).

In the United States, CLRVD was first reported in Alabama from cotton in 2019. Plants infected with CLRVD showed symptoms including intense dark green to bluish foliage, reddening of stems and petioles, curling and drooping of leaves, internodal shortening, and moderate to severe stunting. The genome sequences from Alabama and Georgia isolates were characterized. The isolates present in the United States differ from those causing CBD in South America and other regions. The disease caused by CLRVD in the United States is hence referred to as cotton leafroll dwarf disease (CLRDD; Brown et al., 2019). It has been observed that cotton plants infected in younger stages could suffer complete yield loss, whereas the losses decrease when plants are infected at the mature stages of plant development (Parkash et al., 2021). Although the incidence of the virus in some commercial cotton fields in Georgia and Alabama is 80%–100%, no significant yield losses have been reported (Mahas et al., 2022).

2 | GEOGRAPHICAL DISTRIBUTION

CBD was first reported around 1949 in the Central African Republic. The disease spread towards Benin (previously known as Dahomey), Chad, Cameroon, Congo, and Ivory Coast (Cauquil, 1977; Cauquil & Follin, 1983; Cauquil & Vaissayre, 1971). Later, CBD was introduced in Brazil and Argentina (Corrêa et al., 2005; Costa & Carvalho, 1962) and Asian countries including India (Mukherjee et al., 2012), Thailand (Sharman et al., 2015), and Timor-Leste (Ray et al., 2016; Figure 1a).

More recently, CLRVD was reported in China (Feng et al., 2017), South Korea (Igori et al., 2022), and Uzbekistan (Kumari et al., 2020; Figure 1a). In North America, symptoms associated with CBD were first observed in the autumn of 2017 on the Gulf Coast of Alabama, and the identity of CLRVD was confirmed in 2019 (Avelar et al., 2019). Currently, CLRVD is widespread in all major cotton-growing regions in the United States (Aboughanem-Sabanadzovic et al., 2019; Alabi et al., 2020; Ali & Mokhtari, 2020; Ali et al., 2020; Avelar et al., 2019; Faske et al., 2020; Ferguson & Ali, 2022; Iriarte et al., 2020; Price et al., 2020; Tabassum et al., 2019; Thiessen et al., 2020; Wang et al., 2020).

The incidence of CLRVD infection in different states in the United States as estimated based on symptomatology ranges from 2% to 100% (Aboughanem-Sabanadzovic et al., 2019; Alabi et al., 2020; Ali & Mokhtari, 2020; Avelar et al., 2019, 2020). In Georgia, a disease incidence of less than 10% was recorded, but CLRVD was detected in many plant tissues with and without symptoms in commercial fields and research plots (Figure 1b; Tabassum et al., 2020, 2021).

3 | SYMPTOMATOLOGY

Symptoms including leaf rolling, crumpling, and a bushy top were reported in the province of Chaco in north-west Argentina (Agrofoglio et al., 2017). Plants infected in the mature stages had normal morphology in the lower plant parts (stem and internodes) but a bushy phenotype in the top, with deformed apical leaves (Agrofoglio et al., 2017). Symptomless CLRVD infections in cotton are very common in the United States (Bag et al., 2021; Tabassum et al., 2020, 2021). In addition, CLRVD-induced symptoms are highly variable at different locations in the United States. On the Gulf Coast of Alabama, symptoms such as leaf deformation with bluish-green discoloration, vein clearing, leaf curling and rolling, and dwarf, stacked internodes were observed during the autumn of 2017 (Avelar et al., 2019). In Georgia, CLRVD-infected cotton plants exhibit a multitude of symptoms depending on the plant growth stage (Bag et al., 2021; Figure 2a,b). Plants infected early (within 50 days after emergence) exhibit symptoms such as reddening of leaves, petioles, and stems, puckering, crinkling, deformation of the leaf lamina, wilting, and downward leaf drooping with V-shaped lamina folding (Figure 2c–f). Infected plants often wilt and some may recover but do not produce any bolls as they mature (Parkash et al., 2021). Plants infected later in the season at maturity exhibit more turgidity with a leathery texture, crinkling, and square/leaf dropping along with wilting (Figure 2g–j). Often, these plants have more vegetative growth in the upper branches of the stem, exhibiting stacked internodes and ceasing reproductive development (Figure 2k,l). Terminal whips or accentuated verticality and a bushy phenotype are also observed,



FIGURE 2 Symptoms observed on cotton plants infected with cotton leafroll dwarf virus (CLRDV) grown in commercial cotton fields in Georgia, USA. (a) Leaf deformation with bluish-green discoloration. (b) Vein clearing, leaf curling, rolling, and deformation. (c) Reddening of leaf, petioles, and stem. (d) Puckering and crinkling. (e) Wilting and downward leaf drooping (V-shaped lamina folding). (f) Deformation of the leaf lamina. (g) Leaf turgidity with a leathery texture. (h) Crinkling. (i) Square/leaf dropping. (j) Wilting. (k, l) Vegetative growth in the upper branches of the stem and stacked internodes and ceased reproductive growth. (m) Bushy phenotype. (n) Terminal whips or accentuated verticality. (o) Parrot beak fruits. (p) Plant infected in the early stage. (q, r) Symptoms diminish in early infected plants but plants remain dwarf without any harvestable bolls. (s, t) Volunteer cotton stalks.

if the pedigree involved crosses with Tamcot SP-37 or its progeny Miscot T8-27 (Bell et al., 2002). In other countries, symptoms such as red leaves, red wilt, and anthocyanosis are associated with bronze wilt and are considered a physiological disorder induced by numerous abiotic factors (Gade et al., 2013). Studies that were conducted to understand the aetiology of bronze wilt and its causal biotic or abiotic factors were inconclusive due to the inconsistency of the disease symptoms and severity (Padgett et al., 2004). The combined role of biotic (virus) and abiotic factors in causing bronze wilt disease has never been evaluated. Due to the similarity of CLRDD and bronze wilt symptoms, the cause of symptoms observed in cotton in Georgia and the surroundings is still under discussion.

4 | PHYSIOLOGY OF CLRDV-INFECTED COTTON

Because CLRDV is a relatively new cotton pathogen in the United States, minimal research has been conducted on the physiological response of cotton plants to CLRDV infection. However, leaf reddening in response to other stresses is a result of anthocyanin production, which is speculated to attenuate solar radiation or to reduce reactive oxygen species levels (Close & Beadle, 2003). Similar to plants exposed to other wilt-inducing stresses like drought (Chastain et al., 2014, 2016), recent research has shown that plants with CLRDV symptoms significantly reduce stomatal conductance and the net photosynthetic rate under field conditions (Parkash et al., 2021). Even in the earliest stages of the disease, when severe wilting is not yet observable, stomatal conductance and the net photosynthetic rate were reduced by 94% and 84%, respectively. Because declines in conductance limit transpirational cooling, leaf temperature also significantly increased by 0.5–3.8°C at advanced stages of the disease (Parkash et al., 2021). Using stomatal conductance as a reference indicator of stress, Medrano et al. (2002) and Parkash et al. (2021) were also able to show that the electron transport rate through photosystem II was less sensitive to CLRDV-induced stress than carbon assimilation. The possibility that these differences in sensitivity lead to oxidative stress and subsequent increases in anthocyanin production should be explored further.

5 | HOST RANGE

Crops and weeds are vital components in virus disease epidemiology and spread into new geographical locations. While cotton is the primary host of CLRDV, weeds and other crops that serve as hosts for this virus can influence its spread and aid in reoccurrence in agricultural landscapes. In Georgia, where the winters are mild, CLRDV was detected in a large number of overwintering cotton stalks and regrowth from these stalks (Figure 2s,t; Sedhain et al., 2021). CLRDV was also detected in 23 weed species growing near cotton fields in Georgia (Sedhain et al., 2021). In greenhouse experiments, CLRDV was detected by reverse transcription (RT)-PCR

following aphid-mediated transmission in hibiscus (*Hibiscus acetosella*), okra (*Abelmoschus esculentus*), *Nicotiana benthamiana*, Palmer amaranth (*Amaranth palmeri*), and prickly sida (*Sida spinosa*) (Pandey et al., 2022). Aphids feeding on CLRDV-infected malvaceous hosts including hibiscus, prickly sida, and okra were able to acquire CLRDV and back-transmitted it to noninfected cotton seedlings (Pandey et al., 2022). These volunteer cotton stalks and weed hosts could potentially act as a “green bridge” and as a source of primary inoculum for the next growing season (Bag et al., 2021; Sedhain et al., 2021).

In addition to cotton, CLRDV also infects a number of crop species worldwide. For example, chickpea (*Cicer arietinum*) was shown to be a host for CLRDV (Kumari et al., 2020). Symptoms induced by CLRDV on chickpea are similar to those observed on cotton, including chlorosis, stunting, necrosis, yellowing, and reddening (Kumari et al., 2020). In addition, CLRDV capsid protein gene sequences share 89.4% to 100% homology with chickpea stunt disease-associated virus (CpSDaV) isolates from India (Mukherjee et al., 2016). The virus was also transmitted from cotton to chickpea, suggesting that CLRDV and CpSDaV are strains of the same virus (Mukherjee et al., 2016). Natural infection of CLRDV has also been observed on *Hibiscus syriacus* exhibiting vein-clearing symptoms in South Korea upon mixed infection with other viruses (Igori et al., 2022). CLRDV was reported infecting cacao (*Theobroma cacao*) trees in Bahia state in north-eastern Brazil (Ramos-Sobrinho et al., 2022), suggesting its movement to different cultivated crop species.

6 | MORPHOLOGY AND GENOME ORGANIZATION

As a primary confirmation of Koch's postulates, CLRDV was detected in infected plants using electron microscopy. Virion particles were partially purified from young bark tissues from cotton plants with CLRDV symptoms (Figure 2c,d). The virion preparation was examined under a transmission electron microscope as described by Al Rwahnih et al. (2021). A minimal number of spherical virion particles with a diameter of 25–35 nm were observed (Figure 3a), suggesting the titre of the virus particles in the sample was low. RT-PCR further confirmed the presence of CLRDV in the same bark tissues following the protocol described in Tabassum et al. (2020). Previously Takimoto et al. (2009) conducted anatomical studies on CLRDV-infected cotton petioles and midrib tissues that exhibited more callose depositions and inclusion bodies in phloem cells (Takimoto et al., 2009).

The CLRDV genome organization resembles those of other poleroviruses (Avelar et al., 2020; Corrêa et al., 2005; Distéfano et al., 2010; Tabassum et al., 2020, 2021). The virus genome comprises seven open reading frames (ORFs) with an intergenic region between ORF2 and ORF3a (Figure 3b). ORF0 encodes the P0 protein (28.9 kDa), a silencing suppressor (Delfosse et al., 2014). ORF1 encodes the P1 protein (70.1 kDa), predicted to be expressed through leaky scanning. ORF1–2 encodes a fused protein P1–P2 replication-related protein of 118.7 kDa through ribosomal frameshift. ORF3–5 are expressed through subgenomic RNAs.

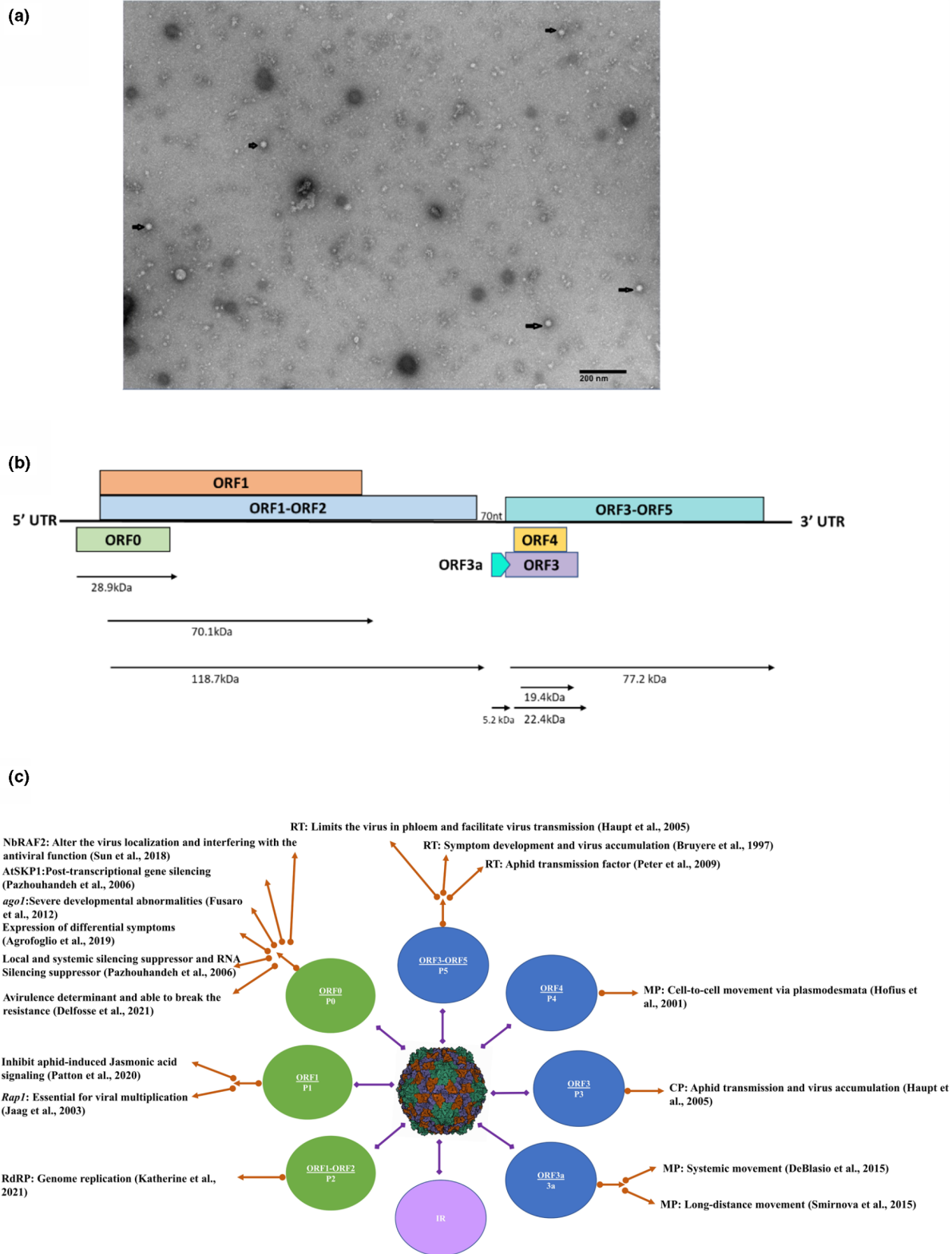


FIGURE 3 (a) Transmission electron micrograph of virion particles of cotton leafroll dwarf virus (CLRVDV) from bark tissues of an infected cotton plant with symptoms. The presence of CLRVDV in these bark tissues was further confirmed by reverse transcription-PCR. (b) Schematic representation of the genome of CLRVDV and open reading frames (ORFs). (c) Functional overview of proteins associated with CLRVDV, including a schematic representation and their functions. An artificially coloured cryo-electron microscopy image of rice yellow mottle virus (family *Solemoviridae*) is shown in the centre (ICTV; <https://ictv.global/report/chapter/solemoviridae/solemoviridae> and Opalka et al., 2000). The functional role played by each viral protein (shown in green, blue, and lavender-coloured circles) encoded by CLRVDV and other members of the genus *Polevirus* are depicted at the periphery corresponding to their functional characterization in that virus species.

ORF3 encodes the P3 (capsid) protein (22.4 kDa), ORF4 encodes the P4 (movement) protein (19.4 kDa), and P5 is translated through an in-frame read through the P3 stop codon. The P3–P5 protein (77.2 kDa) is essential for aphid transmission and virus accumulation in plants (Agrofoglio et al., 2017; Distéfano et al., 2010; Silva et al., 2008; Figure 3b).

ORF0 is a highly variable genomic region of CLRDV (Cascardo et al., 2015; Delfosse et al., 2014); the P0 protein is the most diverse protein. The P0 protein contains a conserved F-box (LPxx[L/I]) domain located near the N-terminus and a conserved sequence ([K/R]IYGEDGXXXFWR) at the C-terminus (Delfosse et al., 2021) considered essential for viral silencing suppressor activity (Pazhouhandeh et al., 2006; Zhuo et al., 2014). Silencing suppression activity varies in different species and can be systemic or local, and a few proteins have null activity (Delfosse et al., 2021). In isolates collected from the United States, five unique amino acid substitutions were present; however, their role in disease development and expression is unknown. The amino acid substitution from isoleucine (I) to valine (V) at the 72nd position (I72V), in the F-box domain, differentiates between the typical and atypical genotypes of CLRDV (Delfosse et al., 2014). The significance of this substitution in symptom development and virulence is not yet determined. The sequenced isolates from Georgia and Alabama resemble the South American resistance-breaking atypical genotypes with valine (V) at the 72nd position, whereas isolates from Texas have both typical (I) and atypical (V) residues at the 72nd position (Tabassum et al., 2020, 2021).

7 | DIVERSITY AND EVOLUTION OF CLRDV

Since the first report of CLRDV in Alabama, isolates from different host species and geographical locations in the United States were sequenced and compared with the isolates of other parts of the world to further understand the diversity and evolution of CLRDV (Avelar et al., 2020; Ramos-Sobrinho et al., 2021; Tabassum et al., 2020, 2021). A study by Tabassum et al. (2021) revealed that the P0 proteins of CLRDV isolates from Texas and Alabama were >90% identical to those of the CLRDV isolates from Georgia. However, Georgia isolates have >10% divergence in their amino acid sequences compared to other South American CLRDV sequences. These data might be helpful in further understanding the diversity, degree of genetic differentiation, and population dynamics of CLRDV. A recent study suggests that eight newly determined full-length genome sequences of CLRDV isolated from cotton plants in Alabama, Florida, and Texas were phylogenetically related with a CLRDV genotype from Alabama (Ramos-Sobrinho et al., 2021). Furthermore, these isolates have been shown to cluster into a monophyletic group among the CLRDV populations in North America. These data further suggest that horizontal gene flow may have occurred between CLRDV isolates during the course of evolution.

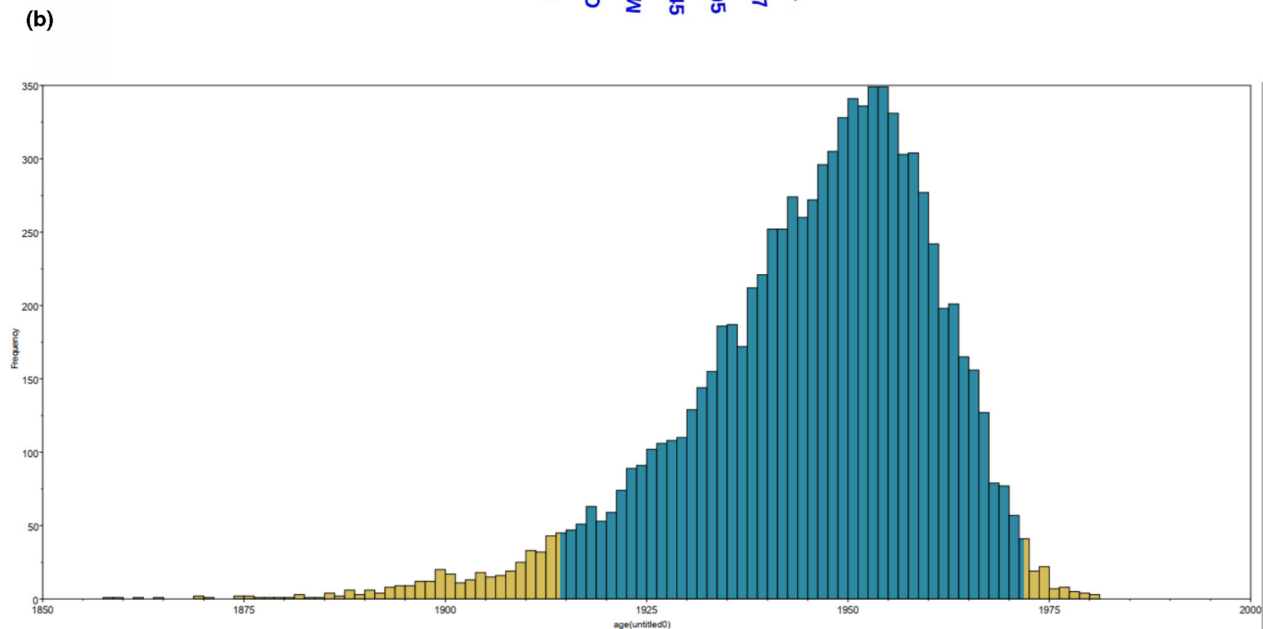
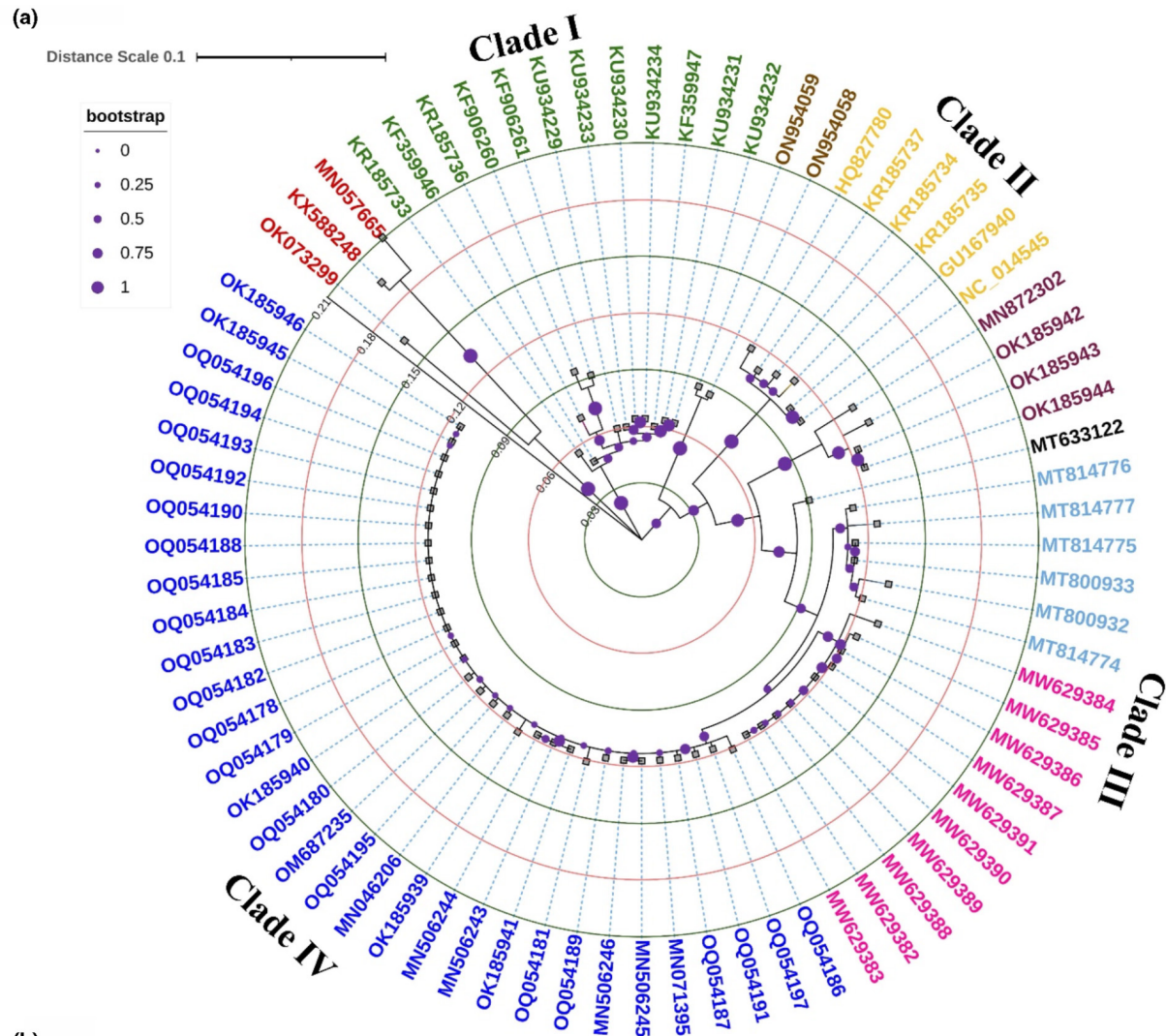
A more in-depth analysis of ORF0 of various CLRDV isolates was performed with the sequence information available as of 2023-01-10 ($n=76$) using MEGA-XI and iTOL (Table S1; Letunic & Bork, 2021; Tamura et al., 2021). CLRDV isolates were separated into four major clades mostly based on geographical location (Figure 4a). The Asian clade consisted of three CLRDV isolates, of which two infect the family Malvaceae (*H. syriacus* and *Malvaviscus arboreus*) in South Korea and one was reported from soybean aphid (*Aphis glycines*) in China. The CLRDV P0 sequences ($n=20$) from South American cotton isolates diverged into three separate clades: (i) one clade containing “typical” CLRDV genotypes (mostly from Brazil), (ii) one clade containing “atypical” CLRDV genotypes (mostly from Argentina; da Silva et al., 2015), and (iii) one clade containing two “atypical” isolates from cacao (*T. cacao*) trees in Brazil (Ramos-Sobrinho et al., 2022). The most diverse and most extensive clade consisted of 53 sequences from North America. Among the CLRDV isolates reported from the United States, isolates associated with cotton ($n=43$) and weed ($n=10$) species were segregated into two distinct clusters. Such segregation suggests genetic recombination, which might help the viruses to broaden their host range and foster successful establishment in the ecosystem (Vassilakos et al., 2016).

The same set of sequences was also used to study the amino acid substitution rates and evolutionary pattern of CLRDV using BEAST v. 1.10.4 and Tracer v. 1.7.1 (Rambaut et al., 2018; Suchard et al., 2018). BEAST analysis showed that the amino acid substitution rate of P0 protein from different hosts combined was 2.194×10^{-3} substitutions per site per year. However, when P0 from only weeds was considered, the amino acid substitution rate was higher (6.313×10^{-10} substitutions per site per year) (Table 1). Genome-wide mutations and recombination in viruses allow them to jump from one host to another (Calvo et al., 2014). The higher amino acid substitution rate in cultivated and noncultivated host species emphasizes the potential threat to cotton cultivation and other crops in the future.

Based on the CLRDV ORF0 sequences available in GenBank from 2005 to 2022, BEAST analysis estimated the age (root) of the CLRDV population in the year 1945 during the period 1938–1962 when CLRDV symptoms were first observed and documented in the Central African Republic (Cauquil, 1977; Cauquil & Follin, 1983; Costa & Carvalho, 1962). However, the 95% highest posterior density credible interval spans from 1914 to 1971 (Bryant et al., 2007; Figure 4b). These data provide insight into virus transmission among cotton and other species and suggest that CLRDV was probably introduced in South America from the African continent (as a CBD).

8 | INSECT VECTOR AND TRANSMISSION

The cotton or melon aphid *Aphis gossypii* is a major agricultural pest found worldwide in over 170 countries (CABI Compendium, 2021). The earliest known investigations of CLRDV transmission were conducted in Africa in the early 1970s when CLRDV was commonly known as CBD. Aphids collected from cotton plants with symptoms in the field were able to transmit the virus to cotton (Cauquil



& Vaissayre, 1971). Studies conducted in Brazil determined that susceptible cultivars required fewer viruliferous aphids to develop symptoms while more resistant cultivars required a minimum of 10

aphids to develop symptoms (Takimoto, 2003). *A. gossypii* transmits CLRDV to cotton plants in a circulative and nonpropagative manner (Michelotto & Busoli, 2003). Both winged and wingless morphs can

FIGURE 4 (a) The evolutionary history was inferred by using the maximum-likelihood method and a JTT matrix-based model with 1000 bootstrap replicates. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. This analysis involved 76 nucleotide sequences. All positions with less than 95% site coverage were eliminated. The blue colour indicates cotton leafroll dwarf virus P0 (CLR DV-P0) sequences obtained from cotton plants in Alabama, Florida, Georgia, Oklahoma, and Texas, in 2018–2022. The light pink colour indicates CLR DV-P0 sequences from weed species in Georgia. The CLR DV-P0 sequences obtained from cotton in Georgia/Alabama in 2018 are represented in teal. The typical CLR DV-P0 sequences from Brazil are shown in mustard yellow, whereas atypical CLR DV-P0 sequences from cotton are shown in green. Four sequences from Texas forming a subclade are shown in purple. Two sequences (accessions ON954058 and ON954059) shown in golden brown were isolated from cacao (*Theobroma cacao*) trees in Brazil. Three CLR DVs (accessions UID85580, QLJ58259, and ARU09826), isolated from *Hibiscus* sp., *Malvaviscus* sp., and aphid species, are shown in red. The phylogenetic tree was visualized using iTOL v. 4 (Letunic & Bork, 2021). (b) Graph representing the evolution of CLR DV. The virus epidemic may have started around 1945. Analysis was conducted in BEAST v. 1.10.4 and the results were further visualized using Tracer v. 1.7.1.

TABLE 1 Analysis of the rate of mutation and the age (root) of the CLR DV P0 protein using the BEAST tool (v. 1.10.4).

Sequence source	Mean rate	95% HPD interval	S	Variation	ESS/no. of samples	Age (root)	95% HPD interval
All 76 sequences	2.194×10^{-3}	1.293×10^{-3} to 3.081×10^{-3}	0.1015	0.0103	8,871/9,001	1945	1914 to 1971
Only weed species	6.313×10^{-10}	1.266×10^{-23} to 1.398×10^{-10}	1.129×10^{-12}	1.275×10^{-22}	566/9,001	–	–

Abbreviations: ESS, effective sample size, that is, the number of independent samples or the chain length; HPD, highest posterior density; Stdev, standard deviation of the mean.

transmit the virus after a 48-h inoculation access period for up to 12 days (Michelotto & Busoli, 2006, 2009).

A. gossypii is an annual pest of cotton in the south-eastern United States. Seasonal dynamics of cotton aphid populations and their potential role in the spread of CLR DV were investigated in field plots in Georgia and Alabama. A higher incidence of *A. gossypii* was observed during June and July. *A. gossypii* was detected in all test plots irrespective of the intensity of insecticide use surveyed in 2019. CLR DV was detected in 60% to 100% of the samples tested (Mahas et al., 2022), suggesting a correlation between vector and disease spread in the region.

Soybean aphids (*A. glycines*) collected in 2017 in China were found to harbour CLR DV, although cotton is a nonpreferred host of this aphid (Feng et al., 2017). *Aphis craccivora* and the green peach aphid *Myzus persicae* transmit CpSDaV (Horn et al., 1995), which is suspected to be another strain of CLR DV (Mukherjee et al., 2016). These findings indicate that more than one aphid species could transmit CLR DV and might contribute to the introduction and spread of the virus to different hosts and geographical regions. In the United States, *A. craccivora*, *Aphis fabae*, *Macrosiphum euphorbiae*, *Protaphis middletonii*, and *Rhopalosiphum rufiabdominale* are known to colonize cotton (Blackman & Eastop, 2000; Mahas et al., 2022). However, transmission experiments with these aphids need to be conducted to confirm their ability to transmit CLR DV. Due to the presence of sweet potato whitefly (*Bemisia tabaci*) on cotton in the south-eastern United States, its ability to transmit CLR DV on cotton was tested. *B. tabaci* could neither acquire nor transmit the virus, suggesting *A. gossypii* is the only efficient vector transmitting CLR DV onto cotton in the United States (Heilsnis et al., 2022).

9 | DIAGNOSIS

The symptoms of CLR DV-infected cotton plants are complex and depend on the plant growth stage at the time of infection, the variety, and the geographical location, making it difficult to diagnose solely on the basis of symptomology. Serological tests performed on CBD samples from north-eastern Argentina suggested its relationship to barley yellow dwarf virus serotypes RPV and PAV members of the family *Luteoviridae* (Lenardon, 1994). Recently, antibodies raised against CLR DV capsid protein antigens were used in double antibody sandwich-ELISA to detect CLR DV in *Commelina* sp. weed samples (Hoffmann et al., 2022).

After the CLR DV genome was sequenced, PCR-based methods were developed for detection. Initially, degenerate primers targeting ORF0 and ORF3 were used to detect the virus (Corrêa et al., 2005). Later, specific primers targeting ORF3 were developed for detection assays (Silva et al., 2008). With the emergence of CLR DV in the United States, PCR assays using primers designed specifically targeting the US isolates were developed (Tabassum et al., 2020). Another set of degenerate primers targeting ORF3 were designed to detect several polero- and luteoviruses from Argentina, Brazil, and India, including CLR DV isolates (Sharman et al., 2015). SYBR Green-based RT-quantitative PCR (RT-qPCR) assays targeting ORF3 were developed for more precise and quick detection (Pandey et al., 2022).

High-throughput sequencing plays a significant role in the early detection of CLR DV on plants with symptoms in commercial fields in Alabama, Florida, and Texas (Avelar et al., 2019, 2020; Ramos-Sobrinho et al., 2021). CLR DV was found as coinfections with other viruses in cotton plants exhibiting leaf roll and vein yellowing

symptoms (Yang et al., 2021) in China and on *H. syriacus* exhibiting vein clearing symptoms (Igori et al., 2022) in South Korea.

10 | COTTON LEAFROLL DWARF VIRUS TISSUE TROPISM

CLRDV is phloem-limited, and during initial studies conducted in Georgia, detection in whole leaf tissues gave inconsistent results due to the low virus titre (Figure 3a). To determine the best tissue to be sampled for accurate detection of CLRDV in cotton, virus titre was estimated in different tissues from plants exhibiting symptoms associated with CLRDV. The copy number of the virus in samples was estimated by the method described by Kavalappara et al. (2022). A partial capsid protein gene amplified and cloned in a Topo-TA PCR cloning vector (Invitrogen) was used as standard. Six 10-fold serial dilutions of the linearized plasmids were prepared and a standard curve was made. The number of copies of standard was calculated based on the spectrophotometric determination of the plasmid DNA concentration using a previously reported formula (Rotenberg et al., 2009). In general, CLRDV titre was higher in the upper parts of infected cotton plants than in the lower parts. CLRDV titre was highest in the main stem, followed by the branches and petioles. Fruits carried the lowest CLRDV titre among various tissues tested (Table 2). Thus, young phloem tissues in the upper canopy would be the ideal candidate for sample collection for accurate and efficient detection.

11 | ECONOMIC IMPORTANCE

In Argentina and Brazil, yield losses of up to 80% were reported due to CLRDV infection in susceptible varieties (Distéfano et al., 2010; Silva et al., 2008). Yield reductions of up to 99% due to a decrease in boll number and boll mass resulting from a lower number of seeds

TABLE 2 Estimated copy number of cotton leafroll dwarf virus (CLRDV) in different parts of infected cotton plants.

Part of the plant		Estimated copy number per ng of total RNA
Underground	Roots	1.78×10^5
Lower plant parts	Main stem	5.01×10^5
	Branches	4.47×10^5
	Petioles	2.63×10^3
	Fruit bracts and skin	2.00×10^3
Upper plant parts	Main stem	8.91×10^5
	Branches	6.15×10^6
	Petioles	1.58×10^5
	Fruit bracts and skin	1.26×10^4

Note: Values are averages from eight CLRDV-infected plants as determined by reverse transcription-PCR. Samples from each plant were run in triplicate.

per boll have been recorded on individual plants (Parkash et al., 2021). However, overall yield losses are variable in the United States (Avelar et al., 2019; Mahas et al., 2022). Many commercial cotton fields in the south-eastern United States, where CLRDV was first detected in 2019, have met their production goals (authors' personal observations). It is unknown whether variation in disease and yield loss among locations is due to varietal, environmental, crop age, or vector-related factors. Most of the cotton varieties cultivated in the United States are tolerant to CLRDV; the virus may be present, but the host plants do not exhibit any symptoms. Furthermore, only a small number of commercial cotton varieties exhibit disease symptoms such as reddening of leaves, wilting, and downward leaf drooping with V-shaped lamina when the plant is infected by the virus early in the season. Consequently, CLRDD seems to be a minor concern at present. However, given the previous virus outbreaks and the yield losses in South American countries, monitoring of the evolving virus species/strains and its epidemics in commercial cotton varieties may be a good preventive measure. A thorough study of virus–host interactions will help in developing management strategies for future outbreaks.

12 | DISEASE MANAGEMENT

Vector management is one of the major components of integrative pest management strategies for virus management. However, aggressive aphid sprays (weekly application) during 2019–2020 did not reduce the occurrence of CLRDV in an experimental plot in Georgia. It was also observed that insecticide application did not prevent aphid colonization or decrease CLRDV incidence in both Alabama and Georgia. Moreover, it could adversely impact other beneficial insects as well as elevate the risk of insects developing insecticide resistance. In addition, adjusting the planting date was also found to be ineffective in reducing the incidence of CLRDD (Mahas et al., 2022).

CBD was effectively mitigated in South America by deploying varieties possessing a single dominant resistance gene, *Rghv1* (Pupim Junior et al., 2008). Morello et al. (2010) developed BRS-293, a mid-season high-yielding cultivar that is moderately resistant to typical and atypical CLRDV but was found to be susceptible to the prevalent CLRDV in the south-eastern United States (Brown et al., 2019). While assessing resistant populations obtained from cotton cultivar Delta Opal, possessing a single dominant resistance gene, *Cbd*, Fang et al. (2010) identified two tightly linked simple-sequence repeat markers associated with the *Cbd* gene, localized on chromosome 10. However, genetic analyses have indicated that varieties carrying the *Cbd* gene were susceptible to CLRDV (Brown et al., 2019), suggesting that breeding for resistance would necessitate screening to identify new sources of resistance.

13 | CONCLUSION

CLRDD is an emerging viral disease in cotton-producing regions in the United States. Several weed species and overwintering cotton

stalks might act as a potential green bridge and source of virus inoculum. Early and late season symptoms are yield-limiting in the infected plant but not detrimental over a large acreage due to low disease incidence since its first report in 2018. Earlier recorded yield losses, the increase in host range, and its worldwide distribution are still concerning. At present, CLRDD may not be yield-limiting in the United States, but it can cause significant yield loss in individual infected plants, as observed in commercial fields and experimental plots in Georgia (Parkash et al., 2021) and in other cotton-producing countries. Basic research on virus–host interactions using a reverse genetic approach will be advantageous to devise appropriate management strategies and breeding approaches for future crops.

CLRVD is phloem-limited, but little is known about its interactions with phloem proteins and interference with host phloem activities (Ψ_w , Ψ_p , Ψ_s , phloem loading, and unloading), highlighting that further research is needed. Systemic infection with plant viruses can affect the phloem tissue and its transcriptome (Collum & Culver, 2017). CBD viruses trigger severe symptoms and yield losses to which resistant germplasm was developed, which was soon overcome by the resistance-breaking atypical strain causing mild symptoms. Reasons for the varied host response to different virus strains are not well known, and could include abiotic factors, virus–host interactions, or a combination of both, resulting in leaf drooping, wilting, reddening, stunting, and stacking internode symptoms. The CLRVD P0 protein is a silencing suppressor; it contains a highly variable region with five amino acid substitutions that are uniquely present in the isolates collected in Georgia, USA. The effects of these amino acid substitutions in P0 on symptom development and virulence need further investigation. The number of aphid vectors is very high from early June to mid-July in Georgia. Aggressive sprays (once weekly) reduced the aphid populations but did not inhibit the occurrence of CLRVD and negatively influenced beneficial insects. The soilborne fungus *Neozygites fresenii* acts as a biological (natural) control and collapses the aphid population in the region, reducing the dependence on chemical control. The development and deployment of resistant varieties remain the most efficacious strategy for CLRDD management. Further investigations of the disease symptomatology, virus interactions with crop and noncrop hosts, vectors, the virus population, and cultural practices to develop and implement integrated disease management strategies are necessary.

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CONFLICT OF INTEREST STATEMENT

The authors read the manuscript and declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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