



RESEARCH COMMUNICATION

Characterisation of begomoviruses causing yellow mosaic disease in zucchini (*Cucurbita pepo* L.) in South India

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Abstract

Zucchini (*Cucurbita pepo* L.) is a valuable cucurbitaceous crop cultivated widely in subtropical and temperate regions. However, its productivity is increasingly threatened by begomoviruses, especially in South India. The present study investigates the incidence, molecular characterisation, vector transmission and host range of squash leaf curl China virus (SLCCNV) and tomato leaf curl Karnataka virus (ToLCKV) infecting zucchini in Bengaluru and Mysuru districts of Karnataka. Field surveys conducted during 2020–21 revealed a high incidence of yellow mosaic and leaf curl symptoms in affected fields. Molecular analysis confirmed the presence of bipartite SLCCNV and monopartite ToLCKV in symptomatic plants. Whitefly (*Bemisia tabaci*) transmission assays determined that a minimum of 10 min acquisition period and 30 min inoculation periods were necessary for efficient virus transmission. Host range studies demonstrated that squash leaf curl China virus–Zucchini [Bengaluru-1] (SLCCNV-ZUB1) could infect pumpkin, ridge gourd and bottle gourd, while other cucurbits and solanaceous crops remained uninfected. Complete genome sequencing revealed > 91 % nucleotide identity with known SLCCNV isolates and 97.9 % identity of tomato leaf curl Karnataka virus–Zucchini [Bengaluru-5] (ToLCKV-ZUB5) with ToLCKV infecting tomato. Recombination analyses identified significant breakpoint events suggesting viral evolution from related begomoviruses. This is the first report of ToLCKV infecting zucchini in South India, highlighting the emergence of new begomoviral variants and their epidemiological significance in cucurbit production.

Keywords: begomovirus; *Bemisia tabaci*; monopartite; recombination; zucchini

Introduction

Zucchini also known as courgette, baby marrow or summer squash (*Cucurbita pepo* L.), is an important cucurbitaceous crop that is commonly cultivated in subtropical and temperate regions. It belongs to the family Cucurbitaceae (1) and originates from North America (2). It has high economic value primarily due to its use as immature fruits (3). It is a rich source of calcium, phosphorus, vitamin A and helps in digestion, prevents heart disease and lowers the risk of diabetes in people because of its high fibre and antioxidant contents (4). Cultivation of zucchini is primarily limited by plant viral diseases, which cause significant yield losses worldwide (5). Many regions of the world have well documented the occurrence of RNA viruses on zucchini (6). Among them, whitefly transmitted squash leaf curl China virus (SLCCNV) is causing a serious problem on zucchini in India (7). The cultivation of cucurbits is severely constrained by SLCCNV, tomato leaf curl New Delhi virus (ToLCNDV) and tomato leaf curl Palampur virus (ToLCPaV). The SLCCNV has limited hosts and infects cucurbitaceous crops in the past two decades (5). Recently, its prevalence has increased in cucurbits

across several countries, including India (8–10), Pakistan (11), China (12), the Philippines (13), Thailand (14) and East Timor (15). The other associated begomovirus in zucchini is tomato leaf curl Karnataka virus (ToLCKV). These belong to family Geminiviridae, having circular ssDNA with either monopartite or bipartite genomes and approximately 2.6 kb in size. Five to six proteins of DNA-A component are transcription activator protein (TrAP), the replication associated protein (Rep), replication-enhancer protein (REn) and coat protein (CP). The DNA-A encoded protein, AV2 and AC4 is involved in movement of virion and in the suppression of pathogenicity of post-transcriptional gene silencing (16). The DNA-B component encodes the genes for shuttle protein and movement protein are present in DNA-B (17). The non-coding region referred to as the common region (CR) is found DNA-A and DNA-B (18).

Over the last three decades, many begomoviruses spread by whiteflies have evolved into destructive diseases, particularly in the tropical and subtropical areas, where they affect crops and cause economic losses (19). The spread and severity of infection are influenced by interactions among the virus, the vector and the host

plant (20). The emergence and spread of begomovirus is increasingly posing a threat to cucurbit production (21). A strong correlation exists between the resurgence of *Bemisia tabaci* populations and the emergence of begomoviral diseases (22, 23).

The begomovirus that affects zucchini in India has a complex interaction with its vector, *B. tabaci*. The introduction of new cryptic species with a wide host range, high fecundity, high transmission rate and virus spreading to non-hosts is evident from serious disease outbreaks. SLCCNV infection on squash has been reported on the coat protein gene sequence (24). However, there are limited studies on the molecular identity, comprehensive characterisation and virus–vector interactions of begomoviruses infecting zucchini in South India. In India, bipartite begomovirus such as SLCCNV causing yellow mosaic and leaf curl disease in summer squash has been reported (20, 25). Despite increasing field incidence, the begomovirus strains infecting zucchini particularly ToLCKV- have not been comprehensively characterised in South India.

Therefore, the present study was undertaken to molecularly characterise begomoviruses associated with yellow mosaic and leaf curl disease of zucchini collected from Bengaluru and Mysuru districts of Karnataka, with special emphasis on the identification and characterisation of ToLCKV. Based on available published literature, this study provides the first detailed molecular evidence of ToLCKV infecting zucchini in South India.

Materials and Methods

Survey for yellow mosaic in zucchini

The survey was conducted in the Bengaluru and Mysuru districts of Karnataka, South India during June and July month of 2020–21. During the survey, the incidence of yellow mosaic and leaf curl on zucchini was recorded (Supplementary Table 1). To determine the disease incidence, plants (1000 Nos.) were visually observed following a W pattern across the rows and sampling was performed in 31 different fields. Plants showing characteristic begomovirus-like symptoms such as yellow mosaic, leaf curl, chlorosis, vein clearing, blistering and leaf deformation were recorded. Disease incidence was calculated as the percentage of symptomatic plants relative to the total number of plants observed. Symptomatic leaf samples were collected from each field for molecular confirmation of virus infection.

Whitefly transmission and virus culture maintenance

The isolate collected from Kurubarahalli village, Hoskote, Bengaluru district, South India, was designated as squash leaf curl China virus–Zucchini [Bengaluru-1] (SLCCNV-ZUB1) based on nucleotide sequence identity of the complete DNA-A genome with reference SLCCNV sequences available in the NCBI database. Another isolate collected from Budigere village, Devanahalli taluk, Bengaluru district, was designated as tomato leaf curl Karnataka virus–Zucchini [Bengaluru-5] (ToLCKV-ZUB5) following complete genome sequencing and comparative sequence analysis with published ToLCKV isolates.

Seeds of zucchini (Cora F1 hybrid) were sown in portrays containing soil and manure (1 : 1) and maintained under insect-free glasshouse conditions. Non-viruliferous whiteflies (*B. tabaci*) reared on cotton and brinjal plants in insect-proof cages were used for virus transmission experiments. After inoculation, plants were sprayed with Imidacloprid (0.05 %) to eliminate whiteflies and maintained in insect-proof glasshouse conditions for symptom expression. Plants

showing symptoms of yellow mosaic were selected for subsequent virus transmission experiments.

Virus–vector transmission relationships

Virus transmission experiments were conducted to determine the acquisition access period (AAP), inoculation access period (IAP) and minimum number of whiteflies required for transmission of SLCCNV–ZUB1. Whiteflies were allowed a 24 hr AAP on virus-infected zucchini twigs. Viruliferous whiteflies were released in groups of 1, 2, 5, 10, 15, 20, 25 and 30 adults per plant for a 24 hr IAP. To determine the optimum AAP, whiteflies were allowed to feed on infected twigs for 5, 10, 15, 30 min and 1, 2, 3, 6, 12, 16 and 24 hr, followed by a fixed IAP of 24 hr. To determine the optimum IAP, whiteflies were given a 24 hr AAP and then allowed to feed on healthy zucchini plants for the same time intervals. Each treatment consisted of 10 plants and the experiment was repeated twice. After transmission, whiteflies were killed by spraying Imidacloprid (0.05 %). Plants were maintained in insect-proof glasshouse conditions and observed weekly for symptom development for up to 3 months.

Host range

The healthy seedlings of 19 crop plants belonging to diverse families were raised in an insect proof cage under glasshouse conditions. Seeds were sown in nursery polybags containing 2 : 1 mixture of soil and compost. Healthy seedlings of seven-day-old were inoculated with 10 whiteflies for a 24 hr IAP following a 24 hr AAP. The seedlings which are inoculated were kept in an insect proof cage until symptoms appeared.

Virus genome amplification and sequencing

Total genomic DNA was extracted from 100 mg of symptomatic and asymptomatic zucchini leaf tissue using the CTAB method (26). DNA integrity was assessed on 1 % agarose gel, while DNA quality and concentration were estimated spectrophotometrically using a NanoDrop by measuring OD260/OD280 ratios. DNA samples were stored at -20 °C until further use. Initial polymerase chain reaction (PCR) amplification targeting the coat protein gene of begomoviruses was performed using DNA-A-specific primers (27). The PCR reactions were carried out in a 25 µL volume containing 2.5 µL 10× PCR buffer, 1.0 µL MgCl₂ (25 mM), 2.0 µL dNTPs (2.5 mM), 2.0 µL each of forward and reverse primers (10 µM), 0.1 µL Taq DNA polymerase, 2.0 µL template DNA and sterile distilled water. The PCR cycling conditions consisted of an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 45 sec, extension at 72 °C for 1.5 min and a final extension at 72 °C for 10 min. Which resulted in amplification of 1.2 kb partial genome from all the five virus infected zucchini samples (data not shown). The sequencing of this genome revealed that the sequences had highest homology with ToLCKV and SLCCNV and were designated as SLCCNV- ZUB1, ZUB2, ZUMY1, ZUMY2 and ToLCKV-ZUB5 isolates. The complete genome (DNA A and DNA-B) amplification for SLCCNV isolates and ToLCKV (DNA A) through rolling circle amplification (28) using a TempliPhi illustra amplification kit (GE Healthcare, Piscataway, NJ) was performed. The linear fragments of 2.8 kb were obtained by digesting the RCA product using BamH1 (DNA A) and XbaI (DNA B). The fragments were cloned into pUC19 plasmid with respective sites. After transformation of the ligated products, the clones that showed positive were confirmed by PCR following restriction digestion. The clones were sequenced from Chromous Biotech India Pvt. Ltd., Bangalore, Karnataka, India.

Sequence analysis and detection of recombination

The complete genome sequences of zucchini infecting begomovirus were subjected for sequence similarity searches at the National Center for Biotechnology Information (NCBI) database using BLASTn (29). The full-length genome of selected begomoviruses which showed maximum blast score were retrieved from the NCBI database. The complete nucleotide sequences of full-length genomes were aligned using CLUSTALW (30) and percentage pair wise nucleotide (nt) identity matrix plot was done using the Sequence Demarcation Tool (SDT). The phylogenetic tree was formed and evolutionary distances between the sequences were estimated by MEGA X software (31) using the maximum likelihood method with 1000 bootstrapped replications. Recombination analysis was done by utilising the Recombination Detection Program version 4 (RDP4) (32) with default RDP settings.

Statistical analysis

All transmission and host range experiments were conducted with a minimum of 10 biological replicates per treatment and repeated twice. Disease incidence and transmission efficiency data were expressed as percentages. Mean values and standard deviations were calculated and differences among treatments were analysed using analysis of variance (ANOVA).

Results

Survey, collection and identification of virus isolates

A roving survey was conducted in Bengaluru and Mysuru districts of Karnataka, India during 2020–21 to record the incidence of yellow mosaic and leaf curl disease on zucchini. During the survey, incidence of the disease was noted at every stage of crop growth. The virus infected zucchini plants showed the typical symptom of yellowing, mosaic and chlorosis (Fig. 1a). The symptoms of mild to severe leaf curl was also observed in the field (Fig. 1b). The incidence of yellow mosaic and leaf curl disease ranged from 21.2 to 77.5 % in Mysuru district and 28.7 to 73.7 % in Bengaluru Rural district. The existence of begomoviral infection in zucchini was confirmed by amplifying a 1300 bp fragment of the coat protein (CP) gene from leaf samples taken from several sites.

Whitefly transmission

A 100 % virus transmission was achieved when AAP and IAP of 24 hr were given. The virus isolates, SLCCNV-ZUB1 and ToLCKV-ZUB5 were transmitted by *B. tabaci* to zucchini and symptom appeared after 8 days post inoculation. The initial symptoms of small chlorotic

specks with mild mosaic were observed followed by yellow mosaic and mottling in SLCCNV-ZUB1 inoculated plants (Fig. 2a). In ToLCKV-ZUB5 inoculated plants, symptoms appeared as severe leaf curl with stunted growth (Fig. 2b).

The relationships of SLCCNV-ZUB1 with its vector *B. tabaci* was determined. Single whitefly was unable to transmit SLCCNV-ZUB1 isolate to zucchini after AAP and IAP of 24 hr. A minimum of 7 whiteflies was found to be efficient for virus transmission (70 %) with an incubation period of 8 days. The transmission efficiency increased to 100 % when 10 adults per test plant were used. Increase in number of whiteflies increased the efficiency of disease transmission under glass house conditions. *Bemisia tabaci* adults required minimum AAP of 10 min to become viruliferous with 10 % transmission efficiency. A 100 % transmission efficiency was achieved with increased AAP of 12 hr. An IAP of 30 min on healthy zucchini plants achieved 10 % transmission efficiency. Increase in IAP from 1 hr to 6 hr increased the transmission from 40 to 70 %. The transmission efficiency of 100 % was achieved with IAP 12 hr proving the importance of latent period in virus transmission. An increase in AAP and IAP increased the per cent disease transmission of adult whiteflies (Table 1).

Host range of squash leaf curl China virus

Host range studies with 19 plants from different crops were inoculated with whiteflies after virus acquisition (Table 2) and kept in polyhouse for symptom expression. On inoculation of SLCCNV-ZUB1 isolate to different plants, 100 % virus transmission was obtained on pumpkin, 8–15 days post inoculation. Yellow vein mosaic symptom was induced by the virus on pumpkin (Fig. 3a), bottle gourd (Fig. 3b) and ridge gourd (Fig. 3c), no transmission was induced on other cucurbitaceous and solanaceous crops. Each host plant species included 10 biological replicates and the experiment was conducted twice.

Virus genome amplification and sequencing

To determine the occurrence of begomovirus on zucchini, the leaf samples of 20 different locations were collected. The DNA of the collected samples were isolated and analysed by PCR with specific primers components. Of the total samples collected, only 5 samples had positive for DNA-A tests, indicating that begomovirus infection was present. and no amplification was obtained in non-symptomatic plants. Similarly, attempts to amplify DNA B component by PCR were successful in four samples. This suggests that in the present study, both mono and bipartite viruses infect zucchini in South India. The components of bipartite begomovirus

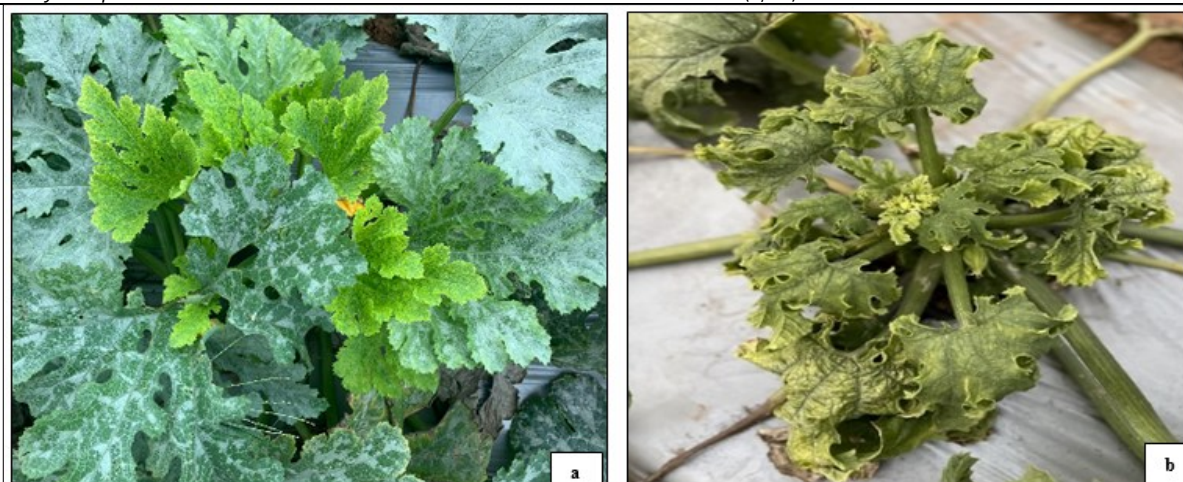
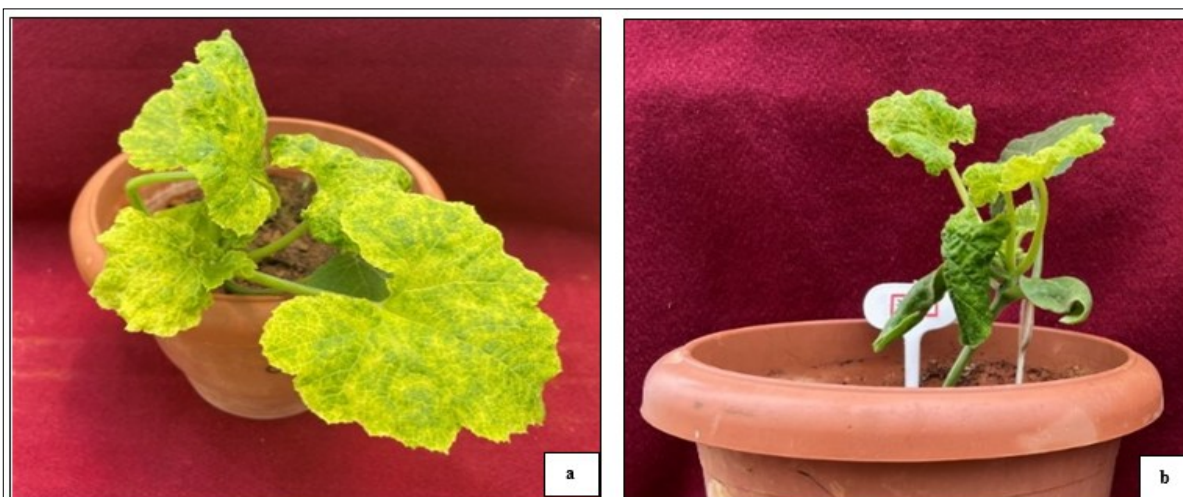
Table 1. Determination of acquisition access period and inoculation access period of SLCCNV-ZUB1 on zucchini seedlings by whiteflies

Minimum acquisition access period				Minimum inoculation access period			
AAP*	IAP**	Transmission		AAP*	IAP**	Transmission	
		Plants infected/ plants inoculated	Percentage of plants infected (%)			Plants infected/ plants inoculated	Percentage of plants infected (%)
5 min	24 hr	(0/10)	0	24 hr	5 min	(0/10)	0
10 min		(1/10)	10		10 min	(0/10)	0
15 min		(1/10)	10		15 min	(0/10)	0
30 min		(3/10)	30		30 min	(1/10)	10
1 hr		(5/10)	50		1 hr	(4/10)	40
2 hr		(6/10)	60		2 hr	(6/10)	40
3 hr		(6/10)	60		3 hr	(8/10)	60
6 hr		(7/10)	70		6 hr	(10/10)	70
12 hr		(10/10)	100		12 hr	(10/10)	100
16 hr		(10/10)	100		16 hr	(10/10)	100
24 hr		(10/10)	100		24 hr	(10/10)	100

*AAP: Acquisition access period; IAP: **Inoculation access period. Ten whiteflies were released on each plant.

Table 2. Transmission of squash leaf curl China virus–Zucchini [Bengaluru-1] (SLCCNV-ZUB1) by insect on different hosts

Host	Common name	Family	% infection	Symptoms expressed	DOSE (days)	PCR
Crop plants						
<i>Lagenaria siceraria</i> (Mol.) Standl.	Bottle gourd	Cucurbitaceae	100 (10/10)	Chlorosis and leaf curling	8-15	+
<i>Luffa acutangula</i> (L.) Roxb. <i>Lagenaria</i>	Ridge gourd	Cucurbitaceae	10/10 (100)	Puckering and downward leaf curling	8-12	+
<i>Momordica charantia</i> L.	Bitter gourd	Cucurbitaceae	0 (0/10)	-	-	-
<i>Trichosanthes cucumerina</i> L.	Snake gourd	Cucurbitaceae	0 (0/10)	-	-	-
<i>Secchium edule</i> L.	Chayote	Cucurbitaceae	0 (0/10)	-	-	-
<i>Luffa cylindrica</i> (L.) Roem.	Sponge gourd	Cucurbitaceae	0 (0/10)	-	-	-
<i>Benincasa hispida</i> (Thunb.) Cogn.	Ash gourd	Cucurbitaceae	0 (0/10)	-	-	-
<i>Coccinia cordifolia</i> L.	Ivy gourd	Cucurbitaceae	0 (0/10)	-	-	-
<i>Cucumis sativus</i> L.	Cucumber	Cucurbitaceae	0 (0/10)	-	-	-
<i>Cucumis sativus</i> L.	Gherkin	Cucurbitaceae	0 (0/10)	-	-	-
<i>Cucurbita moschata</i> Duch. ex Poir	Pumpkin	Cucurbitaceae	100 (10/10)	Mosaic and vein clearing	10-12	+
<i>Citrullus vulgaris</i> Schrad.	Watermelon	Cucurbitaceae	0 (0/10)	-	-	-
<i>Cucumis melo</i> L.	Musk melon	Cucurbitaceae	0 (0/10)	-	-	-
<i>Praecitrullus fistulosus</i> L.	Round melon	Cucurbitaceae	0 (0/10)	-	-	-
<i>Lycopersicon esculentum</i> Mill.	Tomato	Solanaceae	0 (0/10)	-	-	+
<i>Capsicum annuum</i> L.	Bell pepper	Solanaceae	0 (0/10)	-	-	-
<i>Capsicum annuum</i> L.	Chilli	Solanaceae	0 (0/10)	-	-	-
<i>Solanum melongena</i> L.	Egg plant	Solanaceae	0 (0/10)	-	-	-
<i>Nicotiana benthamiana</i> Domin.	Tobacco	Solanaceae	0 (0/10)	-	-	-
Weeds						
<i>Ageratum conyzoides</i> L.		Asteraceae	0 (0/10)	-	-	-
<i>Chenopodium amaranticolor</i> Coste & Ryne		Chenopodiaceae	0 (0/10)	-	-	-
<i>Croton bonplandianum</i> Baill.		Euphorbiaceae	0 (0/10)	-	-	-
<i>Euphorbia geniculata</i> Orteg.		Euphorbiaceae	0 (0/10)	-	-	-
<i>Malvastrum coromandelianum</i> (L.) Garckle		Malvaceae	0 (0/10)	-	-	-
<i>Parthenium hysterophorus</i> L.		Asteraceae	0 (0/10)	-	-	-

**Fig. 1.** Symptoms showing yellow mosaic and leaf curl disease on zucchini in field. a: zucchini plant exhibiting yellow mosaic symptoms infected with squash leaf curl China virus (SLCCNV) under natural condition; b: zucchini plant showing symptoms of leaf curl under natural condition.**Fig. 2.** Symptoms showing yellow mosaic and leaf curl disease on zucchini after the virus transmission. a: zucchini plant showing yellow mosaic; b: zucchini plant showing severe leaf curl with stunted growth.

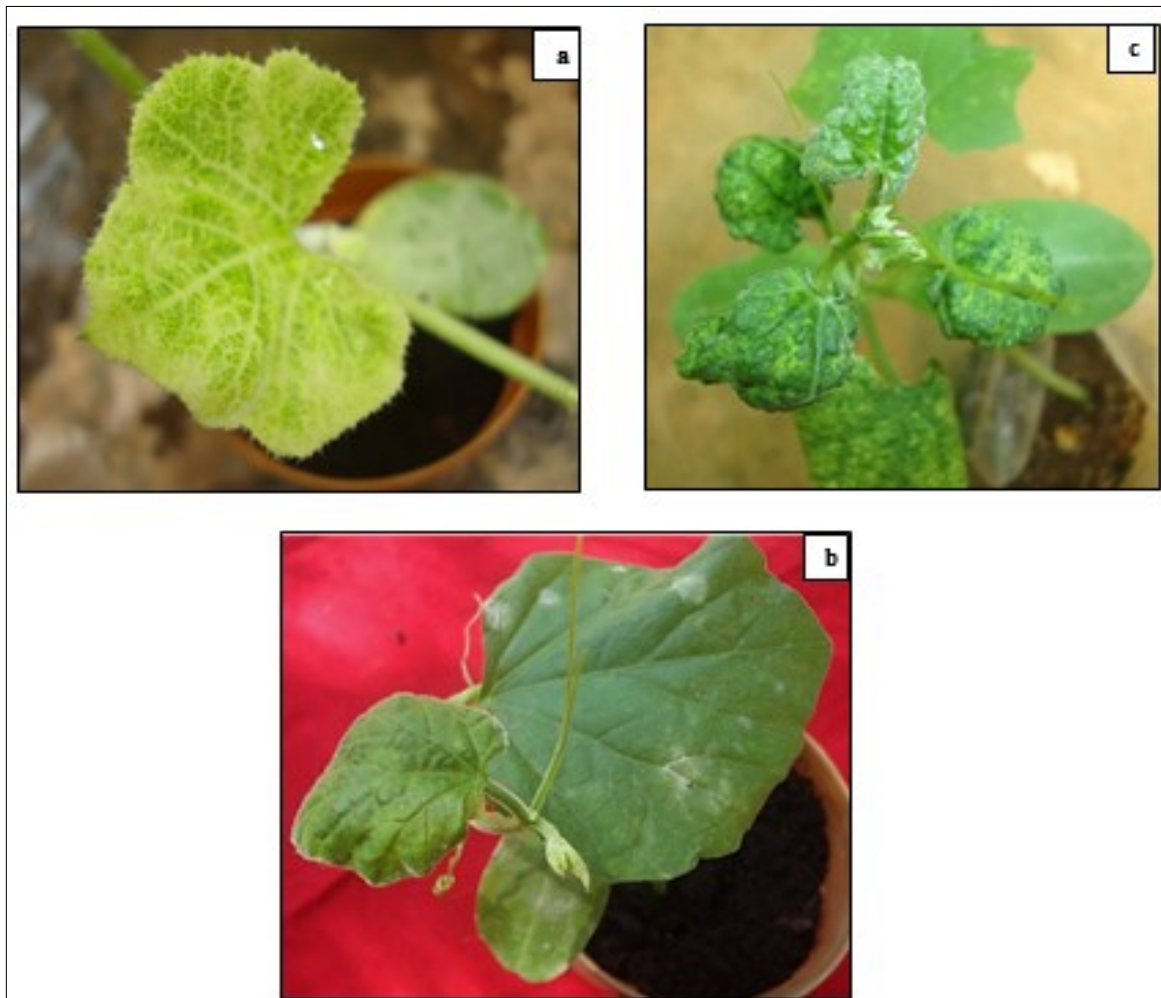


Fig. 3. Symptoms induced by squash leaf curl China virus-Zucchini [Bengaluru-1] (SLCCNV-ZUB1) on different crops. a: mosaic and vein clearing on pumpkin; b: puckering and upward leaf curling on ridge gourd; c: chlorosis and leaf curling on bottle gourd.

(SLCCNV-ZUB1, SLCCNV-ZUB2, SLCCNV-ZUMY1 and SLCCNV-ZUMY2) and the DNA-A component of monopartite begomovirus, ToLCKV-ZUB5 were determined in both orientations and the nucleotide length ranged between 2683 to 2736 for SLCCNV isolates and 2766 for ToLCKV-ZUB5 isolate. The infection by SLCCNV-ZUB1 in bottle gourd, ridge gourd and pumpkin under test inoculation was also confirmed in PCR using specific primers. Sequence analysis of (ZUB1) isolate revealed DNA-A and DNA-B component of old world (OW) bipartite begomovirus whereas the sequence analysis of DNA-A component of ZUB5 isolate was similar to new world (NW) monopartite begomovirus. The ORFs of SLCCNV-ZUB1 isolate encoding coat protein (AV1) and pre coat protein (AV2) had nucleotide sequences ranging from 281–1051 (771 nt) and 121–459 (339 nt), respectively on the virion sense strand. The five ORFs, encoding replication-initiator protein (Rep), the transcriptional

activator protein (TrAP), the replication enhancer protein (REn), AC4 and AC5 had nucleotide sequences ranging from 1500–2585 (1086 nt), 1193–1597 (405 nt), 1048–1458 (411 nt), 2252–2428 (177 nt) and 331–796 (486 nt). The sequence of DNA-B revealed the presence of nuclear shuttle protein (BV1) and movement protein (BC1) with nucleotide sequences ranging from 468–2173 (807 nt) and 1328–2173 (846 nt), respectively (Table 3).

Genome organisation of DNA-A and DNA-B component of begomovirus

Comparative analysis of sequence of isolates, ZUB1, ZUB2, ZUMY1, ZUMY2 and ZUB5 was performed with 40 selected begomoviruses retrieved from NCBI database. Analysis of Sequence by comparison and SDT of complete nucleotide sequence of DNA-A (ZUB1, ZUB2, ZUMY1, ZUMY2 and ZUB5) showed a maximum nucleotide identity > 91.1 % with SLCCNV isolates and < 84 % sequence identity with

Table 3. Features of begomoviruses isolated from zucchini

ORFs ^z	SLCCNV			ToLCKV		
	Stop codon	Start codon	Size of ORFs (nt)	Stop codon	Start codon	Size of ORFs (nt)
DNA A						
AV2 ^y	459	121	339	491	144	348
AV1	1051	281	771	1074	304	771
AC5	796	311	486	-	-	-
AC3	1458	1048	411	1481	1077	405
AC2	1597	1193	405	1222-	1222-	-
AC1	2585	1500	1086	2614	1529	1086
AC4	2428	2252	177	2457	2614	158
DNA B						
BV1	1274	468	807	-	-	-
BC1	2173	1328	846	-	-	-

ORFs^z: Open reading frame indication of genes.

several other previously reported sequences used in the analysis. Sequence analysis of the DNA-A component of the virus infecting zucchini from Bengaluru showed a maximum nucleotide identity of 97.9 % with ToLCKV isolate MN020537 reported from tomato in Maharashtra, India. The complete DNA-A genome of SLCCNV-ZUB1 isolate and ToLCKV-ZUB5 was submitted to NCBI Genbank with Accession No. OR208615 and OR208616, respectively. The DNA-B of 4 isolates were also compared with publicly available begomovirus nucleotide sequences as described previously. The results revealed that all the isolates shared high levels of nucleotide identity (> 82.8 %) with SLCCNV infecting zucchini. Percent nucleotide identity among the DNA-A and DNA-B isolates was also analysed (Supplementary Table 2). The sequence analysis of DNA A and DNA B genomes of SLCCNV isolates infecting zucchini from Bengaluru (SLCCNV-ZUB1 and SLCCNV-ZUB2) and Mysuru (SLCCNV-ZUMY1 and SLCCNV-ZUMY2) revealed 91.1 to 98.5 % and 82.8 to 98.8 % nucleotide sequence identity, respectively with several SLCCNV isolates infecting cucurbits. DNA A (OR208615) and DNA B (OR208614) of the ZUB1 isolate, respectively, had the highest nucleotide identities, 98.5 % to SLCCNV (EU573715) on pumpkin and 97 % to SLCCNV (MF377397) on jasmine. The isolate, ToLCKV infecting zucchini from Bengaluru (ToLCKV-ZUB5) possess a monopartite genome, DNA-A (OR208616) with the highest nucleotide identity of 97.9 % to ToLCKV (MN020537) on tomato in Maharashtra, India. The ZUB1 isolate closely clustered with SLCCNV on pumpkin and summer squash and ZuB5 isolate with ToLCKV infecting tomato in India (Fig. 4a) and it is well supported using species demarcation tool (Fig. 4b and 5b). It is confirmed from the present study that the begomovirus isolates (SLCCNV-ZUB1, SLCCNV-ZUB2, SLCCNV-ZUMY1 and SLCCNV-ZUMY2) was a bipartite begomovirus, considered as a new variant of SLCCNV isolated in zucchini from South India described as ZUB1 strain. The virus isolate ZUB5 was a monopartite begomovirus and is a new variant of ToLCKV described as ToLCKV-ZUI5. By the current species strain demarcation criteria for begomoviruses, (89 % and 93 % identity cut off) the conclusion was made (31). The complete nucleotide sequences of SLCCNV-ZUB1 and DNA-A of ToLCKV-ZUB5 is in the NCBI database (OR208615, OR208614 and OR208616).

Recombination analysis

In this present study, the neighbour-network and RDP analysis was performed for the five begomovirus isolates (DNA-A and DNA-B) with other selected begomoviruses infecting different crops using RDP4 software integrated with different programs (RDP, GENECOV, Max Chi, Chimaera, Si Scan, 3Seq) (Table 4). The recombinant events were significant if it is detected by more than 3 methods, which are implemented in the RDP program (33). The analysis by RDP showed one recombination break point was detected in DNA A genome of SLCCNV-ZUB1, SLCCNV-ZUB2, SLCCNV-ZUMY1 and SLCCNV-ZUMY2 isolates (Fig. 6). The recombination break points for four SLCCNV isolates were derived from ToLCNDV-KU196750 and SLCCNV-MF967019 infecting chilli and pumpkin, acting as major and minor parents. Similarly, for ToLCKV isolate, breakpoints were detected.

The majority of DNA-A genome was derived from ToLCKV- tomato and mung bean yellow mosaic virus (MYMIV-MF818048) infecting tomato and cucumber, as major and minor parents, respectively. There were no recombination break points in DNA-B genome.

Discussion

The emergence of begomovirus in the vegetables is causing a threat to cucurbits production. The begomovirus evolution has been characterised due to continuous mutation in the virus. However, the changes in the climate and the cropping system can also contributed for the virus emergence (23). The yellow mosaic disease in cucurbits causes considerable yield loss throughout Indian subcontinent. The symptoms of SLCCNV infection on zucchini is clearly resembled like pumpkin and summer squash leaf curl disease (5, 8). The symptoms of ToLCKV infection on zucchini was like any other begomovirus infection reported in tomato and other solanaceous crops, it is clearly indicating zucchini as alternate host. We report the infection of ToLCKV begomoviruses for the first time on zucchini in India by vector transmission assays and PCR detection of begomoviruses in both naturally infected and artificially inoculated plants.

The mode of whitefly transmission reveals like that of begomoviruses previously identified. The biological characterisation of the virus revealed minimum AAP for whiteflies was 10 min. Successful transmission of SLCCNV infecting summer squash by *B. tabaci* was reported (5). A100 % transmission was recorded, demonstrating that the latent period was met in 12 hr or less, which was also a hallmark of begomovirus vector transmission (34). These results were in accordance with the earlier reports on virus vector relationships of begomovirus (34–36). The results of studies on identification of susceptible hosts to the virus indicated that, SLCCNV-ZUB1 was successfully transmitted to pumpkin, bottle gourd and ridge gourd. But plant species such as bitter gourd, chayote, sponge gourd, ash gourd, ivy gourd, cucumber, gherkin, musk melon, watermelon, round melon, tomato, tobacco, bell pepper, chilli and eggplant did not show visible symptoms proving that these crop plants were non hosts for SLCCNV-ZUB1 isolates. The study of the host range showed that pumpkin, bottle gourd and ridge gourd, can act as a reservoir host and cause an increase in SLCCNV epidemics in cucurbitaceous crops. The SLCCNV had a narrow host range among begomoviruses infecting cucurbits (8, 12).

The SDT analysis of SLCCNV DNA A resulted in nucleotide identity of 91.1 to 98.5 % among the five SLCCNV isolates infecting zucchini. The difference in nucleotide in begomovirus isolates and its sub clustering with other SLCCNV strains reveals emergence of begomovirus through mutation and recombination (37). The nucleotide sequence analysis showed a maximum nucleotide identity range from 85.8 to 98.8 % for DNA-B. Current begomovirus species demarcation standards proves that the begomovirus isolates infecting zucchini (SLCCNV-ZUB1, SLCCNV-ZUB2, SLCCNV-ZUMY1 and SLCCNV-ZUMY2) were considered as a novel variant of

Table 4. DNA-A break point analysis components of different isolates of zucchini and their putative parental sequences

Begomovirus isolates	Major parent	Minor parent	p-Values (0.05)					
			RDP	GENECOV	MAX Chi	Chimaera	SiScan	3 Seq
SLCCNV-ZUB1	ToLCNDV Chilli	SLCCV Pumpkin	2.68x10 ⁻⁰³	1.664x10 ⁻⁰²	1.355x10 ⁻⁰⁷	1.693x10 ⁻⁰⁵	6.737x10 ⁻²⁴	1.804x10 ⁻¹³
SLCCNV-ZUB2	ToLCNDV Chilli	SLCCV Pumpkin	2.68x10 ⁻⁰³	1.664x10 ⁻⁰²	1.355x10 ⁻⁰⁷	1.693x10 ⁻⁰⁵	6.737x10 ⁻²⁴	1.804x10 ⁻¹³
SLCCNV-ZUMY1	ToLCNDV Chilli	SLCCV Pumpkin	2.68x10 ⁻⁰³	1.664x10 ⁻⁰²	1.355x10 ⁻⁰⁷	1.693x10 ⁻⁰⁵	6.737x10 ⁻²⁴	1.804x10 ⁻¹³
SLCCNV-ZUMY2	ToLCNDV Chilli	SLCCV Pumpkin	2.68x10 ⁻⁰³	1.664x10 ⁻⁰²	1.355x10 ⁻⁰⁷	1.693x10 ⁻⁰⁵	6.737x10 ⁻²⁴	1.804x10 ⁻¹³
ToLCKV-ZUB5	ToLCKV Tomato	MYMIV Pumpkin	NS	1.871x10 ⁻⁰⁶	NS	NS	NS	3.790x10 ⁻⁰³

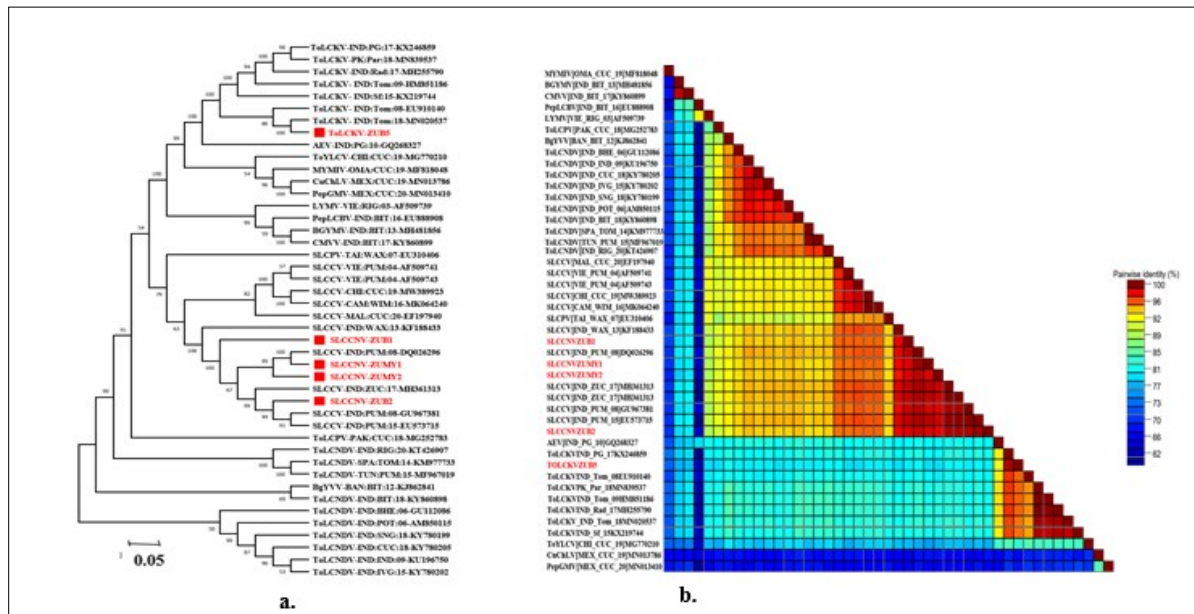


Fig. 4. Phylogenetic relationship, percentage genome scores and nucleotide identity analysis of the DNA-A component of squash leaf curl China virus (SLCCNV) isolates and the ToLCKV isolate from zucchini with selected begomovirus isolates. a: Phylogenetic tree constructed using the neighbour-joining method in MEGA 11 with 1000 bootstrap replicates; b: Graphical representation of percentage genome scores and nucleotide identity plot generated using Species Demarcation Tool (SDTV1.0).

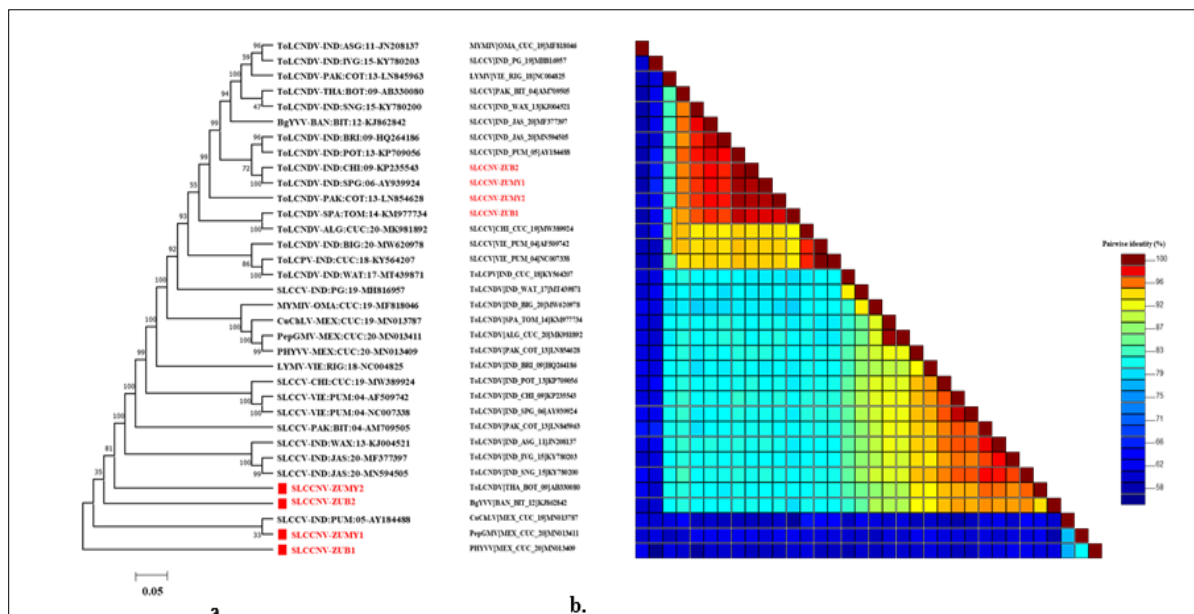


Fig. 5. Phylogenetic relationship, percentage genome scores and nucleotide identity analysis of the DNA-B component of squash leaf curl China virus (SLCCNV) isolates from zucchini with selected begomovirus isolates. a: Phylogenetic tree constructed using the neighbour-joining method in MEGA 11 with 1000 bootstrap replicates; b: Graphical representation of percentage genome scores and nucleotide identity plot generated using Species Demarcation Tool (SDTV1.0).

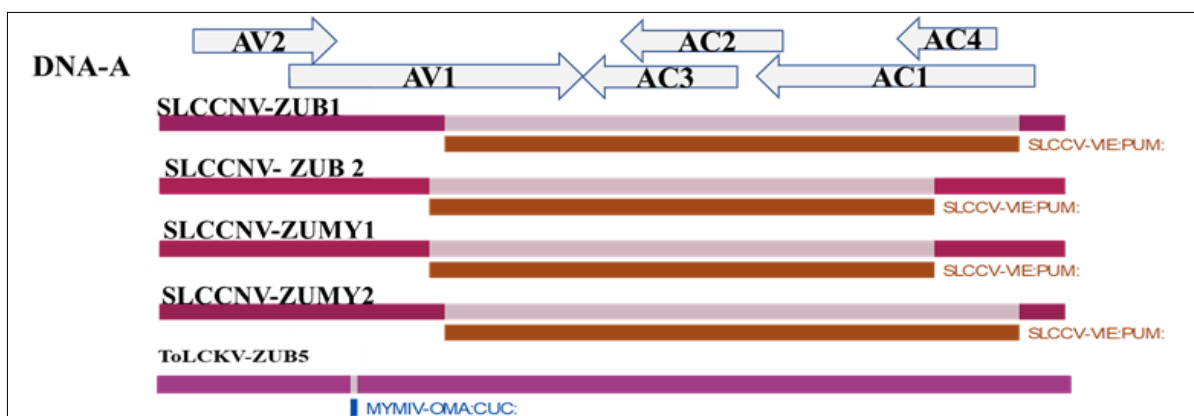


Fig. 6. Recombination analysis of the DNA-A component of squash leaf curl China virus (SLCCNV) isolates and the ToLCKV isolate from zucchini with selected begomoviruses infecting different crops, carried out using recombinant detection program (RDP), GENECONV, MaxChi, Chimera, SiScan and 3Seq methods integrated in RDP4.

SLCCNV. Phylogenetic study revealed that SLCCNV isolates infecting zucchini were closely associated with numerous isolates of SLCCNV infecting diverse hosts (38). The DNA-A obtained was represented as SLCCNV-[ZUB1] and ToLCKV-[ZUB5].

The recombination event in DNA-A component of SLCCNV revealed diversification mechanisms in begomoviruses that contributes to the genetic variability (39). Plant virus evolution occurs through factors i.e., virus-vector and host virus interactions, increased hosts vector and virus through high level of viral replication (5). The major mechanism of diversification in begomovirus is in recombination (34, 40). It is most important at the species level (40, 41) or at strain level (30). This led to the evolution of the begomoviruses and only one recombination event was determined in all the SLCCNV isolates depicting inter-species and intra-species recombination which has given rise to chimeric genome infecting different crop plants. The data given here clearly demonstrated that SLCCNV infection was more common wherever zucchini is cultivated in India. Insect transmission experiments, as well as genome analysis, revealed that zucchini plants exhibiting characteristic begomovirus symptoms such as yellow mosaic caused by the infection of SLCCNV. Further recombination of virus entire genome revealed as a recombinant one and that predicts high risk in production of cucurbits.

Conclusion

The findings from the present study revealed that squash leaf curl China virus–Zucchini [Bengaluru-1] (SLCCNV-ZUB1) was a bipartite begomovirus variant with a restricted host range, while tomato leaf curl Karnataka virus–Zucchini [Bengaluru-5] (ToLCKV-ZUB5) was a monopartite begomovirus infecting zucchini for the first time in India. The virus–vector studies demonstrated efficient transmission of both viruses by *Bemisia tabaci* with short acquisition and inoculation access period (IAP), emphasising the role of whitefly in rapid disease spread. Sequence analysis and recombination studies indicated the emergence of new variants through mutation and recombination, contributing to genetic diversification and potential epidemics in cucurbit cultivation. Overall, the study highlights the threat posed by begomoviruses to zucchini production in South India and underlines the need for integrated management strategies focusing on host resistance and vector control.

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Authors' contributions

STG and RR conducted the study and carried out the molecular work. RR, MS, VA, LTN and VSC participated in the design and coordination of the study. AKR and ATC prepared the manuscript and performed the statistical analysis. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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