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RESEARCH ARTICLE

Evolutionary dynamics and recombination patterns in begomoviruses infecting *Abelmoschus esculentus*: A phylogenetic and population structure analysis

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Abstract

Okra (*Abelmoschus esculentus* (L.) Moench) is a major vegetable crop cultivated globally, particularly in India and Nigeria, but it is highly susceptible to begomovirus infections transmitted by the whitefly (*Bemisia tabaci*). The predominant virus, *Bhendi yellow vein mosaic virus* (BYVMV), along with related viruses such as *Bhendi yellow vein India virus* (BYVIV), *Tomato leaf curl New Delhi virus* (ToLCNDV), *Okra leaf curl virus* (OkLCuV) and *Okra mosaic virus* (OMV), causes severe yield losses. This study examines the genetic diversity and recombination patterns of begomoviruses infecting okra using 94 viral genome sequences (DNA-A, DNA-B and betasatellites) retrieved from public databases. Phylogenetic analysis revealed distinct viral clades, while nucleotide substitution analysis showed that transitions occurred more frequently than transversions. Recombination analysis identified several breakpoints in the replication (Rep) and coat protein (CP) genes, with 29 and 11 breakpoints, respectively. Genetic diversity parameters showed high variation, with nucleotide diversity values of 0.13025 for DNA-A, 0.20899 for DNA-B and 0.10672 for betasatellites. The AC1 gene exhibited the highest mutation rate. Haplotype analysis identified 50 haplotypes for DNA-A, 8 for DNA-B and 36 for betasatellites, with DNA-A and betasatellites showing nearly complete haplotype diversity. Neutrality tests suggested selective pressure on the virus populations, possibly due to population expansion or purifying selection. These findings enhance understanding of begomovirus evolution and underscore the need for continuous monitoring and management of viral diseases in okra cultivation.

Keywords: begomoviruses; evolution; mutations; phylogeny; recombination

Introduction

Abelmoschus is a genus in the Malvaceae family encompassing some of the most important vegetable crops and its origin is uncertain, with early botanical and biogeographical work suggesting an Ethiopian (East African) origin and more recent morphological and germplasm-based studies suggesting an Asian origin as well (1). Today, *Abelmoschus* species are found in tropical, subtropical and warm temperate regions worldwide. The genus consists of 11 species, 3 subspecies and 4 varieties, with a habitat range spanning from the Himalayan region to southern peninsular India. One of its most important members is *Abelmoschus esculentus* (commonly known as okra or bhindi), which is an allopolyploid with chromosome number 2n = 8x = 72. India is the largest producer of *A. esculentus*, followed by Nigeria (2–4).

Okra is a cultigen primarily cultivated for its immature, edible green pods, which are characterized by their slimy texture due to the presence of mucilage. The use of medicinal plants is deeply rooted in Indian tradition and remains highly valued today. Mucilage extracted from okra pods has been reported to be effective in preparing infusions for treating dysentery, diarrhoea and various inflammatory conditions affecting the stomach, bowels and kidneys. It is also used to manage catarrhal infections, ardour urinae and dysuria, serving as a diuretic, antipyretic and plasma substitute and is even utilized in the treatment of gonorrhoea. The immature pods and roots are decocted for their demulcent and emollient properties. In Latin America, the leaves are used in remedies for tumours, while the seeds are recognized in Indian traditions for their antispasmodic and stimulant effects, as well as for tumour treatment in Latin America. Additionally, flowers and leaves are decocted in Indian ethnomedicine for the treatment of bronchitis and pneumonia (5–8).

The nutritional value and numerous health benefits of okra are significant. It offers protection against free radical damage and is rich in essential nutrients, including vitamins A, E and C, as well as important minerals such as sodium, potassium, magnesium and calcium. Additionally, it contains trace elements like zinc, iron and nickel. The seeds are particularly abundant in phenolic compounds, including flavonoids and catechins, which are known antioxidants, along with a significant amount of dietary fibre (4).

Several reports highlight the prevalence of various diseases affecting *A. esculentus*. Insect pest infestation is a significant factor that hinders the high yielding potential of these plants. Key insect pests include fruit and shoot borers, aphids, whiteflies and ants.

Among these, whiteflies, particularly *Bemisia tabaci*, are noteworthy as they serve as vectors for begomoviruses. Other species, such as *Trialeurodes ricini* and *Trialeurodes vaporariorum*, can also transmit begomoviruses. The most common viruses affecting *A. esculentus* include Bhendi yellow vein mosaic virus (BYVMV), Bhendi yellow vein India virus (BYVIV), Tomato leaf curl New Delhi virus (ToLCNDV), Okra enation leaf curl virus (OELCuV), Okra leaf curl virus (OkLCuV) and Okra mosaic virus (OkMV). Among these, BYVMV poses the most serious threat, as it has been reported from major okra-growing regions worldwide, leading to yield losses of approximately 30–100 %, depending on the age of the plant at the time of infection. The first report of OELCuV emerged in the early 1980s from Bangalore, with yield losses due to this virus estimated at around 80–90 % (9, 10). Symptoms of OELCuV include curling of new leaves, shortened and thickened leaves, twisted petioles, bent stems and thick, deformed lower leaf surfaces. BYVMV, OkMV, OkLCuV and OELCuV all belong to the family *Geminiviridae* and the genus *Begomovirus* (11, 12). Yellow vein mosaic disease (YVMD), transmitted by whiteflies, poses a serious threat to okra production, affecting both fruit yield and quality. While chemical control of the vector population can help manage the disease, using resistant varieties offers a more cost-effective and environmentally friendly solution (13).

According to the latest release from the International Committee on Taxonomy of Viruses (ICTV), the family *Geminiviridae* comprises 15 genera and 522 species. Among these, the genus *Begomovirus* is the largest, containing 455 species that infect a wide range of dicotyledonous plants, resulting in various diseases. The most common symptoms observed include mosaics, mottles, yellowing, leaf curling and deformation, reduced plant growth, vein clearing and decreased fruit production and quality. Begomoviruses have a genome composed of circular single-stranded DNA packaged in pseudo-icosahedral-shaped virions. Their genomes can be either monopartite, predominantly found in Old World viruses with few examples in the New World or bipartite, primarily associated with New World viruses. The genome size of monopartite begomoviruses ranges from 2.5 to 3.1 kb, while each component of bipartite begomoviruses measures approximately 2.6 and 2.8 kb (14, 15).

In monopartite begomoviruses (Old World viruses), DNA-A comprises 6 open reading frames (ORFs). In the virion sense (rightward direction), *AV1/AR1* encodes the coat protein (CP) and *AV2/AR2* encodes the precoat protein. In the complementary sense (leftward direction), 5 ORFs are present: *AC1/AL1* for the replication initiator protein (Rep), *AC2/AL2* for the transcription activator protein (TrAP), *AC3/AL3* for the replication enhancer protein (REn) and *AC4/AL4* and *AC5/AL5* for other proteins essential to the virus lifecycle (16). Notably, AV2 is absent in Old World viruses. In the rightward orientation, the gene *BV1/BR1* encodes the nuclear shuttle protein, while the leftward ORF *BC1/BL1* encodes the movement protein (MP). These proteins are essential for transporting viral DNA into the nucleus and facilitating the movement of the virus within the host plant, respectively (11, 14, 17, 18).

Begomoviruses are also associated with additional circular single-stranded components known as satellites, which are further classified into alphasatellites, betasatellites and deltasatellites (19). The first DNA satellite was identified in 1997 in association with *Tomato leaf curl virus* (ToLCV). Alphasatellites are approximately half the size of begomovirus-DNA and encode a single Rep gene, similar to the Rep found in nanoviruses, allowing for autonomous replication; however, they rely on begomoviruses for their spread. Previously, these were referred to as DNA1 (20, 21). Betasatellites share replication origins with their helper Begomoviruses (DNA-A) and encode a pathogenicity determinant known as beta C1, which suppresses gene silencing. They also contain a conserved region (SCR) essential for replication (22). When co-inoculated with monopartite begomoviruses, betasatellites exacerbate symptoms in infected plants, leading to severe effects such as leaf malformation and stunting. Alphasatellites and betasatellites share only the stem-loop structure with begomoviruses, necessary for replication (19, 20).

Although okra begomoviruses have caused substantial crop losses across India, there remains a critical need for a systematic study of these viruses and their associated satellites in different okra-growing regions. This study aims to conduct a thorough investigation into the population dynamics and genetic diversity of okra begomoviruses, alongside phylogenetic and recombination analyses, to enhance our understanding of the impact of these viral diseases on okra cultivation throughout the country.

Materials and Methods

Sequence retrieval and alignments

A total of 94 full length sequences collected over the past 15 years were analysed to investigate the begomoviruses associated with diseases in *A. esculentus*. The dataset comprises 50 DNA-A sequences, 36 betasatellite sequences and 8 DNA-B sequences. The sequences were retrieved from the NCBI database using the search terms 'okra, bhendi, begomovirus, India within the NCBI Taxonomy Browser (https://www.ncbi.nlm.nih.gov/) on June 7, 2024 (Supplementary Table 1 and Supplementary Table 2). The sequences, along with the 6 open reading frames (ORFs) of DNA-A, were aligned using the Clustal W algorithm in MEGA 11 (23, 24).

Phylogenetic relationship

The optimal nucleotide substitution model was selected based on the lowest Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC) scores (25). Phylogenetic trees were constructed for the selected sequences using MEGA 11, employing the Maximum Likelihood (ML) algorithm, the Neighbor-Joining method and bootstrap values from 1000 replicates. Transition and transversion rates, along with transition/transversion bias (R), were calculated for all 6 ORFs, DNA-A, DNA-B and beta-satellites by applying the same approach (24, 26).

Recombination analysis

To identify potential recombination events, a comprehensive analysis of the aligned sequences was performed using RDP4.1. Seven different algorithms namely RDP, BOOTSCAN, MaxChi, GENECONV, SISCAN, CHIMERA and 3 Seq were utilized with default settings and a maximum p-value cut-off of 0.05, adjusted for multiple comparisons using Bonferroni correction (27, 28). Recombination events identified by at least 3 of the 7 algorithms were considered significant to minimize the likelihood of false positives (18, 29).

Genetic variability and population structure

To assess genetic variability and population structure of the viruses, several parameters were calculated using DnaSP v6.12 for DNA-A, the six open reading frames (ORFs) of DNA-A, DNA-B and betasatellites. The parameters included the total number of segregating sites (*s*), which indicates genetic variation within the

population; the total number of mutations (η); nucleotide diversity (*π*), reflecting the genetic diversity within the population; and the average number of nucleotide differences between sequences (*k*), providing insights into genetic variation. Additionally, Watterson's estimate of the population mutation rate based on the total number of mutations (*θ-η*) and Watterson's estimate based on the total number of segregating sites (*θ-w*) were calculated, offering different perspectives on the population's mutation dynamics. Finally, the number of haplotypes (*H*) indicates the level of genetic variation, while haplotype diversity (*Hd*) measures the probability that two randomly chosen haplotypes from the population will differ (30, 31).

Neutrality test

Neutrality tests (Tajima's D, Fu & Li's D* and F*) were performed to infer selection pressures (32, 33). These analyses were conducted using DnaSP v6.12 for DNA-A, ORFs, DNA-B and betasatellites (31, 32).

Results

Phylogenetic relationship and transition/transversion rates

The phylogenetic history was inferred using both the maximum likelihood and Neighbor-Joining methods and optimal trees were constructed. The DNA-A sequences of begomoviruses associated with diseases in *A. esculentus* were grouped into four distinct clusters: the first group contained BYVIV, the second OELCuV, the third BYVMV and the fourth ToLCNDV (Fig. 1) The best-fit models for each dataset were determined based on pairwise multiple sequence alignments using ClustalW, reflecting distinct evolutionary patterns for each sequence type. The GTR+G model (General Time Reversible model with gamma distribution) was most appropriate for DNA-A sequences. Subsequently, T92+G (Tamura 1992 model with gamma distribution) and GTR+G was suitable for DNA-B and betasatellites respectively.

Additionally, R values were calculated for all datasets including all 6 DNA-A ORFs associated with DNA-A. For DNA-A, the observed transition substitutions ranged from 10.58 to 15.98, while transversion substitutions ranged from 5.01 to 7.56, yielding an R value of 0.96. In contrast, DNA-B exhibited transition substitution rates ranging from 9.39 to 16.89 and transversion rates from 4.73 to 7.34, with an R value of 1.048 (Fig. 2). Among the six ORFs, the *AC3* gene had the highest transition substitution rates, ranging from 6.91 to 22.69, while the *AV2* gene showed the highest transversion substitution rates, ranging from 8.42 to 9.41. Betasatellites exhibited transition substitution rates between 8.78 and 16.37 and transversion substitution rates between 4.32 and 8.51, with a calculated R value of 0.882 (Table 1).

Recombination analysis

Recombination analysis identified potential breakpoints and parental viruses in begomoviruses and their associated satellites infecting *A. esculentus* in India (Fig. 3). This analysis revealed several distinct recombination events. To ensure reliability, only recombination events supported by at least three different methods were considered for further analysis. In total, 29 recombination breakpoints were identified in DNA-A. To validate earlier findings, recombination breakpoints in the 6 ORFs of DNA-A were also analysed. The highest frequency of intra-species recombination was observed in *AC1* and *AV1*, while the rest displayed fewer breakpoints, with only 2 to 3 identified. No notable recombination breakpoints were detected in the *AV2* gene. Additionally, 10 and 18 recombination breakpoints were identified in DNA-B and betasatellites respectively (Table 2).

Genetic structure and demographic analysis

To explore further, begomoviruses and betasatellite isolates from *A. esculentus*, genetic variability was quantified by calculating

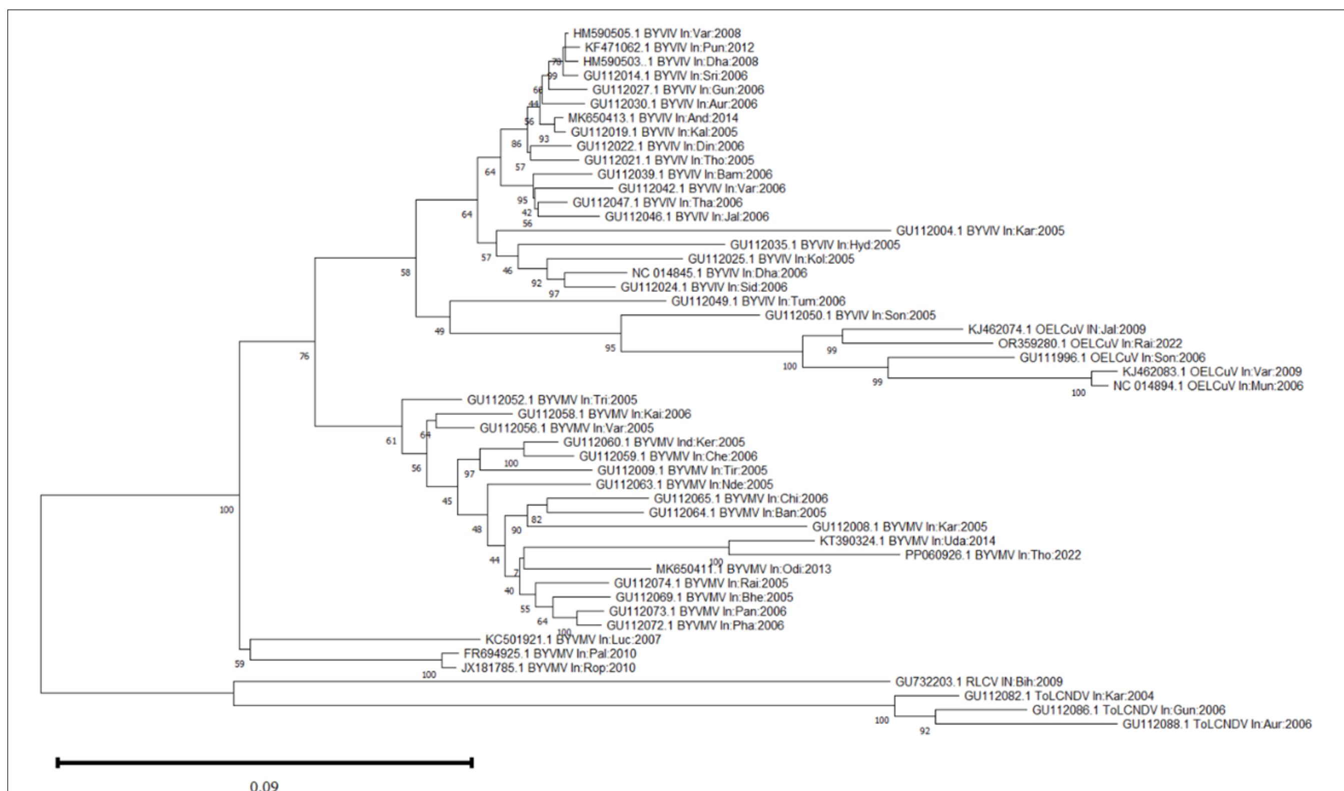

Fig. 1. Maximum likelihood phylogenetic tree of begomoviruses infecting *Abelmoschus esculentus* in India, based on 50 DNA-A sequences clustered into 4 distinct clades, aligned with Clustal W in MEGA 11 and supported by 1000 bootstrap replicates.

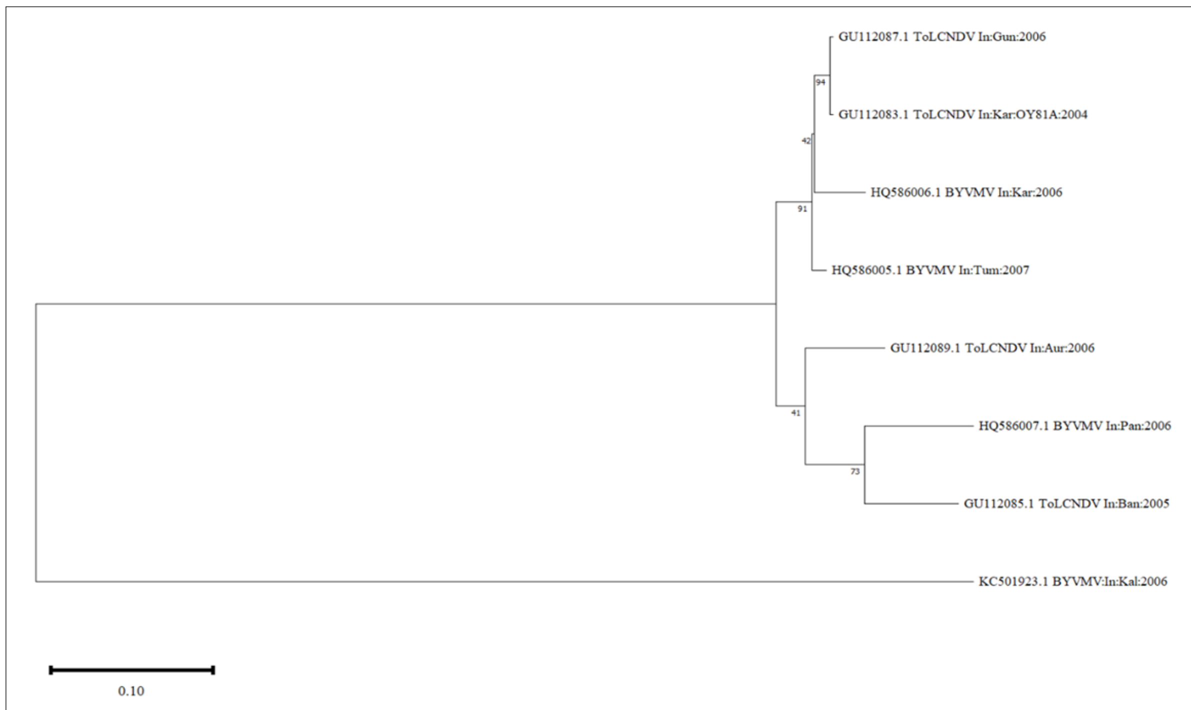

Fig. 2. Phylogenetic tree created using maximum likelihood method illustrating the genetic relationships among 8 begomoviruses DNA-B sequences associated with *Abelmoschus esculentus* in India aligned with Clustal W in MEGA 11 and supported by 1000 bootstrap replicates.

Table 1. Substitution rates and Transition/Transversion bias among DNA-A, DNA-B, ORFs and beta-satellite

Virus component	Transitional substitution rate	Transversional substitution rate	Transition/Transversion bias (R)
DNA-A	10.58-15.98	5.01-7.56	0.96
AC1	10.22-15.03	5.09-7.48	1.079
AC2	8.35-20.85	4.66-7.14	1.045
AC3	6.91-22.69	4.17-8.12	0.902
AC4	10.72-14.95	4.95-7.41	1.033
AC5	9.55-23.21	3.42-6.37	1.379
AV1	10.5-17.64	4.69-6.6	1.108
AV2	0.97-13.22	8.42-9.41	0.411
DNA-B	9.39-16.89	4.73-7.34	1.048
Betasatellite	8.78-16.37	4.32-8.51	0.882

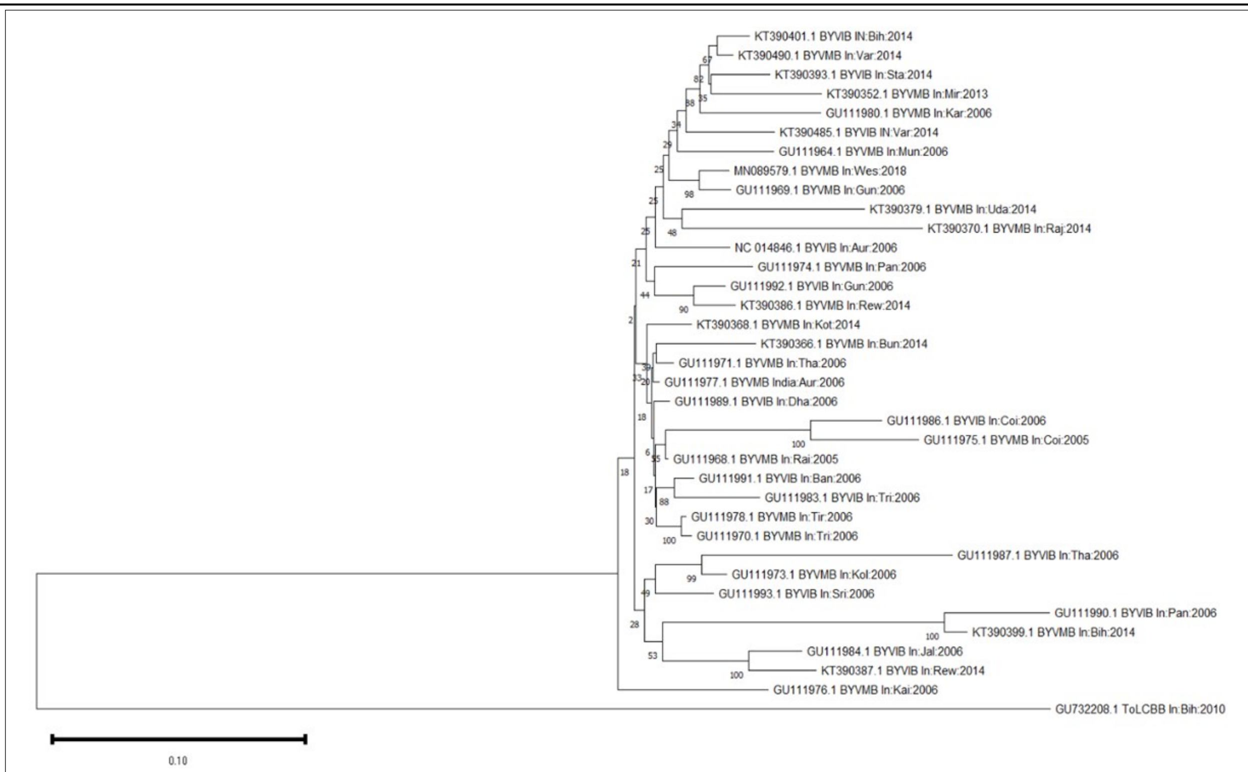

Fig. 3. Phylogenetic tree constructed using maximum likelihood method for begomoviruses associated with diseases in *Abelmoschus esculentus* in India aligned using Clustal W an algorithm in MEGA 11 and bootstrap values based on 1000 replicates.

Table 2. Recombination analysis for DNA-A, associated ORFs, DNA-B and betasatellites determined using different algorithms showing both major and minor parents, recombinants and calculated *p*-value

Events	Breakpoints		Recombinant	Parents		Method	p-value
	Begin	End		Major	Minor		
DNA-A							
1	2850	1871	KJ462074.1_OEL	GU112035.1_BYIV	KJ462083.1_MeY	R,G,B,M,C,S,**T**	8.590 E-92
2	1130	2492	GU112025.1_BYV	Unknown	MK650413.1_BYIV	R,G,B,M,S,**T**	3.837 E-79
3	1224	226	PP060926.1_BYV	Unknown	GU112072.1_BYV	R,G,B,M,S,**T**	7.422 E-83
4	1874	2644	GU112060.1_BYV	KF471062.1_BYV	Unknown	R,G,B,M,C,S,**T**	7.138 E-75
5	1562	170	MK650411.1_BYV	GU112027.1_BYV	KT390324.1_BYV	R,G,B,M,C,S,**T**	4.798 E-68
6	1294	2430	GU111996.1_OEL	KJ462083.1_MeY	MK650413.1_BYIV	**R**,G,M,C,S,T	1.800 E-28
7	684	1420	KT390324.1_BYV	GU112069.1_BYV	KJ462083.1_MeY	**R**,G,B,M,C,S,T	2.218 E-64
8	1320	2622	GU112024.1_BYIV	GU112065.1_BYV	MK650413.1_BYIV	R,G,B,M,S,C,**T**	1.252 E-61
9	1880	649	GU112073.1_BYV	GU112042.1_BYIV	Unknown	G,B,M,S,C,**T**	4.441 E-81
10	86	649	GU732203.1_RLC	GU112073.1_BYV	Unknown	R,**G**,B,M,S,C,T	1.312 E-34
11	1350	1873	KC501921.1_BYV	GU112052.1_BYIV	NC_014894.1_OEL	R,G,B,M,S,C,**T**	9.981 E-43
12	1117	1873	MK650413.1_BYIV	GU112052.1_BYIV	Unknown	R,G,B,M,S,C,**T**	2.593 E-25
13	790	2037	GU112049.1_BYIV	GU112047.1_BYIV	NC_014894.1_OEL	R,B,**M**,S,C,T	7.570 E-24
14	560	1126	PP060926.1_BYV	GU112063.1_BYV	KJ462083.1_MeY	R,G,M,C,S,**T**	5.080 E-19
15	1116	1873	GU112058.1_BYV	GU112082.1_ToLC	KJ462083.1_MeY	R,G,B,M,C,S,**T**	1.628 E-15
16	708	894	GU112082.1_ToLC	GU112086.1_ToLC	GU112047.1_BYV	**R**,G,B,M,S,C,T	2.483 E-13
17	1989	2392	OR359280.1_OEL	GU732203.1_RLC	GU112046.1_BYIV	R,G,**M**,S,C,T	2.639 E-10
18	2657	2786	GU112063.1_BYV	GU112030.1_BYIV	Unknown	**R**,M,C	6.615 E-09
19	650	724	GU112050.1_BYIV	NC_014894.1_OEL	GU112046.1_BYIV	R,**G**,B,M,C	1.624 E- 08
20	2148	2565	GU732203.1_RLC	Unknown	NC_014894.1_OEL	**R**,G,M,S,C,T	4.653 E-08
21	2852	187	GU111996.1_OEL	Unknown	GU112046.1_BYIV	R,**G**,B,M,C	1.715 E-07
22	1381	1873	GU112088.1_ToLC	Unknown	GU112082.1_ToLC	R,G,M,**C**,S,T	1.87 E-07
23	2228	2542	GU112046.1_BYIV	GU112047.1_BYIV	Unknown	G,B,M,C,S,**T**	1.554 E-06
25	1874	1987	KJ462074.1_OEL	GU112042.1_BYIV	OR359280.1_OEL	**R**,G,B,S,T	5.971 E-06
26	271	855	GU112042.1_BYIV	MK650413.1_BYIV	Unknown	M,C,S,**T**	8.356 E-05
28	676	988	GU732203.1_RLC	GU111996.1_OEL	GU112063.1_BYIV	M,S,**T**	1.496 E-03
29	835	1326	GU112082.1_ToLC	GU112086.1_ToLC	Unknown	B,M,C,S,**T**	4.199 E-03
DNA-B							
1	2270	294	GU112089.1_ToLC	HQ586007.1_BYV	GU112087.1_ToLC	**R**,G,B,M,C,S	3.264 E-35
2	2130	2274	HQ586007.1_BYV	Unknown	GU112083.1_ToLC	**R**,G,B,M,C,T	3.815 E-10
3	450	1346	GU112085.1_ToLC	GU112089.1_TLC	HQ586005.1._BYV	R,B,M,S,C,**T**	2.059 E-09
4	228	291	GU112085.1_ToLC	Unknown	HQ586007.1_BYV	R,**G**,B,M,C,T	7.149 E-09
5	295	1498	GU112089.1_ToLC	HQ586005.1_BYV	HQ586007.1_BYV	R,B,M,C,S,**T**	8.374 E-08
6	2262	422	HQ586006.1_BYV	HQ586005.1_BYV	Unknown	R,G,B,M,**C**,S,T	1.523 E-05
7	130	414	HQ586006.1_BYV	Unknown	HQ586005.1._BYV	**G**,C,S	3.085 E-07
9	2235	2274	GU112085.1_ToLC	Unknown	GU112087.1_ToLC	G,M,C,S,**T**	1.170 E-03
10	640	1025	HQ586005.1_BYV	HQ586006.1_BYV	Unknown	R,G,B,M,S,**T**	1.299 E-03

				Betasatellite			
1	906	1370	KT390399.1_BYVB	GU111990.1_ BYVIB	KT390490.1_BYVB	R,G,B,M,C,S,**T**	3.201 E-27
2	139	652	GU111976.1_BYVB	KT390366.1_BYVB	Unknown	R,B,M,C,S,**T**	3.053 E-23
3	80	262	GU111983.1_BYVB	GU111986.1_BYVIB	Unknown	R,**G**,B,M,C,S,T	2.219 E-19
4	780	1062	GU111984.1_BYVIB	GU111977.1_BYVB	GU111980.1_BYVB	R,G,B,M,S,C,**T**	4.418 E-05
5	93	928	KT390379.1_BYVB	Unknown	GU111964.1_BYVB	R,G,B,M,S,C,**T**	9.071 E-11
6	519	1486	GU111986.1_BYVIB	GU111993.1_BYVIB	KT390379.1_BYVB	G,B,M,C,S,**T**	2.284 E-11
7	178	552	KT390379.1_BYVB	GU111993.1_BYVIB	Unknown	R,G,B,M,S,C,**T**	7.266 E-13
8	1198	1310	GU111990.1_BYVIB	Unknown	GU111992.1_BYVIB	R,**G**,B,M,S,C,T	2.349 E-11
9	140	779	KT390387.1_BYVIB	Unknown	GU111984.1_BYVIB	G,B,M,**T**	2.470 E-11
10	744	218	NC_014846.1_BYVIB	GU111992.1_BYVIB	KT390366.1_BYVB	R,G,B,**M**,C,S,T	2.866 E-10
11	1457	910	KT390386.1_BYVB	GU111977.1_BYVB	GU111992.1_BYVIB	R,G,B,M,S,**T**	3.974 E-10
12	861	218	KT390366.1_BYVB	Unknown	GU111969.1_BYVB	R,G,**M**,S,C,T	6.990 E-11
13	178	556	KT390399.1_BYVB	Unknown	GU111968.1_BYV	R,**M**,C,S	2.946 E-08
14	1016	26	GU111969.1_BYVB	GU111992.1_BYVIB	Unknown	R,**G**,M,T	4.49 E-06
15	1189	290	GU111964.1_BYVB	GU111971.1_BYVB	Unknown	**M**,C,S	5.272 E-06
16	992	1382	GU111993.1_BYVIB	KT390490.1_BYVB	Unknown	M,S,**T**	5.940 E-05
17	358	597	GU111974.1_BYVB	Unknown	NC_014846.1_BYVIB	M,S,**T**	8.782 E-04
18	570	751	KT390379.1_BYVB	Unknown	GU111992.1_BYVIB	R,M,S,**T**	1.886 E-03
				AC1			
1	806	1102	GU112004.1_CLC	GU112021.1_BYVIV	Unknown	R,G,B,M,C,S,**T**	2.473 E-50
2	272	1054	GU112035.1_BYIV	GU112073.1_BYV	GU112039.1_BYV	R,G,B,M,S,**T**	1.375 E-41
3	828	1102	GU111996.1_OEL	GU112014.1_BYV	Unknown	R,G,M,C,S,**T**	3.77 E-41
4	824	1083	GU112058.1_BYV	GU112060.1_BYV	KJ462074.1_OEL	R,G,B,M,C,S,**T**	4.919 E-37
5	1.98	548	KC501921.1_BYV	GU112058.1_BYV	GU112050.1_BYIV	R,G,B,M,C,S,**T**	1.469 E-29
6	294	1060	GU112039.1_BYV	GU112060.1_BYV	KJ462074.1_OEL	R,G,B,M,C,S,**T**	4.821 E-28
7	285	823	OR359280.1_OEL	GU112058.1_BYV	KJ462074.1_OEL	R,G,M,S,**T**	2.617 E-23
8	581	1101	FR694925.1_BYV	KJ462074.1_OEL	GU732203.1_RLC	R,G,M,C,S,**T**	8.402 E-22
9	965	1052	GU112063.1_BYV	GU112059.1_BYV	GU112049.1_BYIV	**R**,G,M,C,S,T	8.444 E-16
10	376	550	GU112074.1_BYV	GU112065.1_BYV	GU112049.1_BYIV	**R**,G,M,C,S,T	1.211 E-14
11	68	128	GU112049.1_BYIV	GU112046.1_BYIV	Unknown	R,G,M,C,**T**	4.439 E-12
12	294	562	GU112065.1_BYV	GU112008.1_BYIV	KJ462074.1_OEL	R,G,B,**M**,C,T	1.111 E-13
13	466	1056	GU732203.1_RLC	Unknown	GU112009.1_BYV	R,G,M,S,**T**	6.414 E-10
14	1048	1102	GU112009.1_BYV	GU112072.1_BYV	GU112049.1_BYIV	**R**,G,B,C,T	9.887 E-11
15	128	240	GU112035.1_BYIV	GU112064.1_BYV	Unknown	R,G,**M**,C,T	1.139 E-08
16	294	482	GU112088.1_ToL	Unknown	GU112004.1_CLC	R,M,**C**,S,T	3.380 E-07
17	573	1102	GU112008.1_BYIV	Unknown	GU112059.1_BYV	R,**M**,C,S,T	6.310 E-07
18	108	484	GU112064.1_BYV	GU112072.1_BYV	Unknown	R,G,M,C,**T**	8.025 E-07
19	684	1097	GU112074.1_BYV	Unknown	GU112072.1_BYV	M,C,**T**	2.625 E-06
20	294	403	GU732203.1_RLC	Unknown	GU112050.1_BYIV	R,M,C,**T**	8.666 E-06
21	544	791	GU112052.1_BYIV	GU112049.1_BYIV	Unknown	M,C,S,**T**	9.266 E-05
23	483	1034	GU112082.1_ToLC	GU112088.1_ToL	GU112086.1_ToL	M,C,**T**	1.107 E-04
24	32	388	MK650411.1_BYV	Unknown	GU112058.1_BYV	M,S,**T**	3.391 E-04
25	1102	173	GU112072.1_BYV	GU112025.1_BYV	GU112046.1_BYIV	M,S,**T**	3.904 E-04
				AC2			
1	144	327	MK650411.1_BYV	GU112008.1_BYIV	GU112022.1_BYVIV	R,G,**M**,S,T	2.864 E-07
2	171	417	GU112022.1_BYVIV	GU112065.1_BYV	Unknown	M,S,**T**	3.143 E-04
3	141	196	GU732203.1_RLC	Unknown	GU112088.1_ToL	C,S,**T**	4.881 E-02

				AC3			
1	217	453	GU111996.1_OEL	Unknown	GU112021.1_BYVIV	M,C,S,**T**	1.449 E-05
2	228	453	GU112065.1_BYV	GU112024.1_BYIV	Unknown	M,C,S,**T**	1.449 E-05
3	268	462	GU112008.1_BYIV	GU112021.1_BYVIV	Unknown	M,S,**T**	2.226 E-04
				AC4			
1	19	184	GU111996.1_OEL	NC_014894.1_OEL	GU112004.1_CLC	R,G,M,C,S,T	1.047 E-21
2	24	174	KT390324.1_BYV	GU112088.1_ToL	Unknown	M,C,S,T	2.790 E-05
				AV1			
1	328	768	KT390324.1_BYV	GU112063.1_BYV	KJ462074.1_OEL	G,B,M,**T**	1.274 E-46
2	418	766	GU112050.1_BYIV	GU112025.1_BYV	NC_014894.1_OEL	R,G,B,M,C,S,**T**	1.082 E-19
3	27	204	PP060926.1_BYV	KJ462074.1_OEL	GU112025.1_BYV	**R**,G,M,C,S,T	1.827 E-09
4	42	325	GU112049.1_BYIV	Unknown	GU112009.1_BYV	**R**,G,M,C,S,T	2.950 E_08
5	774	450	GU112063.1_BYV	GU112008.1_BYIV	GU112025.1_BYV	R,G,**M**,S,T	7.744 E-07
6	566	173	GU112008.1_BYIV	Unknown	GU112064.1_BYV	**M**,S,T	1.832 E-06
7	328	758	NC_014845.1_BYV	GU112009.1_BYV	Unknown	M,C,S,**T**	1.109 E-01
8	708	352	GU112082.1_ToLC	GU112059.1_BYV	GU112086.1_ToLC	M,**C**,S	2.047 E-06
9	774	428	KC501921.1_BYV	GU112009.1_BYV	Unknown	R,M,C,S,**T**	5.193 E-02
10	386	764	MK650411.1_BYV	Unknown	GU112009.1_BYV	M,S,**T**	1.476 E-05
11	454	672	GU112082.1_ToLC	GU112035.1_BYIV	GU112086.1_ToLC	B,S,**T**	2.788 E-02

Okra enation leaf curl virus (OELCuV), Bhendi yellow vein India virus (BYVIV), Bhendi yellow vein mosaic virus (BYVMV), Mesta yellow vein mosaic virus (MeYVMV), Radish leaf curl virus (RLCV), Tomato leaf curl New Delhi virus (ToLCNDV), Bhendi yellow vein mosaic betasatellite (BYVMB), Bhendi yellow vein India betasatellite (BYVIB) and Cotton leaf curl Alabad virus (CLCuAV).

nucleotide diversity (π) for DNA-A, DNA-B and betasatellites, yielding values of 0.130, 0.209 and 0.107 respectively. Mutations were also analysed across all open reading frames (ORFs) within the DNA-A component, with the highest levels of genetic variability observed in *AC1* and *AC4*. Notably, DNA-B sequences displayed greater genetic variability than betasatellite sequences across the entire isolate set, emphasizing the dynamic evolution of DNA-B in these viral complexes. The average number of nucleotide differences (*k*) among the DNA-A sequences was 341.25, compared to 542.964 for DNA-B and 125.719 for beta-satellite sequences. Among the DNA-A coding regions, *AC1* exhibited the highest k value at 161.158. To further assess mutational contributions to genetic variability, we calculated the total number of mutations (η) for DNA-A, DNA-B and beta-satellites, which were 2057, 1939 and 1301 respectively. The *AC1* gene exhibited both the highest number of mutations (881) and the highest k value. These findings underscore the significant role of mutational dynamics in driving the diversification of these viral entities.

The analysis of haplotype distribution across DNA-A, its open reading frames (ORFs), DNA-B and betasatellite revealed a total of 50 haplotypes for DNA-A. Among the ORFs, *AV1* and *AC1* exhibited the highest number of haplotypes. In contrast, only 8 haplotypes were detected for DNA-B, while 36 were identified for the beta-satellite. Haplotype diversity (Hd) for the isolates, particularly within the ORFs of DNA-A approached 1, indicating high diversity. Notably, the betasatellite also had an Hd value of 1. These results suggest that the majority of the viral isolates are unique (Table 3).

Neutrality tests, including Tajima's D, Fu & Li's D* and Fu & Li's F*, were conducted to evaluate the selection pressures on DNA-A and its associated ORFs, as well as DNA-B and the betasatellite. Significant statistical deviations were observed across all datasets, with most showing markedly negative values (Table 4). These negative values indicate substantial genetic polymorphism specific to the sequences, suggesting that nucleotide diversity may result from transient polymorphisms being purged by purifying selection or from population expansion. Overall, the results indicate that the begomoviruses associated with *A. esculentus* are under selective pressure.

Discussion

This study elucidates the genetic variability, recombination and population structure of begomoviruses infecting *A. esculentus*. The

Table 3. An overview of the genetic composition of the begomoviruses associated with *Abelmoschus esculentus*, highlighting key elements such as DNA-A, DNA-B, the 6 genes encoded by DNA-A and the associated betasatellite sequences

Virus component	S	η	π	k	θ–η	θ–W	h	Hd
DNA-A	1480	2057	0.13025	341.25	0.17528	0.12611	50	1
AC1	637	881	0.15118	161.158	0.18451	0.13341	49	0.999
AC2	230	312	0.10538	41.837	0.17714	0.12934	45	0.993
AC3	322	464	0.1118	44.832	0.25951	0.18009	47	0.998
AC4	149	186	0.15504	44.496	0.14469	0.11591	36	0.951
AV1	344	466	0.113	82.833	0.14193	0.10477	50	1
AV2	85	124	0.11958	18.775	0.17633	0.12087	29	0.925
DNA-B	1653	1939	0.20899	542.964	0.28785	0.24539	8	1
Betasatellite	852	1301	0.10672	125.719	0.38333	0.17441	36	1

reports of diverse *Begomovirus* species infecting okra point towards the widespread threat posed by these viruses to okra cultivation, particularly in India and other tropical regions.

The phylogenetic analyses of DNA-A, DNA-B and betasatellites revealed the clustering of begomoviruses into distinct evolutionary groups, suggesting that these viruses have evolved through multiple divergent lineages. The four main groups identified -BYVIV, OELCuV, BYVMV and ToLCNDV demonstrate the high diversity of begomoviruses infecting okra plants. This could be attributed to the extensive recombination events observed across the viral genomes. The presence of multiple transition/transversion biases and numerous haplotypes in DNA-A and betasatellite sequences indicate frequent genetic exchanges and diversification.

Table 4. Results of various neutrality tests performed on DNA-A, DNA-B, the open reading frames (ORFs) of DNA-A and the betasatellite

Virus component	Neutrality test		
	Tajima's D	Fu & Li's D	Fu & Li's F
DNA-A	-0.94079	-1.23002	-1.34035
AC1	-0.6601	-1.41255	-1.34689
AC2	-1.47075	-2.08552	-2.21648
AC3	-2.0782	-3.6101	-3.62497
AC4	0.25798	-0.85855	-0.52266
AC5	-1.92315	-1.21644	-1.75066
AV1	-0.74238	-0.16574	-0.46158
AV2	-1.15072	-0.58839	-0.9555
DNA-B	-1.5075	-1.45509	-1.64025
Betasatellite	-2.28499	-3.03191	-3.29469

Recombination events were widespread across the viral genomes, with a significant number of breakpoints identified in DNA-A, DNA-B and betasatellites. This confirms that recombination plays a critical role in the evolution of begomoviruses, allowing them to adapt to different hosts, evade plant defences and enhance their transmission efficiency. The high frequency of recombination within the replication (Rep) and coat protein (CP) genes suggests that these regions are hotspots for genetic exchange, which may contribute to the emergence of new viral strains with enhanced virulence or broader host ranges.

Notably, the absence of significant recombination breakpoints in the *AV2* gene of DNA-A suggests that this region may be more conserved or subject to stronger functional constraints. This may be due to its critical role in viral pathogenicity and RNA-silencing suppression. This pattern aligns with previous studies on *Begomovirus* recombination, where specific regions of the viral genome are more prone to recombination while others remain conserved. This selective recombination may drive the generation of genetic diversity necessary for viral adaptation, while maintaining the integrity of essential viral functions.

The genetic variability analyses indicated high levels of nucleotide diversity (π) and haplotype diversity (*Hd*) across DNA-A, DNA-B and betasatellite sequences, with the Rep gene showing the highest genetic variability. This suggests that the replication-associated protein (Rep) may be under strong positive selection pressure, likely due to its central role in viral replication and interaction with host factors. The significant nucleotide diversity within the viral population reflects the dynamic evolution of these viruses, driven by mutations, recombination and selection pressures exerted by the host immune system and environmental conditions. Moreover, the higher genetic variability in DNA-B compared to betasatellites suggests that the DNA-B component, which encodes movement-related proteins, is more susceptible to evolutionary changes. This could be due to its direct involvement in viral transport within the host, where mutations may enhance viral mobility, leading to increased infection efficiency. In contrast, betasatellites, while still showing high diversity, may evolve at a slower rate due to their auxiliary role in enhancing viral pathogenicity.

The neutrality tests consistently showed negative values across DNA-A, DNA-B and betasatellites, suggesting that begomoviruses associated with *A. esculentus* are under strong purifying selection or have undergone recent population expansion. The negative values imply that deleterious mutations are being removed from the viral population, possibly due to selective pressures exerted by the host's immune response. Alternatively, the negative Tajima's D values could indicate that these viral populations are expanding rapidly, likely due to the broad cultivation of *A. esculentus* in virus-prone regions, which provides a large and consistent host reservoir for viral replication and spread.

The high genetic variability and frequent recombination events observed in begomoviruses pose significant challenges for the management of viral diseases in *A. esculentus*. The emergence of new viral strains through recombination could potentially overcome existing resistant okra varieties, making it difficult to develop durable resistance. The role of insect vectors, particularly *B. tabaci*, in spreading begomoviruses further complicates disease control efforts. Integrated pest management strategies, including vector control, crop rotation and the development of resistant cultivars, are essential to mitigate the impact of these viral diseases.

Future research should focus on characterizing the functional consequences of recombination in begomoviruses, particularly in relation to host adaptation and virulence. Additionally, understanding the interactions between begomoviruses and their insect vectors at the molecular level could provide novel insights into disrupting viral transmission. Finally, the development of molecular tools for rapid diagnosis and the deployment of resistant okra varieties remains key strategies for minimizing the economic losses associated with viral infections in okra.

Conclusion

This study reveals the significant genetic diversity, recombination events and evolutionary dynamics of begomoviruses infecting *A. esculentus*, underscoring the complexity of viral infections in okra. The phylogenetic analyses revealed distinct viral lineages, with high levels of recombination contributing to the emergence of new viral strains, particularly in key genes like Rep and CP. These findings indicate the challenge of managing viral diseases in okra, as the frequent genetic exchanges and high nucleotide diversity make it difficult to develop long-lasting resistance. Furthermore, the study demonstrates that strong purifying selection or population expansion influences the viral population structure. Given the role of insect vectors in spreading these viruses, integrated pest management, vector control and the development of resistant cultivars are essential strategies for reducing the impact of

begomoviruses on okra cultivation. Future research should focus on elucidating the functional implications of recombination and advancing viral management efforts through rapid diagnostic tools and molecular breeding approaches.

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Authors' contributions

CS conceptualized the study, conducted the research, performed data analysis and wrote the manuscript. AJ contributed to data collection, methodology and manuscript drafting. RK helped with the data analysis. YK supervised the study, provided guidance throughout the research process and reviewed the manuscript for critical revisions. All authors read and approved the final version of the manuscript.

Compliance with ethical standards

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