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Gemini Viruses-emerging threat to crops

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Abstract

Geminiviruses represent a new threat to global food security and sustainability by causing heavy losses on food, feed and cash crops. During the last two decades, epidemics of re-emerging and newly emerging geminiviruses have caused huge crop losses and threatened crop production, particularly in the tropics and subtropics region. Geminiviruses (family Geminiviridae) are a large group of plant viruses that possess small circular single-stranded (ss)DNA genomes that are encapsidated in twinned virions. *Geminiviridae* is second largest family of plant viruses. Begomoviruses have emerged as major problems in crops like cassava, cotton, grain legumes and vegetables. These geminiviruses cause economically important diseases of food, feed and fiber crops worldwide and the extent of yield loss caused by geminiviruses can reach up to 100 per cent. Epidemics caused by re-emerging and newly emerging geminiviruses are becoming frequent even in regions that were earlier free from these viruses. Major contributory factors for the emergence and spread of new geminivirus diseases are the evolution of variants of the viruses, appearance of the whitefly 'B' biotype and the increase in the vector population. Management of geminiviruses is a worldwide challenge because of the widespread distribution of economically important diseases. Application of insecticide to manage vectors is the most widely used measure but can cause undesirable effect on environmental and human health. Management is most effective with an integrated pest management (IPM) approach.

Keywords: Gemini Viruses-emerging, global food security

Introduction

Geminiviruses are a large family of ss DNA viruses that can infect a wide range of plants in most parts of the world (Zerbini *et al.*, 2017; Mansoor *et al.*, 2006) [17, 29]. The family Geminiviridae is divided into nine genera: Becurtovirus, Begomovirus, Curtovirus, Eragrovirus, Mastrevirus, Topocovirus, Turncurtovirus, Capulavirus and Grablovirus (Zerbini *et al.*, 2017; Varsani *et al.*, 2017) [17, 67]. More than three hundred species with single-stranded circular DNA (ss DNA) genomes that are encapsidated in twinned icosahedral particles with approximately 22 nm×38 nm in dimension having either one (monopartite) or two (bipartite) ssDNA molecules of 2 600–2 800 nucleotides (nt) in *Geminiviridae* (Yang *et al.* 2016) [72]. Present century witnesses severe disease epidemics resulted in annual losses on cassava plants have been estimated at more than US\$1 billion (Legg and Alvarez, 2017; Legg *et al.*, 2015), US \$5 billion for cotton in Pakistan between 1992-97 (Briddon and Markham, 2000) [3], US \$300 million for grain legumes in India (Varma *et al.*, 1992) [62] and US \$140 million in Florida for tomato alone (Moffat, 1999) [32]. Cassava mosaic disease (CMD) occurs in severe form in Sri Lanka and Southern India, and disease outbreak was also reported in Cambodia in 2016 (Alagianagalingam and Ramakrishnan, 1966; Saunders *et al.*, 2002; Wang *et al.*, 2016) [2, 50, 71]. Despite concerted efforts to manage geminiviruses and their vectors, menacing disease epidemics caused by newly emerging or re-emerging geminiviruses are becoming frequent and appearing even in new regions, previously free from such virus diseases.

Taxonomy

Geminiviruses have been grouped into nine genera on bases of their vector, host range and genomic characteristics. They are transmitted by different insects in four families of homopterans (whiteflies, leafhoppers, aphids and treehoppers). Members of the genus *Begomovirus* are transmitted by whiteflies, member of genera *Mastrevirus*, *Curtovirus*, *Becurtovirus* and *Turncurtovirus* are transmitted by leafhoppers, one member under genus *Capulavirus* is transmitted by an aphid, and members of the single species under genus *Topocovirus* are transmitted by treehoppers.

Vectors for only known members of the genera *Eragrovirus* and *Grablovirus* remain unknown.

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Table 1: Different genera of Geminiviruses

S N	Genera-species	Virus	Vector
1.	Begomovirus->320	African cassava mosaic virus, Bean golden mosaic virus, Cotton leaf curl Kokhran virus and Tomato yellow leaf curl virus.	Whiteflies
2.	Capulavirus -4	Alfalfa leaf curl virus	Aphid
3.	Becurtovirus -2	Beet curly top Iran virus and Spinach curly top Arizona virus	Leaf hoppers
4.	Curtovirus-3	Beet curly top virus	Leaf hoppers
5.	Mastrevirus->30	Maize streak virus and Wheat dwarf virus	Leaf hopper
6.	Eragrovirus -1	Eragrostis curvula streak virus	
7.	Turncurtovirus-1	<i>Brassica rapa</i> or <i>Raphanus sativus</i> in Iran	Leaf hopper
8.	Grablovirus -1	Grapevine red blotch virus	
9.	Topocuvirus-1	Tomato Pseudocurly top virus	Tree hopper

(Zerbini *et al.*, 2017)

Genome

In Geminivirus genome consists of one (monopartite) or two (bipartite) DNA molecules that is individually encapsidated in a geminate (quasi)-icosahedral virion (Davies and Stanley, 1989; Lazarowitz and Shepherd, 1992) [26]. Bipartite viruses have two components called as DNA-A and DNA-B, (e.g. Mung bean yellow mosaic India virus; MYMIV) and monopartite genome, containing DNA-A only (e.g. Tomato yellow leaf curl virus (TYLCV) or some species of Tomato leaf curl virus; ToL CV). Both the DNA-A and DNA-B

components are essential for systemic infection, although DNA-A alone can also induce disease symptom. A novel sub-viral agents associated with monopartite viruses called as DNA satellite (e.g. DNA-β) have been identified, which are essential for production of disease symptom in monopartite viruses (Dry *et al.*, 1997; Nawaz-ul-Rehman and Fauquet, 2009) [9]. A conserved nonanucleotide sequence “TAATATTAC” within a stem-loop structure in all of the DNA components essential for the initiation of rolling circle replication (RCR) (Stanley, 1995) [59].

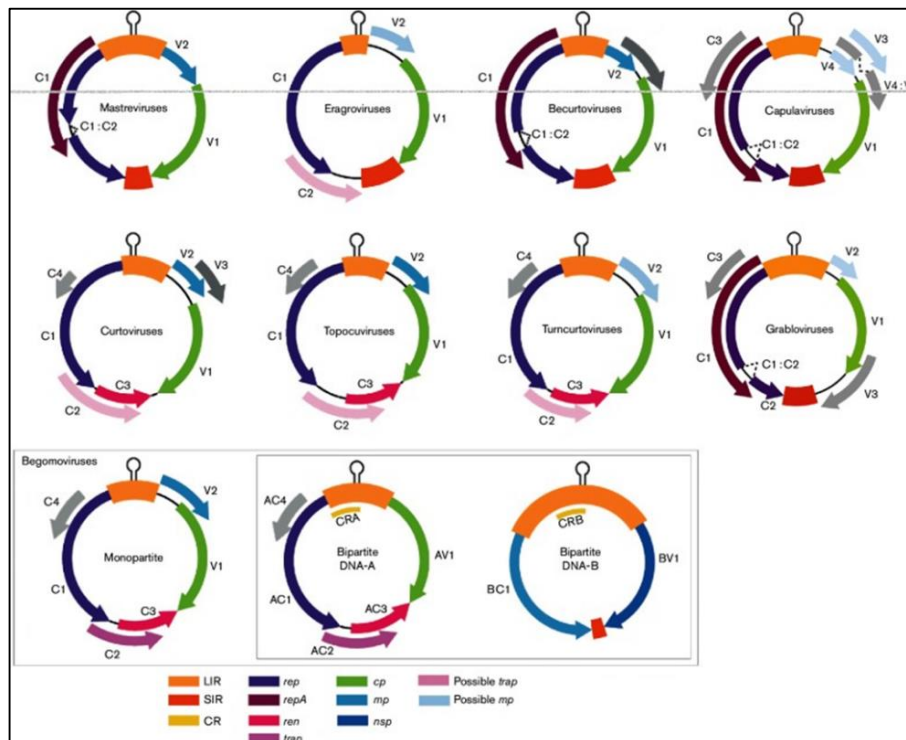


Fig 1: Genome organization of isolates in various geminivirus lineages. The ORFs (V1, V2, V3, C1, etc.) are colour-coded according to the function of their protein products (rep, replication-associated protein; ren, replication activator protein; cp, capsid protein; mp, movement protein; nsp, nuclear shuttle protein). LIR, long intergenic region; SIR, short intergenic region; CR, common region. The hairpin which includes the origin of replication is indicated in the LIR (modified from [4]). (Zerbini *et al.*, 2017) [73].

Geminiviruses

The Geminiviridae family includes a rapidly increasing number of viruses that infect plants and frequently produce very significant reductions in production of economically important crops of both monocotyledonous and dicotyledonous plants (Moffat, 1999) [32]. The diseases caused by geminiviruses affect the yield of infected crop plants (Fauquet *et al.*, 2008; Martin and Shepherd, 2009) [12, 30] and sometimes the crop failure reaches up to 100% (Czosnek *et al.*, 1990; Nakhla and Maxwell, 1998; Polston *et al.*, 1999) [7, 35, 40].

Bhindi

The bhindi yellow vein mosaic virus (BYVMV) was first reported from Bombay in India (Kulkarni, 1924) [21]. Bhindi is severely infected by *Begomovirus*. In India, monopartite *begomoviruses* such as *Bhendi yellow vein Madurai virus* (BYVMV), *Bhendi yellow vein Maharashtra virus* (BYVMaV), *Bhendi yellow vein Bhubaneswar virus* (BYVBhV) and *Okra enation leaf curl virus* (OEL Cu V) have been reported (Fauquet *et al.*, 2008; Brown *et al.*, 2012; Venkataravanappa *et al.*, 2012b, 2013a, b) [12, 4]. Okra isolate (OY131) of BYVMV was isolated from bhindi plant in Palem

region of New Delhi showing reduced leaves size, vein twisting and yellow vein mosaic, and a bushy appearance of plant. BYVMV disease is caused by a complex consisting of the monopartite begomovirus BYVMV and a satellite DNA- β component. Alone BYVMV produces only mild leaf curling in host. However, the typical symptom of this disease is produce when BYVMV is agro-inoculated with DNA- β to bhindi (Jose and Usha, 2003) ^[18].

Cotton

For the first time cotton leaf curl disease was observed in northwest India (Rishi and Chauhan, 1994). Thereafter, it spread throughout northern India in a short interval of 4 to 5 years (Monga *et al.*, 2004) ^[33]. Cotton leaf curl disease in India is caused by many monopartite begomoviruses associated with a betasatellite (Kirthi *et al.*, 2002) ^[20]. Four *begomoviruses* are associated with this disease in India, namely, *Cotton leaf curl Rajasthan virus* (CL Cu RV), *Cotton leaf curl Multan virus* (CLCuMuV), *Cotton leaf curl Kokhran virus* (CL Cu KV) and *Tomato leaf curl Bangalore virus* (Ahuja *et al.*, 2007) ^[1]. The CP gene sequence of another Indian isolate, *Cotton leaf curl virus- Hissar 2*, was reported from Haryana, India, that showed 97.3% amino acid sequence identity with Pakistan cotton leaf curl virus (Sharma *et al.*, 2005) ^[54]. At recently two new isolates has been identified, CLCuV-SG01 and CLCuVSG02 from Rajasthan, which are reportedly recombinants with other begomoviruses (Kumar *et al.*, 2010). Cotton leaf curl disease (CLCuD)-affected plants show curling of leaves, enations, thickening of veins and stunting. The typical symptom is the dark green colour of veins that is easily observed in transmitted light. Almost no yield is obtained when plants get severely affected, resulting in enormous losses to the growers. Cotton production is severely constrained by cotton leaf curl disease (CLCuD), which is considered as the most complex and economically important disease of cotton (Zubair *et al.*, 2017; Naveen *et al.*, 2017; Sattar *et al.*, 2017) ^[74, 37].

Chili

The complete genome of a Begomovirus and cognate DNA- β satellite component was sequenced and analyzed from chili plant infected with chili leaf curl disease (ChLCD), from Varanasi, India (Chattopadhyay *et al.*, 2008) ^[6]. In India, several begomoviruses infect chilli; four begomoviruses infecting chilli, *viz.*, *Chilli leaf curl virus* (Chi LCV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Tomato leaf curl Joydebpur virus* (To LCJV) and *Chilli leaf curl Palampur virus* (ChiL CPV) (Khan *et al.*, 2006; Shih *et al.*, 2006; Kumar *et al.*, 2011a, b) ^[19, 22, 55, 56], has been characterized. The presence of a begomoviruses confirmed by PCR while the BLAST search of GenBank revealed close similarity of the sequence with the *Chilli leaf curl virus* (Pakistan: Multan) (ChiL CuV-[Pk:Mul]; AF336806) (Shih *et al.*, 2003) ^[56]. In India, *Tomato leaf curl New Delhi virus* (ToL CNDV) was reported to be associated with chilli leaf curl disease occurring in Lucknow with a diverse group of beta satellites found in crops and weeds (Khan *et al.*, 2006; Kumar *et al.*, 2015) ^[19, 22]. A very high incidence of ChLCV in chili was observed in Meerut district of Western Uttar Pradesh in 2011 with typical symptoms of crowding and yellowing of leaves, upward leaf curling, and stunting of whole plants. Chilli plants severely affected by this disease did not produce fruits and remain stunted. A very high disease incidence (up to 100% of plants during December 2004) in farmer's fields in Narwa and Tinwari villages at Jodhpur District Rajasthan was

also reported (Senanayake *et al.*, 2007). A disease incidence up to 100% during December 2008 in Vellanad region of Kerala was also reported. Severe upward curling, leaf thickening, stunted plant growth and vein clearing were observed at Jodhpur (Rajasthan) (Senanayake *et al.*, 2012).

Legumes

Geminiviruses have been isolated from many legume species in India. The yellow mosaic disease (YMD) of legumes was first time reported by Nariani (1960) ^[36]. Since then, it has been identified as a most important disease of legumes in India and other south Asian countries. Yellow mosaic and golden mosaic diseases of grain legumes and leguminous vegetables are challenging problems in the tropics and subtropics regions. The prominent bright yellow and golden mosaic symptoms of disease, which appear as blooms, could not have been missed if they had occurred earlier, even if the infections were of negligible magnitude, thereby suggesting the recent origin of legume geminiviruses. It is a major constraint in the production of legumes across the Indian subcontinent (Varma and Malathi, 2003) ^[16]. This disease affects several legume crops *viz.*, mungbean, blackgram, pigeonpea, soybean, mothbean and common bean (Varma and Malathi, 2003) ^[16]. YMD of legumes in India have been reported to be caused by four species of *begomoviruses* (Qazi *et al.*, 2007) ^[42]. *Mungbean yellow mosaic India virus* (MYMIV) and *Mungbean yellow mosaic virus* (MYMV) are prevalent and the *Dolichos yellow mosaic virus* and *Horsegram yellow mosaic virus*, occurred rare (Fauquet and Stanley, 2003; Maruthi *et al.*, 2006) ^[11]. A bipartite *begomovirus* isolate causing YMD in blackgram produced differential symptom in different leguminous hosts and had DNA-A, a variant of MYMV, and DNA-B, a variant of MYMIV (Haq *et al.*, 2011) ^[16].

Cassava

Cassava mosaic disease (CMD) had been reported in India in 1966 (Alagianagalingam and Ramakrishnan, 1966) ^[2]. CMD has subsequently become prevalent in southern India (Calvert and Thresh 2002) ^[5], resulting in yield losses of between 10–15%. Two bipartite begomoviruses have been recognized to be the causative agents for CMD in India; *Indian cassava mosaic virus* (ICMV) and *Sri Lankan cassava mosaic virus* (SLCMV). Phylogenetic analysis of several CMD-affected cassava samples revealed recombination among the population of cassava infecting-begomoviruses in India (Rothenstein *et al.*, 2006) ^[47]. Later, in a biodiversity study, while ICMV was found restricted to only certain regions, SLCMV was found to be rather widespread in southern India. A virus free cassava, generated by meristem tip culture, was used to study the whitefly transmissibility of the viruses in cassava using cassava adapted whiteflies; symptoms appeared in 85 per cent of the plants after 25th day of inoculation (Duraisamy *et al.*, 2012) ^[10].

Chickpea chlorotic dwarf virus

The mastrevirus *Chickpea chlorotic dwarf virus* (CpCDV) is a part of the disease complex that causes chickpea stunt disease. CpCDV was first reported in India (Horn *et al.*, 1993) ^[17] and is transmitted by the leafhopper *Orosius orientalis*. In the early 1990s, a disease characterized by chlorosis veinal necrosis and severe stunting was shown to be caused by Chickpea chlorotic dwarf virus (CpCDV). CpCDV, a tentative mastrevirus species (van Regenmortel *et al.*, 2000; Fauquet and Stanley, 2003) ^[12], could become a major

problem not only in chickpea but also other leguminous crops such as pea (*Pisum sativum*) and lentil (*Lens esculenta*) in which the virus has been reported to cause severe symptoms under experimental conditions.

Papaya

The papaya leaf curl disease has major impact on papaya cultivation in North India. The disease caused by papaya leaf curl virus (PLCV). By using PCR-based diagnosis, confirmed the presence of geminivirus in papaya in Uttar Pradesh state (Singh *et al.*, 2007) [25]. In another incidences in Uttar Pradesh, PCR and Southern blot hybridization with geminiviral specific DNA probes also prove the causal agent of papaya leaf curl disease as PLCV (Sangeeta *et al.*, 1998). However, the mixed infection of PCLV along with other virus in papaya is also reported. A mixed infection of papaya leaf curl virus (PLCV), a bipartite geminivirus, and papaya ring spot virus (PRSV) used to hamper the production of papaya in several parts of world. Therefore, a duplex PCR for reliable and rapid detection of these viruses has been standardized (Usharani *et al.*, 2013). In a recent effort to confer resistance against geminiviruses infecting papaya, siRNAs were designed using computational tools, which could possibly be used against a wide range of viral isolates and/or strains (Saxena *et al.*, 2011).

Tobacco

Leaf curl disease of tobacco (TbLCD) is endemic in India. A monopartite begomovirus, a betasatellite and an alphasatellite were found associated with this disease in Pusa, Bihar and isolate was named as Tobacco leaf curl Pusa virus [India: Pusa: 2010]. A betasatellite, associated with TbLCD, was reported to be a variant of Tomato leaf curl Bangladesh betasatellite [IN:Raj:03], sharing 90.4% sequence identity. An alphasatellite, detected in the diseased plants, had 87% nucleotide sequence identity with Tomato leaf curl alphasatellite. Using sequence analysis, the begomovirus was revealed to be a product of recombination of multiple begomovirus complexes (Singh *et al.*, 2011) [23].

Tomato

Tomato leaf curl disease (ToLCD) was first time reported in northern India by Vasudeva and Sam Raj. Tomato plant appears to be an ideal haven for begomoviruses. Tomato leaf curl disease (ToLCD) is a common disease of tomato crop all over India. Tomato leaf curl is becoming a major concern due to involvement of six different species of *begomovirus*, viz., *Tomato leaf curl Bangalore virus* (ToLCBV), *Tomato leaf curl Bangladesh virus* (ToLCBDV), *Tomato leaf curl Karnataka virus* (ToLCKV), *Tomato leaf curl Gujarat virus* (ToLCGV), *Tomato leaf curl NewDelhi virus* (ToLCNDV), and *Tomato leaf curl Sri Lanka virus* (ToLCVSLV) (Fauquet *et al.*, 2003) [12]. Most of these viruses produce symptoms characteristic of leaf curl diseases, including severe reduction in leaf size, crinkling of interveinal areas, downward curling, interveinal and marginal chlorosis, occasional development of enations, purple discolouration of the abaxial surface of leaves, shortening of internodes, development of small branches and reduced fruiting. Bright yellow spots on leaves is also produced by some of these viruses therefore, It is, natural that most of the tomato diseases caused by begomoviruses have been collectively described as either leaf curl or yellow leaf curl based on their biological properties and subtle differences in symptoms. The

sequence analysis of CPgene of Indian tomato leaf curl viruses (ToLCVs) reported its highly diverse nature. The Indian isolates of ToLCV contain two components, A and B, which are required for systemic movement and symptom development in host plant. In one of the studies, the genome organization of ToLCV-India was reported similar to other whitefly-transmitted geminiviruses (WTGs) having bipartite genomes (Padidam *et al.*, 1995) [39].

Potato

First-time natural occurrence of a geminivirus from potatoes in India was reported by immune electron microscopy. This viral agent was named as potato apical leaf curl begomovirus (Garg *et al.*, 2001) [14]. The nucleotide sequence data revealed that the cause is a virus closely related to *Tomato leaf curl New Delhi virus* (ToLCNDV) (Gawande *et al.*, 2007) [15]. The emergence of PALCV as a severe problem may reflect the earlier sowing times for potato that result in the presence of active whiteflies during the cropping season. The infected potato plants usually show vein thickening, crinkling, stunting and leaf distortion.

Brinjal

Ar Recent, from central India (Nagpur) a bipartite begomovirus has been reported from brinjal indicating yellow mosaic and mottling symptoms. The DNA-A and DNA-B component of the virus shared high identities with the respective DNA components of Tomato leaf curl New Delhi virus (ToL CNDV). Hence, this revealed that the ToLCNDV has started invading a new host, eggplant. Its pathogenicity was demonstrated in brinjal and tomato by agroinfiltration (Pratap *et al.*, 2011) [41].

Cucurbits

Begomoviruses are emerging as a serious problem to cultivation of cucurbitaceous crops in India. In the Indian subcontinent, yellow vein mosaic of *Cucurbita pepo* (pumpkin) has been known for over 50 years (Varma, 1963) [63]. The disease was mainly confined to central-western India until the 1980s but in 1990 a severe epidemic of leaf curl in muskmelon (*Cucumis melo*) and yellow vein mosaic in pumpkin, caused by begomoviruses, occurred in northern India (Varma, 1990) [66]. This epidemic was occurred due to the increase in whitefly populations early in the cucurbit growing season. In 2001 over 50% of pumpkin, watermelon (*Citrullus vulgaris*), muskmelon, and bottle gourd (*Lagenaria siceraria*) plants were severely affected by begomoviruses in commercial cucurbit growing areas (A Varma, I Bhat and S SSohrab, unpublished results). Author reported more than 98 per cent samples were infected with Begomovirus (Nagendran *et al.*, 2017) [34]. Chlorotic curly stunt disease of bottle gourd from Delhi and adjoining state of Haryana was reported to caused by a begomovirus on the basis of whitefly transmission and sequence identity of coat protein (CP) and replication initiator protein (Rep) genes (Sohrab *et al.*, 2010) [58]. The yellow mosaic disease (YMD) reduces the several nutrients and antioxidant in bitter melon (*Momordica charantia*) (Raj *et al.*, 2005). The PCR and southern hybridization confirmed the causative agent of disease as begomovirus. The virus was given a specific name as bitter melon yellow mosaic virus (BGYMV) (Raj *et al.*, 2005) [43]. Later on it was demonstrated that bitter melon yellow mosaic virus (BGYMV) was transmitted by whitefly (Rajinimala *et al.*, 2005) [44].

Conclusion

This review highlights the importance of diseases caused by geminiviruses. Geminiviruses are endemic in Indian crops probably due to tropical climate and intensive crop cultivation. The broad overlapping host range may be due to mixed cropping systems and Polyphagous nature of the whitefly vector prevalent in the country. Recently the emergence of a large number of beta satellites and alpha satellites associated with begomoviruses in India is also observed. Extensive efforts to understand the biology and the epidemiology of geminivirus diseases have allowed for considerable progress in managing geminivirus diseases. The most desirable measure entails breeding and deployment of geminivirus-resistant genotypes. However, such cultivars are not available for some crops (cucurbits, grapevines and peppers) and, in the case of some resistant cultivars, resistance-breaking geminivirus strains have emerged (cotton and tomato). Development of resistant varieties along with agronomic, fertilizer, insecticidal control and biotechnological methods can be used alone and in combination to manage severe disease which is still a challenge even after extensive research. Application of insecticides to manage insect vectors of geminivirus continues to be an important management measure for diseases and promising new chemistries are available (e.g., cyazypyr). But, insecticide-resistant insect vectors, especially *Bemisia tabaci*, are an ongoing problem. Thus, although substantial progress has been made, effective management of geminivirus diseases remains challenging, especially in tropical and subtropical parts where subsistence farmers grow overlapping crops and apply excessive amounts of insecticide. Clearly, no single measure will provide long-term sustainable management, and an integrated pest management (IPM) approach is required.

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