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Identification of Begomoviruses from Three Cryptic Species of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) in Nepal

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Abstract: The *Bemisia tabaci* species complex consists of at least 44 cryptic species, which are potential vectors of approximately 320 begomovirus species, most of which are significant plant viruses. However, the relationship of begomovirus transmission through vectors at the cryptic species level is uncertain. In our previous study, three cryptic species (Asia I, Asia II 1, and Asia II 5) of *B. tabaci* were identified from 76 *B. tabaci* samples collected across 23 districts in Nepal. Using the same individuals we identified seven different begomovirus species (Squash leaf curl China virus [SLCCNV], Tomato leaf curl New Delhi virus [ToLCNDV], Okra enation leaf curl virus [OELCuV], Synedrella leaf curl virus [SyLCV], Tomato leaf curl Kerala virus [ToLCKeV], Ageratum enation virus [AEV], and Tomato leaf curl Karnataka virus [ToLCKV]) by PCR using universal begomovirus primers. The begomoviruses were detected in 55.26% of whitefly samples, and SLCCNV was the most prevalent species (27.63%). Among the three cryptic species of *B. tabaci*, the virus detection rate was highest in Asia I (60%), followed by Asia II 1 (58.82%) and Asia II 5 (53.06%). Most viruses were detected in all three species, but AEV and ToLCKV were found only in Asia I and Asia II 1, respectively. Geographic analysis showed that SLCCNV was distributed in the whole country, which is similar to the distribution of the Asia II 5 species, but OELCuV and SyLCV were detected only in the middle region of Nepal. Our results provide important information on the begomovirus profile in Nepal which can be beneficial for plant virus risk assessment and develop the management strategies to reduce the damage of whitefly transmitted viruses.

Keywords: *Bemisia tabaci*; begomovirus; cryptic species; geographic distribution; vector insect

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1. Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a vector of at least 320 plant viruses of the genera *Begomovirus*, *Carlavirus*, *Crinivirus*, *Ipomovirus*, *Polerovirus* and Torradovirus [1–4]. Particularly, *B. tabaci* is the only vector of Begomovirus, such as the Tomato yellow leaf curl virus (TYLCV), Tomato leaf curl New Delhi virus (ToLCNDV), Squash leaf curl China virus (SLCCNV), and African cassava mosaic virus (ACMV) [5]. These viruses can be transmitted in a persistent, circulative, and non-propagative manner [1,6]. Furthermore, *B. tabaci* is geographically distributed worldwide with great genetic diversity, forming a species complex consisting of at least 44 morphologically indistinguishable cryptic species [7–15]. For example, two cryptic species—Middle East Asia Minor 1 (MEAM1) and Mediterranean (MED)—are highly invasive and cause significant economic damage to various crops worldwide, whereas most other cryptic species are indigenous to specific regions (i.e., Asia and Africa) [16]. In addition, this species has a broad host range of at

least 600 different plant species [17]. These characteristics of *B. tabaci* indicate that this species has significant potential to transmit plant viruses into various crops and spread them widely into most countries across the world.

Previous studies reported the specific interaction between the *B. tabaci* cryptic species and the transmission rate of begomoviruses [18–20]. For example, two cryptic species of *B. tabaci*—MEAM1 and MED—efficiently transmit the TYLCV, which is highly virulent in the tomato plant [18]. Similarly, the Cotton leaf curl Multan virus (CLCuMuV) transmission by Asia II 1 was higher than those of the cryptic species MEAM1, MED, and Asia I [21]. Bedford et al. [22] and Sánchez-Campos et al. [23] also suggested that different species from the *B. tabaci* species complex exhibited differential capacities to transmit begomoviruses.

Prevalence and diversity studies of plant viruses are usually based on infected plant samples with virus-like disease symptoms. However, a vector-based plant virus identification technique has been conducted in several studies and provides several advantages [24,25]. Using this technique, which relies on PCR assay of vector insects, researchers can easily detect the viruses and survey their geographic distribution. More importantly, this technique provides information on the relationships between vectors and plant viruses.

Information on the occurrence, distribution, and economic loss of begomovirus in Nepal is limited. Until now, only a few begomoviruses, such as Mungbean yellow vein mosaic Indian virus (MYMIV) [26], Pea leaf distortion virus (PLDV) [27], Ageratum enation virus (AEV) [28] and Ageratum yellow vein virus (AYVV) [29] have been reported in Nepal. Ghimire et al. [30] reported the occurrence of Tomato yellow leaf curl virus (TYLCV) disease in most tomato growing pockets in Nepal and recorded more than 40% yield loss. There are many vegetable and fruit crops showing various begomovirus-like symptoms in Nepal. In addition, *B. tabaci* which is a vector of begomoviruses is abundant in agricultural fields in Nepal. However, the presence of begomovirus species and their potential vectors are still unknown in Nepal.

In our previous study on the genetic and geographic distribution of *B. tabaci* species complex in Nepal, we found three cryptic species: Asia I, Asia II 1, and Asia II 5 [31]. We expected that the geographic distribution of begomoviruses that were ingested by different cryptic species of *B. tabaci* can be determined by analyzing whitefly samples in Nepal. Thus, we analyzed the begomovirus profile in the three cryptic species of *B. tabaci* in Nepal using a vector-based virus detection technique. This study provides important information on the geographic distribution of begomovirus in Nepal and the potential relationships between *B. tabaci* cryptic species and begomoviruses.

2. Materials and Methods

2.1. Whitefly Samples

We collected seventy-six *B. tabaci* samples during the summer of 2017–2019 across 23 districts from east to west Nepal (Table 1) for identification of *B. tabaci* cryptic species and their geographic distribution, which were reported in our previous study [31]. Samples were collected from different host plants, including vegetables (*Abelmoschus esculentus*, *Cucurbita pepo*, *Cucumis sativus*, *Phaseolus vulgaris*, and *Sechium edule*,), oilseed crop (*Sesamum indicum*), flowers (*Bougainvillea spectabilis*, *Hibiscus rosa-sinensis*, *Ixora* sp., and *Salvia officinalis*), tuber crop (*Smallanthus sonchifolius*) and fruit plant (*Psidium guajava*). All the samples were preserved in ethanol (95%) and stored at −20 °C for further analysis. We used the same individuals of *B. tabaci* for this study.

Table 1. Details of *Bemisia tabaci* samples were collected from different regions in Nepal along with the most identical sequences information from NCBI including their percent identities, numbers of identical bases and accession numbers.

S.N.	Samples Name	Collection Sites	Collection Dates	Plant Viruses Detected in *B. tabaci*				
				Virus Name	Accession Numbers [1]	% Identity (NCBI)	Numbers of Identical Bases	Accession Numbers [2]
1	Ktm-Cuc-10	Kritipur, Kathmandu	18 July 2017	ToLCNDV	MZ345131	98.74	1022/1035	AY428769
2	Lal-Pum-11	Lele, Lalitpur	19 July 2017	SyLCV	MZ345128	99.61	1034/1038	MK784250
3	Ktm-Cuc-13	Syuchatar, Kathmandu	28 July 2017	OELCuV	MZ345122	99.33	1037/1044	MK784275
4	Ktm-Tom-15	Syuchatar, Kathmandu	28 July 2017	OELCuV	MZ345123	99.33	1037/1044	MK784275
5	Ktm-Pum-17	Dahachwok, Kathmandu	12 August 2017	OELCuV	MZ345124	99.33	1037/1044	MK784275
6	Ktm-Tom-18	Dahachwok, Kathmandu	12 August 2017	SyLCV	MZ345129	99.81	1036/1038	MK784250
7	Gor-Pum-19	Gorkha, Gorkha	13 August 2017	OELCuV	MZ345125	99.33	1037/1044	MK784275
8	Kas-Tom-20	Pokhara, Kaski	13 August 2017	SyLCV	MZ345130	99.61	1034/1038	MK784250
9	Naw-Pum-24	Daldale, Nawalparasi	17 July 2019	SLCCNV	MZ362879	97.98	1018/1039	DQ026296
10	Naw-Okr-25	Gaidakot, Nawalparasi	17 July 2019	OELCuV	MZ345126	99.62	1039/1043	KT390454
11	Naw-Pum-26	Gaidakot, Nawalparasi	17 July 2019	SLCCNV	MZ362880	98.27	1018/1039	DQ026296
12	Chi-Okr-27	Tandi, Chitwan	17 July 2019	OELCuV	MZ345127	99.62	1040/1044	KT390454
13	Chi-Pum-28	Tandi, Chitwan	17 July 2019	SLCCNV	MZ357192	98.07	1017/1037	MN294705
14	Chi-Bri-29	Ramnagar, Chitwan	25 July 2019	AEV	MZ291461	99.42	1031/1037	JX436473
15	Gor-Cum-32	Manakamana, Gorkha	17 July 2019	ToLCNDV	MZ345132	98.65	1021/1035	KM383742
16	Gor-Bri-34	Ghyalchwok, Gorkha	22 May 2019	SLCCNV	MZ357193	98.94	1026/1037	MN294705
17	Pyu-Cum-35	Khaira, Pyuthan	15 August 2019	ToLCNDV	MZ345133	97.97	1014/1035	KM383742
18	Dan-Bri-36	Ghorahi, Dang	23 July 2019	ToLCKV	MZ291462	99.23	1030/1038	LN878125
19	Dan-Tom-37	Ghorahi, Dang	23 July 2019	ToLCNDV	MZ345134	97.1	1005/1035	KM383742
20	Dan-Pum-38	Ghorahi, Dang	23 July 2019	SLCCNV	MZ357194	98.07	1017/1037	MN294705
21	Ban-Pum-39	Koholpur, Banke	23 July 2019	ToLCKeV	MZ328173	98.94	1031/1042	KF551575
22	Ban-Cum-40	Koholpur, Banke	23 July 2019	ToLCKeV	MZ328174	98.94	1031/1042	KF551575
23	Ban-Bri-41	Koholpur, Banke	23 July 2019	SLCCNV	MZ357195	98.36	1020/1037	MT081229
24	Kan-Cuc-45	Mahendranagar, Kanchanpur	24 July 2019	SLCCNV	MZ357196	98.26	1019/1037	MN294705
25	Kav-Pum-46	Banepa, Kavre	28 July 2019	SLCCNV	MZ357197	98.36	1020/1037	MT081229
26	Mor-Pum-47	Biratnagar, Morang	1 August 2019	SLCCNV	MZ357198	98.36	1020/1037	MT081229
27	Sun-Pum-49	Dharan, Sunsari	1 August 2019	SLCCNV	MZ357199	97.69	1013/1037	EU573715
28	Sun-Bri-51	Dharan, Sunsari	1 August 2019	ToLCNDV	MZ345135	98.74	1022/1035	KM383742
29	Dhk-Pum-52	Guthitar, Dhankuta	1 August 2019	SLCCNV	MZ357200	97.78	1014/1037	EU573715
30	Ilm-Tom-53	Fikkal, Ilam	2 August 2019	SLCCNV	MZ357201	97.69	1013/1037	EU573715
31	Ilm-Pum-54	Fikkal, Ilam	2 August 2019	SLCCNV	MZ357202	97.69	1013/1037	EU573715
32	Ilm-Bri-55	Fikkal, Ilam	2 August 2019	SLCCNV	MZ357203	98.26	1019/1037	MN294705
33	Pan-Pum-58	Lalikharka, Panchthar	3 August 2019	SLCCNV	MZ357204	98.26	1019/1037	MN294705
34	Jha-Bri-59	Kakadvitta, Jhapa	3 August 2019	SLCCNV	MZ357205	97.97	1016/1037	MN294705
35	Jha-Pum-60	Kakadvitta, Jhapa	3 August 2019	SLCCNV	MZ357206	97.69	1013/1037	EU573715
36	Lal-Tom-64	Godamchaur, Lalitpur	18 July 2019	ToLCNDV	MZ345136	98.74	1022/1035	AY428769
37	Ktm-Cha-67	Banasthali, Kathmandu	6 June 2019	SLCCNV	MZ357207	98.17	1018/1037	MT081229
38	Ktm-Tom-69	Naikap, Kathmandu	29 July 2019	ToLCNDV	MZ345137	98.84	1023/1035	AY428769
39	Ktm-Pum-71	New Baneshwor, Kathmandu	15 June 2019	SLCCNV	MZ357208	98.46	1021/1037	MT081229
40	Ktm-Tom-72	Dahachwok, Kathmandu	30 July 2019	ToLCNDV	MZ345138	98.84	1023/1035	AY428769
41	Bha-Cuc-74	Sanga, Bhaktapur	28 July 2019	SLCCNV	MZ357209	98.26	1019/1037	MT081229
42	Bha-Bri-75	Sanga, Bhaktapur	28 July 2019	SLCCNV	MZ357210	98.26	1019/1037	MT081229

[1] The 6th column refers to the GenBank accession numbers assigned in this work. [2] The 9th column refers to the closest database isolates in the GenBank.

2.2. DNA Extraction and Polymerase Chain Reaction

Total genomic DNA was extracted from individual whitefly using a PureLink Genomic DNA Mini Kit (Invitrogen, CA, USA), as described in the commercial kit. A single adult of *B. tabaci* was placed in a 1.5 mL centrifuge tube containing digestion buffer (180 µL) and homogenized with a plastic homogenizer. By adding 20 µL proteinase K, the sample was incubated at 55 °C for 4 h. Furthermore, DNA was purified using genomic spin column, wash buffer 1 and 2, as described in the kit. Begomovirus acquisition of *B. tabaci* was determined using universal begomovirus primers Begomo1 (5′CCGTGCTGCTGCCCCCATTGTCCGCGTCAC-3′) and Begomo2 (5′CTGCCACAACCATGGATTCACGCACAGGG-3′) [32]. The PCR product size was around 1100 bp long. For positive and negative controls, we used DNA from the TYLCV-viruliferous *B. tabaci* and non-viruliferous *B. tabaci*, respectively, which were reared at Insect Molecular Physiology Laboratory, Kyungpook National University, Daegu, Korea. The total PCR volume of 30 µL contained 15 µL of Solg^{TM} 2x Taq Pre-Mix (Solgent, Daejeon, Korea), 2 µL of each primer (10 pmol/µL), 3 µL of the DNA solution, and 8 µL of distilled water. The mixtures were amplified in a Veriti 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA) with an initial denaturation at 95 °C for 2 min, followed by 35 cycles of 94 °C for 40 s denaturation, 65 °C for 1 min annealing, 72 °C for 30 s extension, and final extension of 7 min at 72 °C. The gel electrophoresis was performed using 1%

agarose gel, stained with ethidium bromide solution, and visualized under ultraviolet light. Finally, the amplified PCR products from the gel were purified using the Wizard PCR preps DNA purification system (Promega, Madison, WI, USA).

2.3. Sequence Alignment and Phylogenetic Analyses

Total 42 purified PCR DNA samples were sequenced by using the ABI Prism 3730XL DNA Analyzer (50 cm capillary) (DNA Sequencer) and a BigDye® Terminator v3.1 cycle Sequencing kit (Applied Biosystems) at the Solgent Sequencing Facility (Solgent, Daejeon, Korea). CLUSTAL W [33] was used to align the raw nucleotide sequences, and the resulting alignment was manually edited. Sequences were identified by BLAST searches to the GenBank database of the National Center for Biotechnology Information (NCBI) [34]. Phylogenetic analyses were performed by using the MEGA 6.0 program [35]. The maximum likelihood method was implemented to construct the phylogenetic tree. The robustness of the phylogenetic tree was assessed by 1000 bootstrap replicates [36].

3. Results

3.1. Identification of Begomovirus from Different Cryptic Species of Bemisia tabaci and Their Distribution

Begomoviruses were detected from all three cryptic species sampled in Nepal (Table 1). We identified 7 different begomoviruses: the SLCCNV, ToLCNDV, Okra enation leaf curl virus (OELCuV), Synedrella leaf curl virus (SyLCV), Tomato leaf curl Kerala virus (ToLCKeV), AEV, and Tomato leaf curl Karnataka virus (ToLCKV) from three cryptic species of *B. tabaci* collected from 18 districts in Nepal (Figures 1 and 2).

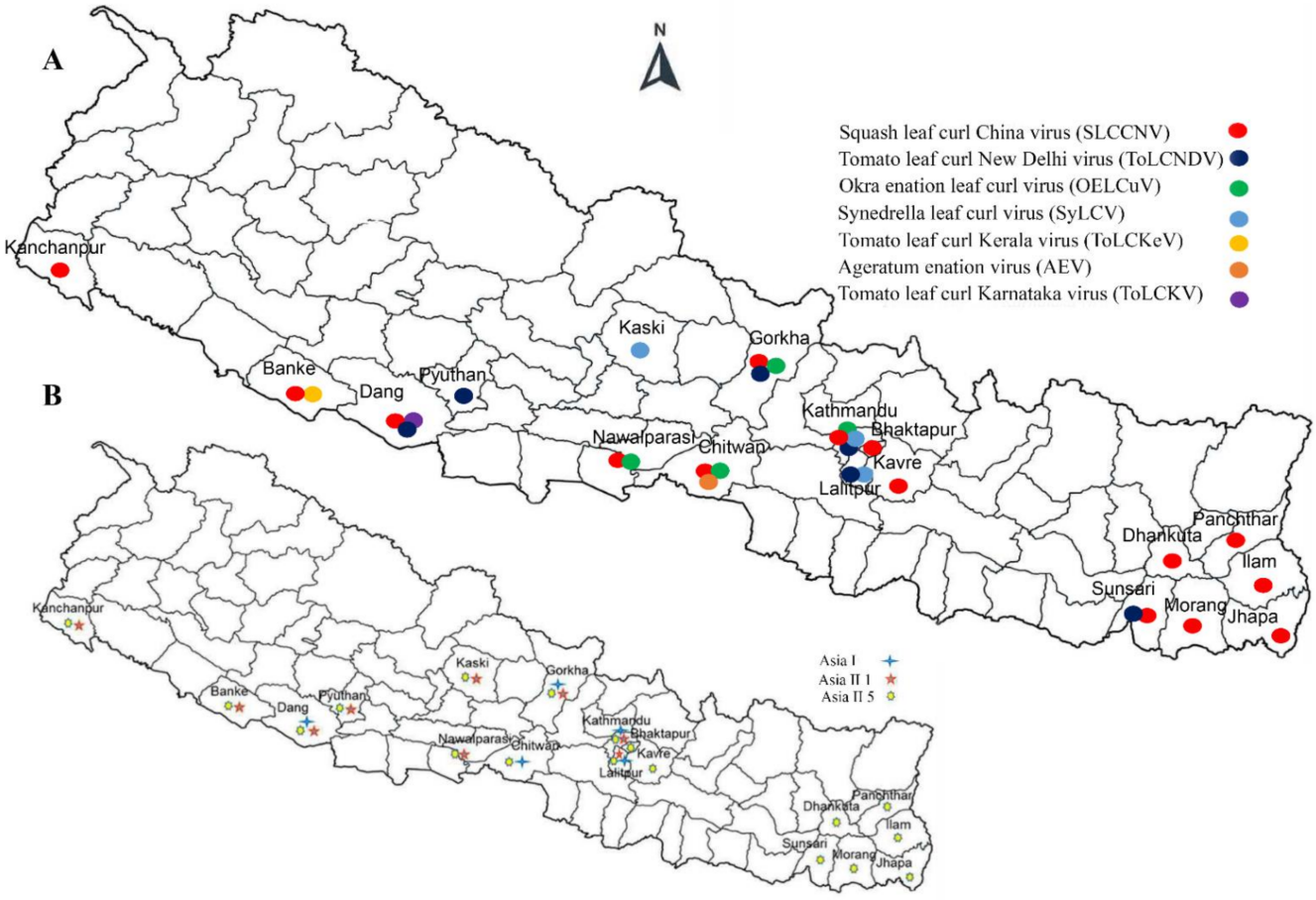

Figure 1. Map of Nepal showing the distribution of the begomoviruses (**A**) and distribution of *Bemisia tabaci* cryptic species (**B**) [31] in the country. The seven different colors represent the detected begomoviruses species from the different cryptic species of *B. tabaci* (**A**) whereas different symbols represent *B. tabaci* cryptic species (**B**).

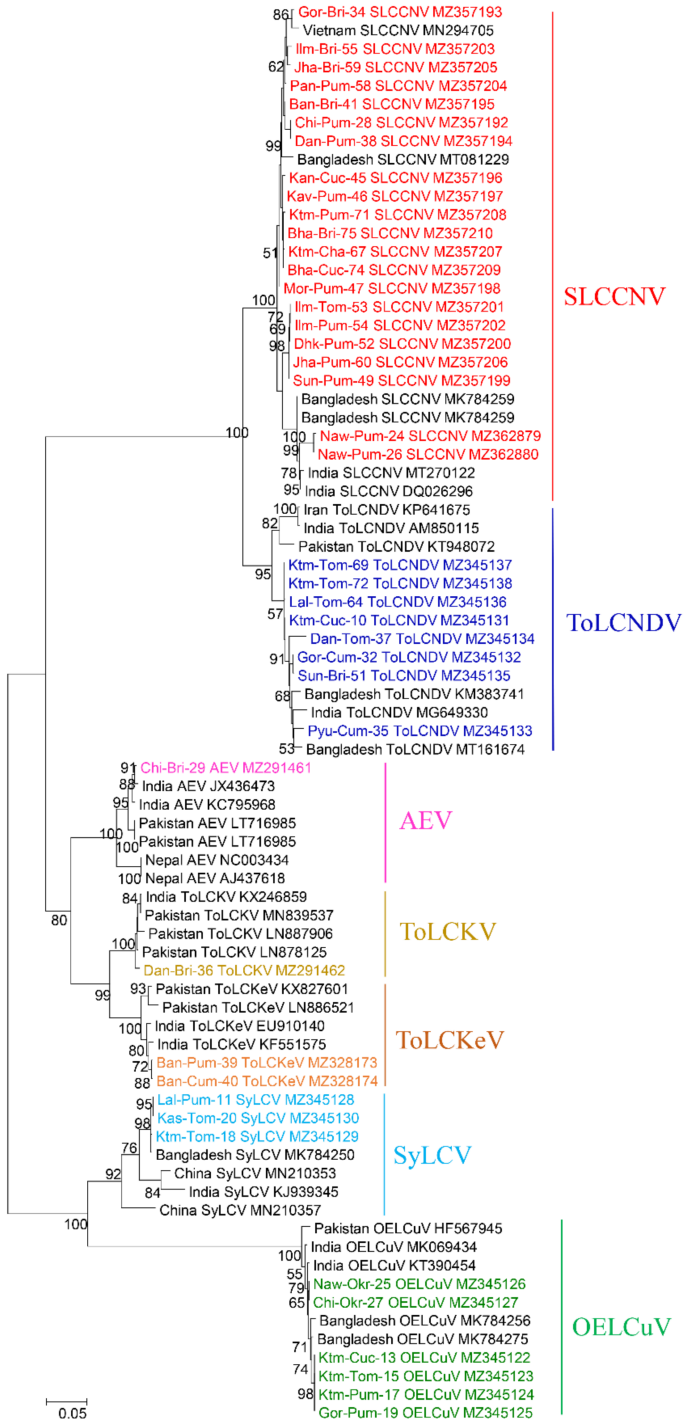

Figure 2. Maximum likelihood phylogenetic tree of begomoviruses sequences detected from three different cryptic species of *Bemisia tabaci* in Nepal. Different colors indicate begomovirus sequences from our samples (42 sequences), and black color indicates reference sequences obtained from the GenBank database (35 sequences). The phylogenetic tree was built in Mega6 using the HKY+G model.

Among them, the detection rate of begomoviruses was highest for SLCCNV (27.63%; 21/76) followed by ToLCNDV (10.53%; 8/76), OELCuV (7.89%; 6/76), SyLCV (3.95%; 3/76), and ToLCKeV (2.63%; 2/76). Both AEV and ToLCKV were detected equally at the lowest rate of 1.32% (1/76) (Figure 3). The total detection rate of the begomoviruses in *B. tabaci* was 55.26% (42/76) (Figure 3). The virus detection rate was similar in three cryptic species such as Asia I (60%; 6/10), Asia II 1 (58.82%; 10/17), and Asia II 5 (53.06%; 26/49).

The detection rate of SLCCNV and TOLCNDV were highest in Asia II 5, whereas OELCuV was detected in high frequency in Asia I and Asia II 5. SLCCNV, ToLCNDV, and OELCuV were detected in all three cryptic species, whereas AEV and ToLCKV were found only in Asia I and Asia II 1, respectively (Figure 4).

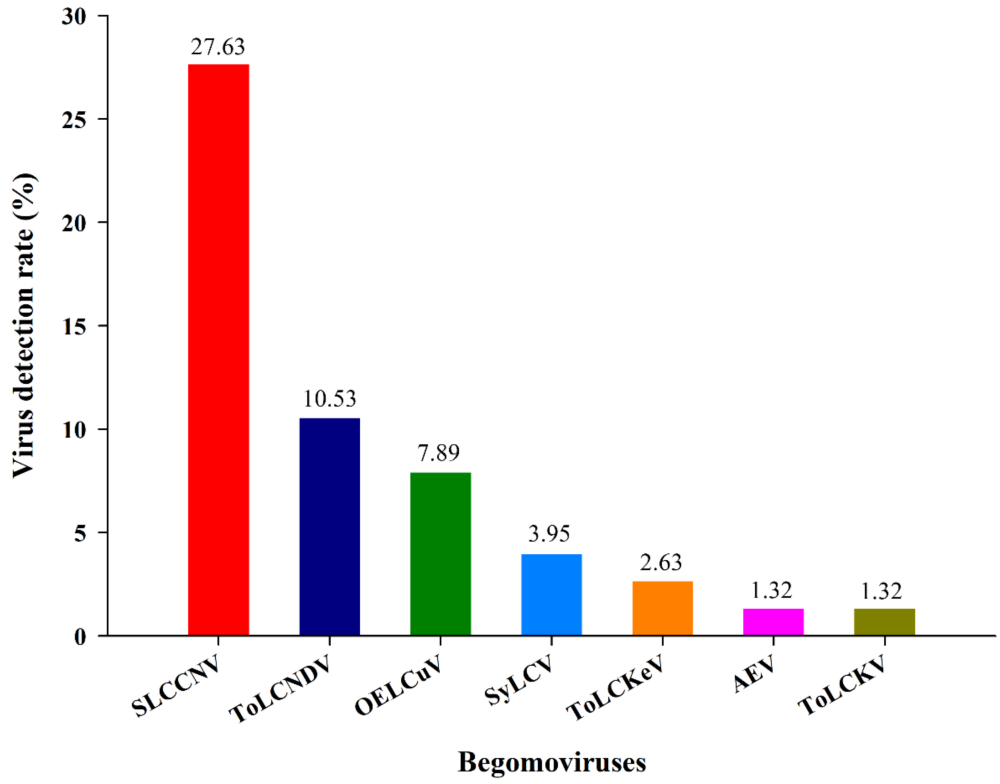

Figure 3. Detection rates of different begomoviruses from *Bemisia tabaci* in Nepal.

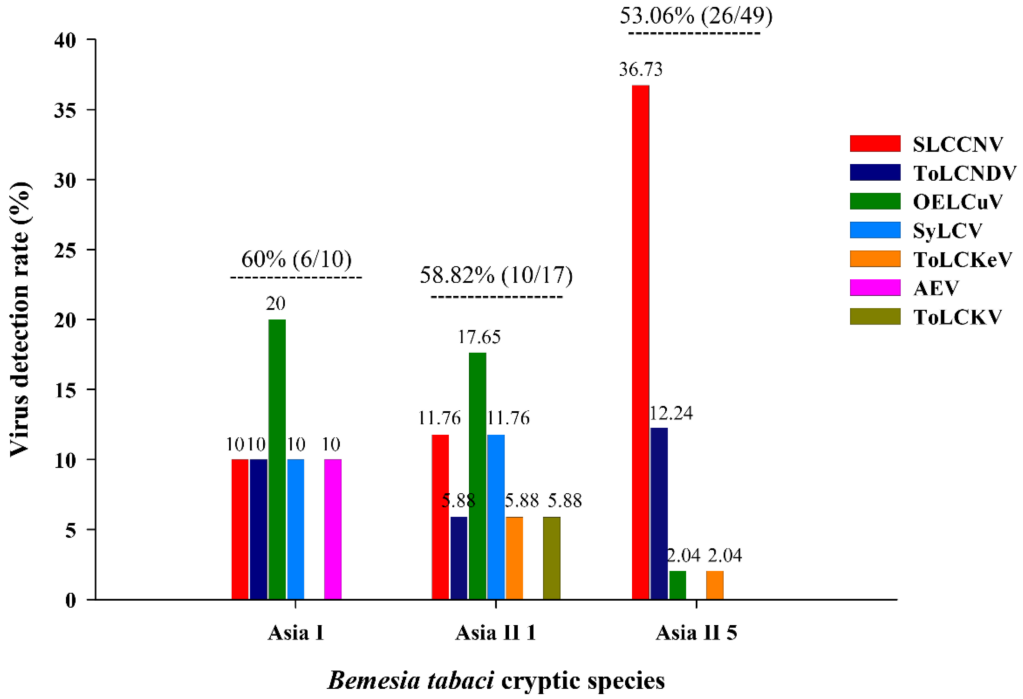

Figure 4. Detection rates of different begomoviruses from three cryptic species of *Bemisia tabaci* in Nepal.

The geographic distribution of SLCCNV was widespread in Nepal, but both OELCuV and SyLCV were found in the middle region of Nepal, whereas AEV was found in only one location in the Chitwan district located in the central-southern part of Nepal (Figure 1). The nucleotide sequence variations of the seven identified begomoviruses were in the range of 97.10%–99.81% with the related sequences in the GenBank database (Table 1).

3.2. Identification of the Begomoviruses in B. tabaci Collected from Different Host Plants

We collected *B. tabaci* from 14 plant species: 7 vegetable crops (*Abelmoschus esculentus*, *Cucurbita pepo*, *Cucumis sativus*, *Sechium edule*, *Solanum lycopersicum*, *Solanum melongena*, and *Phaseolus vulgaris*), 4 flower plants (*Hibiscus rosa-sinensis*, *Ixora* sp., *Bougainvillea spectabilis*, *Salvia officinalis*), 1 fruit plant (*Psidium guajava*), 1 oilseed crop (*Sesamum indicum*), and 1 tuber crop (*Smallanthus sonchifolius*). The begomoviruses were identified in *B. tabaci* infesting only in the vegetable crops, but they were not identified in the flower plants, fruit, oilseed, and tuber crop. In addition, SLCCNV was identified in *B. tabaci* infesting in *C. pepo*, *C. sativus*, *S. edule*, *S. lycopersicum*, and *S. melongena*. On the other hand, ToLCNDV was identified in *C. sativus*, *S. lycopersicum*, and *S. melongena*, and OELCuV was identified in *A. esculentus*, *C. pepo*, *C. sativus*, and *S. lycopersicum*. Finally, AEV and ToLCKV were identified in the *B. tabaci* samples infesting only *S. melongena* (Table 2).

Table 2. List of the begomoviruses detected from the three cryptic species of *Bemisia tabaci* collected from various host plants in Nepal.

S.N.	Begomovirus	*B. tabaci* Cryptic Species (Number of Virus Detected Samples)	Host Plants for *B. tabaci*
1	Squash leaf curl China virus (SLCCNV)	Asia I (1), Asia II 1 (2), Asia II 5 (18)	*Cucurbita pepo*, *Cucumis sativus*, *Sechium edule*, *Solanum lycopersicum*, *Solanum melongena*
2	Tomato leaf curl New Delhi virus (ToLCNDV)	Asia I (1), Asia II 1 (1), Asia II 5 (6)	*Cucumis sativus*, *Solanum lycopersicum*, *Solanum melongena*
3	Okra enation leaf curl virus (OELCuV)	Asia I (2), Asia II 1 (3), Asia II 5 (1)	*Abelmoschus esculentus*, *Cucurbita pepo*, *Cucumis sativus*, *Solanum lycopersicum*
4	Synedrella leaf curl virus (SyLCV)	Asia I (1), Asia II 1 (2)	*Cucurbita pepo*, *Solanum lycopersicum*
5	Tomato leaf curl Kerala virus (ToLCKeV)	Asia II 1 (1), Asia II 5 (1)	*Cucurbita pepo*, *Cucumis sativus*
6	Ageratum enation virus (AEV)	Asia I (1)	*Solanum melongena*
7	Tomato leaf Curl Karnataka virus (ToLCKV)	Asia II 1 (1)	*Solanum melongena*

4. Discussion

We identified seven different begomoviruses (SLCCNV, ToLCNDV, OELCuV, SyLCV, ToLCKeV, AEV, and ToLCKV) from whitefly samples collected from 18 different locations in Nepal. Among them, only AEV was previously recorded in Nepal [28]. However, the other six begomoviruses were identified for the first time in this study in Nepal. The overall begomovirus detection rate in *B. tabaci* was 55.26%. While the total percentage of whiteflies containing begomoviruses was similar among the three species, yet the prevalence of the specific begomoviruses differed within the species.

Our results indicated the different geographic distribution of identified begomoviruses. SLCCNV was distributed in the whole country while the other six viruses were distributed in the middle and west regions of Nepal. In comparison with our previous study [31], the begomovirus profile is highly associated with the geographic distribution of three cryptic species of *B. tabaci*. Particularly, investigation at the eastern region of Nepal showed that distribution of only Asia II 5 which ingested mostly SLCCNV. This result suggests that a strong relationship between begomovirus species and *B. tabaci* cryptic species.

The bipartite SLCCNV is one of the major begomoviruses in Asian countries, including China, India, Pakistan, Malaysia, Vietnam, and Phillippines in the Cucurbitaceae crops [37–39]. Our finding is the first report of SLCCNV presence in Nepal. This virus was

detected in the Asia I, Asia II 1, and mostly from Asia II 5 species, which is the most abundant cryptic species of *B. tabaci* in Nepal [31]. In a previous study, Khatun et al. [40] detected SLCCNV only in the Asia I species. Previously, Dolores and Valdez [39] detected this virus only in squash and pumpkin but not in cucumber and melon. Similarly, Khatun et al. [40] detected this virus in *B. tabaci* collected from *S. melongena* and *Nicotiana tabacum*. However, we detected this virus in *B. tabaci* collected from *C. pepo*, *C. sativus*, *S. edule*, *S. lycopersicum*, and *S. melongena*. This result suggested that the host range of SLCCNV is not restricted only in Cucurbitaceae crops but also poses a potential threat to non-cucurbitaceae crops.

The Tomato leaf curl virus (ToLCV) is one of the most important economic viruses for solanaceous crops in Asia [25,40–44]. In India, 21 different types of ToLCV were recorded [43]. Three types of ToLCV (ToLCNDV, ToLCKeV, and ToLCKV) were found in this study. Among them, ToLCNDV was detected in all three cryptic species from host crops—*C. sativus*, *S. lycopersicum*, and *S. melongena.* Geographically, it was also distributed from the east (Sunsari district) to the west (Banke district) part of Nepal. Our results are similar to those found by Khatun et al. [40]. They also detected ToLCNDV in three cryptic species (Asia I, Asia II 1, and Asia II 5) of *B. tabaci* that infested *P. vulgaris*, *S. melongena*, *Dahlia* sp., and *S. lycopersicum*. The ToLCKeV is another important virus infecting solanaceous crops. It is widely distributed in India and Pakistan [25,45]. In Nepal, the ToLCKeV was detected in two cryptic species of *B. tabaci*—Asia II 1 and Asia II 5—in the host plants *C. pepo* and *C. sativus*. We identified it only from the Banke district, which is located very close to India. We suppose that ToLCKeV was disseminated via *B. tabaci* from India, as it was detected near India. The ToLCKV is also an important virus infecting solanaceous crops and is widely distributed in India and Pakistan [25,46]. We detected ToLCKV in only one sample in Asia II 1 from the host plant *S. melongena*. We identified it from only one location in Dang, located midwest-southern part of Nepal.

The OELCuV virus was firstly identified in India and later in Pakistan, Bangladesh, Iran, and African countries [40,47–54]. It caused severe disease in okra and an 80% yield loss in India [52]. We detected this virus in the central and western parts of Nepal, including Chitwan, Nawalparasi, Gorkha, and Kathmandu. All three cryptic species of *B. tabaci*, Asia I, Asia II 1, and Asia II 5 from host plants *A. esculentus*, *C. pepo*, *C. sativus*, *S. lycopersicum* were infected by this virus in Nepal. However, OELCuV was associated with only Asia I and Asia II 5 in Bangladesh [40]. Hameed et al. [55] reported that the OELCuV is related to the Cotton leaf curl virus, which is more efficiently transmitted by Asia II 1 [56]. Therefore, the OELCuV is a significant potential threat to okra production in Nepal.

The SyLCV is an old-world monopartite begomovirus reported from India, China, and Bangladesh [40]. We found SyLCV from Kaski, Kathmandu, and Lalitpur districts. It was detected in the Asia I and Asia II 1 species of *B. tabaci* in the host plants *C. pepo* and *S. lycopersicum*. In Bangladesh, this virus was found in the species Asia I of *B. tabaci* in the host plant *S. melongena* [40]. Therefore, it suggests that SyLCV has a diverse host range and can be acquired by multiple cryptic species of *B. tabaci*.

The AEV is a monopartite begomovirus and was first identified in Nepal in the late 1990s [28,57]. Currently, it is widely distributed in northern India [28,58–61] and Pakistan [57]. This virus mainly infects the weed plants under the genus *Ageratum* and also infects agricultural crops [57]. As we did not collect the *B. tabaci* samples from weed plants, we found this virus from only one sample collected from Chitwan district, southern Nepal, which is near the border to India. We detected AEV in the Asia I species of *B. tabaci* collected from the host plant *S. melongena*, suggesting a new potential host plant, which needs to be validated in the future. Other plants, including *S. lycopersicum* [62], *Carica papaya* [63], and *Brassica rapa* var. *rapa* [57], were also recorded as the host plants of AEV.

Our results suggest that a vector-based plant virus detection technique provides important information to understand the distribution of begomoviruses. Furthermore, future analysis of plant samples, as well as the characterization of identified viruses such as full genome sequences (DNA A/B) and associated satellites, are required to determine the precise profile of begomoviruses in Nepal.

5. Conclusions

Our study provides the begomovirus profile and their geographic distribution in Nepal using a vector-based PCR analysis at the cryptic species level of *B. tabaci*. Overall, we detected seven different begomovirus species, including SLCCNV, ToLCNDV, OELCuV, SyLCV, ToLCKeV, AEV, and ToLCKV. SLCCNV was distributed in the whole country while the other six viruses were distributed in the middle and west regions of Nepal. The results of this study can be beneficial for virus disease risk assessment and devising management plans to limit the spread of the whitefly transmitted viruses in Nepal.

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References

1. Hogenhout, S.A.; Ammar, E.-D.; Whitfield, A.E.; Redinbaugh, M.G. Insect Vector Interactions with Persistently Transmitted Viruses. *Annu. Rev. Phytopathol.* **2008**, *46*, 327–359. [CrossRef]
2. Navas-Castillo, J.; Fiallo-Olivé, E.; Sánchez-Campos, S. Emerging Virus Diseases Transmitted by Whiteflies. *Annu. Rev. Phytopathol.* **2011**, *49*, 219–248. [CrossRef] [PubMed]
3. Krause-Sakate, R.; Watanabe, L.F.M.; Gorayeb, E.S.; Da Silva, F.B.; Alvarez, D.D.L.; Bello, V.H.; Nogueira, A.M.; De Marchi, B.R.; Vicentin, E.; Ribeiro-Junior, M.R.; et al. Population Dynamics of Whiteflies and Associated Viruses in South America: Research Progress and Perspectives. *Insects* **2020**, *11*, 847. [CrossRef] [PubMed]
4. Nigam, D. Genomic Variation and Diversification in Begomovirus Genome in Implication to Host and Vector Adaptation. *Plants* **2021**, *10*, 1706. [CrossRef]
5. Varma, A.; Mandal, B.; Singh, M.K. *Global Emergence and Spread of Whitefly (Bemisia tabaci) Transmitted Geminiviruses*; Thompson, W.M.O., Ed.; Springer: Dordrecht, The Netherlands, 2011; ISBN 9789400715240.
6. Rubinstein, G.; Czosnek, H. Long-term association of tomato yellow leaf curl virus with its whitefly vector *Bemisia tabaci*: Effect on the insect transmission capacity, longevity and fecundity. *J. Gen. Virol.* **1997**, *78*, 2683–2689. [CrossRef]
7. Kanakala, S.; Ghanim, M. Global genetic diversity and geographical distribution of *Bemisia tabaci* and its bacterial endosymbionts. *PLoS ONE* **2019**, *14*, e0213946. [CrossRef]
8. Hu, J.; Zhang, X.; Jiang, Z.; Zhang, F.; Liu, Y.; Li, Z.; Zhang, Z. New putative cryptic species detection and genetic network analysis of *Bemisia tabaci* (Hempitera: Aleyrodidae) in China based on mitochondrial COI sequences. Mitochondrial DNA Part A DNA Mapping. *Seq. Anal.* **2018**, *29*, 474–484. [CrossRef]
9. Firdaus, S.; Vosman, B.; Hidayati, N.; Supena, E.D.J.; Visser, R.G.; Van Heusden, A.W. The *Bemisia tabaci* species complex: Additions from different parts of the world. *Insect Sci.* **2013**, *20*, 723–733. [CrossRef]
10. Esterhuizen, L.L.; Mabasa, K.G.; van Heerden, S.W.; Czosnek, H.; Brown, J.K.; van Heerden, H.; Rey, M.E.C. Genetic identification of members of the *Bemisia tabaci* cryptic species complex from South Africa reveals native and introduced haplotypes. *J. Appl. Èntomol.* **2013**, *137*, 122–135. [CrossRef]
11. Chowda-Reddy, R.; Kirankumar, M.; Seal, S.E.; Muniyappa, V.; Valand, G.B.; Govindappa, M.R.; Colvin, J. *Bemisia tabaci* Phylogenetic Groups in India and the Relative Transmission Efficacy of *Tomato leaf curl Bangalore virus* by an Indigenous and an Exotic Population. *J. Integr. Agric.* **2012**, *11*, 235–248. [CrossRef]
12. Alemandri, V.; De Barro, P.; Bejerman, N.; Caro, E.B.A.; Dumón, A.D.; Mattio, M.F.; Rodriguez, S.M.; Truol, G. Species within the *Bemisia tabaci* (Hemiptera: Aleyrodidae) Complex in Soybean and Bean Crops in Argentina. *J. Econ. Èntomol.* **2012**, *105*, 48–53. [CrossRef] [PubMed]
13. Boykin, L.M.; Armstrong, K.; Kubatko, L.; De Barro, P.J. Species Delimitation and Global Biosecurity. *Evol. Bioinform.* **2011**, *8*, EBO.S8532–37. [CrossRef]

14. De Barro, P.J.; Liu, S.-S.; Boykin, L.; Dinsdale, A.B. *Bemisia tabaci*: A Statement of Species Status. *Annu. Rev. Èntomol.* **2011**, *56*, 1–19. [CrossRef]
15. Dinsdale, A.; Cook, L.G.; Riginos, C.; Buckley, Y.M.; De Barro, P. Refined Global Analysis of *Bemisia tabaci* (Hemiptera: Sternorrhyncha: Aleyrodoidea: Aleyrodidae) Mitochondrial Cytochrome Oxidase 1 to Identify Species Level Genetic Boundaries. *Ann. Entomol. Soc. Am.* **2010**, *103*, 196–208. [CrossRef]
16. Rosell, R.C.; Bedford, I.D.; Frohlich, D.R.; Gill, R.J.; Brown, J.K.; Markham, P.G. Analysis of Morphological Variation in Distinct Populations of *Bemisia tabaci* (Homoptera: Aleyrodidae). *Ann. Èntomol. Soc. Am.* **1997**, *90*, 575–589. [CrossRef]
17. Oliveira, M.; Henneberry, T.; Anderson, P. History, current status, and collaborative research projects for *Bemisia tabaci*. *Crop. Prot.* **2001**, *20*, 709–723. [CrossRef]
18. Guo, T.; Guo, Q.; Cui, X.-Y.; Liu, Y.-Q.; Hu, J.; Liu, S.-S. Comparison of transmission of *Papaya leaf curl China virus* among four cryptic species of the whitefly *Bemisia tabaci* complex. *Sci. Rep.* **2015**, *5*, 15432. [CrossRef] [PubMed]
19. Wei, J.; Zhao, J.-J.; Zhang, T.; Li, F.-F.; Ghanim, M.; Zhou, X.-P.; Ye, G.-Y.; Liu, S.-S.; Wang, X.-W. Specific Cells in the Primary Salivary Glands of the Whitefly *Bemisia tabaci* Control Retention and Transmission of Begomoviruses. *J. Virol.* **2014**, *88*, 13460–13468. [CrossRef]
20. Li, M.; Hu, J.; Xu, F.-C.; Liu, S.-S. Transmission of *Tomato Yellow Leaf Curl Virus* by two invasive biotypes and a Chinese indigenous biotype of the whitefly *Bemisia tabaci*. *Int. J. Pest. Manag.* **2010**, *56*, 275–280. [CrossRef]
21. Pan, L.; Chen, Q.; Guo, T.; Wang, X.; Li, P.; Wang, X.; Liu, S. Differential efficiency of a begomovirus to cross the midgut of different species of whiteflies results in variation of virus transmission by the vectors. *Sci. China Life Sci.* **2018**, *61*, 1254–1265. [CrossRef]
22. Bedford, I.D.; Briddon, R.W.; Brown, J.K.; Rosell, R.C.; Markham, P.G. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. *Ann. Appl. Biol.* **1994**, *125*, 311–325. [CrossRef]
23. Sánchez-Campos, S.; Navas-Castillo, J.; Camero, R.; Soria, C.; Díaz, J.A.; Moriones, E. Displacement of Tomato Yellow Leaf Curl Virus (TYLCV)-Sr by TYLCV-Is in Tomato Epidemics in Spain. *Phytopathology* **1999**, *89*, 1038–1043. [CrossRef]
24. Maruthi, M.N.; Alam, S.N.; Kader, K.A.; Rekha, A.R.; Cork, A.; Colvin, J. Nucleotide Sequencing, Whitefly Transmission, and Screening Tomato for Resistance against Two Newly Described Begomoviruses in Bangladesh. *Phytopathology* **2005**, *95*, 1472–1481. [CrossRef] [PubMed]
25. Islam, W.; Lin, W.; Islam, S.U.; Arif, M.; Li, X.; Yang, Y.; Ding, X.; Du, Z.; Wu, Z. Genetic diversity of begomoviruses in Pakistan captured through a vector based survey. *Microb. Pathog.* **2018**, *118*, 91–97. [CrossRef] [PubMed]
26. Shahid, M.S.; Ikegami, M.; Natsuaki, K.T. First report of *Mungbean yellow mosaic India virus* in Nepal. *New Dis. Rep.* **2012**, *25*, 30. [CrossRef]
27. Shahid, M.S.; Pudashini, B.J.; Khatri-Chhetri, G.B.; Briddon, R.W.; Natsuaki, K.T. Molecular characterization of a distinct monopartite begomovirus associated with betasatellites and alphasatellites infecting *Pisum sativum* in Nepal. *Virus Genes* **2016**, *53*, 300–306. [CrossRef] [PubMed]
28. Briddon, R.W.; Bull, S.E.; Amin, I.; Idris, A.M.; Mansoor, S.; Bedford, I.D.; Dhawan, P.; Rishi, N.; Siwatch, S.S.; Abdel-Salam, A.M.; et al. Diversity of DNA β, a satellite molecule associated with some monopartite begomoviruses. *Virology* **2003**, *312*, 106–121. [CrossRef]
29. Shahid, M.S.; Yoshida, S.; Khatri-Chhetri, G.B.; Briddon, R.; Natsuaki, K.T. Complete nucleotide sequence of a monopartite *Begomovirus* and associated satellites infecting *Carica papaya* in Nepal. *Virus Genes* **2013**, *46*, 581–584. [CrossRef]
30. Ghimire, S.R.; Subedi, P.P.; Green, S.K. Status of Tomato Yellow Leaf Curl Virus in Tomato in the Western Hills of Nepal. *Nepal Agric. Res. J.* **2001**, *4*, 1–4. [CrossRef]
31. Acharya, R.; Shrestha, Y.K.; Sharma, S.R.; Lee, K.-Y. Genetic diversity and geographic distribution of *Bemisia tabaci* species complex in Nepal. *J. Asia-Pacific Èntomol.* **2020**, *23*, 509–515. [CrossRef]
32. Malik, A.H.; Briddon, R.W.; Mansoor, S. Infectious clones of *Tomato leaf curl Palampur virus* with a defective DNA B and their pseudo-recombination with *Tomato leaf curl New Delhi virus*. *Virol. J.* **2011**, *8*, 173. [CrossRef]
33. Thompson, J.D.; Higgins, D.G.; Gibson, T.J. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **1994**, *22*, 4673–4680. [CrossRef] [PubMed]
34. Schaffer, A.A.; Aravind, L.; Madden, T.L.; Shavirin, S.; Spouge, J.L.; Wolf, Y.I.; Koonin, E.V.; Altschul, S.F. Improving the accuracy of PSI-BLAST protein database searches with composition-based statistics and other refinements. *Nucleic Acids Res.* **2001**, *29*, 2994–3005. [CrossRef] [PubMed]
35. Tamura, K.; Stecher, G.; Peterson, D.; Filipski, A.; Kumar, S. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. *Mol. Biol. Evol.* **2013**, *30*, 2725–2729. [CrossRef]
36. Felsenstein, J. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution (N. Y.)* **1985**, *39*, 783–791.
37. Kon, T.; Dolores, L.M.; Bajet, N.B.; Hase, S.; Takahashi, H.; Ikegami, M. Molecular characterization of a strain of *Squash leaf curl China virus* from the Philippines. *J. Phytopathol.* **2003**, *151*, 535–539. [CrossRef]
38. Revill, P.; Ha, C.V.; Porchun, S.C.; Vu, M.T.; Dale, J. The complete nucleotide sequence of two distinct geminiviruses infecting cucurbits in Vietnam. *Arch. Virol.* **2003**, *148*, 1523–1541. [CrossRef] [PubMed]
39. Dolores, L.M.; Valdez, R.B. Identification of squash viruses and screening for resistance. *Phytopathology* **1988**, *24*, 43–53.

40. Khatun, M.F.; Hwang, H.-S.; Shim, J.-K.; Kil, E.-J.; Lee, S.; Lee, K.-Y. Identification of begomoviruses from different cryptic species of *Bemisia tabaci* in Bangladesh. *Microb. Pathog.* **2020**, *142*, 104069. [CrossRef] [PubMed]
41. Agnihotri, A.K.; Mishra, S.P. Temporal and spatial dynamics of tomato leaf curl disease at Chitrakoot region in India. *J. Pharmacogn. Phytochem.* **2018**, *SP1*, 531–536.
42. Zaidi, S.S.E.A.; Shafiq, M.; Amin, I.; Scheffler, B.; Scheffler, J.A.; Briddon, R.; Mansoor, S. Frequent Occurrence of *Tomato Leaf Curl New Delhi Virus* in Cotton Leaf Curl Disease Affected Cotton in Pakistan. *PLoS ONE* **2016**, *11*, e0155520. [CrossRef]
43. Shelat, M.; Murari, S.; Sharma, M.C.; Subramanian, R.B.; Jummanah, J.; Jarullah, B. Prevalence and distribution of *Tomato leaf curl virus* in major agroclimatic zones of Gujarat. *Adv. Biosci. Biotechnol.* **2014**, *5*, 1–3. [CrossRef]
44. Rishi, N. Current status of begomoviruses in the Indian subcontinent. *Indian Phytopathol.* **2004**, *57*, 396–407.
45. Pandey, P.; Mukhopadhya, S.; Naqvi, A.R.; Mukherjee, S.K.; Shekhawat, G.S.; Choudhury, N.R. Molecular characterization of two distinct monopartite begomoviruses infecting tomato in India. *Virol. J.* **2010**, *7*, 337. [CrossRef] [PubMed]
46. Chatchawankanphanich, O.; Maxwell, D.P. *Tomato leaf curl Karnataka virus* from Bangalore, India, Appears to be a Recombinant Begomovirus. *Phytopathology* **2002**, *92*, 637–645. [CrossRef] [PubMed]
47. Kumar, R.; Esakky, R.; Palicherla, S.R. The emergence of okra enation leaf curl virus—an important begomovirus, infecting okra in several states across India. *Arch. Phytopathol. Plant. Prot.* **2019**, *52*, 234–238. [CrossRef]
48. Mishra, G.P.; Singh, B.; Seth, T.; Singh, A.K.; Halder, J.; Krishnan, N.; Tiwari, S.K.; Singh, P.M. Biotechnological Advancements and Begomovirus Management in Okra (*Abelmoschus esculentus* L.): Status and Perspectives. *Front. Plant. Sci.* **2017**, *8*, 1–16. [CrossRef] [PubMed]
49. Bananej, K.; Kraberger, S.; Varsani, A. Okra Enation Leaf Curl Virus in Papaya from Iran Displaying Severe Leaf Curl Symptoms. *J. Plant. Pathol* **2016**, *98*, 637–639.
50. Kon, T.; Rojas, M.R.; Abdourhamane, I.K.; Gilbertson, R.L. Roles and interactions of begomoviruses and satellite DNAs associated with okra leaf curl disease in Mali, West Africa. *J. Gen. Virol.* **2009**, *90*, 1001–1013. [CrossRef] [PubMed]
51. Ghanem, G.A.M. Okra leaf curl virus: A monopartite begomovirus infecting okra crop in Saudi Arabia. *Arab J. Biotechnol* **2003**, *6*, 139–152.
52. Singh, S.J. Assessment of losses in okra due to enation leaf curl virus. *Indian J. Virol.* **1996**, *12*, 51–53.
53. Swanson, M.; Harrison, B. Serological relationships and epitope profiles of isolates of okra leaf curl geminivirus from Africa and the Middle East. *Biochim.* **1993**, *75*, 707–711. [CrossRef]
54. Atiri, G.I. The occurrence and importance of okra mosaic virus in Nigerian weeds. *Ann. Appl. Biol.* **1984**, *104*, 261–265. [CrossRef]
55. Hameed, U.; Zia-Ur-Rehman, M.; Herrmann, H.W.; Haider, M.S.; Brown, J.K. First report of *Okra enation leaf curl virus* and associated cotton leaf curl Multan beta satellite and cotton leaf curl Multan alpha satellite infecting cotton in Pakistan; a new member of the cotton leaf curl disease complex. *Plant. Dis.* **2014**, *98*, 1447–1448. [CrossRef] [PubMed]
56. Saleem, H.M.; Bedford, I.D.; Evans, A.A.; Markham, P.G. Geminivirus transmission by different biotypes of the whitefly *Bemisia tabaci* (Gennadius). *Pak. J. Zool.* **2003**, *35*, 343–351.
57. Tahir, M.; Amin, I.; Haider, M.S.; Mansoor, S.; Briddon, R.W. *Ageratum enation virus*—A Begomovirus of Weeds with the Potential to Infect Crops. *Viruses* **2015**, *7*, 647–665. [CrossRef] [PubMed]
58. Kumar, J.; Gunapati, S.; Singh, S.P.; Gadre, R.; Sharma, N.C.; Tuli, R. Molecular characterization and pathogenicity of a carrot (*Daucus carota*) infecting begomovirus and associated betasatellite from India. *Virus Res.* **2013**, *178*, 478–485. [CrossRef]
59. Kumar, Y.; Hallan, V.; Zaidi, A.A. Chilli leaf curl Palampur virus is a distinct begomovirus species associated with a betasatellite. *Plant. Pathol.* **2011**, *60*, 1040–1047. [CrossRef]
60. Kumar, Y.; Bhardwaj, P.; Hallan, V.; Zaidi, A.A. Detection and characterization of *Ageratum enation virus* and a nanovirus-like satellite DNA1 from zinnia causing leaf curl symptoms in India. *J. Gen. Plant. Pathol.* **2010**, *76*, 395–398. [CrossRef]
61. Raj, S.K.; Snehi, S.K.; Khan, M.S.; Tiwari, A.K.; Rao, G.P. Detection of *Ageratum enation virus* from cat's whiskers (*Cleome gynandra* L.) with leaf curl symptoms in India. *J. Gen. Plant. Pathol.* **2010**, *76*, 292–294. [CrossRef]
62. Swarnalatha, P.; Mamatha, M.; Manasa, M.; Singh, R.P.; Krishnareddy, M. Molecular identification of Ageratum enation virus (AEV) associated with leaf curl disease of tomato(*Solanum lycopersicum*) in India. *Australas. Plant. Dis. Notes* **2013**, *8*, 67–71. [CrossRef]
63. Singh-Pant, P.; Pant, P.; Mukherjee, S.K.; Mazumdar-Leighton, S. Spatial and temporal diversity of begomoviral complexes in papayas with leaf curl disease. *Arch. Virol.* **2012**, *157*, 1217–1232. [CrossRef] [PubMed]