Annual Review of Phytopathology

Kitaviruses: A Window to Atypical Plant Viruses Causing Nonsystemic Diseases

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Annu. Rev. Phytopathol. 2023. 61:97–118

First published as a Review in Advance on May 22, 2023

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

https://doi.org/10.1146/annurev-phyto-021622-121351

Keywords

cilevirus, higrevirus, blunervirus, citrus leprosis, *Brevipalpus* mites, negevirus

Abstract

Kitaviridae is a family of plant-infecting viruses that have multiple positive-sense, single-stranded RNA genomic segments. Kitaviruses are assigned into the genera *Cilevirus*, *Higrevirus*, and *Blunervirus*, mainly on the basis of the diversity of their genomic organization. Cell-to-cell movement of most kitaviruses is provided by the 30K family of proteins or the binary movement block, considered an alternative movement module among plant viruses. Kitaviruses stand out for producing conspicuously unusual locally restricted infections and showing deficient or nonsystemic movement likely resulting from incompatible or suboptimal interactions with their hosts. Transmission of kitaviruses is mediated by mites of many species of the genus *Brevipalpus* and at least one species of eriophyids. Kitavirus genomes encode numerous orphan open reading frames but RNA-dependent RNA polymerase and the transmembrane helix-containing protein, generically called SP24, typify a close phylogenetic link with arthropod viruses. Kitaviruses infect a large range of host plants and cause diseases of economic concern in crops such as citrus, tomato, passion fruit, tea, and blueberry.

1. INTRODUCTION

Citrus leprosis disease: a citrus disorder caused by at least six viruses, with major losses associated with CiLV-C and CiLV-C2 in sweet oranges

Transcriptome shotgun assembly (TSA): archive of computationally assembled transcript sequences from expressed sequence tags or high-throughput sequencing

Kitaviridae, order *Martellivirales*, class *Alsuviricetes*, is a family of plant-infecting viruses that was recognized by the International Committee of Taxonomy of Viruses in 2019 (88). Its name is a tribute to Dr. Elliot Watanabe Kitajima, a leading plant virologist who has dedicated 50 years to identifying and characterizing viruses transmitted by tenuipalpid mites of the genus *Brevipalpus* (2, 44, 47).

Diseases such as leprosis and zonate chlorosis in citrus (*Citrus* spp.) and leprosis in privet (*Ligustrum* spp.) were first reported in South America between 1919 and 1940 and are now recognized as caused by kitaviruses (16–18, 40, 101, 114, 121). In a century of progress from biological observations to the metagenomic era, the early attempts to discover the viral etiology of these diseases (28, 31, 32, 45, 49, 51, 66) and, more recently, the in-depth molecular characterization of the involved viruses are at the foundation of the creation of the family *Kitaviridae* (38, 42, 67, 72, 86–88, 90, 93, 103). This journey has been closely intertwined with research on *Brevipalpus*-transmitted viruses beyond the family *Kitaviridae* (see the sidebar titled *Brevipalpus*-Transmitted Viruses). Lessons learned from the exercise of comparative pathobiology mainly involving the study of viral diversity associated with citrus leprosis disease have been an undeniable contribution (reviewed in 12, 44, 47).

Kitaviruses have multiple positive-sense (+), single-stranded (ss) RNA segments in their genome and are assigned into three genera. Genus *Cilevirus* contains the largest number of molecularly and biologically characterized members, and genera *Higrevirus* and *Blunervirus* have one and three recognized species, respectively. Partial and near-complete genomes of tentative new kitaviruses of the three genera, including those mined in the transcriptome shotgun assembly (TSA) database, have been detected in plants around the world. This expansion brings a novel insight that undermines the previous view of *Kitaviridae* as a geographically restricted family and gives rise to an understanding of a globally scattered family with members on at least five continents. At the genus level, however, kitaviruses seem to be unequally distributed. Cileviruses and the solely recognized higrevirus are reported in Hawaii (USA) and American countries, mostly in South America, and tentative novel cileviruses and higreviruses have been found in plants from

BREVIPALPUS-TRANSMITTED VIRUSES

Dichorhaviruses (genus *Dichorhavirus*, family *Rhabdoviridae*) and kitaviruses of the genus *Cilevirus* are historically recognized as *Brevipalpus*-transmitted viruses (BTV). According to the cytopathic effects observed in their host cells, BTVs were subdivided into the nuclear and cytoplasmic types (44). Although they have contrasting molecular biology features (39), viruses of these two groups produce nonsystemic infections showing rather similar symptoms, and, in some cases, they share vectors and ecological niches. Dichorhaviruses and cileviruses have even been found in mixed infections (36, 106). Besides the kitaviruses CiLV-C and CiLV-C2, four dichorhaviruses produce citrus leprosis (26, 27, 33, 91, 108). As the transmission of the Brazilian isolates of the higrevirus HGSV2 by *Brevipalpus* mites was recently confirmed (101), this virus is the newest member of the BTV-C.

Genus *Brevipalpus* (Acari: Tenuipalpidae) comprises nearly 300 species of flat mites, which colonize hundreds of plant species around the world (2, 14, 23). *Brevipalpus* reproduce by sexual or thelytokous parthenogenesis, but those vectoring kitaviruses are almost exclusively asexually reproduced (2, 122). The life cycle includes development from eggs to larvae, protonymphs, deutonymphs, and adults, with a quiescent phase between them (112). Under favorable conditions, the *Brevipalpus yothersi* life cycle lasts approximately 30 days and an adult may lay 30–50 eggs (55).

Iran (74) and China (64) (TSA: GIKT01083416). In contrast, the largest number of blunerviruses, i.e., tea plant necrotic ring blotch virus (TPNRBV; *Blunervirus camelliae*) (42, 71) and three other tentative species (76, 124), have been detected in Asia. The blunerviruses blueberry necrotic ring blotch virus (BNRBV; *Blunervirus vaccinii*) has been reported only in the United States (22, 87, 98), whereas tomato fruit blotch virus (ToFBV; *Blunervirus solani*) has been found on four continents (30, 68, 78, 97).

Genomic comparisons between kitaviruses of the same genera have relatively low nucleotide sequence identity values, i.e., lower than 60% and 50% among cileviruses and blunerviruses, respectively (42, 92). This suggests the existence of phylogenetic gaps within and among genera, meaning that a diversity of unknown kitaviruses might exist. At the same time, although among isolates of some species different phylogenetic lineages and some uniqueness in their genomic organizations have been detected, intraspecies variability is low even between isolates collected from geographically distant places (25, 73, 78, 90, 93, 101, 102, 107, 120). Taken together, the data hint that although the spread of some kitaviruses likely could have been biased by anthropic actions possibly associated with the trade and exchange of their plant hosts, e.g., the cileviruses citrus leprosis virus C (CiLV-C; *Cilevirus leprosis*) and citrus leprosis virus C2 (CiLV-C2; *Cilevirus colombiaense*) in citrus and ToFBV in tomato (*Solanum lycopersicum*), there are regional diversity hot spots where kitaviruses of different genera have evolved from ancestors likely spread by continental drift.

Globally, kitaviruses naturally infect mono- and dicotyledonous plants, including herbaceous and woody plants comprising weeds, ornamentals, and crops (41, 50, 81, 104). Regardless of whether they are found in their natural hosts or experimental hosts, kitaviruses show a limited or null capacity to systemically move (39, 88). Damages include small areas of tissues ranging from chlorotic to necrotic lesions in which most kitaviruses remain restricted (**Figure 1**). In some virus–host combinations, e.g., the cilevirus passion fruit green spot virus (PfGSV; *Cilevirus passiflorae*) in passion fruit plants (*Passiflora* spp.), the infection, although nonsystemic, can reach large areas of the leaves along the veins (93), and in the case of the blunervirus TPNRBV in tea plants (*Camellia sinensis*), some segments of the viral genome can be detected in systemic tissues that remain asymptomatic (96). The activity of kitavirus-encoded movement protein (MP) has been experimentally confirmed (58, 61). However, the systemic movement, at least for the cilevirus CiLV-C, may be negatively impacted by the consequences of an incompatible plant–virus interaction in which hormone-mediated pathways are triggered, producing a hypersensitive-like response (3, 4).

In this review, we introduce data describing the multiplicity of viruses assigned to the family *Kitaviridae* and discuss evolutionary processes that might be involved in their diversification through their comparison with phylogenetically related arthropod viruses belonging to unclassified taxa that group negeviruses and other nege/kita-like viruses. We also debate putative causes of the incompatible interaction of kitaviruses with their host plants based on a comprehensive evaluation of studies about the activity of kitavirus MPs, plant–cilevirus transcriptomic interaction, and viral protein expression. Advances in the knowledge of kitavirus–vector interaction are contextualized within the dynamic of the tritrophic mite–plant–virus interaction in the citrus leprosis pathosystem. Because most of the information available corresponds to cilevirus studies, this review has been conceived as a starting point for paving a wider reference framework for forthcoming research.

Movement protein (MP): virus-encoded movement proteins allow the transport of virus genomes to and through plasmodesmata

Hypersensitive-like response: reaction typified by cell death and prevention of pathogen spread where typical effector and resistance proteins are missing/unknown

2. DISEASES CAUSED BY KITAVIRUSES AND THEIR ECONOMIC IMPACT

Despite nonsystemic infections, diseases caused by kitaviruses in economically important crops represent threats that, in some cases, become a high economic burden for growers. Tea, blueberry

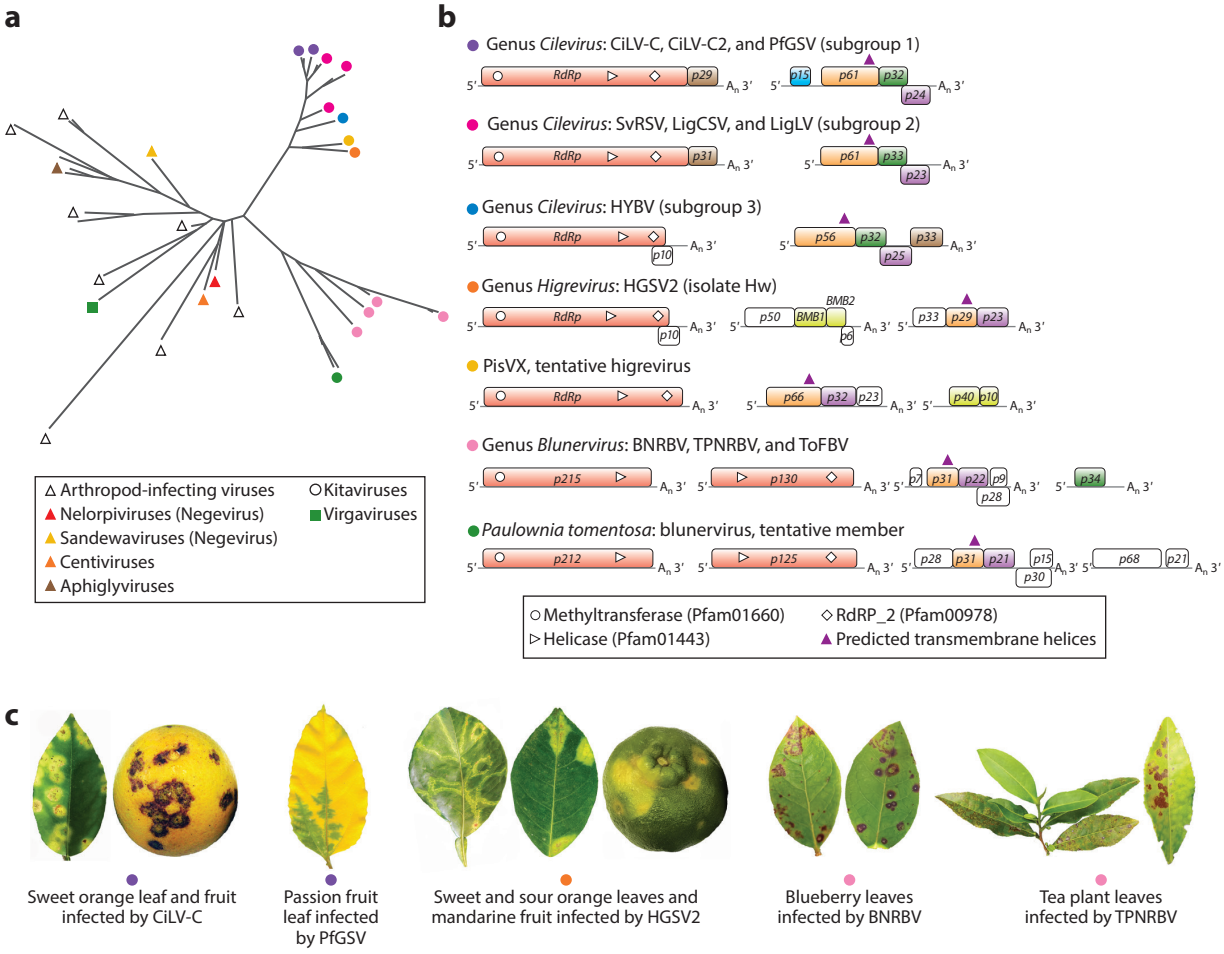

Figure 1

Diversity of kitaviruses. (*a*) Phylogenetic reconstruction using the RNA-dependent RNA polymerase (RdRp) protein of kitaviruses; kitavirus-related arthropod viruses of the taxa negevirus, aphiglivirus, centivirus; and other nege/kita-like viruses. The unrooted tree also includes RdRp of virgaviruses, representing plant-infecting viruses of the order *Martellivirales*. Tree leaves corresponding to kitaviruses are depicted with circles filled according to the description presented in panel *b*. (*b*) Genome organization of some kitaviruses. Boxes indicate open reading frames (ORFs), and the fill color indicates orthologs and/or conservation of structural or functional features in their encoded proteins across the family. Light red, RdRp; purple, negevirus SP24-like protein; green, movement protein of the 30K family; light green, proteins of the binary movement block (*BMB*); brown, putative coat protein; orange, putative glycoprotein; unfilled boxes indicate ORFs with unknown or unpredictable functions. The genomic segments of each virus are arranged from left to right in descending order according to their lengths. RNA2 genomic segment represented for cileviruses of subgroup 2 corresponds to that of SvRSV, RNA3–4 for BNRBV, TPNRBV, and ToFBV correspond to that of BNRBV isolate Georgia. (*c*) Symptoms produced by some kitaviruses in relevant hosts. Abbreviations: A_n, poly A tail; BNRBV, blueberry necrotic ring blotch virus (isolate Georgia, RNA1-RNA4: JN651148-JN651151); CiLV-C, citrus leprosis virus C (GenBank accession numbers of the isolate CRD RNA1 DQ352194 and RNA2 DQ352195); CiLV-C2, citrus leprosis virus C2 (isolate Colombia, RNA1 JX000024 and RNA2 JX000025); HGSV2, hibiscus green spot virus 2 (isolate WAI 1-1, RNA1 HQ852052, RNA2 HQ852053, and RNA3 HQ852054); HYBV, hibiscus yellow blotch virus (isolate OUGC, RNA1: MT472637, RNA2: MT472637); LigCSV, Ligustrum chlorotic spot virus (isolate SPa1, RNA1: OK626447 and RNA2: OK626448); LigLV, Ligustrum leprosis virus isolate Cdb1 (RNA1 OK626451 and RNA2 OK626452); PfGSV, passion fruit green spot virus (isolate Snp1, RNA1 MK804171 and RNA2 MK804172); PisVX, Pistachio virus X (RNA1 MT334620, RNA2 MT334618, and RNA3 MT334619); SvRSV, Solanum violifolium ringspot virus (isolate Prb1, RNA1 OK626439 and RNA2 OK626440); ToFBV, tomato fruit blotch virus (RNA1-RNA4: MK517477-MK517480); TPNRBV: tea plant necrotic ring blotch virus (RNA1-RNA4: MG781152-MG781155). *Paulownia tomentosa* blunervirus (TSA: *Paulownia tomentosa*, GEFV01158142, GEFV01018191, and GEFV01018861).

(*Vaccinium* spp.), and tomato plants are affected by blunerviruses, but calculated economic losses have not been reported. BNRBV has been detected in the southeastern United States since 2006 (22, 87). Infected plants show necrotic rings or irregularly shaped circular blotches (**Figure 1**) with green centers on the upper and lower surfaces of the leaves, which eventually coalesce, leading to premature defoliation and yield reduction (87). TPNRBV is relatively abundant among viruses infecting tea trees in China (96). Symptomatic leaves are commonly mature and located at the bottom of the trees. They show discoloration and necrotic ring blotches and usually drop prematurely. Under field conditions, tea cultivars have different degrees of resistance to TPNRBV, with the light albinistic ones being the most susceptible (96). ToFBV-infected tomato plants show symptoms of circular or uneven ripening blotches and dimpling in the fruits (30, 78).

The higrevirus hibiscus green spot virus 2 (HGSV2) was identified infecting hibiscus (*Hibiscus arnottianus*) shrubs and an isolated citrus (*Citrus volkameriana*) tree in Hawaii, USA, in 2012 (72). In 2022, HGSV2 was shown to be the causal agent of zonate chlorosis, a citrus disorder known in Brazil for decades (18, 101). The disease affects many citrus species and hybrids, causing mainly chlorotic irregular lesions in fruits and leaves (**Figure 1**), and seems to have been restricted to coastal regions in Brazil since the mid-twentieth century (29, 101).

Passion fruit green spot and passion fruit sudden death are diseases produced by PfGSV that, in the absence of phytosanitary control, can devastate passion fruit groves (49, 110). PfGSV, so far only detected in South America (48, 93, 102, 105), produces chlorotic spots in young leaves, green spots in senescent leaves and mature fruits, and necrotic lesions, which sometimes exhibit deep slits, in the stems of passion fruit plants (**Figure 1**). The stem lesions can coalesce, encircling the branch, leading to the death of the distal ends and, progressively, of the plants. Citrus leprosis prevalently caused by CiLV-C, and also by CiLV-C2 in Colombia, is the most economically important disease involving kitaviruses. Widely distributed across Latin America (25, 89), the disease erodes the sustainability of citrus orchards by potentiating losses caused by premature fruit drop, and because of the complexity and high costs of controlling *Brevipalpus* mites, the vector of a wide variety of kitaviruses (35, 75, 99, 100). Citrus leprosis symptoms are nonsystemic chlorotic spots that usually evolve into necrotic lesions on fruits, leaves, and branches of citrus trees (**Figure 1**). Eventually, infection leads to the death of young citrus trees because of the high rate of defoliation and dieback. Citrus growing areas in the states of São Paulo and Minas Gerais, Brazil, make up the largest sweet-orange belt in the world, which is responsible for ~30% of the orange fruits and half of the orange juice global production (118). In that region, the annual impact of premature fruit drop exclusively caused by citrus leprosis has increased yearly since 2016, reaching 2.83% of the harvest in 2021–2022, which represents more than 370 thousand tons of fruits and losses of up to US$60 million (24, 75). Additionally, citrus leprosis management mainly involves the use of miticides to control *Brevipalpus* mites, which costs approximately US$54 million spent annually and may represent ~5% of the maintenance costs of an adult sweet-orange grove (10).

3. PHYLOGENY, EVOLUTION, AND GENOMIC ORGANIZATION OF KITAVIRUSES

Phylogenomic studies based on the RNA-dependent RNA polymerase (RdRp) domains have shown that kitaviruses are phylogenetically closer to arthropod-infecting viruses than to any other plant-infecting virus, including those in the class *Alsuviricetes*, which, besides *Kitaviridae*, contains several other families of plant viruses within the orders *Martellivirales*, *Hepelivirales*, and *Tymovirales* (53, 92) (**Figure 1**). It has been suggested that kitavirids emerged from ancestors likely shared with arthropod-infecting viruses of the proposed taxa negevirus, centivirus, aphiglyvirus, and other nege/kita-like groups of viruses (52, 92, 119), making kitaviruses a robust example of interkingdom horizontal virus transfer (37, 94).

Untranslated regions (UTRs): viral genomes may play roles as promoters for complementary strand synthesis, translational enhancers, signals for polyadenylation, and/or packaging

Transmembrane helix (TMH): found in membrane-spanning protein domain. It may mediate transport and sorting of membrane-anchored proteins

Orphan open reading frame (ORFan): open reading frame coding for proteins with unrecognized homologs in any other species

The genome of a typical negevirus is a nonsegmented, 3′-end polyadenylated, (+)ssRNA molecule of 9–10 kb (119). It contains three open reading frames (ORF1–3) separated by two intergenic regions and two untranslated regions (UTRs) at the 5′- and 3′-ends of the molecule. *ORF1*, the largest gene, is placed at the 5′-end of the genome and encodes the RdRp with the domains methyltransferase (Pfam01660), FtsJ-like methyltransferase (Pfam01728), helicase (Pfam01443), and RdRP_2 (Pfam00978). *ORF2* and *ORF3* encode two virion structural proteins: a glycoprotein showing the domain DiSB-ORF2_chro (Pfam16506), and a membrane protein termed SP24 (structural protein of 24 kDa; Pfam16504) (82). Besides the phylogenetic relationships between the RdRp among negeviruses and kitaviruses, assessments of amino acid (aa) identities and predicted structural traits strongly suggested that proteins encoded by the *ORF2–3* of the negeviruses have homologs within the kitavirus proteome (54).

Accordingly, on the basis of a well-argued extant of an arthropod virus ancestor, the main evolutionary events shaping the genomes of present-day kitaviruses have involved genome segmentation and acquisition from either other viruses or hosts of specialized proteins, such as the MP, to deal with intrinsic features of plant biology (59, 77, 87, 95). The ubiquity of RNA from different origins across kitavirus genomes has led to considering them natural genetic chimeric systems (76).

Kitaviruses of the three genera have divided genomes in which homologs of genes found in negeviruses are always located on two different RNA segments (**Figure 1**). Although there is a structurally based homology between *ORF2* of negeviruses and cileviruses, the gene is not readily detected in other kitaviruses, but proteins with motifs reminiscent of glycoproteins, such as transmembrane helices (TMHs) and potential glycosylation sites, are detected in blunerviruses and higreviruses (76). In contrast, homologs of the *ORF3* (*p24*) exist in all kitaviruses and the proteins they encode are the most conserved within the family. In kitaviruses in which homologs of *ORF2–3* are present, the two ORFs are displayed on the same RNA segment but not necessarily in contiguous loci, e.g., RNA2 of cileviruses. The genome of kitaviruses often harbors orphan ORFs (ORFans) and taxonomically restricted ORFs (93, 95). These genes likely provide alternative adaptative mechanisms to cope with challenging environments, e.g., the change of natural host range from arthropods to plants. Particularly, blunerviruses encode two to three distinct helicase domains of the SF1 class in different RNA segments (87, 124). They seem to have evolved from different phylogenetic lineages, and their role during viral replication is not fully understood (87).

By and large, genomic RNAs of kitaviruses are flanked by a poly-A tail at their 3′-end, but exceptions may exist, e.g., an isolate of the blunervirus BNRBV (87). UTRs at the 3′-end of each component of kitaviruses display high nucleotide sequence identity across isolates of the same species, and the length of the conserved stretch may vary even within each species (76, 92). The existence of an RNA cap structure at the 5′-end of RNAs of kitaviruses has been suggested based on the presence of methyltransferase domains in viral RdRp, but specific studies for its detection are lacking.

3.1. Genomic Organization of Cileviruses

The cilevirus genome is divided into two molecules and three subgroups of viruses can be distinguished. The first subgroup encompasses CiLV-C (67), CiLV-C2 (103), and PfGSV (93). RNA1 of these viruses has 8.7–8.9 kb and, in the sense 5′-3′, comprises ORFs *RdRp* and *p29*. *RdRp* is the largest ORF with 7.5 kb and encodes in a single polypeptide the domains methyltransferase, helicase, and RdRp, and reminiscent domains of UvrD_C_2 (Pfam13538) and FtsJ can also be detected. The gene *p29* codes for the putative coat protein and is considered a taxonomically restricted ORF. RNA2 in this subgroup is 4.7–5.0 kb in length and typically has four ORFs: *p15*, *p61*, *p32*, and *p24*. Located at the 5′-end, the ORF *p15* encodes scarcely conserved polypeptides.

Despite this, *p15* should not be considered an ORFan, as the 3D structures of proteins P15 share elements of common ancestry with the protein ORF49 of radinoviruses and the phosphoprotein C-terminal domain of mononegavirids (95). ORFs *p15* and *p61* are separated by a stretch of variable length, 0.8–1.0 kb, in which numerous small ORFs (<350 nts), scattered in a virus-specific array, are detected (95). Some of these ORFs putatively encode peptides with predicted TMHs (95). The ORFs *p61* and *p24* encode the putative glycoprotein P61 and the protein P24, respectively. In silico analyses of the P61 proteins show the presence of a signal peptide (SP), several putative N-glycosylation sites, and two to three adjacent TMHs located near their C-termini, but the domain DiSB-ORF2_chro present in negeviruses is absent. ORF *p32* is downstream of *p61* and encodes an MP with the domain 3A (Pfam00803). ORF *p24* is at the 3′-end of the molecule and encodes P24, a homolog of SP24 of negeviruses.

Signal peptide (SP): a short peptide at the N-terminus of proteins targeting them to the secretory pathway

Solanum violifolium ringspot virus (SvRSV; *Cilevirus solani*), Ligustrum leprosis virus (LigLV; *Cilevirus australis*), and Ligustrum chlorotic spot virus (LigCSV; *Cilevirus ligustri*) represent the second genomic array (subgroup 2) within the genus *Cilevirus* (92). The tentative cileviruses pistachio virus Y (PisVY) (74) and vinca ringspot virus (ViRSV; GenBank accession numbers OQ116675 and OQ116676) (E.W. Kitajima, unpublished data), detected in Iran and Chile, respectively, are included in this subgroup as well. The genomic organization of these viruses is essentially similar to that shown for cileviruses of subgroup 1, except for the lack of 1.0–1.5 kb at the 5′-end of the RNA2. Thus, the RNA2 of cileviruses in subgroup 2 is shorter, 3.5–3.6 kb long, comprising the orthologs of the genes *p61*, *p32*, and *p24* but missing *p15* and sequences of the intergenic region *p15–p61* present in subgroup 1 viruses. Subgroup 2 viruses show small ORFs that potentially encode peptides with predicted TMHs and are more abundant in LigLV (92).

The third subgroup of cileviruses is represented by a sole member, only detected in Hawaii, USA. RNA1 of hibiscus yellow blotch virus (HYBV; *Cilevirus oahuense*) is 8.4 kb long, the shortest among cileviruses, with an organization resembling that observed in the RNA1 of the higrevirus hibiscus green spot virus 2 (83). HYBV RNA1 contains two ORFs, one coding for the RdRp, and the partially overlapping ORFan *p10* of 255 nts, which potentially encodes a protein harboring a TMH. RdRp has the domains methyltransferase, helicase, and RdRP_2 as observed in other cileviruses. The main difference in HYBV RNA1 is the absence of an ortholog of the ORF *p29*, which in contrast, is found at the 3′-end of its RNA2 molecule. In composition, the RNA2 of HYBV is partially equivalent to RNA2 shown by cileviruses of subgroup 2 but additionally presents *p29* downstream of *p24*. With a unique genomic organization, HYBV has been considered an evolutionary link between members of the genera *Higrevirus* and *Cilevirus* (83). RdRp-based phylogenetic reconstructions place HYBV in a relatively basal position, closer to higreviruses, within the evolutionary branch comprising cileviruses (92).

Altogether, marked dissimilar genomic arrangements in combination with relatively low identity values in interspecies comparisons may suggest the existence of three evolutionary lineages within the genus *Cilevirus*, perhaps three subgenera, or support the creation of two novel genera.

3.2. Genomic Organization of Higreviruses

The genome of hibiscus green spot virus 2 (HGSV2; *Higrevirus waimanalo*) is divided into three molecules (72). RNA1 is 8.3 kb long and harbors two partially overlapped ORFs coding for RdRp and a protein of 11 kDa (P11). RdRp has 2,645 aa, including residues in methyltransferase, helicase, and RdRP_2 domains. ORFan *p11* is of unknown function and has two predicted TMHs. RNA2 is tetracistronic and 3.2 kb long, and at its 5′-end, there is an ORFan encoding a predicted 50-kDa protein of unknown function that shows two TMHs near its C-terminus. ORF *p39* encodes a protein with a helicase motif, whereas *p9* and *p6* code for polypeptides with two and one

TMH, respectively. ORFs *p39* and *p9* are also termed *BMB1* and *BMB2*, and they are necessary and sufficient to mediate cell-to-cell viral movement (59). RNA3 in the isolate Hawaii (Hw) of HGSV2 is 3.1 kb long, shows a large 5′-end UTR of 460 nts, and has three ORFs putatively encoding proteins of 33, 29, and 23 kDa, respectively. The protein of 23 kDa is an ortholog of the negevirus SP24, and the other two proteins show very low identity values with known proteins (72). Predicted proteins P33 and P29 show one and three TMHs at their N- and C-termini, respectively. Other isolates of HGSV2, characterized in Brazil, show the general genome structure of the isolate Hw but exhibit two rather than three ORFs in their RNA3 (101). The largest ORF in the RNA3 of the South American isolate of HGSV is 1.7 kb long and seems to be a fusion between the ORFs *p33* and *p29* detected in HGSV2_Hw. The predicted P65 protein from the Brazilian isolates shows one and three TMHs near its N- and C-termini, respectively, resembling the putative pattern of TMHs that might be observed in proteins P33 and P29 of HGSV2_Hw if they were translated from a single ORF (101).

The partially characterized, tentative higreviruses pistachio virus X (PisVX) and phellodendron-associated higre-like virus (PaHLV) were detected in pistachio (*Pistacia vera*) and *Phellodendron amurense* plants collected in Iran and China, respectively (64, 74). PisVX has three RNA molecules, but they show different compositions and arrangements from those observed in HGSV2. Its RNA1, with 8.1 kb, is monocistronic and codes for the RdRp that comprises similar functional domains observed in HGSV2. The genome segment equivalent to RNA2 of HGSV2 in PisVX is 2.4 kb long, shorter than its RNA3, and has two ORFs, which are orthologs to *BMB1* and *BMB2* in HGSV2, respectively (59). RNA2 in PisVX has a large 5′-UTR and seems to be a truncated version of the HGSV2 RNA2 molecule. RNA3 is 3.7 kb long and three ORFs are detected: *p66*, *p31*, and *p23*. ORF *p31* encodes an ortholog of the SP24 of negeviruses, and *p66* and *p23* are ORFans. P66 has five predicted TMHs, one near the N-terminus and four at its C-terminus. PaHLV genome shows a similar organization to that of PisVX, sharing ∼72% nucleotide sequence identity (64). From a synteny perspective, the RNA3 of PisVX and PaHLV shows an intermediary array between those exhibited by the RNA3 of the higrevirus HGSV2, RNA2 of cileviruses, and, to a lesser degree, RNA3 of the blunervirus BNRBV.

3.3. Genomic Organization of Blunerviruses

The genomes of the blunerviruses BNRBV (22, 87), TPNRBV (42), and ToFBV (30) are divided into four molecules. RNA1 and RNA2 have 5.7–6.0 kb and 3.5–4.1 kb, respectively, and are monocistronic. Peculiarly, these two ORFs encode proteins containing helicase domains. Besides the helicase, the protein from the RNA1 comprises the methyltransferase domain, and the protein encoded by the RNA2 includes the RdRP_2 domain. RNA3 is 2.5–2.8 kb long and has four to five ORFs of flexible sizes, but, invariably, the *ORF3* encodes the SP24 homolog. Proteins encoded by the *ORF2* in BNRBV and ToFBV have adjacent TMHs near their C-termini, whereas the equivalent ORF in TPNRBV shows only reminiscent TMHs at the same position. Most of the remaining ORFs in the RNA3 of these viruses are ORFans or encode proteins with very low similarity with proteins in public databases. The predicted protein encoded by the *ORF5* in ToFBV shows ∼30% identity to a hypothetical protein from *Acinetobacter baumannii* (30), whereas the P28 encoded by *ORF4* in BNRBV shows reminiscent sequences of the minor capsid protein VI domain (Pfam02993). RNA4 is 1.7–2.1 kb long and generally harbors a single ORF that encodes the putative MP containing the 3A motif. The RNA4 molecule possesses large 5′- and 3′-end UTRs reaching up to ∼1 kb in the 3′-UTR of TPNRBV (42).

Tentative blunerviruses have been identified in the tree of heaven (*Ailanthus altissima*), apple (*Malus domestica*), and princess tree (*Paulownia tomentosa*) collected in China (76, 124). RNA1–2

of *Paulownia tomentosa* blunervirus are equivalent to those in BNRBV (87). RNA3 has five ORFs in an arrangement resembling that shown by the RNA3 of ToFBV (30), but only P21 encoded by *ORF3*, with the SP24 motif, has moderate similarity to other kitavirus proteins. P31 (*ORF2*) displays TMHs near its C-terminus, P30 (*ORF4*) contains a reminiscent potato leaf roll virus readthrough protein domain (Pfam01690), and P15 (*ORF5*) presents two TMHs. Remarkably, the virus lacks a recognized MP. RNA4 has two ORFans; P68 predicted protein displays a sequence motif moderately similar to the nuclear localization signal of 2b proteins of cucumoviruses, known to have silencing suppressor functions (76).

The genome of Ailanthus blunervirus 1 has five segments (124). Notably, RNA1 is 7.5 kb long, the largest among blunerviruses, and encodes a sole protein provided with three domains equivalent to those detected in cileviruses and higreviruses. Proteins from RNA1–2 have moderate similarity with proteins encoded in RNA1–2 of TPNRBV. P33 encoded by the RNA4 has an SP and TMHs near its C-terminus, and P21 is an ortholog of SP24. RNA5 has three ORFans, and *ORF3*-encoded protein shows six TMHs. The genome of apple blunervirus is also divided into five segments, but the structure of its RNA1–2 resembles that observed in BNRBV (124). SP24 ortholog is encoded in the RNA3. Proteins with similarity to known MPs are not detected and the predicted protein encoded in the RNA5 has a reminiscent helicase domain.

4. INTRASPECIES VARIABILITY OF KITAVIRUSES

Except for CiLV-C, the number of fully or partially sequenced isolates of most kitaviruses is low, making thorough variability studies unfeasible. Among other consequences, this insufficient understanding of genome variability within each species has led to the empirical setting of the sequence identity threshold used as one of the criteria for new species demarcation within each genus as well as a lessened capacity to correlate biological traits with given haplotypes or viral lineages.

From an overall perspective on available data, isolates collected from distant geographic regions of some kitaviruses show high nucleotide sequence conservation, and, in other cases, isolates from relatively close localities display substantial divergencies and contrasting genetic traits. RNA1–4 of BNRBV isolate RL (red lesion), for example, have 86–90% nucleotide sequence identity with those of the isolate Georgia, both collected in the southern United States (22, 87). Each RNA segment of the isolate RL presents poly-A tails, which are not observed in the isolate Georgia (87). The isolate RL causes red concentric ringspots that expand across leaves and frequently have green centers, but they neither turn necrotic nor are associated with the premature foliar drop, which is typical of isolate Georgia (87). Whether or not these distinct symptoms are only a direct consequence of viral molecular traits remains to be determined. In contrast, RNAs of ToFBV isolates collected in Europe, Australia, and Brazil are very conserved, having 96–99.7% nucleotide sequence identity (30, 68, 78, 97). However, despite the high similarity, there are many amino acid substitutions in the proteins of the Australian isolate, suggesting an older introduction of ToFBV in that country (30). Very low variability, less than 5%, has also been observed between the isolates of TPNRBV collected in China, Japan, and Iran (42, 71). High sequence conservation is also characteristic of components RNA1–3 among the isolates of HGSV2 found in Brazil and Hawaii, USA (72, 101). Values commonly above 90% nucleotide sequence identity are noteworthy considering that one of the Brazilian isolates corresponds to a virus whose sequence was recovered from an herbarium sample collected in 1937 (101). The main difference between these isolates lies in the ORF composition of their RNA3 segments.

Assessment of a dozen genomic sequences of PfGSV collected in South America indicates that their RNA1 is less variable (5%) than their RNA2 (7%) (93, 105) and that the highest divergence

is in RNA2 at the 5′-end, upstream from the ORF *p61*. In that region, isolate-specific arrays of ORFans have been found and the relationships of these ORFs with the wide natural host range of PfGSV have been speculated (93). Isolates of CiLV-C2 have been found infecting citrus and hibiscus (*Hibiscus rosa-sinensis*) in Colombia (103) and passion fruit (84) and hibiscus in the United States (73, 107). Isolates collected in hibiscus and passion fruit show less than 5% nucleotide sequence variability, but they show a more accentuated divergence, i.e., 80–86% nucleotide sequence identity, with the RNA1–2 of the isolate infecting citrus in Colombia (73). It is unclear whether strains of CiLV-C2 infecting hibiscus and passion fruit can infect citrus and vice versa.

The variability study of CiLV-C has involved the analysis of more than 400 citrus samples collected from Mexico to Argentina, mostly in Brazil, in the period 1932–2020 (25, 90). The population of CiLV-C consists of two main lineages, called CRD (Cordeirópolis, where the strain was first identified) and SJP (São José do Rio Preto, where the strain was first identified), unevenly distributed from North to South America. A third lineage, whose present-day existence is unknown, was detected in an herbarium citrus sample collected in Asunción, Paraguay, in 1937 (25). It is thought that the ancestors of these lineages likely originated in contact with the native vegetation of South America before the introduction of citrus to the continent (25). Viruses from these lineages share about 85% nucleotide sequence identity across their genomes and show signs of interclade recombination events involving the 5′-end of their RNA2 segments. The subpopulations CRD and SJP are genetically well-differentiated ($Fst \geq 0.92$), have very low genetic diversity ($\pi \sim 0.01$), and are under purifying selection ($\omega < 0.5$) (25). It is speculated that low diversity within each lineage may result from continuous bottlenecks as a consequence of the limited number of plant cells reached during the infection, and the transmission by *Brevipalpus yothersi* mites (25, 90). Viruses of the lineage SJP reach up to nine times more viral molecules than CRD in plant tissues, which suggests a potential adaptive advantage of the SJP strain and may partially explain why this lineage is becoming prevalent in the Brazilian citrus belt (9).

5. VIRION MORPHOLOGY AND CYTOPATHOLOGY OF KITAVIRUS-INFECTED CELLS

All kitaviruses have split genomes, but whether RNA segments are packaged individually or assembled into physically separate virions is still unknown. Regardless of that, virion particles of kitaviruses accumulate in the cytoplasm of cells in the symptomatic tissues, but the extension of cytopathic effects and the morphology of the virions may vary from one another.

Particles of CiLV-C have been purified (31), but most of the studies of virion morphology of kitaviruses have been obtained from observations of infected cells. Virions of CiLV-C, CiLV-C2, PfGSV, and SvRSV are bacilliform and 40–70 nm wide and 100–120 nm long (44, 92, 103), whereas those of HGSV2 isolate Hw are short bacilliform (30 × 50 nm) (72) and those of ToFBV are slender short bacilliform particles of approximately 25 nm in width and approximately 100 nm in length (46). Particles of LigLV and LigCSV are quasi-spherical and 40–53 nm wide and 55–65 nm long (92). In the cases of HGSV2 isolate Brazil (101), HYBV (83), and TPNRBV (42), spherical virus-like particles (VLPs) that are 55 nm, 66 nm, and 88 nm in diameter, respectively, have been observed, but these results require further confirmation.

In negeviruses, both SP24 and the glycoprotein are components of the mature assembled spherical or teardrop-shaped enveloped virion particles (82, 119). The composition of kitavirus particles is unknown. However, in cileviruses, immunogold labeling assays use antirecombinant CiLV-C P29 antibodies labeled viroplasms and also short, bacilliform particles of CiLV-C. This suggests that besides composing the viroplasms, P29 takes part in their virions, likely as their coat protein (21). Orthologs of P29 are restricted to members of the genus *Cilevirus*; they are of variable size,

from 28.6 kDa in PfGSV to 33.3 kDa in HYBV, and show low (20–35%) to moderate (45–65%) levels of amino acid sequence conservation (25, 83, 92, 93). Transiently expressed P29 proteins of CiLV-C in *Nicotiana benthamiana* plants form stable VLPs (85). These VLPs are icosahedral and have a diameter of 15.8 ± 1.3 nm, a morphology that diverges from that of CiLV-C virions, suggesting the contribution of other viral proteins and/or the packaged viral genome in the shape of the native virus particles.

Subgenomic RNA (sgRNA): molecules generated during the viral process of transcription

Whether the diversity of virion morphologies within the genus *Cilevirus*, as well as across kitaviruses, may be related to the role of P29 or any other kitavirus protein as structural proteins remains to be studied. However, whatever the morphology of the particles, mature virions of several cileviruses and the blunervirus ToFBV are wrapped with a lipid membrane, which seems to be reminiscent of nege/kita-like arthropod viruses. Remarkably, *Kitaviridae* is the sole plant virus family in the class *Alsuviricetes* comprising enveloped virions (37).

Extensive observations of infected plant cells have shown that replication/assembly of kitaviruses occurs in the cytoplasm and, in most of the species, their particles accumulate in the lumen of the endoplasmic reticulum (ER) and the perinuclear spaces (42, 44, 46, 92). Frequently, amorphous, vacuolar electron-dense viroplasms of wide-ranging sizes are present (32). However, nonvacuolated viroplasms have been observed in privet leaves infected by the cilevirus SvRSV (92), whereas viroplasms were unnoticed in tomato pericarp fruit cells infected by ToFBV (46) and hibiscus leaves infected by HYBV (83). In cilevirus-infected cells, what seems to be the budding process has been observed in the ER next to viroplasms (44). In citrus leaves, some cells of the parenchyma in the mesophyll region of the lesion caused by CiLV-C undergo intense hypertrophy and hyperplasia, whereas others, at the edge of the lesions, scattered in halos, endure plasmolysis (70).

6. KITAVIRUS–PLANT INTERACTION: CILV-C AS A MODEL

Overall, the most appealing feature of the kitavirus pathobiology is the lack of long-distance movement, i.e., their infections occur in smaller or larger necrotic or chlorotic spots, or a combination of the two types of symptoms, but always in locally restricted lesions. It should be pointed out, however, that in field-collected samples of tea plants infected by the blunervirus TPNRBV, the infection of systemic tissues has been reported (42). Nevertheless, in a controlled experiment with TPNRBV-infected plants showing typical symptoms on basal leaves, no symptoms were observed on upper new leaves after one year. In systemic tissues, only parts of the viral RNAs were detected (96), suggesting that the long-distance movement of the virus is likely unfit at best.

Insights into kitavirus–plant interaction have arisen from the integration of studies with both natural and experimental host ranges of the cilevirus CiLV-C and, to a lesser extent, with the cilevirus CiLV-C2 and the higrevirus HGSV2. Details of the blunervirus–plant interplay are almost entirely unknown. Outside the genus *Citrus*, CiLV-C naturally infects *Commelina benghalensis* (79) and *Swinglea glutinosa* (63) and can be experimentally transmitted to dozens of plant species, including the model plants *Nicotiana benthamiana* and *Arabidopsis thaliana* (5, 41). In citrus, sweet oranges are highly susceptible, mandarins (*Citrus deliciosa*, *Citrus reshni*, and *Citrus reticulata*) have a lower degree of susceptibility, and lemons (*Citrus limon*) and limes (*Citrus aurantifolia*) are rarely or not affected (11, 13).

In lesions of susceptible sweet oranges, fruits support higher CiLV-C loads than stems and leaves, and the levels of RNA molecules containing *p29* are always higher than those containing *RdRp* (7). As also detected in other cileviruses of subgroup 1, CiLV-C transcription produces one subgenomic RNA (sgRNA) from the RNA1, i.e., sgRNA *p29*, and three sgRNAs from segment 2, which are supposedly the primary source for translation of P29, P61, MP, and P24, respectively (86, 93, 103). Because of the transcription of sgRNA molecules, the detection of 3′-end ORFs

encompasses targets in sgRNA, in addition to those in genomic RNA and antigenomic RNA (agRNA) molecules. In controlled infection of *Arabidopsis* plants using CiLV-C-viruliferous *B. yothersi* mites, *p29* accumulates at the highest absolute values among viral genes (3).

Antigenomic RNA (agRNA): molecules generated during the viral process of replication

Subcellular localization studies revealed that P29, P24, P15, and P61 of CiLV-C colocalize with the ER (62). P29, P15, and P24 interact with actin filaments; P24, P15, and P61 interact with the Golgi complex; and P15 colocalizes with the nucleus (62). Bimolecular fluorescence complementation assays indicated that P29, P24, and P15 self-interact, P15 forms heterodimers with MP, and P29 interacts with P15, P24, and MP (62). Hetero-association of P29 with several proteins and its plasticity to form homodimers in both head-to-tail and head-to-head interfaces suggest that this protein may play a central role in viral functional complexes and is, perhaps, a structural component of the virion particles (62). In line with this, besides detecting CiLV-C bacilliform particles using anti-P29 polyclonal antibodies, intense labeling was observed in the viroplasms found in the cytoplasm of infected cells (21). Transient-expressed enhanced green fluorescent protein (eGFP)-fused P24 in *N. benthamiana* remodels the ER and promotes the formation of vesicle-like spherical structures, reinforcing the hypothesis that P24 might have a structural role, likely involved in the formation of the viral matrix protein (62). P15 can also stimulate vesicle biogenesis through ER remodeling, which potentially suggests its role in virus replication (62). Its expression delays plant growth and enhances necrosis in young leaves of *N. benthamiana*, and a role as a gene silencing suppressor has also been suggested (60). P61 disturbs the ER network (62) and, despite the typical features predicted in silico, its role as a glycoprotein lacks confirmation.

6.1. Cell-to-Cell Movement of Kitaviruses

The very presence of viroplasms and virions distributed in plant cells along the lesions is itself an element grounding the extant of kitavirus-encoded factors that provide local viral movement. On top of this, in cileviruses and blunerviruses, a key piece of evidence of local viral movement activity is the recognition of 3A motif in one of their proteins. However, the lack of systemic movement in kitavirus infection has led to questioning the efficiency and extent of the functionality of these proteins. Studies to address this issue have shown that CiLV-C MP traffics along the ER and colocalizes with the plasmodesmata (PD) (62). MPs from CiLV-C and CiLV-C2 functionally replace the 30K-superfamily MP in the movement-defective alfalfa mosaic virus (AMV) and restore the AMV cell-to-cell and systemic movement in *Nicotiana tabacum* (61). Because a direct interaction between AMV CP and MP is not required in this system, it is believed that the cilevirus MPs assist the transport of viral ribonucleoprotein (vRNP) complexes rather than virion particles (61). Moreover, cilevirus MPs induce the formation of tubules and redirect P29 to the PD-associated cell periphery (61). Altogether, evidence indicates two possible routes for the cell-to-cell transport of cileviruses: (*a*) the P29-independent ER route, in which the MP anchors the vRNP complex to the ER membranes and facilitates the passage through PD by tubule formation, and (*b*) the microfilament route, where the MP-P29 interaction is required and the association of P29 with actin anchors the vRNPs along the microfilaments, guiding them to the cell periphery (61).

Synteny of ORFs and structural and functional features of proteins encoded by the RNA2 segment of HGSV2 indicated the presence of a transport gene module evolutionarily related to the triple gene block system (72, 113). Functional characterization of RNA2:*ORFs 2–4* using a transport-deficient potato virus X (PVX) in *N. benthamiana* revealed that *ORF2* and *ORF3* are two MPs necessary and sufficient to mediate cell-to-cell movement of PVX, representing a novel specialized transport module called binary movement block (BMB) (59). Because the complementation assays with the *ORFs 2–3*, termed *BMB1* and *BMB2*, were performed in the absence of any other higrevirus-encoded protein, it is thought that the BMB machinery transport vRNPs, at least in the PVX model system (59). BMB1 protein contains the NTPase/helicase domain, localizes to

the cytoplasm and the nucleoplasm, and likely participates in the formation of vRNPs. BMB2 is an integral membrane protein that induces constrictions of the ER tubules, similar to reticulons (56, 57, 59). The intracellular transport of BMB2 to PD can occur via lateral translocation along the ER membranes (58). This protein promotes the generation of membrane bodies in the vicinity of PD, increasing its size-exclusion limit (59). BMB1 interacts in vitro and in vivo with BMB2, which is essential for BMB2-directed targeting of BMB1 to PD-associated membrane bodies and probably provides intra- and intercellular transports of vRNPs (8, 59).

6.2. Plant Response to CiLV-C Infection

New light is shed on plant–kitavirus interplay in transcriptomic and biochemical studies of CiLV-C-infected plants. A first insight into the data suggested that the concomitant activation of plant defense mechanisms and the interference with interlinked essential cellular functions may contribute to counteract the viral multiplication and limit the virus movement.

Upon virus infection, *Arabidopsis* plants trigger the RNA silencing and the salicylic acid (SA)-mediated pathways and upregulate genes involved in the production of reactive oxygen species (ROS) and the hypersensitive response (HR) but downregulate jasmonic acid (JA)-dependent genes (3, 4). Accordingly, abundant ROS production and the appearance of dead cells are detected in the lesion tissues using histochemical assays (4). Similar responses are found in CiLV-C-infected sweet-orange plants (4, 70). These data suggest that local lesions caused by CiLV-C result from an incompatible interaction, a hypersensitive-like response, that somehow restricts the viral multiplication (3, 4). However, CiLV-C infections in mutant *Arabidopsis* plants containing RNA silencing core gene disruptions, e.g., *dcl2/4*, *rdr1/6*, and *nahG*-expressing *Arabidopsis* plants reach higher viral titers and produce larger and earlier chlorotic lesions than in wild-type plants, but the virus is detected in neither systemic leaves nor the roots (P.L. Ramos-González, unpublished data). Hence, although several antiviral mechanisms may counteract CiLV-C infection, at least individually, they are not responsible for restricting the CiLV-C systemic movement. The effectiveness of enhanced antiviral activity to restrain viral systemic movement produced by double genotypes combining *nahG* and knockdown of any RNA silencing core enzymes remains to be determined.

CiLV-C proteins have been transiently expressed in *N. benthamiana* plants using agroinfiltration (3). Ectopic expression of P61 consistently produced a ROS burst, increased the levels of SA, reduced the levels of JA, upregulated the expression of SA- and HR-related genes, and induced cell death in infiltrated leaf areas (3). Because such responses were not observed with the other expressed proteins, it is suggested that P61 may be at the epicenter of the hypersensitive-like response triggering processes during CiLV-C infection and might indicate the putative existence of a corresponding plant resistance (R) protein (3). However, the hypothesis of direct recognition of P61 by any R protein seems to be weak because cell death and local infections are invariably observed in every natural or experimental host of CiLV-C (41). Otherwise, the reaction against P61 would be triggered by interference with plant-conserved mechanisms.

In plants, the innate immune system and ER stress are interconnected processes, and they have in common that they can trigger cell death upon plant pathogen infections (1, 111). To restore ER homeostasis, plants induce unfolded protein response (UPR) (69). The inability to revert the ER stress may lead to a chronic condition that can trigger plant cell death (111, 123). P61 of CiLV-C accumulates in the ER and disrupts its membrane system (62). During both P61 ectopic expression and CiLV-C infection, UPR marker genes, e.g., those coding for bZIP60, ER luminal binding proteins, and calreticulins, are induced (3). Transient expression of the P61-truncated version, lacking its SP, drastically reduces the cell death phenotype and the expression of HR and ER marker genes, suggesting that ER localization is necessary for triggering the hypersensitive-like

***nahG*:** gene of bacterial origin encoding salicylate hydroxylase, an enzyme that metabolizes salicylic acid to catechol

Endoplasmic reticulum (ER) stress: cellular disturbance characterized by the accumulation of aberrant proteins in the endoplasmic reticulum, overwhelming its folding capacity

Unfolded protein response (UPR): response to overcome imbalanced endoplasmic reticulum folding capacity that includes degradation of misfolded proteins and increase of chaperone synthesis

response (G.D. Arena, unpublished data). Conversely, the expression of P61 in *nahG*-expressing *N. benthamiana* plants produces a stronger cell death phenotype in the infiltrated areas and upregulates HR and UPR marker genes to levels higher than those observed in wild-type plants. In agreement with this, in plants transiently expressing P61 the spliced form of the transcriptional factor *bZIP60* mRNA could be detected (G.D. Arena, unpublished data). UPR induction by the nucleus-targeted form of bZIP60 is a pathway also activated during plant infection by other viruses (65). Altogether, these findings support the involvement of ER stress on the development of cell death upon P61 expression and suggest the contribution of SA-mediated pathways to alleviating the ER stress triggered by this protein.

7. KITAVIRUS TRANSMISSION AND VIRUS–VECTOR INTERACTION

The absence of systemic infection of kitaviruses certainly impacts their spread not only between two infection loci on the same plant but also among plants. There is evidence of neither seed nor vegetative propagation of cileviruses and blunerviruses (96, 98). Graft and mechanical transmissions of CiLV-C have succeeded under controlled conditions, albeit with difficulty, and the virus also produces local infections (28, 32). All existing information on the natural transmission of kitaviruses relies on the action of mite vectors.

Vector-mediated transmission of the blunervirus ToFBV is strongly associated with plant colonization by the eriophyid mite *Aculops lycopersici* (78). In blueberry plants infected by BNRBV, both *Calacarus* sp. (20, 22) and *Brevipalpus* sp. mites have been observed, but the virus could only be transmitted to blueberry plantlets by the eriophyids (A.D. Tassi & D. Carrillo, unpublished data). In contrast, the transmission of HGSV2 and cileviruses to many natural and experimental viral host plants by three species of *Brevipalpus* mites has been unequivocally proved (34). *B. yothersi* mainly transmits cileviruses of subgroup 1 (15, 49, 90, 104, 109) and *Brevipalpus papayensis* can also transmit CiLV-C, although at a lower efficiency (80). *B. papayensis* and *Brevipalpus obovatus* transmit LigCSV and SvRSV, respectively, and *Brevipalpus tucuman* mites have been collected from privets infected by LigLV, but viral transmission needs to be confirmed (38, 92). Likewise, mites of the species *B. yothersi* and *B. obovatus* were found in hibiscus plants infected by HYBV, but the virus could only be molecularly detected in *B. yothersi* (83). The transmission of Brazilian isolates of HGSV2 to *Arabidopsis* plants succeeded using viruliferous *B. yothersi* and *B. papayensis* (101).

A thorough study of the CiLV-C–*B. yothersi* interaction revealed that both female and male mites transmit the virus regardless of the presence of the endosymbiont *Cardinium* sp. (116). Virus vertical transmission was undetected, and all the active stages of *B. yothersi* can acquire and inoculate CiLV-C. However, the effectiveness of CiLV-C transmission by *B. yothersi* not only seems to be virus-strain specific but also varies among mite populations. The transmission rate of CiLV-C strain SJP using single *B. yothersi* mites of five populations ranged from 31.4% to 77.9%, whereas for the strain CRD, the transmission efficiency varied from 18.6% to 56%. These data suggest a putative role of the vector population in the uneven distribution of these strains in Brazil (A.D. Tassi, unpublished data).

During feeding of *Brevipalpus* mites on CiLV-C-infected plants, acquired virions circulate across the mite body and are consistently found between cells at the basal part of the ceca and anterior podocephalic gland and close to the coxal gland, ovaries, and muscles but never in the synganglion (43). These particles may appear aligned up to 10 μm long in the intercellular spaces, and the rows are frequently interrupted by septate junctions. Longer acquisition time seems to lead to higher viral loads in mites, but neither recognizable viral particles nor viroplasms could ever be observed inside cells (43, 117).

Viral multiplication in *Brevipalpus* cells is a challenging aspect of cilevirus–mite interaction. Although the absence of virus particles inside mite cells suggests no replication, the detection of agRNAs of CiLV-C and CiLV-C2 in viruliferous mites seemed contradictory (104). However, technical issues such as self-priming during reverse transcription of RNA molecules and the likely detection of viral molecules of plant origin suggested a cautious interpretation of such molecular test results. In novel analyses using both vector and nonvector mites reared on CiLV-C infected plants, genomic RNAs and agRNAs were detected when mites were collected directly from the lesions (A.D. Tassi, unpublished data). However, when strand-specific RT-PCR and RT-qPCR with both biotinylated and tagged primers (19, 115) and viruliferous *Brevipalpus* mites reared without contact with external viral sources were used, agRNAs were detected in low quantities and remained stable throughout the 21 days of the experiment (A.D. Tassi, unpublished data). Additionally, the assessments of the ratio *p29*sgRNA:RNA1 of CiLV-C in mites were markedly lower than those detected in plants (7). Based on these results, the hypothesis raised is that, although CiLV-C uses the paracellular route to cross the epithelial cell barrier moving from the midgut lumen up to the stylet channel (117), the virus can replicate in yet unidentified cells, in which the replication occurs at a low rate.

Transcriptomic analyses of *Arabidopsis* plants infested by nonviruliferous or CiLV-C-viruliferous *Brevipalpus* mites indicated that the tritrophic interaction rewires the plant defensive response potentiating the biotrophic and alleviating the herbivory-specific defenses (4, 6). Upon infestation with viruliferous mites in *Arabidopsis*, and in sweet-orange plants as well, expression levels of SA-specific transcripts are higher than those observed during the interaction with nonviruliferous mites. Meanwhile, JA-dependent responses are drastically reduced during the CiLV-C infection, suggesting that the virus might favor mite colonization (6). Accordingly, oviposition of viruliferous *Brevipalpus* mites is increased in CiLV-C-infected *Arabidopsis* leaves and reduced in SA-deficient *Arabidopsis* mutants when compared to wild-type plants (4, 6). As in CiLV-C-infected *Arabidopsis*, *B. yothersi* oviposition significantly increases in sweet-orange fruits infected with CiLV-C (A.D. Tassi, unpublished data). Altogether, these data suggest the improvement of mite fitness in the presence of CiLV-C and support the hypothesis that CiLV-C may act as a helper (effector-like) factor of mites favoring the infestation by suppressing herbivory plant defenses (3, 4).

SUMMARY POINTS

1. Kitavirus genomes are likely natural recombinant products; the molecular and biological characteristics of kitaviruses reveal a prominent ancestral relationship with arthropod viruses of the taxon negevirus.
2. Cell-to-cell movement of kitaviruses is supported by 30K movement proteins in viruses of the genus *Cilevirus* and by a specialized transport system known as the binary movement block in higreviruses.
3. Kitaviruses have a limited or null capacity to systemically move in their hosts. Locally restricted infection of CiLV-C may result from the viral incapacity to circumvent plant effective defenses. Ectopic expression of CiLV-C P61 mimics plant responses observed during viral infection, putting P61 at the epicenter of the hypersensitive-like response triggering processes against CiLV-C.
4. Kitavirus infections are associated with mite infestations; plant-to-plant transmission of higreviruses and cileviruses depends on a small number of *Brevipalpus* species.

FUTURE ISSUES

1. Reevaluate the current taxonomic organization and limits of taxa within and beyond the family *Kitaviridae* considering newly discovered viruses.
2. Determine the role of small ORFans that often occur at the 5′-end of the kitaviruses.
3. Characterize the structure–function relationship of the putative glycoproteins of kitaviruses, e.g., P61 of CiLV-C, and investigate the physical interactome of these proteins with plant and vector proteins.
4. Determine the type of interaction of kitaviruses with their vectors and identify the molecular determinants of the virus–vector interaction.
5. Assess the contribution of plant defenses in kitavirus locally restricted infections. Determine the role of the endoplasmic reticulum stress in cell death upon kitaviruses infection.
6. Identify unknown vectors of blunerviruses, characterize the eriophyid–virus interaction, and recognize factors mediating the cell-to-cell movement of some blunerviruses in the absence of classical movement proteins, e.g., *Paulownia tomentosa* blunervirus.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

The authors thank Dr. Xinyuan Hao from the Tea Research Institute, Chinese Academy of Agricultural Sciences, Hangzhou, China, who kindly donated pictures of the tea plant infected by TPNRBV. The authors are also grateful to CAPES, CNPq, and FAPESP for scholarships, research fellowships, and research grants associated with this work (CAPES PNPD20132154-33141010001P4-PNPDIBSP, 88882.157041/2017-01; CNPq 312528/2020-5; FAPESP 2017/50222-0, 2017/50334-3, 2018/12252-8, 2019/02137-0, 2019/25078-9, and 2020/15413-2).

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