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Research article

Characterization of Begomovirus and *Potyvirus* causing mixed infections on chili plant (*Capsicum frutescens* L.) in South Lampung, Indonesia

Selvi Helina*, Fauziah Rizky Nurfadillah, Hasriadi Mat Akin, Muhammad Nurdin, Purnomo, Puji Lestari

Department of Plant Protection, Faculty of Agriculture, University of Lampung, Prof. Dr. Ir. Sumantri Brojonegoro Street, Rajabasa, Lampung 35144, Indonesia

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Abstract

Importance of the work: Chili (*Capsicum frutescens* L.) productivity in Indonesia is threatened by mixed virus infections that complicate diagnosis and control

Objectives: To characterize viruses associated with symptomatic *C. frutescens* plants in South Lampung using molecular techniques.

Materials and Methods: DNA and RNA were extracted from infected samples and subjected to polymerase chain reaction amplification using specific primers targeting *Begomovirus* and *Potyvirus*. Sequencing was followed by homology analysis using the Basic Local Alignment Search Tool. In addition, phylogenetic analysis was conducted and pathogenicity tests were performed on healthy chili plants.

Results: The presence was confirmed of both *Begomovirus* and *Potyvirus* in the analyzed samples, with no amplification detected for specific primers of *Cucumber mosaic virus* or *Tobacco Infectious Chlorosis Virus*. The *Begomovirus* isolate shared 93% identity with *Pepper Yellow Leaf Curl Virus* from Kertha, Indonesia. The *Potyvirus* isolate had 100% identity with *Potato Virus Y* from Ukraine. Phylogenetic analysis confirmed these relationships. The pathogenicity tests identified symptoms ranging from mild to severe mosaicing, leaf curling and chlorosis.

Main finding: The occurrence was confirmed of mixed infections involving *Begomovirus* and *Potyvirus* in *C. frutescens* from South Lampung, Indonesia and highlighted the importance of molecular diagnostics in understanding virus diversity and supporting disease control strategies.

* Corresponding author.
E-mail address: selvi.helina@fp.unila.ac.id (S.Helina)

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Introduction

Capsicum frutescens L., commonly known as bird's eye chili, is a highly valued horticultural commodity in Indonesia (Anggraeni et al., 2025). Its wide ecological adaptability allows for extensive cultivation across various agroclimatic zones, including highlands and lowlands (Datau et al., 2015). The fruit of this plant is widely used not only as a culinary spice but also for its bioactive properties, as it contains numerous secondary metabolites such as capsaicin, capsanthin, carotenoids, alkaloids, essential oils and resins, contributing to the nutritional and economic value of the chili crop (Arifin, 2010). In recent years, consumer demand for chili has steadily increased due to its culinary versatility and health-related properties, thus establishing it as a priority commodity in the national agricultural development agenda (Lukas et al., 2023)

Despite its economic significance, the productivity of *C. frutescens* in Indonesia has faced serious challenges (Lukas et al., 2023). According to the Central Bureau of Statistics, in 2021 there was a reported decline in chili production of approximately 1.5 million t, equivalent to 8.09%, compared to the previous year (Mahardika and Yuliarni, 2018). This substantial reduction in yield has necessitated the importation of chili from neighboring countries, such as Malaysia and Australia, to fulfill domestic demand (Widowati et al., 2023). Various factors have contributed to this decline, among which viral infections stand out as one of the most destructive (Lukas et al., 2023; Widowati et al., 2023).

Plant viruses are among the most important biotic stressors in horticultural crop production, particularly in solanaceous crops such as chili (Hancinsky et al., 2020). Viral infections interfere with plant metabolism, often causing physiological disturbances that result in visual symptoms such as chlorosis, mosaic patterns, leaf distortion, stunting and in severe cases, plant death (Hamdayanty and Hardina, 2023). These effects lead to considerable yield losses, which in chili cultivation can exceed 90% under severe infection conditions (Saleh and Rahayuningsih, 2014). In addition, the presence of mixed viral infections, where two or more distinct viruses infect the same host plant, further complicates symptomatology, diagnosis and management strategies (Singhal et al., 2020; Sánchez-Tovar et al., 2025).

Mixed viral infections have been recognized increasingly as a major concern in crop virology, as these infections can arise from the simultaneous or sequential presence of multiple viruses within a single plant host, leading to interactions that may be synergistic, additive, or antagonistic (Syller, 2012; Alcaide et al., 2020; Sánchez-Tovar et al., 2025). For example, in synergistic interactions, the combined effect of co-infecting viruses is greater than the sum of their individual effects, potentially leading to more severe symptoms and increased viral loads and such conditions have been observed in numerous cropping systems and are particularly problematic due to their unpredictable impact on disease progression and epidemiology (Tuhumury and Amanupunyo, 2013; Septariani et al., 2014).

In chili cultivation, two common major viral genera involved in mixed infections are *Begomovirus* (family: Geminiviridae) and *Potyvirus* (family: Potyviridae). *Begomoviruses* are transmitted primarily by the whitefly *Bemisia tabaci*, a polyphagous and highly mobile vector capable of rapid virus dissemination across wide areas (Marianah, 2020). In Indonesia, several species within this genus, including *Ageratum yellow vein virus* (AYVV) in papaya plant, have been reported to cause severe yellowing and leaf curl symptoms (Helina et al., 2024). On the other hand, *Potyviruses*, such as *Potato Virus Y* (PVY), are transmitted through aphids in a non-persistent manner and also via mechanical means (Miftakhurrohmah et al., 2013). *Potyvirus* infections are characterized by vein clearing, mottling and mosaic symptoms which often overlap with those of *Begomovirus*, thus complicating diagnosis.

The concurrent infection of chili plants by *Begomovirus* and *Potyvirus* may exacerbate disease severity and broaden symptom expression, making it difficult to distinguish between the two or identify the primary causal agent based solely on visual assessment (Laili and Damayanti, 2019). Furthermore, viral evolution driven by recombination and mutation under mixed infection pressure may give rise to novel variants with altered pathogenicity, host range and transmission efficiency (Fortes et al., 2025). Therefore, a comprehensive molecular approach is necessary for the accurate detection and characterization of such infections.

Molecular diagnostic tools, particularly polymerase chain reaction (PCR), have become essential in plant virology for detecting and identifying virus species based on their nucleic acid sequences (Olmos et al., 2007; Cassedy et al., 2021). PCR allows for sensitive and specific amplification of viral genomic regions, enabling researchers to detect even low-titer infections that may be missed by serological or visual inspection methods (Naully and Septriliyana, 2022). Furthermore, sequence analysis followed by phylogenetic tree construction facilitates the elucidation of evolutionary relationships among virus isolates and helps to determine their species-level identity and geographical lineage (Tallei et al., 2016).

The current study conducted molecular characterization on symptomatic *Capsicum frutescens* samples collected from South Lampung, Indonesia, with the aim of identifying the viruses responsible for observed symptoms and determining whether mixed infections involving *Begomovirus* and *Potyvirus* were present. PCR-based detection, nucleotide sequencing and phylogenetic analysis were used to confirm the presence of these viruses and explore their genetic relationships with previously reported isolates. This research could provide important insights into the diversity, co-infection patterns and epidemiological implications of viral pathogens affecting chili production in one of Indonesia's key agricultural regions.

Materials and Methods

Study area and sample collection

Field surveys were conducted for the collection of symptomatic chili pepper (*Capsicum annuum* L.) samples in Sidosari, Natar, South Lampung Regency, Lampung Province, Indonesia. This region is a major horticultural production center in southern Sumatra and features a tropical climate characterized by high temperatures (average 27–32°C) and relative humidity (70–85%), which favor the proliferation of plant viruses and their insect vector (Regassa, 2021).

Sampling was performed systematically across multiple chili cultivation fields in different subdistricts of South Lampung to capture variability across diverse agroecosystems. A stratified random sampling approach was applied. Each field was divided into quadrats (10 m × 10 m) and plants exhibiting virus-like symptoms were selected randomly from each quadrat to ensure representative sampling. Symptomatic plants were identified based on visual assessment of typical viral disease manifestations, including leaf curling, upward leaf rolling, chlorosis, mosaic patterns, vein yellowing and leaf deformation. Both mild and severe symptoms were considered, in order to capture potential single and mixed viral infections, particularly those associated with *Begomovirus*, *Potyvirus*, *Cucumovirus* and *Tobamovirus*.

Each sampled plant was carefully uprooted or excised to collect young leaves and shoots, placed in sterile, labeled plastic bags and immediately stored in a portable cooler with ice packs to maintain a low temperature and to minimize RNA/DNA degradation. Then, samples were transported to the Laboratory of Biotechnology, Faculty of Agriculture, Universitas Lampung, Indonesia for subsequent molecular analysis based on nucleic acid extraction, virus detection and identification using PCR-based techniques.

DNA and RNA isolation, complementary DNA synthesis and polymerase chain reaction amplification

DNA and RNA extraction

Nucleic acid extraction was performed on leaf tissue from the chili plants exhibiting viral infection symptoms. Two types of extractions were conducted: DNA extraction to isolate viral DNA; and RNA extraction to isolate viral RNA. In the DNA extraction, total DNA was extracted using a Plant Genomic DNA Mini Kit (Geneaid Biotech Ltd.; Taiwan) following the manufacturer's protocol. In the RNA extraction, total RNA was extracted using a Plant RNA Mini Kit (Geneaid Biotech Ltd.; Taiwan) according to the kit instructions. The quality and concentration of the extracted nucleic acids were assessed using a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE, USA) and the RNA was stored at –80°C until further use.

Complementary DNA synthesis

The total RNA samples were reverse-transcribed into complementary DNA (cDNA) using a ReverTra Ace® qPCR RT Kit (Toyobo Co., Ltd., Osaka, Japan) following the manufacturer's protocol. The reverse transcription reaction mixture consisted of 4 µL of RT Buffer, 1 µL of RNase Inhibitor, 1 µL of ReverTra Ace enzyme, 8 µL of RNase-free H_2O, 1 µL of Oligo$(dT)_{20}$ primer, 2 µL of dNTPs mixture and 3 µL of RNA template, making a total volume of 20 µL. The reaction was carried out by incubating the mixture at 37°C for 15 min to allow for reverse transcription, followed by enzyme inactivation at 98°C for 5 min. Subsequently, the resulting cDNA was stored at –20°C until used for downstream PCR analysis.

Detection method using polymerase chain reaction amplification

PCR amplification was performed to detect the presence of viral genomes using specific primer pairs targeting both DNA and RNA viruses. For DNA virus detection, the SPG1/SPG2 primer pair was used to amplify the AC1/AC2 gene region of *Begomovirus*. For RNA viruses, three primer pairs were used: MJ1/MJ2 targeting the coat protein gene of *Potyvirus;* CMV-F/CMV-R for *Cucumber Mosaic Virus* (CMV); and *Tobacco Infectious Chlorosis Virus* coat protein forward (TICV-CF) and *Tobacco infectious chlorosis virus* replication-associated

protein reverse (TICV-CR) for *Tobacco Infectious Chlorosis Virus* (TICV). The PCR reactions were prepared using standard reaction mixtures recommended by the respective protocols. Thermal cycling was conducted under the conditions of: an initial denaturation at 95°C for 1 min followed by 40 amplification cycles consisting of denaturation at 95°C for 15 s; annealing at 52°C for 15 s; and extension at 72°C for 10 s, with a final extension at 72°C for 5 min.

Electrophoresis and visualization

The amplified PCR products were separated using electrophoresis on a 1% agarose gel stained with ethidium bromide. Electrophoresis was carried out at 50 V for approximately 50 min. The resulting DNA bands were visualized and documented using a ultraviolet transilluminator (Bio-Rad Laboratories, Hercules, CA, USA).

Data analysis

PCR products were submitted to PT Genetics Science Jakarta for nucleotide sequencing. The resulting nucleotide sequences were then analyzed using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website to identify viral homology and to assess genetic similarity with reference sequences available in the GenBank database (www.ncbi.nlm.nih.gov/genbank). Isolates showing high sequence similarity (approaching 100%) to known viral strains were selected for further analysis. Multiple sequence alignment was performed using the MEGA11 software (Pennsylvania State University, University Park, PA, USA) and phylogenetic relationships were performed through the construction of phylogenetic trees using the maximum likelihood method Kimura two -parameter model. Bootstrap analysis with 1,000 replications was conducted to evaluate the reliability of the clustering patterns (Tamura et al., 2021).

Mechanical inoculation tests were performed on healthy chili plants to confirm the pathogenicity of the detected viruses. A sap extract was prepared by homogenizing 1 g of symptomatic chili leaves in 5 mL of phosphate buffer with the addition of 0.1 g of zeolite. The resulting solution was applied to the leaves of test plants using sterile cotton swabs. Then, the inoculated plants were maintained under controlled conditions and incubated for 1 wk. Symptom development was monitored and recorded to confirm the infectivity and symptom expression associated with the identified viral pathogens.

Results

Detection and identification of mixed virus infections in chili pepper plants

Field observations of chili plants exhibiting symptoms of mixed viral infections revealed a diverse range of manifestations, including leaf curling, yellowing accompanied by spot formation, thickening of dark green pigmentation that resulted in a wavy leaf appearance, upward leaf curling, and yellow mosaic patterns. Based on these symptomatic characteristics, four representative samples of infected chili leaves were collected for further analysis (Fig. 1).

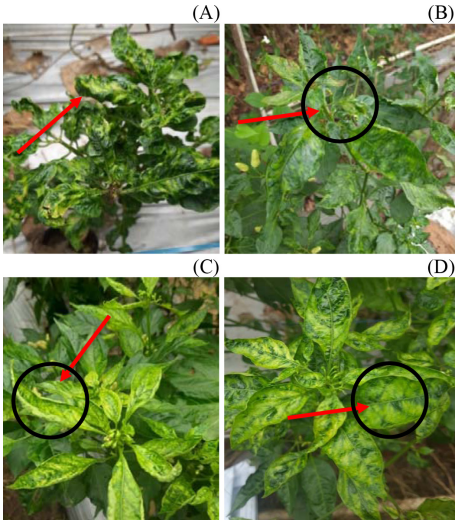

Fig. 1 Variation of virus infection symptoms of chili plants observed in the field (indicated by red arrows and circled areas): (A) leaf curling; (B) chlorosis followed by curling of young leaves; (C) yellow mosaic on young leaves with upward leaf curling; and (D) dark green stripes around leaf veins (midrib).

PCR and reverse transcriptase (RT)-PCR analyses were performed using four pairs of primers: SPG1/SPG2 for the detection of *Begomovirus* from DNA extracts; and MJ1/MJ2, CMV-F/CMV-R and TICV-F/TICV-R for RNA virus detection using RT-PCR. The results showed successful amplification with only two primer sets. The SPG1/SPG2 primers targeting the AC1/AC2 gene of *Begomovirus* produced a DNA band of approximately 912 bp, while the MJ1/MJ2 primers, targeting the coat protein gene of *Potyvirus*, generated a DNA band of approximately 320 bp. In contrast, no amplification was observed with CMV-F/CMV-R and TICV-F/TICV-R primers (Fig. 2). These findings indicate that the chili plant samples were infected with viruses from the genera *Begomovirus* and *Potyvirus*.

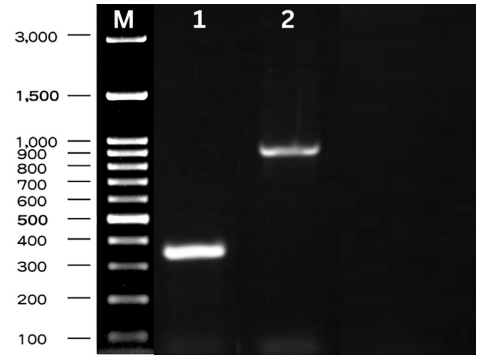

Fig. 2 Agarose gel electrophoresis of PCR amplicons using universal primers. Lane M: DNA marker; lane 1: Potyvirus (~320 bp) amplified with MJ1/MJ2; lane 2: Begomovirus (~912 bp) amplified with SPG1/SPG2.

Phylogenetic analysis and genetic relationship of viral isolates from South Lampung

Phylogenetic analysis based on nucleotide sequences using the neighbor-joining method revealed that the *Begomovirus* isolate from South Lampung was clustered within the same clade as *Pepper Yellow Leaf Curl Virus* (Genbank accession number LC895550), as shown in Fig. 3. The *Begomovirus* isolate from South Lampung and the PYLCV isolate from Kertha (LC381257) shared a nucleotide sequence similarity of 93%, suggesting that they belonged to different species within the *Begomovirus* genus. Sequence similarity between the South Lampung isolate and other *Begomovirus* species was in the range 63–74% (Table 2).

In contrast, phylogenetic analysis of the *Potyvirus* isolate (Genbank accession number LC895551) from South Lampung showed that it was clustered together with *Potato Virus Y* (PVY) from Ukraine (MN092711), with sequence similarity of 100%, indicating that they were the same species but represented different strains (Fig. 4), with a high degree of genetic identity despite differences in host range. Homology with other *Potyvirus* isolates was in the range 96–98%, further supporting their classification within the same species (Table 2). According to Fauquet et al. (2008), a nucleotide similarity value above 89% is indicative of the same viral species. In addition, Tallei et al. (2016) noted that smaller genetic distances reflected closer phylogenetic relationships. Therefore, it could be concluded that the *Potyvirus* isolate from South Lampung was a strain of *Potato Virus Y* closely related to the PVY isolate from Ukraine.

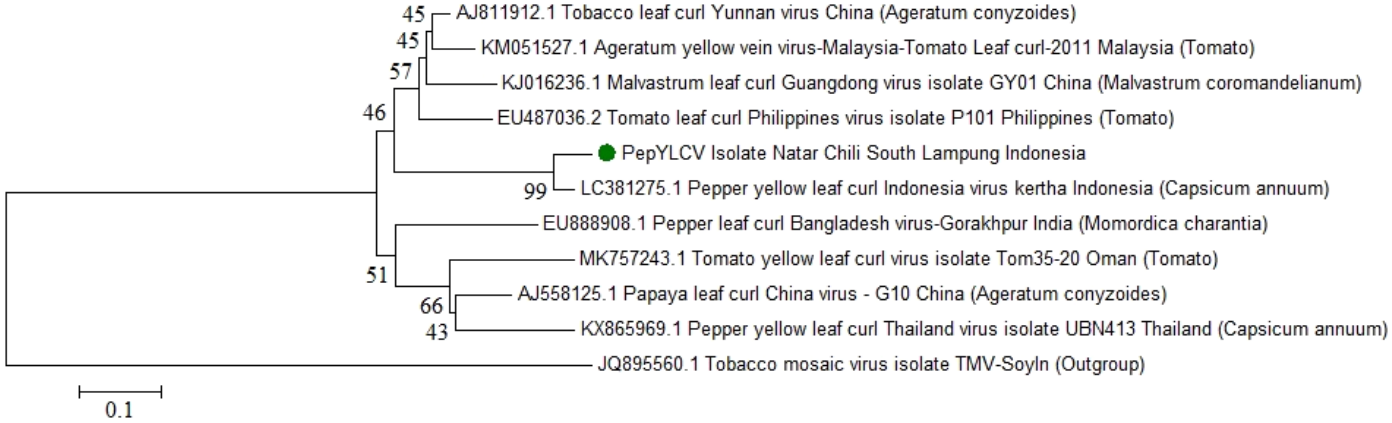

Fig. 3 Maximum likelihood phylogenetic tree of *Begomovirus* detected from chilibased on AC1/AC2 gene sequences. Bootstrap support values (1,000 replicates) are shown at the nodes. Tobacco mosaic virus (TMV) was used as the outgroup; the scale bar indicates nucleotide substitutions per site.

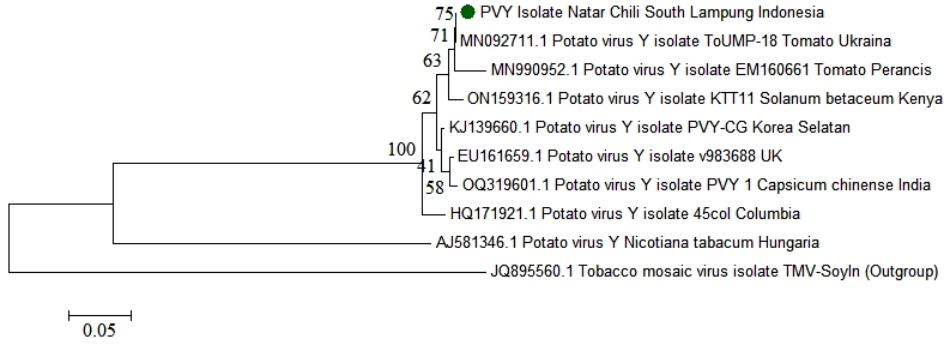

Fig. 4 Maximum likelihood phylogenetic tree of *Potyvirus* detected from chili based on coat protein gene sequences. Bootstrap support values (1,000 replicates) are shown at the nodes. Tobacco mosaic virus (TMV) was used as the outgroup; the scale bar indicates nucleotide substitutions per site.

The pairwise alignment heatmap analysis indicated a high degree of nucleotide similarity (90–100%) between the PepYLCV isolate from Natar, South Lampung and several other isolates, namely PepYLCV from Indonesia, TYLCV from Oman, PaLCuV from China and PepYLCV from Thailand. This finding, as shown by the red coloration in the heatmap (Fig. 5), suggests a close genetic relationship between the South Lampung isolate and members of the Asian Begomovirus group, specifically those from Southeast and Western Asia. In contrast, isolates from Bangladesh, the Philippines and Malaysia (AYVV) had lower similarity values (70–80%), indicating greater genetic divergence. Collectively, based on these results, the PepYLCV isolate from Natar belongs to the Old World Begomovirus complex, with a probable evolutionary origin or dispersal route associated with regions in Asia.

Similarly, the pairwise alignment heatmap analysis of the *Potyvirus* (PVY) isolate from chili plants in Natar, South Lampung revealed a high level of nucleotide similarity (90–100%) with PVY isolates from *Nicotiana tabacum* (Hungary), PVY isolate v938368 (United Kingdom) and PVY isolate 45col (Colombia) (Fig. 6). The red coloration in the heatmap reflects this strong genetic relatedness among the isolates. Conversely, PVY isolates from South Korea, Ukraine, France, Kenya and India had lower similarity values (60–80%), representing greater genetic variation within the group. Overall, these findings indicated that the PVY isolate from South Lampung is part of a globally distributed PVY group, sharing close genetic relations with isolates from Europe and the Americas. This pattern suggests a possible evolutionary origin or the spread of the virus facilitated by the migration of host plant material across regions.

The observed diversity of symptoms is unlikely to be the result of a single viral infection. The manifestation of various symptoms may be influenced by multiple factors, including the simultaneous presence of two or more virus species infecting the same plant (Sutrawati et al., 2012). According to Miftakhurrohmah et al. (2013), severe viral infections could induce mosaic symptoms accompanied by morphological alterations in leaf shape and surface, such as leaf reduction in size and undulating margins. Mixed infections are defined as the presence of more than one virus species within a single host plant. These infections may arise from concurrent viral invasions or from sequential infections by different viruses. Mixed infections can exhibit either synergistic or antagonistic interactions (Alcaide et al., 2020). Laili and Damayanti (2019) reported that mixed infections could generate symptom expressions that were distinct from those caused by individual viral infections.

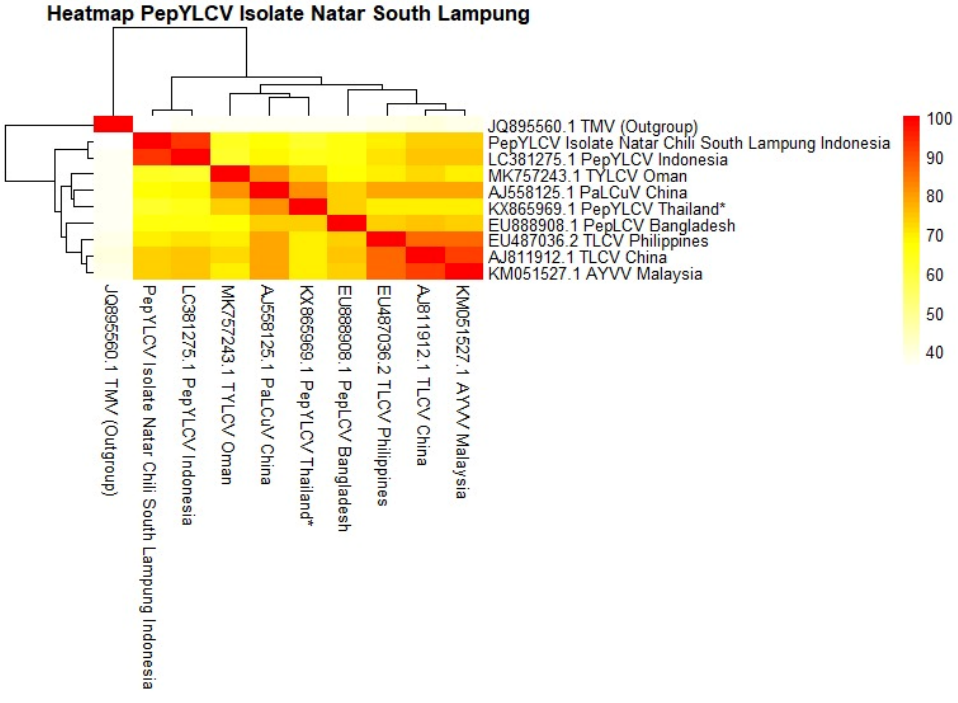

Fig. 5 Heatmap of pairwise sequence identity among the *Begomovirus* isolates from South Lampung and reference *Begomovirus* isolates from various countries. Warmer colors (red) indicate higher sequence identity (greater genetic relatedness) between isolates.

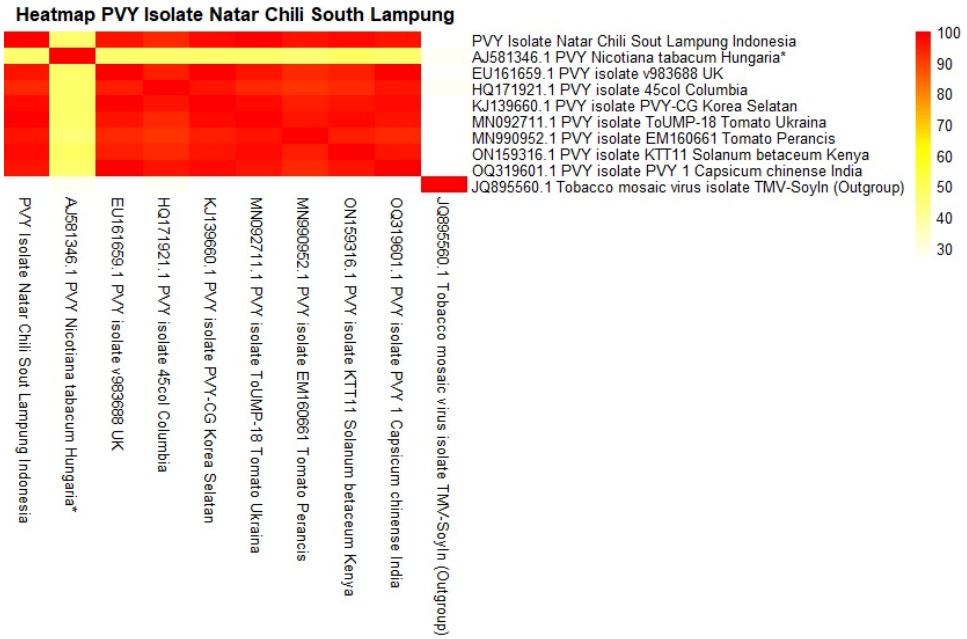

Fig. 6 Heatmap of pairwise sequence identity among the *Potyvirus* isolates from South Lampung and reference *Potyvirus* isolates from various countries. Warmer colors (red) indicate higher sequence identity (greater genetic relatedness) between isolates.

Transmission tests of the *Potyvirus* isolate from South Lampung on chili plants produced a wide range of symptom expressions, including mild to severe mosaic patterns. Additional symptoms included leaf vein banding and divergence. As the infection progressed, symptoms developed into a yellow mosaic accompanied by leaf malformations such as curling or shrinkage (Fig. 7). Symptom onset was observed at approximately 14 d post-inoculation, consistent with findings by Harveson et al. (2022). The initial symptoms were generally mild mosaics, which gradually intensified into more severe manifestations.

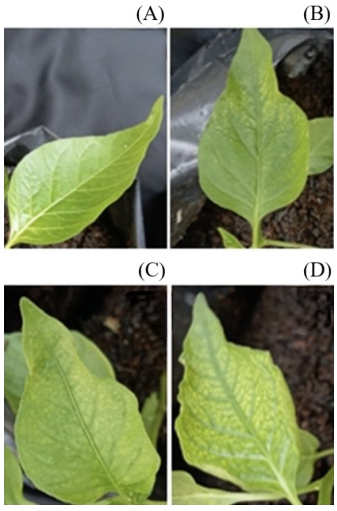

Fig. 7 *Potyvirus* pathogenicity test on chili showing leaf symptoms: (A) healthy plant (control) and infected plants with increasing symptom severity: (B) chili 1, (C) chili 2 and (D) chili 3.

Discussion

This study demonstrated that mixed infections involving *Begomovirus* and *Potyvirus* in chili plants from South Lampung reflected the inherent complexity of viral pathogenesis within agricultural systems. The field observations revealed a diverse spectrum of symptoms, including leaf curling, chlorosis with spotting, rugose dark-green thickening, upward curling and yellow mosaic patterns that are indicative of synergistic interactions between these two phylogenetically distinct viruses. Such symptom variability aligned with other reports, which indicated that co-infections frequently led to more severe, complex and unpredictable phenotypic outcomes compared to single-virus infections (Moreno et al., 2007; Gonzalez et al., 2021; Syller, 2011).

At the molecular level, co-infection by *Begomovirus* and *Potyvirus* was confirmed via PCR and RT-PCR, as evidenced by the amplification of the AC1/AC2 gene (~912 bp) specific to *Begomovirus* and the coat protein gene (~320 bp) specific to *Potyvirus*. The absence of amplification for CMV and TICV indicated their exclusion from the examined disease complex. The concurrent use of DNA-based and RNA-based detection methodologies is essential for identifying co-infecting viruses possessing divergent genomic architectures (Cassedy et al., 2021).

The phylogenetic analyses substantiated the molecular findings. The *Begomovirus* isolate from South Lampung displayed 93% nucleotide similarity to a *Pepper yellow leaf curl virus* (PYLCV) isolate from Kertha; however, it remains a distinct species according to *Begomovirus* taxonomy (species demarcation threshold: 94%), based on Brown et al. (2015). In comparison, the *Potyvirus* isolate had 100% nucleotide identity with a *Potato virus Y* (PVY) strain from Ukraine, suggesting near-complete genetic equivalence despite disparate host origins. Such phenomena are observed frequently among Potyviruses, which have extensive host adaptability and broad genetic plasticity (Wylie et al., 2017).

From an interaction perspective, co-infections of *Begomovirus* and *Potyvirus* tend to amplify disease severity through synergistic mechanisms—a trend also reported for other solanaceous crops such as tomato (Syller, 2011). Synergism enhances viral accumulation, accelerates movement within host tissues and results in greater physiological impairment and yield losses (Pruss et al., 1997; Mascia and Gallitelli, 2016). Dual infections impose additional evolutionary pressures, increasing the likelihood of recombination events that generate novel variants with altered pathogenicity, transmission dynamics, or expanded host range (Jeger, 2023; Sánchez-Tovar et al., 2025). The practice of mixed horticultural cropping in South Lampung, including chili, tomato (*Solanum lycopersicum*), eggplant and other hosts, facilitates cross-host transmission, further elevating epidemic risk.

Epidemiological dynamics are compounded by the presence of two highly efficient vectors *Bemisia tabaci* (whitefly) for *Begomovirus* and aphids for *Potyvirus* (Zaffaroni et al., 2021; McLaughlin et al., 2022; Naveed et al., 2023; Roonjha et al., 2025). Both vectors are active throughout the year in the warm, humid climate of South Lampung, facilitating continuous and overlapping virus transmission cycles. Persistent chili cultivation without adequate crop rotation perpetuates inoculum sources in the field, further complicating management.

The implications for local agriculture are considerable. Complex symptomology hinders a rapid diagnosis and a timely response, while mixed infections are associated with reduced photosynthetic productivity, stunted growth and lower fruit quality and yield (Rodríguez-Verástegui et al., 2022; Yu and Wei, 2025). Consequently, considerable economic losses can be incurred by smallholder farmers, who rely predominantly on chemical vector control strategies, which frequently are inadequate and unsustainable, particularly given the rapid reproduction and persistence of vector populations. The simultaneous presence of two distinct vectors challenges effective management, as reducing one vector does not necessarily diminish the risk from the other.

An integrated management approach is vital. This should entail routine vector monitoring, the deployment of virus-resistant chili cultivars and the adoption of adaptive cultural practices. Implementation of combined molecular diagnostics and ecosystem-based strategies is necessary to disrupt viral transmission cycles and safeguard regional chili production. These findings emphasize the urgent need for coordinated virus surveillance, resistance breeding programs and dual-vector management to ensure the sustainability of chili as a strategic horticultural commodity in Indonesia (Navas-Castillo et al., 2011).

Conflict of Interest

The authors declare that there are no conflicts of interest.

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