



Molecular Detection of Emerging Begomoviruses Associated with Pepper (*Capsicum* spp.) in the Philippines

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Abstract: Several *Begomoviruses* have become significant plant viruses affecting pepper crops worldwide. In the Philippines, begomoviruses infecting pepper were first documented in 2011, but since then, no further studies have been conducted. As new and emerging begomoviral diseases continue to threaten and hamper crop production, it is important to deal with them proactively to mitigate losses. Here, begomoviruses associated with pepper in the country were successfully detected and identified using a combination of conventional molecular methods and next-generation sequencing (NGS). Pepper leaf samples exhibiting disease symptoms were gathered from various locations across the country. Presence of begomovirus infection was initially detected through visual observation of its characteristic symptoms (leaf curling/cupping/crinkling, interveinal yellowing of leaves, and apical leaf size reduction) and subsequently verified through polymerase chain reaction (PCR) using begomovirus-specific degenerate primers, together with rolling circle amplification (RCA). Emerging begomoviruses in pepper were not detected by restriction enzyme digestion but were identified through NGS-derived sequences. Two known and five unknown begomoviruses were identified from NGS sequences and provided evidence of the presence of emerging known begomoviruses in new areas, as well as new begomoviruses of pepper in the Philippines. This is the first study to employ Illumina-NGS technology for the molecular surveillance of begomoviruses infecting pepper in the Philippines.

Keywords: Bioinformatics; next-generation sequencing; phylogenetic analysis; polymerase chain reaction; rolling circle amplification

1. Introduction

Peppers (*Capsicum* spp.) rank among the key spice and vegetable crops grown in tropical and subtropical regions, valued for their characteristic spicy taste and for being excellent sources of vitamins A and C, along with various phenolic acids [1, 2]. They are produced in numerous varieties, exhibiting considerable variation in fruit form, size, color, and pungency [3]. Although considered a minor crop in the Philippines, pepper is cultivated year-round and continues to face increasing market demand [4]. Virus infections remain among the most significant limitations to pepper production globally [5, 6]. To date, 49 virus species have been identified as infecting pepper [6, 7], and approximately 20 of them, representing 15 distinct taxonomic groups, have been documented

to cause substantial losses in pepper-growing areas [6]. The appearance of new viral problems in pepper production is not unexpected, as outbreaks of newly identified viruses or the increasing incidence and severity of established ones are often associated with intensified farming systems and expanding global trade [8]. Because peppers are cultivated across broad geographic regions, they are exposed to diverse pathogens [9]. The movement of contaminated propagative materials through trade promotes the introduction of viruses and their insect vectors into previously unaffected areas [10]. Moreover, large-scale cultivation of single pepper varieties promotes the adaptation, transmission, and survival of begomoviruses across successive cropping seasons [11]. In addition, shifts in climate patterns can alter vector distributions and increase plant vulnerability, thereby heightening the risk of emerging viral diseases in pepper-growing areas.

Several begomoviruses (genus *Begomovirus*, family *Geminiviridae*) have become prominent viral pathogens affecting pepper crops worldwide. The whitefly *Bemisia tabaci* transmits the genus *Begomovirus*, the largest genus of plant viruses, which now comprises over 400 identified species [12, 13]. Members of this genus possess circular, single-stranded DNA (ssDNA) genomes arranged either as a single component (monopartite) or as two separate components (bipartite), each approximately 2.6-3.0 kb in size. These genomes are enclosed within characteristic twinned (geminata) icosahedral particles measuring about 22 x 38 nm [13]. More recent studies have further described the structural organization, genome architecture, and evolutionary dynamics of begomoviruses, emphasizing their genomic plasticity and recombination-driven diversification [14, 15]. In the Western Hemisphere, begomoviruses infecting pepper (*Capsicum* spp.) were initially documented in the United States [16] and subsequently in Mexico [17]. Recent reports confirmed the continued occurrence and expanding diversity of begomoviruses infecting pepper across the Americas, highlighting their ongoing evolution and geographic spread [14, 15]. In Oman, *Tomato yellow leaf curl virus* (TYLCV; recently reclassified as *Begomovirus coheni* by the ICTV [12]) was reported infecting sweet pepper, producing typical begomovirus symptoms such as upward leaf curling, chlorosis, and stunting [18]. In India, TYLCV-associated leaf curl disease in pepper has been widely documented and remains prevalent [19]. In the Philippines, begomoviruses were first detected in pepper in 1994 using a TYLCV-based DNA probe [20]. Subsequent reports confirmed the presence of *Tomato leaf curl Cebu virus* infecting pepper [21].

Accurate detection and identification of viruses are critical for effective disease management, especially in breeding and developing resistant cultivars. To date, only one study [21] has been done on the molecular identification of begomoviruses associated with pepper in the Philippines. As new and emerging begomoviral diseases continue to threaten and hamper pepper crop production, it is imperative to be proactive in dealing with them rather than reactive. Early detection and precise identification of emerging begomoviruses infecting pepper are crucial for implementing appropriate management strategies and minimizing potential yield losses. In several countries, next-generation sequencing (NGS) has been used effectively to detect and identify emerging viral pathogens, including begomoviruses rapidly. In this study, emerging begomoviruses infecting pepper were detected and identified by conventional and next-generation sequencing to assess their occurrence and geographic distribution across the country's crop-producing provinces.

2. Materials and Methods

2.1 Survey and Sample Collection

Field surveys and sampling were conducted to determine the occurrence of *Begomovirus* in the major crop-growing provinces of Luzon (Laguna, Quezon, Bulacan, Batangas, Nueva Ecija, Pangasinan, Ilocos Sur, and Ilocos Norte), Visayas (Cebu, Iloilo, and Leyte), and Mindanao (Butuan and Davao). In total, 160 leaf samples were obtained from 32 pepper farms across the surveyed areas. Leaf samples were randomly collected from five to ten plants exhibiting virus-like symptoms per site. Relevant field data, including GPS coordinates of field location, crop growth stage, observed whitefly populations, cultivar planted, and other agronomic details, were documented. All samples were brought to the Plant Virology Laboratory, Institute of Weed Science, Entomology and Plant Pathology (IWEP), College of Agriculture and Food Science (CAFS), University of the Philippines Los Baños (UPLB), and stored at 40 °C before molecular analysis.

2.2 Field Assessment of Begomovirus Symptoms

Begomovirus infection in pepper fields was evaluated by visual inspection for characteristic leaf curl symptoms, including leaf curling, cupping, or crinkling, accompanied by interveinal yellowing. Other virus-associated symptoms such as mosaic or mottle patterns, chlorosis, leaf distortion, and plant stunting were also noted. All symptomatic plants were photographed, and symptoms were categorized based on symptom type, crop growth stage, and sampling location.

2.3 Molecular Detection of Begomovirus Using Polymerase Chain Reaction (PCR)

Begomovirus infection in symptomatic pepper leaves was confirmed through polymerase chain reaction (PCR) using two sets of degenerate primers, TY1F/TY2R and PAL1v1978B/PAR1c715H. The TY1F/TY2R primer pair amplified an approximately 585 bp fragment corresponding to the coat protein gene [22] while PAL1v1978B/PAR1c715H generated approximately 1.5 kb of the DNA-A component [21, 23]. Total genomic DNA was isolated from symptomatic leaf tissues following the Dellaporta et al. protocol [24]. DNA quality and concentration were assessed spectrophotometrically using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) by measuring the A260/A280 and A260/A230 absorbance ratios; only samples with A260/A280 ratios between 1.8 and 2.0 were used for downstream molecular analyses. PCR reactions were prepared in a 25.0 μ L mixture containing PCR buffer (2.5 μ L 1X), $MgCl_2$ (1.0 μ L, 2 mM), dNTPs (0.5 μ L), forward and reverse primers (1.0 μ L, 10.0 μ M), *Taq* DNA Pol (0.2 μ L, 1U, Invitrogen, Carlsbad, CA, USA), sterile nuclease-free water (16.8 μ L), and DNA template (2.0 μ L, 50.0 ng/ μ L). The composition of the reaction mixture was modified as needed based on the primer pair used. Each PCR assay included DNA from a healthy plant as the negative control, sterile distilled water as the blank control, and DNA from a confirmed ToLCPV-infected plant as the positive control. PCR amplification was carried out in a Veriti™ 96-Well Thermal Cycler (Applied Biosystems, Foster City, CA, USA). For the TY1R/TY2R primer pair, the cycling profile included an initial denaturation at 94 °C for 1 minute, followed by 35 cycles of denaturation at 94 °C for 15 seconds, annealing at 55 °C for 15 seconds, and extension at 72 °C for 1 minute, with final extension at 72 °C for 10 minutes. For the PAL1v1978B/PAR1c715H primers, the protocol began with an extended initial denaturation at 94 °C for 5 minutes, followed by 35 cycles incorporating a longer annealing step at 55 °C for 2 minutes and extension at 72 °C for 2 minutes, and concluded with a final extension at 72 °C for 10 minutes.

2.4 Rolling Circle Amplification (RCA)

Circular single-stranded DNA was amplified from total nucleic acids extracted from symptomatic pepper leaves using the TempliPhi Amplification Kit (GE Healthcare Life Sciences, Chicago, IL, USA) in accordance with the manufacturer's instructions. Briefly, 1.0 μ L of undiluted total DNA was combined with 5.0 μ L of sample buffer, incubated at 95 °C for 3 minutes in a thermoshaker, and immediately chilled on ice. Thereafter, 5 μ L of reaction buffer and 0.2 μ L of enzyme mix were added to each sample, followed by incubation at 30 °C for 18 hours in a Biometra Thermoshaker (Analytik Jena, Jena, Germany). The amplification reaction was terminated by heating at 65 °C for 10 minutes to inactivate the enzyme. The resulting rolling circle amplification (RCA) products were analyzed by agarose gel electrophoresis. Specifically, 1.0 μ L of the RCA product was mixed with 5.0 μ L of 1x loading dye and loaded onto a 0.8% agarose gel pre-stained with GelRed (Biotium, Fremont, CA, USA) (1.0%), then electrophoresed at 95 V for 40 minutes.

2.5 Restriction Enzymes (RE) Digestion

RCA-restriction enzyme digestion analysis [25] was performed on RCA products from representative PCR-positive and PCR-negative pepper samples to detect Begomovirus(es). These four enzymes were selected based on the restriction fragment profiles previously described for begomoviruses [25, 21], where *Sal*I (recognition site: G[^]TCGAC), *Hind*III (A[^]AGCTT), *Eco*RI (G[^]AATTC), and *Bam*HI (G[^]GATCC) are known to generate diagnostic fragments of approximately 2.7 kb (*Sal*I), 0.8 and 1.9 kb (*Hind*III), 1.5 and 1.2 kb (*Eco*RI), and 2.7 kb (*Bam*HI) for a typical ~2.7-kb begomovirus genome, thereby enabling species-level discrimination based on their distinct restriction site distributions. Rolling circle amplification (RCA) products were digested with *Sal*I, *Hind*III, *Eco*RI, and *Bam*HI. Each digestion reaction was performed according to the manufacturer's protocol, using 0.5 μ L of RCA-amplified viral DNA and incubating the reaction at 37 °C for 1 hour in a Biometra ThermoShaker (Analytik Jena, Jena, Germany). The restriction enzyme-digested products were

subsequently analyzed by agarose gel electrophoresis. A total of 10 µL of the digested RCA product was mixed with 2 µL of 6x loading dye and loaded onto a 0.8% agarose gel prepared in 1x TBE (Tris-borate-EDTA) buffer and pre-stained with 1.0% GelRed. Electrophoresis was performed at 95 V for 30 minutes. Detection of potential emerging Begomovirus was based on comparison of the resulting restriction fragment patterns with those of previously characterized begomovirus species.

2.6 Illumina MiSeq Sequencing

A total of 10 RCA-amplified DNA samples (5.0 µL) from pepper (5 from bell pepper and 5 from chili pepper) were submitted to the DNA Sequencing Laboratory at the Philippine Genome Center (PGC) (UP Diliman, Quezon City, Philippines) for next-generation sequencing. The RCA-amplified DNA samples were purified using the Ampure DNA purification kit (Beckman Colter, Brea, CA, USA), followed by library preparation using Nextera XT and deep sequencing on the Illumina MiSeq (2x300 V3) (Illumina, San Diego, CA, USA).

2.7 Viral Genome Assembly and Analysis

NGS sequences were submitted to the Core Facility for Bioinformatics (PGC, UP Diliman, Quezon City) for quality control assessment, de novo assembly, annotation, and phylogenetic analysis. Figure 1 shows the PGC bioinformatics pipeline (PGC) employed for the assembly and analysis of begomoviral genome sequences. Briefly, each sample was trimmed, clipped, and filtered using fastp [26]. Quality-filtered sequencing reads were assembled into contigs using SPAdes [27], and the resulting contigs were subsequently annotated through BLASTn. For contigs that did not map to begomovirus sequences, they were filtered out. From the remaining contigs, “genes” were predicted using GeneMark [28]. These “genes” were re-mapped and re-annotated using BLASTn. The sequences of annotations that were present in the greatest number of samples were then used for alignment and downstream analysis.

2.8 Sequence and Phylogenetic Analyses

Genes mapped to or annotated as a part of the begomovirus genome were then used as the basis for the sequence alignment. The annotations for all samples were then compared, and the annotation with the most samples was used to construct the phylogenetic tree. Representative sequences obtained from the samples were combined into a single multi-FASTA file. The sequences were aligned using MAFFT [29], subsequently trimmed using trimAl [30], and converted into PHYLIP format using an online generic file converter before phylogenetic tree construction. The phylogenetic trees were created using jmodelTest2 and PhyML [31], and iTOL [32] was used for visualization.

3. Results and Discussion

3.1 Symptoms of Begomovirus and other diseases

Field inspections during farm visits revealed begomovirus infection in pepper crops, as indicated by characteristic foliar symptoms. Infected plants typically exhibited upward leaf curling, interveinal chlorosis, generalized yellowing, and crinkling of the young apical leaves. These symptoms were noted in both bell and chili pepper crops across different growth stages. In bell peppers, infection was associated with pronounced leaf deformations, including curling, cupping, puckering, and crinkling, as well as interveinal yellowing or mosaic patterns, particularly during the vegetative to early fruiting stages (Figure 2). Similarly, infected chili pepper plants exhibited leaf curling and crinkling, interveinal chlorosis, reduced apical leaf size (little leaf), shortened internodes, leaf crowding, and overall stunting during the vegetative to early fruiting stages (Figure 3). The symptomatology documented in this study is consistent with previous descriptions of begomovirus infection in pepper [18] and aligns with more recent reports highlighting typical leaf curl, chlorosis, vein thickening, and growth suppression in begomovirus-affected *Capsicum* spp. [15]. Sweet pepper plants infected with *Tomato yellow leaf curl virus* (TYLCV) have been reported to exhibit curling of leaf margins, interveinal and marginal chlorosis, vein thickening, and noticeable leaf thickening, with severe infections leading to pronounced yellowing [18]. In Indonesia, yellow leaf curl disease has been documented in nearly all major pepper-producing regions, where infected plants commonly display little leaf symptoms, yellow mosaic, shortened internodes, and stunted growth [33]. In Costa Rica, begomovirus-infected sweet pepper plants exhibited symptoms ranging from mild to severe, including mosaic patterns, chlorosis, upward or downward

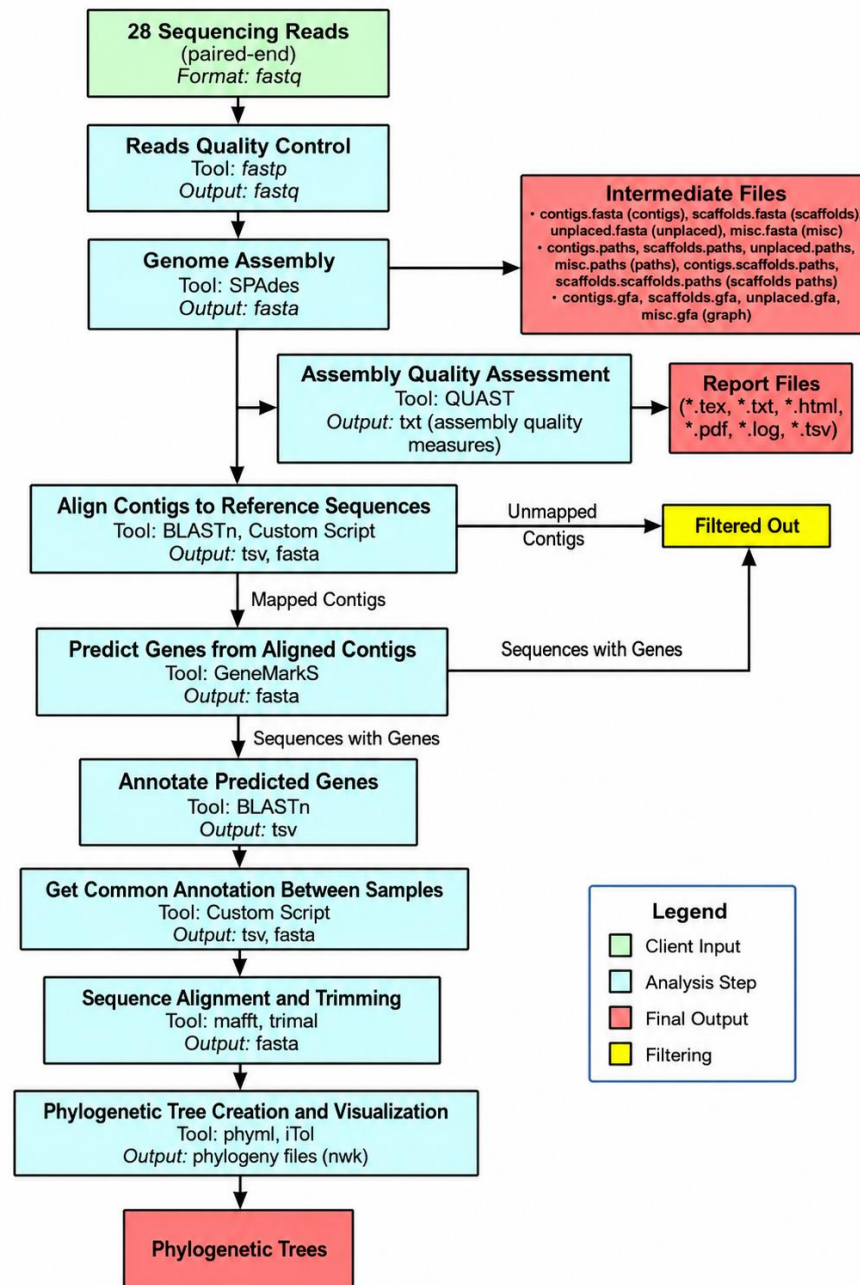


Figure 1. Bioinformatics pipeline (Core Facility of Bioinformatics, PGC) employed for assembly and analysis of begomoviral genome sequences.

leaf curling, vein-associated yellowing, and overall plant stunting. In the Philippines, [21] likewise reported an association between begomoviruses and pepper, describing symptomatic plants with leaf curling, mosaic, yellowing, blistering, and growth reduction. More recent studies further confirm that these foliar distortions, chlorosis, and growth abnormalities remain hallmark symptoms of begomovirus infections in *Capsicum* spp. across diverse agroecological regions [15]. Other symptoms of virus infection were also observed on chili peppers, including mosaic/mottle, curling or severe leaf distortion, particularly on apical or emerging leaves, and shoestring. These virus-like symptoms were primarily observed during the flowering and fruiting stages of chili crops. The manifestations resembled those commonly associated with pepper-infecting viruses such as *Potato virus Y* (PVY), *Cucumber mosaic virus* (CMV), and *Tomato/Tobacco mosaic virus* (ToMV), which have previously been reported in the Philippines [21].



Figure 2. Begomovirus-like symptoms observed in bell peppers. (A-D) Upward leaf curling and crinkling, interveinal chlorosis, and puckering of leaves.



Figure 3. Begomovirus-like symptoms observed in chili peppers. (A-D) Leaf curling and crinkling, interveinal chlorosis, and reduced size of apical leaves.

3.2 Incidence of Begomovirus and Other Diseases

The occurrence of Begomovirus and other virus-like diseases in pepper crops, as determined by visual symptom assessment, varied among sampling sites (Table 1). Begomovirus-like symptoms were observed in 78.1% (125/160) of the total samples across Luzon, Visayas, and Mindanao. In Luzon, begomovirus-like symptoms were present in all samples from Laguna, Quezon, Batangas, and Nueva Ecija, totaling 40 out of 75 samples (53.3%). Begomovirus-like symptoms occurred in 100% of the samples from all provinces in the

Visayas (Cebu, Leyte, and Iloilo) and Mindanao (Butuan and Davao del Sur). Other virus-like diseases were observed exclusively in Luzon, with a recorded incidence of 46.6%. Most of the samples from Bulacan, Batangas, Pangasinan, Ilocos Sur, and Ilocos Norte showed symptoms of other viruses. In Bulacan (San Idelfonso) and Ilocos Sur (Sinait), these symptoms were observed alongside begomovirus-like symptoms. Begomovirus infection in symptomatic pepper plants was verified through PCR amplification using the primer pairs TY1F/TY2R and PAL1v1978/PAR1c715H. Using genomic DNA as the template, amplification of the begomoviral coat protein gene (~585 bp) was obtained in 43.1 % of the samples (69 out of 160) with the TY1F/TY2R primer pair (Table 1). However, the PCR products detected in positive samples were generally weak in intensity. No amplification was observed in the healthy pepper control or in the no-template control containing distilled water, confirming the primers' specificity for begomoviral DNA. The lack of PCR products in the remaining samples indicated the absence of detectable begomovirus infection in those tissues. Conversely, amplification with the PAL1v1978/PAR1c715H primer pair did not produce any detectable DNA bands in the pepper samples upon gel electrophoresis. The expected ~1.5 kb DNA-A fragment was observed only in the positive control derived from a ToLCPV-infected plant.

The faint amplification obtained with the coat protein primers, along with the lack of amplification using the DNA-A primers, may be due to a low concentration of viral DNA in the total plant DNA extracts, potentially falling below the detection threshold of conventional PCR. Similar observations have been reported, where begomovirus amplification was faint or undetectable in samples with low viral titers [34]. Under such conditions, more sensitive approaches, such as rolling circle amplification coupled with PCR (RCA-PCR), are recommended for the reliable detection of viruses at low concentrations [34]. Indeed, RCA-PCR is significantly more sensitive than the standard PCR, enabling detection of Tomato leaf curl New Delhi virus (ToLCNDV) in samples that tested negative by conventional PCR, thereby reducing false-negative results during virus indexing [34]. Similarly, [35] reported a higher detection rate of begomoviruses with RCA-PCR than with PCR alone. In this study, selected representative samples were first processed using rolling circle amplification (RCA) to increase the amount of circular viral DNA before conducting PCR analysis. Specifically, the 20 samples selected for RCA analysis were those that had tested negative by conventional PCR using genomic DNA as template, thus representing cases in which viral titers were potentially below the detection threshold of standard PCR. Using RCA-amplified DNA as the template, 55 % (11 out of 20) of the samples tested positive for Begomovirus with the same primer pairs (Table 1). All positive samples yielded the expected amplicons of approximately 585 bp and 1.5 kb following amplification with the TY1F/TY2R and PAL1v1978/ PAR1c715H primer pairs, respectively. These results confirmed the presence of Begomovirus in several pepper samples and demonstrated that RCA effectively enriched viral DNA, thereby enhancing the sensitivity and reliability of subsequent PCR detection. The findings further indicate that viral DNA levels in the original extracts were below the detection threshold of conventional PCR but became detectable following RCA enrichment. The remaining 45% (9 out of 20) of the samples (9/20) remained negative after RCA-PCR, suggesting the absence of begomovirus infection in those plants. Overall, the results highlight the usefulness of RCA-PCR in detecting Begomovirus present at very low titers in pepper samples.

Published information on the occurrence of begomoviruses infecting pepper, especially in the Philippines, is still scarce. In Spain, *Capsicum annuum* was identified as a host of Tomato yellow leaf curl virus (TYLCV) [36]. Similarly, in the Caribbean region, molecular detection techniques such as PCR, restriction enzyme analysis, and nucleic acid hybridization successfully confirmed TYLCV infection in field-grown *C. annuum* and *C. chinense* in the Dominican Republic, Cuba, and Jamaica [37]. These findings reinforced earlier reports recognizing *Capsicum* species as natural hosts of TYLCV. Evidence of susceptibility in *C. frutescens*, *C. baccatum*, and *C. chinense* was later documented by [38]. More recent studies have further demonstrated the expanding distribution and diversity of begomoviruses infecting pepper in various regions, including Costa Rica [39] and Indonesia [33], as well as broader global assessments highlighting the increasing host range and recombination-driven evolution of begomoviruses in solanaceous crops [15]. In the Philippines, begomoviruses infecting pepper—specifically *Tomato leaf curl Philippines virus* (ToLCPV) and *Tomato leaf curl Cebu virus* (ToLCCeV)—were previously detected in 3 out of 18 samples collected from Laguna (Los Baños), Cebu, and Bukidnon [21].

Table 1. Incidence of Begomovirus and other virus-like diseases in pepper (bell pepper and chili pepper) by sampling location across Luzon, Visayas, and Mindanao, based on visual symptom assessment, and corresponding detection results from conventional PCR and rolling circle amplification (RCA)-PCR using the TY1F/TY2R and PAL1v1978/PAR1c715H primer pairs.

Province	Location (No. of Farm)	No. of samples	Disease/ symptom observed		No. of Positive Samples		
			Begomo- virus-like	Other virus- like	TY1F/ TY2R ^a	TY1F/ TY2R ^b	PAL1v1978/ PAR1c715H ^b
Luzon	9 (15)	75	40	35	10	4/11	5/11
Laguna	Los Baños (2)	10	10	0	5	1/3	1/3
Quezon	Candelaria (1)	5	5	0	0	-	-
Bulacan	San Idelfonso (4)	20	5	15	0	0/2	0/2
Batangas	Lipa (1)	5	5	0	4	1/1	1/1
	Calaca (1)	5	5	0	1	1/1	1/1
Nueva Ecija	Muños (1)	5	5	0	0	0/1	0/1
Pangasinan	Urdaneta (2)	10	0	10	0	0/1	0/1
Ilocos Sur	Sinait (2)	10	5	5	0	1/2	2/2
Ilocos Norte	Bacarra (1)	5	0	5	0	-	-
Visayas	6 (13)	65	65	0	42	5/6	4/6
Cebu	Cebu City (1)	5	5	0	2	2/2	2/2
	Barili (5)	25	25	0	9	-	-
	Dalaguete (2)	10	10	0	6	1/2	0/2
Leyte	Baybay (1)	5	5	0	5	1/1	1/1
Iloilo	Leon (2)	10	10	0	10	1/1	1/1
	Pavia (2)	10	10	0	10	-	-
Mindanao	2 (4)	20	20	0	17	2/3	2/3
Butuan	Ampayon (2)	10	10	0	7	2/2	1/2
Davao del Sur	Digos (2)	10	10	0	10	0/1	1/0
Total	17 (32)	160	125	35	69	11/20	11/20
			(78.1%)	(21.9%)	(43.1%)	(55.0%)	(55.0%)

3.3 Geographical Distribution of Begomovirus

In this study, begomovirus infection was observed in pepper crops, including both bell peppers and chili peppers, across multiple regions of the country (Figure 4). Begomovirus-like infections were found in more than 20 locations of the pepper farms surveyed. In Luzon, begomovirus infections were present in Laguna, Batangas, Bulacan, and Ilocos Sur, while in other provinces surveyed, such as Quezon, Nueva Ecija, Pangasinan, and Ilocos Norte, other virus-like symptoms were also present. In Visayas, begomovirus-like infections were detected in the majority of the farms in Cebu (Cebu City, Barili, and Dalaguete), Iloilo (Pavia and Leon), and Leyte (Baybay). In Mindanao, Begomovirus was present in Butuan and Davao del Sur.

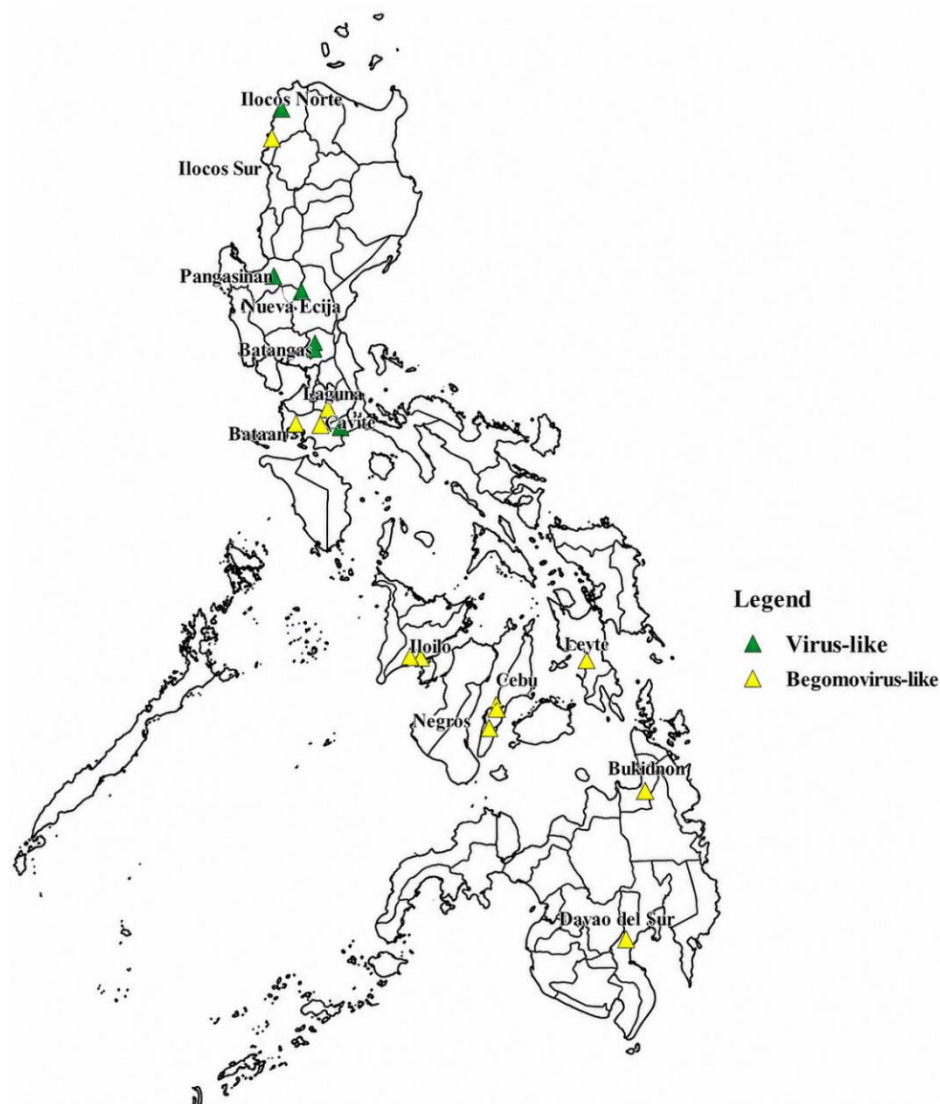


Figure 4. Geographical distribution of *Begomovirus* and other viruses in the provinces surveyed across the Philippines, as detected by visual observation and PCR.

3.4 Emerging Begomoviruses Detected in Pepper Crops by RCA and RE Digestion

RCA was performed on DNA extracts from representative bell pepper and chili pepper samples that were PCR-positive and PCR-negative. All samples showed successful amplification, evidenced by a distinct high-molecular-weight band on the agarose gel corresponding to the begomovirus genome. All RCA-amplified products were digested with *Sall*, *HindIII*, *BamHI*, and *EcoRI* to detect the presence of begomoviruses. However, no digestion product of any specific length was generated after digestion with the four restriction enzymes; hence, the presence of begomoviruses could not be confirmed. These results may suggest the absence of Begomovirus in the sample. However, PCR analysis using RCA-amplified DNA as a template revealed Begomovirus in several pepper samples. Therefore, the complete absence of Begomovirus in the tested samples cannot be conclusively established. The findings may suggest the occurrence of different begomovirus species or viral variants lacking the specific restriction sites targeted in previously reported profiles. These observations can be further validated through next-generation sequencing analysis.