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Determination of Begomovirus on chili plants (*Capsicum* sp.) in Buton and Muna Islands, Southeast Sulawesi, Indonesia

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Abstract. *Widodo CJ, Taufik M, Khaeruni A, Mallaranggeng R. 2023. Determination of Begomovirus on chili plants (*Capsicum *sp.) in Buton and Muna Islands, Southeast Sulawesi, Indonesia. Biodiversitas 24: 741-751.* Begomovirus disease is one of the main factors inhibiting chili cultivation which could harm plants and result in crop failure. This study aimed to determine the symptoms of Begomovirus disease in chili plants in Buton and Muna, Southeast Sulawesi. The methods used are disease incidence survey, insect vector observation, molecular identification using the polymerase chain reaction (PCR) technique, and Sanger sequencing (Applied Biosystem 3500). The survey was conducted in Southeast Sulawesi province, covering Bau-Bau City, Buton Regency, Central Buton, North Buton, Muna, and West Muna. The results showed that the incidence and severity of Begomovirus disease were observed highest in Buton islands, 73% and 49.5% (Buton City), followed by North Buton, Central Buton, and Buton, with the incidence and severity of Begomovirus, respectively, of 71.74% and 59.5%; 69.32% and 51.5%; and 68% and 61.5%. While in Muna Regency, 63.16% and 38.5%, West Muna, 73.79% and 53.50%. On the other hand, the highest percentage of the whitefly population was in Bau-Bau City, 24%, while the lowest was in West Muna, 15.5% supporting this observation. Furthermore, PCR analysis successfully amplified symptomatic isolates from Buton and Muna islands at a band length of 580 bp. On the other hand, Sanger sequencing analysis revealed the existence of the Indonesian Pepper Yellow Leaf Curl Virus (PepYLCIV) with more than 96% genetic homology to the Gene Bank data of MN094866 PepYLCIV *Capsicum annuum* Indonesia and MN738463 PepYLCIV Chili pepper Indonesian. This information is the first report of the presence of Begomovirus on the two islands.

Keywords: Buton, chili, disease incidence, Muna, PCR, sequencing

INTRODUCTION

Chili (*Capsicum* spp.) is an essential type of horticultural crop, widely planted by farmers so that it can be found almost in various places in Indonesia. As a result, increasing production continues and occurs in almost every region. However, one of the inhibiting factors for chili cultivation is Begomovirus disease caused by Geminivirus or Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV). This virus belongs to the Geminiviridae family (International Committee for Taxonomy of Viruses/ICTV 2021 (Fiallo-Olivé et al. 2021), a virus group plant that inhibits the growth of chili cultivation in Indonesia.

Begomovirus disease can infect various cultivated plants and weeds, including chili plants. The chili juveniles can experience decay and be unable to produce when contracting the Begomovirus disease. Begomovirus diseases such as Tomato yellow leaf curl Kanchanaburi virus (TYLCKaV) in Thailand (Koeda et al. 2018), Tomato leaf curls New Delhi virus (ToLCNDV) in India (Simón et al. 2018) have harmed cultivated plants. That virus also harmed cultivated *Cassava mosaic virus* in Sri Langka (Wang et al. 2020). Including sweet potato and cassava di Sudan (Misaka et al. 2020), *Pepper golden mosaic virus* (PepGMV) in America (Barboza et al. 2019), and *Chilli leaf curl virus* in German (PepYLCIV). Plant damage reached 100% due to PepYLCIV in chili plants in Java and Sumatra (Fadhila et al. 2022). *Leguminosae* and *Solanaceae* are the most common Begomovirus hosts (Wilisiani et al. 2014; Wang et al. 2020). *Ageratum conyzoides* and *Physalis* sp. is the most common alternative host in chili cultivation (Kandito et al. 2019; Lopez-Lopez 2014).

Southeast Sulawesi Province, Indonesia consists of a mainland area and an island area. The increase in chili production occurred in several mainland areas, such as Konawe, South Konawe, Kolaka, North Kolaka, and other regions, as well as in island areas, such as Buton and Muna. In the last four years (2018-2021) on the two islands, the chili planting area has increased from 401 ha to 545 ha. The high economic worthiness of chilies causes this situation, and growing them provides farmers with a source of income. However, chili productivity in Buton and Muna continues to decline. Between those two islands, 855.7 quintals of chili were produced in 2018, decreasing to only 19.02 quintals in 2021 (BPS Sultra 2022). It is intriguing that, albeit the planting area has expanded, chili's productivity has declined. Farmers suffered significant losses due to reduced chili productivity, one of which was caused by plant diseases, and the Begomovirus disease is one of the diseases that affect chili plants.

Begomovirus disease in chili plants in Southeast Sulawesi was first reported in Kendari and East Kolaka (Taufik et al. 2018). Symptoms include mottled leaves,

yellowish green, mosaic leaves, reduced leaf width and yellow color, and curly leaves. These symptoms continue to increase along with the expansion of chili cultivation. In addition to symptomatic plants, populations of whitefly vector insects (Hemiptera: Aleyrodidae) were discovered in the chili planting region, which may hasten the spread of disease in the field. Yellow mosaic, golden mosaic, and leaf curl are signs of Begomovirus infection in the host, according to Zerbini et al. (2017). Additionally, Ghosh et al. (2019) and Pinheiro-Lima et al. (2020) described the Begomovirus host, which is primarily dicotyledonous plants and is spread by whitefly insects (*Bemisia tabaci*). The signs of a Begomovirus infection on chili plants have been documented in several prior reports as mosaic leaves and yellow color (Kesumawati et al. 2020), leaf vein clearing (Kandito et al. 2019), thickened leaves (Koeda et al. 2018), curly and wrinkled leaves (Noha et al. 2014), shortening of the internodes and petioles (Shingote et al. 2022) and stunted plants (Kwak et al. 2022).

The Begomovirus disease caused a decrease in the productivity of chili in Buton and Muna but was never reported. Therefore, this study aimed to determine the presence of Begomovirus disease in chili plants in Buton and Muna islands, Southeast Sulawesi.

MATERIALS AND METHODS

Research area

The research was conducted in six districts/cities on Buton and Muna islands, Southeast Sulawesi, Indonesia (Figure 1). The coordinate of the research area in Buton is 5.4758S 122.7917E ± 1.50 m and in Muna is 9.9423S 122.4183E ± 1.70 m.

Data collection

Data was collected on chili cultivation in six areas on Buton and Muna islands, namely Bau-Bau City, Buton, Central Buton, North Buton, and Muna and West Muna, respectively. The data collected was analyzed in the Phytopathology Laboratory, Faculty of Agriculture, Halu Oleo University, Kendari and Virology Laboratory, Gadjah Mada University, Yogyakarta, from February to June 2022. Data collection included: observation of symptoms and sampling, incidence and severity, as well as molecular identification of Begomovirus disease and whitefly vector insect populations in chili planting areas.

Observation of symptoms and sample collection of Begomovirus

The symptoms of the Begomovirus attack on chili plants were observed and determined in six areas on the Buton and Muna islands. Each site is determined by at least two locations for chili planting centers. The chili planting area is at least 100 to 200 m^2. Observations were made by making a map measuring 10 by 10 meters. The observed plant population was 5% of the total and was determined randomly. The symptoms were observed by the typical symptoms of a Begomovirus attack, such as light green-yellow mottled leaves, mosaic leaves, bright yellow leaf color, curly and clustered leaves, and small leaves and stunted plants (Taufik et al. 2018; Fadhila et al. 2022).

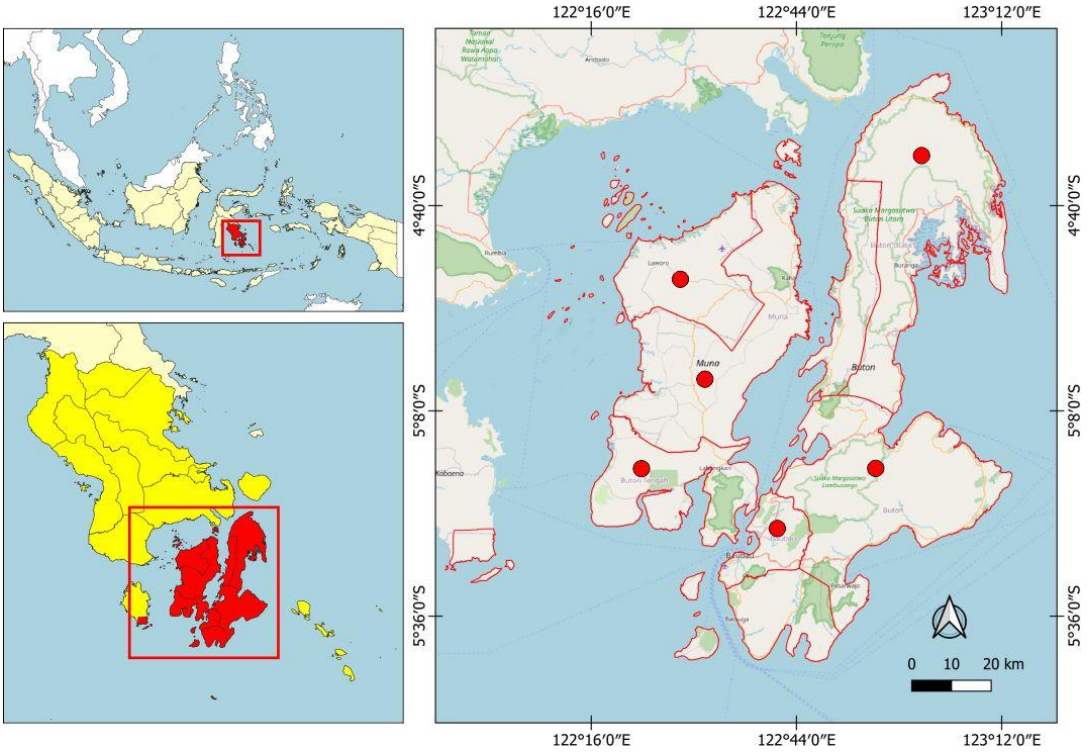


Figure 1. Study area on the determination of Begomovirus in chili plants in six areas in Buton and Muna Islands, Southeast Sulawesi Province, Indonesia. ●: Sampling area

Samples were taken from symptomatic chili plants (the tops of the leaves), and the samples were put into plastic bags containing calcium chloride ($CaCl_2$) and stored at -20°C to identify pathogens. The map is also used to calculate the disease incidence (DI) and severity (DS) of Begomovirus disease and observe the whitefly vector insect population on chili plants in each area in Buton and Muna islands.

Disease incidence (DI) of Begomovirus

The incidence and severity of Begomovirus disease were carried out in six areas of the Buton and Muna islands in predetermined observation plots. The formula calculates disease incidence:

$$DI = \frac{n}{N} x\ 100\%$$

Where:
DI : Disease incidence Begomovirus
n : Number of symptomatic chili plants
N : Number of chili plants observed

Disease severity (DS) of Begomovirus

The severity of the Begomovirus disease was assessed using the score (Gaswanto et al. 2016) that is: 0: asymptomatically, the leaves are yellow, yellow-white, or green-white, smaller than normal leaves, and the plant is stunted or healthy; 1: leaflets look dappled, light green, and yellowish; 2: the color of the leaflets is mottled, light green, and yellowish on more than half the plants; 3: mosaic leaves, only some of which are still green, and there are malformations of the leaf such as wrinkles or asymmetry, and leaf curls; 4: bright yellow leaves, vein clearing, leaf curling upwards, and thickened leaf veins; 5: all leaves are yellow, yellow-white, or green-white, the size is reduced, and the plant is stunted; The scoring results were analyzed using the formula (Khoiri et al. 2021).

$$DS = \frac{\sum (ni\ x\ vi)}{Z \times N} \times 100\%$$

Where:
DS : Disease severity of Begomovirus
i : Score 0 - 5
n : Number of symptomatic plants with certain categories
v_i : The scale of each symptom category
Z : Highest scale value
N : Total Number of plants observed

The value of disease severity (DS) obtained is then grouped based on the category of damage to chili plants (Supartha et al. 2021) can be seen in Table 1.

The population of whitefly vector insects on chili plants

The population of whitefly vector insects was estimated on plant samples based on the prevalence of previous infections as evidenced in imago, nymphs, and eggs. Calculations are carried out carefully and meticulously with the use of a loupe. The population obtained is calculated by the formula (Narendra et al. 2017), e.i.:

KB: Number of whiteflies found/Number of plants observed

The number of insect populations discovered was classified using (Sudiono and Yasin 2006). The population is high when the average is more than 20 individuals per plant, moderate is 10-20 individuals, and low is less than five individuals.

Molecular identification of Begomovirus

The PCR technique and Begomovirus DNA sequencing analysis were performed to identify symptomatic samples (chili leaves) in six areas in Buton and Muna islands. The universal primers for Begomovirus Krusty and Homer (5′-CCNMRDGGHTGTGARGGNCC-3′ and 5'-SVDGCRTGVGTRCANGCCAT-3; (Revill et al. 2003) were used. DNA samples were extracted from angled luffa leaves with curly yellow symptoms using a DNA Geneaid Minikit (Geneaid Biotech Ltd., Taiwan). As much as 0.05 g of leaf samples were placed on porcelain mortars; 400 µL GP1 buffer and five µL RNAse A were added; leaf samples with the mentioned solutions were macerated and put into a 1.5 mL tube. Solutions were homogenized, incubated in a water bath at 60°C for 10 minutes, and inverted every 5 minutes to ensure an equal temperature. The solution was added to 100 µL GP2 buffer, homogenized, and incubated on ice for 3 minutes. Mixtures were moved into 2 mL tubes, placed in a filter column, and centrifuged for 1 minute at 1,000 G. Supernatant was taken and placed in 1.5-mL tubes, and 1.5 times the supernatant volume was added to GP3 buffer. This mixture was then homogenized for 5 seconds. The mixture was placed on the GD column in the 2 mL tube, and the elution buffer was heated to 60°C.

The mix inside the GD column was centrifuged at 10,000 G for 4 minutes. The supernatant was disposed of, and 400 µL of W1 buffer was added and centrifuged at 10,000 G for 3 minutes. The supernatant was disposed of, and 600 µL of wash buffer was added, centrifuged at 10,000 G for 3 minutes, and repeated twice.

Table 1. Category of crop damage due to Begomovirus disease infection

Score	Disease severity	Damage category	
0	0%	5%	Healthy
1	> 5%	≤ 10%	Very low
2	> 10%	≤ 20%	Low
3	> 20%	≤ 40%	Moderate
4	> 40%	≤ 60%	High
5	> 60%	≤ 100%	Very high

The supernatant was disposed of and recentrifuged at 10,000 G for 6 minutes until the column matrix was dry. GD columns were placed in 1.5 mL tubes, and 100 µL of pre-heated elution buffer was added, left for 3 minutes, and centrifuged at 10,000 G for 2 minutes. Extracted solutions were stored at -20°C. Total volumes: 25 mL, 2,5 mL buffer PCR (10 x buffer: 100 mM Tris-HCl, 500 mM KCl, pH 8,3), 0,5 mL dNTP mix (4 mM), 1,0 mL primer (10 mM), 1 unit DNA Taq polymerase, 2 mL DNA template, and 17,8 mL aquabidest. The PCR reaction started with a predenaturation stage at 95°C for 1 minute. Denaturation at 95°C for 14 seconds, an annealing stage at 55°C for 15 seconds, and elongation at 72°C for 10 seconds; repeated for 35 cycles, and the process ended with a final elongation stage at 72°C for 5 minutes. The DNA electrophores were visualized on a 1% agarose gel at 50 V for 50 minutes. The targeted DNA band was 580 bp (Revill et al. 2003).

DNA sequencing analysis of Begomovirus chili on Buton and Muna islands

Begomovirus DNA sequencing in chilies from Buton and Muna islands was carried out using the Sanger sequencing dideoxy nucleotide chain termination method and an ABI Prism 3500-Avant Genetic Analyzer (Applied Biosystems 3500 Genetic Analyzer 2500). DNA analysis using polymer (POP7) and forward and reverse primers). The stages of sequencing are the DNA template sequencing cycle determined by the purification of the first stage, which is 100-200 bp (1-3 ng), 200-500 bp (3-10 ng), 500-1000 bp (10-40 ng), and 1000-2000 bp (20-50 ng). The primer is determined according to the target of only one primer (F or R) for each reaction tube one tube for forward primer and one tube for reverse primer). The following preparation was made, namely (template 1 µL/0.5 µL), (primer (F/R) (3.2 µm/ µL) 1 µL/0.5 µL), (Big Dye Terminator 2 µL/1 µL), (Buffer 5 × 4 µL/2 µL) and (H_2O (nuclease-free water) 12 µL/6 µL). The total volume of 20 µL/10 µL l is mixed thoroughly and centrifuged (*spun down*). The cycle sequencing program was determined on a thermal cycler machine, namely initial denaturation 96^{o}C 1 minute, cycles 24-27 x in (96^{o}C 10 seconds), (50^{o}C 5 seconds) and (60^{o}C 4 minutes). The sequencing results were stored at 4^{o}C until further purification was carried out. The Bigdye X-Terminator Purification Kit was used for the second stage of purification, in which 10 ul of DNA from cycle sequencing was mixed with 45 µL of SAM solution and ten µL of BDX-T (BDX-T was mixed with the vortex for 5 seconds before collection). The mixture was vortexed again for 30 minutes, then centrifuged at 1,000 xg for 5 minutes, and then 10-20 µL of the top liquid was carefully taken and put into the wells provided and closed with septa. The wells were then centrifuged to remove air bubbles.

Sequencing data was collected by electrophoresis using the ABI 3500 Genetic Analyzer, in which a simple plate was inserted into the sequencing tool, and the running plate was set in the form of a name file or plate identity, polymer POP 7, 50 cm capillary). Mode: Sequencing/ Fragment/MixFragm+Seq dan Assay: Fastseq_POP7_50 (target < 500 bp polymer POP7 capillary length 50 cm) and Stdseq_POP7_50 (target 500-1000 bp polymer POP7 capillary length 50 cm). Then the sample is given a name, and the process takes place automatically.

DNA sequencing or scoring/base caller analysis was conducted using the SeqA software available on the European Bioinformatics Institute (EBI) website by opening the SeqA software. Furthermore, the similarity and phylogenetics of the nucleotide and amino acid sequences of isolates from the Buton and Muna islands were determined using the ClustalW software available on the EBI website.

RESULTS AND DISCUSSION

Symptoms, incidence, and severity of Begomovirus disease in Buton and Muna Islands

Symptoms of Begomovirus in chili cultivations in Buton island were pale yellow leaves, wavy leaf edges, thickened leaf bones, and stunted plants. In Buton island, disease incidence and severity were 68% and 61.5%, respectively. Meanwhile, the symptoms of Begomovirus chili cultivations in Muna Island on the leaflets are bright yellow, shriveled, and wavy, with a disease incidence of 73.78% and a severity of 53.5% (West Muna). The observation results of symptoms, incidence, and severity of Begomovirus disease in Buton and Muna Islands are shown in (Table 2).

Begomovirus infection in chili plants in Buton and Muna islands caused various symptoms, including changes in plant height, leaf size and color, and the incidence and severity of the disease. In addition, the population density of whitefly insects also varies (Table 2).

The variations in symptoms are mosaic leaf color, pale yellow and pale green leaf color, malformed leaves, thickened leaf bones, smaller leaves, and stunted plants. However, there are differences in symptoms on the islands of Buton and Muna. On Buton Island, the predominant symptoms of chili plants infected with Begomovirus disease are pale yellow and pale green leaf color, whereas, on Muna Island, it is bright yellow (Figure 2).

The incidence and severity of Begomovirus disease in chili plants were observed highest in Buton island, e.g., 73% and 49.5% (Buton City), followed by North Buton, Central Buton, and Buton, with the incidence and severity of Begomovirus, respectively, of 71.74% and 59.5%; 69.32% and 51.5%; and 68% and 61.5%. On the other hand, in Muna island, West Muna has the incidence and severity of Begomovirus disease in chili plants at moderate levels, e.i. 73.78% and 53.5%, and Muna at 63.16% and 38.5% (Table 2).

Table 2. Variation of symptoms, incidence, and severity of Begomovirus disease of chili from exploration in Buton and Muna islands, Southeast Sulawesi, Indonesia

Isolate	Variation of symptoms in the field	Dominant symptom	Disease incidence (DI) %	Disease severity (DS) %
Bau-Bau City	mc, dk, dkg, mf	Mosaic and wavy leaves	73 ***	49.5 ***
Buton	mc, kup, hjp, dk, dkg, tb, kd	Pale yellow leaves, wavy, thickened leaf bones, and stunted	68 ***	61.5 ***
Central Buton	mc, ku, kr, kj, tb	Mosaic leaves, shriveled, wavy, and thickened leaf bones	69.32 ***	51.5 ***
North Buton	mc, ku, dk, kr	Mosaic leaves, shriveled, wavy, and thickened leaf bones	71.74 ***	59.5 ***
Muna	mc, kj, kr	Mosaic leaves, shriveled, wavy, and thickened leaf bones	63.16 ***	38.5 **
West Muna	mc, ku, kj, kr, cp, mf	Young leaves bright yellow, shriveled, and wavy	73.78 ***	53.5 ***

Description: mc: *mosaic leaf*, dkg: *leaf curling and waving*, dk: *leaf yellowing*; kj: *leaf curling green color*, tb: *leaf vein thickness*, kd: *stunt*, mf: *malformation* mosaic, crinkle, *vein clearing*, cp: *cupping*; ku: yellow, kup: pale yellow color, hjp: pale green. *: low **: moderate, ***: severe

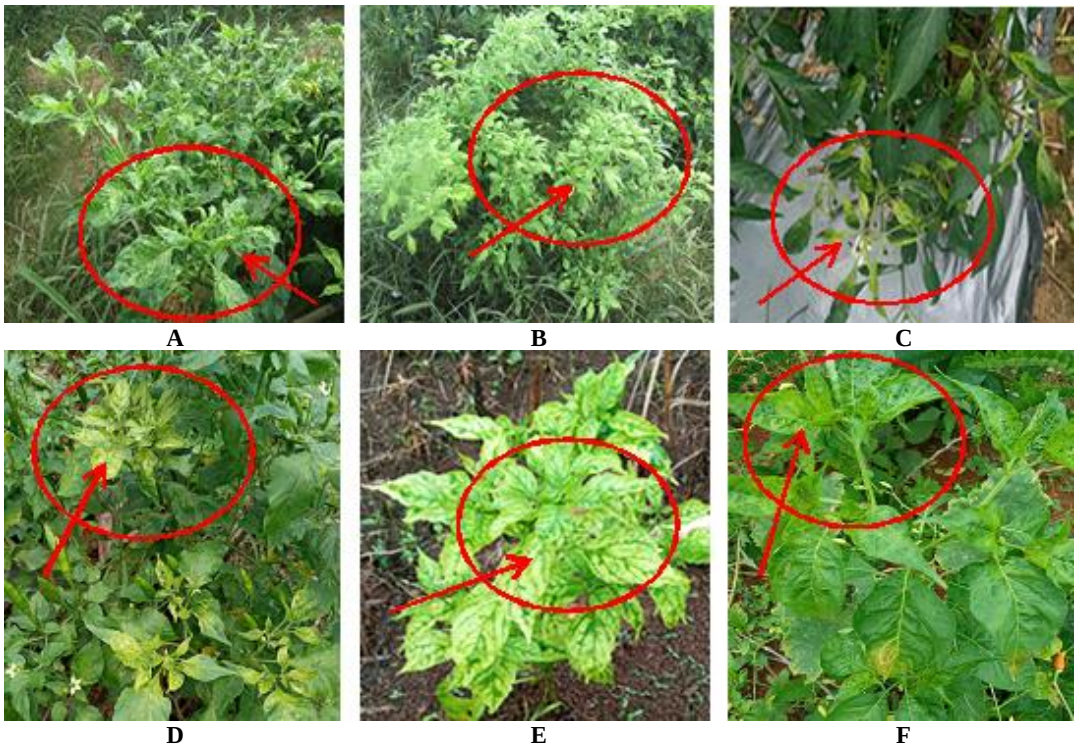


Figure 2. Symptoms of Begomovirus attack on chili plants in six areas in Buton in Muna, Southeast Sulawesi, Indonesia. A. North Buton, B. Bau-Bau City, C. West Muna, D. Central Buton, E. Buton, F. Muna

Table 3. The average population of whitefly vector insects on the islands of Buton and Muna, Southeast Sulawesi, Indonesia

Location	Average whitefly population (%)
Bau-Bau City	24 ***
Buton	22,5 ***
Central Buton	16 **
North Buton	21,5 ***
Muna	15,5 **
West Muna	16 **

Note: *: low **: moderate, ***: severe.

Whitefly vector insect population on chili plants in the Buton and Muna islands

Observations of whitefly vector insect populations on chili cultivation in six areas on Buton and Muna showed varying population levels. On the island of Buton, the whitefly population rate is high: 24% in Bau-Bau City, 22.5% in Buton, 21.5% in North Buton, and 16% in Central Buton. On the other hand, on the island of Muna, the whitefly vector insect population was classified as moderate, e.i. West Muna at 16% and Muna at 15.5% (Table 3).

Molecular identification of Begomovirus in the Buton and Muna islands

The polymerase chain reaction (PCR) method with a pair of Begomovirus universal primers Krusty & Homer (Revil et al. 2003) successfully identified symptomatic leaf samples on chili plants in six places Buton and Muna islands as being infected with the virus. The amplified fragment was 580 bp in size (Figure 3). PCR results showed a positive Begomovirus came from North Buton, Bau-Bau City, Central Buton, Buton, and Muna, but isolates from West Muna had yet to be successfully amplified. However, these samples showed typical symptoms of Begomovirus.

Sequencing analysis of Begomovirus DNA in Buton and Muna Islands

Sequencing analysis of Begomovirus DNA in chili cultivation on Buton and Muna islands, Southeast Sulawesi using the sanger sequencing dideoxy nucleotide chain termination. Then, followed by ABI (Applied Biosystems 3500 Genetic Analyzer 2500) method. Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV) species were identified using Polymer (POP7) DNA analysis and forward and reverse primers. They were grouped with those discovered in the Genk Bank database (Figure 4).

Sanger sequencing analysis Begomovirus, which infects chili plants in Buton and Muna, Southeast Sulawesi, Indonesia, belongs to the Indonesian Pepper Yellow Leaf Curl Virus (PepYLCIV) group. PepYLCIV Buton (B) and PepYLCIV (MN) isolates share genetic similarities with the PepYLCIV group in Genk Bank, such as MNT738466; PepYLCIV Solanum lycopersicum Indonesia, MZ605913; PepYLCIV Pepper Indonesia, MN738463; PepYLCIV Chili Pepper Indonesia, MN094866; PepYLCIV Capsicum annum Indonesia, AB267838; PepYLCIV Ageratum Indonesia, DQ083765; PepYLCIV Thai Pepper, LC051115; PepYLCIV Capsicum annum Indonesia, LC051114; PepYLCIV Capsicum annum Indonesia, KT809346; PepYLCIV Capsicum annum Indonesia and KX900491; PepYLCIV Pepper Thailand. However, PepYLCIV on the islands of Buton and Muna is genetically different from the AY184487 SL group CCNV and JN587811 Pumpkin CCNV1 in India.

Using forward and reverse primers, Sanger sequencing analysis of PepYLCIV DNA in Buton (B) and Muna (MN) islands yielded the same DNA nucleotides/amino acids. Therefore, forward primers nucleotide/amino acid sequences in Buton and Muna islands are shown in the red column, and nucleotide/acid sequences with reverse primers are shown in the green column (Figures 5 and 6).

The nucleotide sequence of PepYLCIV, which infects chili plants on Buton and Muna islands, has a degree of genetic similarity (homology) above 96% with the PepYLCIV group. The GenBank database has previously reported that such as in MN094866 PepYLCIV *Capsicum annum* Indonesia and MN738463 PepYLCIV Chili *pepper* Indonesia. PepYLCIV nucleotide sequence homology results on the two islands prove that the *Pepper yellow leaf curl* Indonesia virus (PepYLCIV) of the genus Begomovirus has been determined in chili plants in Buton and Muna, Southeast Sulawesi.

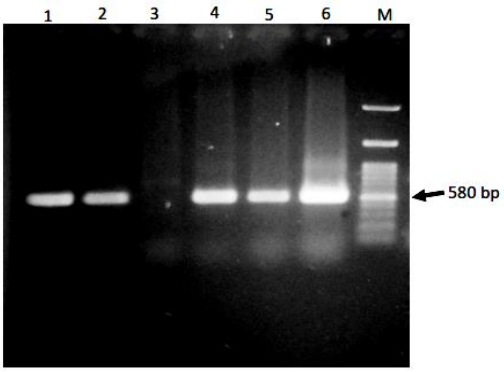


Figure 3. PCR amplification of Begomovirus isolates in Buton and Muna islands, Southeast Sulawesi, Indonesia with a 580 bp DNA fragment size. 1. Isolates North Buton, 2. Bau-Bau City, 3. West Muna, 4. Central Buton, 5. Buton, 6. Muna dan M (Marker 1000 bp)

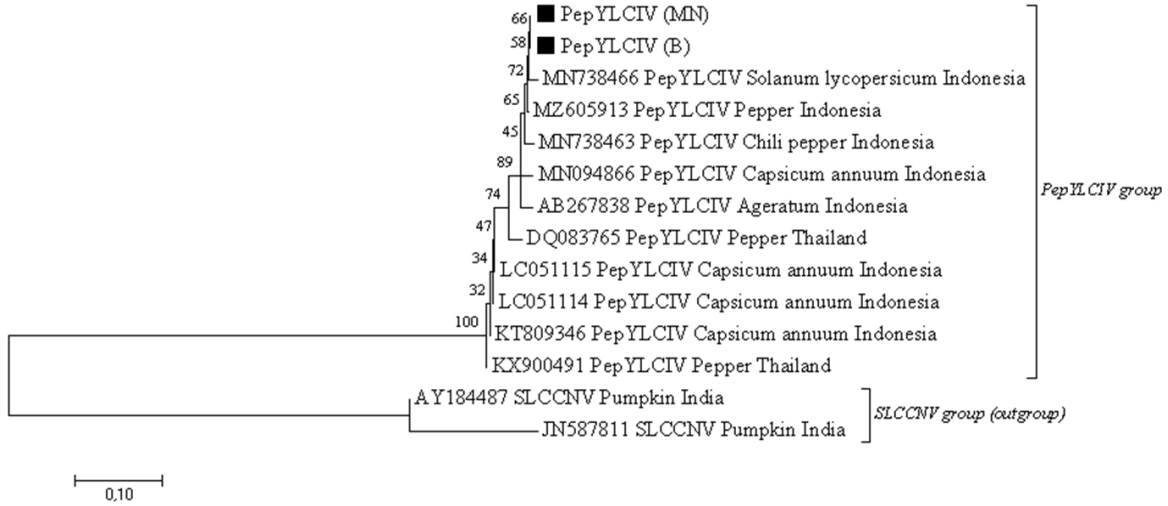
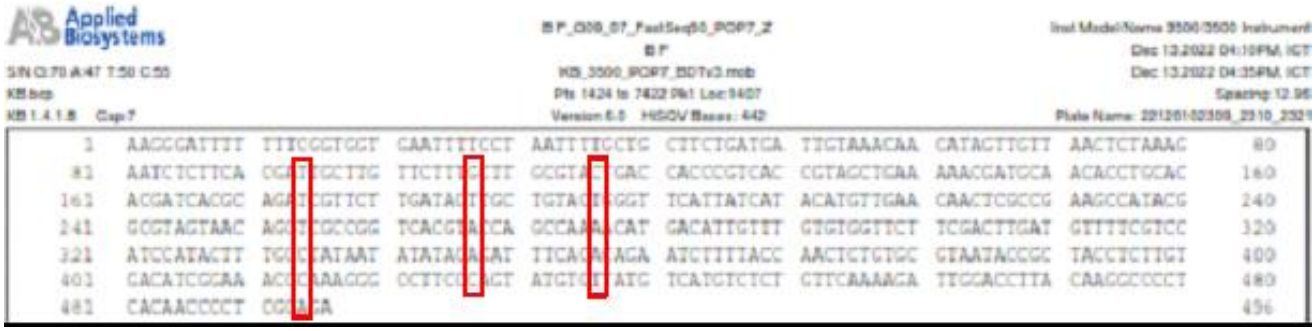


Figure 4. Phylogenetic diagram of PepYLCIV in Buton (B) and Muna (MN), Southeast Sulawesi, Indonesia compared to other PepYLCIV isolates available in the GenBank database

Using a forward primer (F)



Using a primer reverse (R)

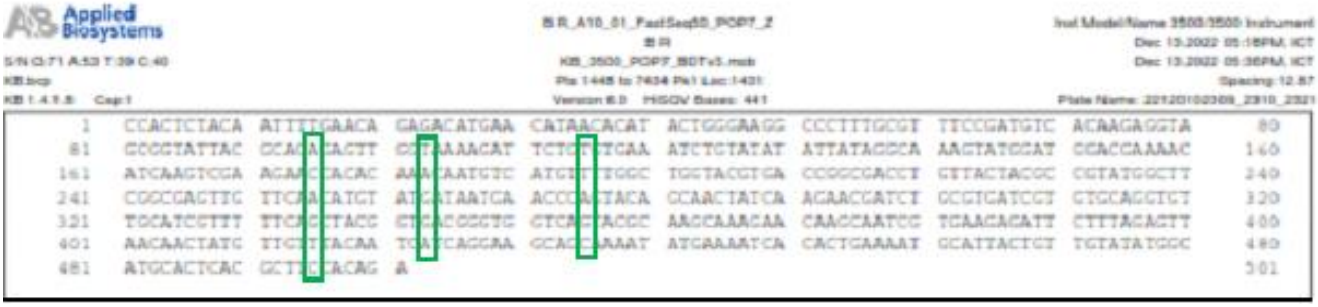
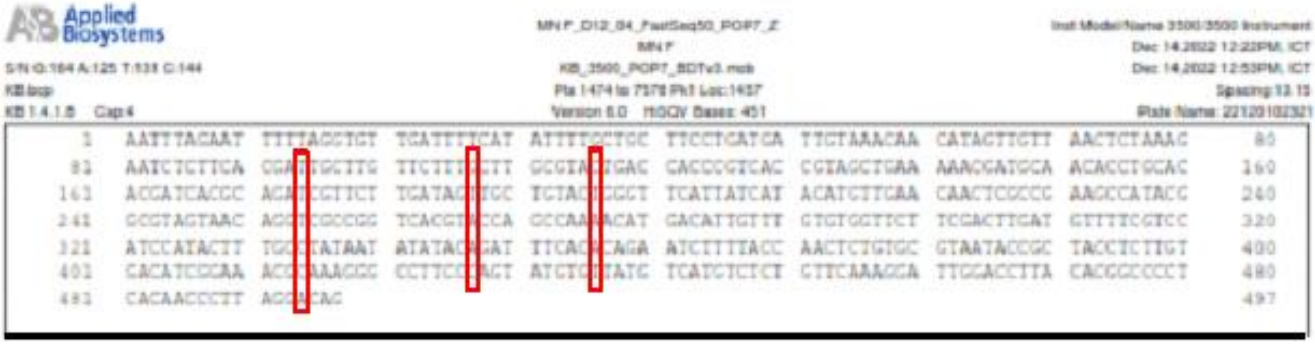


Figure 5. PepYLCIV nucleotide/amino acid sequence in Buton Island, Southeast Sulawesi, Indonesia

Using a primer forward (F)



Using a primer reverse (R)

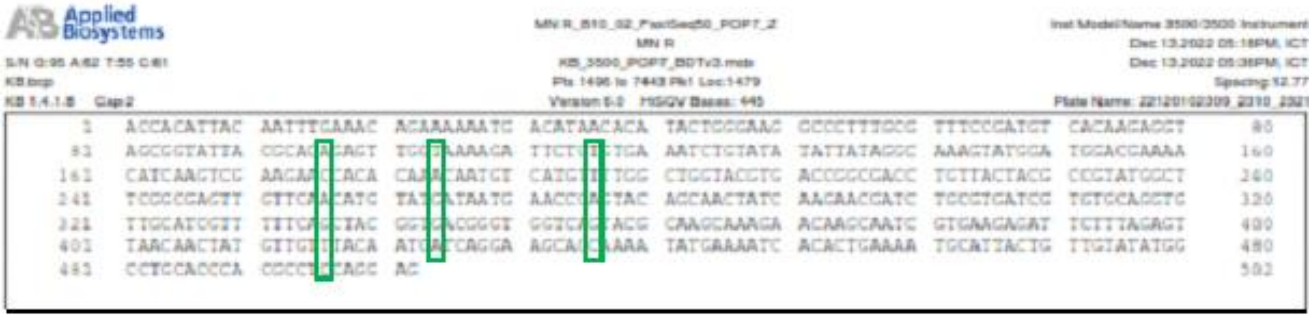


Figure 6. PepYLCIV nucleotide/amino acid sequence in Muna Island, Southeast Sulawesi, Indonesia

Table 4. Degree The percentage of similarity (homology) of the MN (Muna) and B (Buton), Southeast Sulawesi, Indonesia code sample sequences compared to isolates from GenBank

No.	Identity	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	PepYLCIV_(MN)	ID													
2	PepYLCIV_(B)	100,0	ID												
3	MZ605913_PepYLCIV_Pepper_Indonesia	99,8	99,8	ID											
4	AB267838_PepYLCIV_Ageratum_Indonesia	97,6	97,6	97,8	ID										
5	MN738466_PepYLCIV_Solanum_lycopersicum_Indonesia	99,0	99,0	98,8	96,9	ID									
6	MN738463_PepYLCIV_Chili_pepper_Indonesia	98,6	98,6	98,8	97,1	97,6	ID								
7	MN094866_PepYLCIV_Capsicum_annuum_Indonesia	97,8	97,8	97,6	97,3	97,1	97,1	ID							
8	LC051115_PepYLCIV_Capsicum_annuum_Indonesia	96,1	96,1	96,3	95,6	95,1	96,1	95,6	ID						
9	LC051114_PepYLCIV_Capsicum_annuum_Indonesia	95,8	95,8	96,1	95,3	94,8	95,8	95,3	99,8	ID					
10	KX900491_PepYLCIV_Pepper_Thailand	95,6	95,6	95,8	95,1	94,6	95,6	95,1	99,5	99,3	ID				
11	KT809346_PepYLCIV_Capsicum_annuum_Indonesia	95,8	95,8	96,1	95,3	94,8	95,8	95,3	99,8	99,5	99,8	ID			
12	DQ083765_PepYLCIV_Pepper_Thailand	95,8	95,8	96,1	95,8	95,1	96,1	95,6	96,8	96,6	96,3	96,6	ID		
13	AY184487_SLCCNV_Pumpkin_India	-5,1	-5,1	-4,7	-1,9	-4,4	-3,9	-5,6	1,0	1,6	1,6	1,6	-2,1	ID	
14	JN587811 SLCCNV Pumpkin India	-21.2	-21.2	-21.5	-19.0	-20.4	-19.6	-19.9	-14.8	-14.1	-14.1	-14.1	-15.6	86.5	ID

Discussion

Observations of Begomovirus disease attacks on chili plants in six areas in Buton and Muna showed a variety of symptoms ranging from the expression of leaf color, leaf size, plant height, variations in the incidence and severity of Begomovirus disease, and variations in whitefly insect populations around chili cultivations (Table 2). However, the dominant symptoms of Begomovirus on Buton Island are pale yellow and pale green leaf colors, thickening of the leaf veins, and stunted plants. On the other hand, the symptoms of Begomovirus on Muna Island are dominated by symptoms on young leaves, which are mosaic and bright yellow, trim and narrow leaves, and curly leaves (Figure 2). The difference in symptoms on chili plants due to the Begomovirus attack on the two islands could be due to differences in agroecosystems. A hilly topography dominates the agroecosystem on Buton Island, with slightly sandy and sometimes rocky soil. In contrast, on Muna Island, the agroecosystem is lowland with sandy peat soil types.

The study by Febria et al. (2015) explained that differences in agroecosystem conditions could lead to differences in the symptoms, incidence, and severity of Begomovirus disease in chili plants. The appearance of differences in symptoms and severity can be caused by infection with Begomovirus alone or in combination with other types of Begomovirus in the same host or are still closely related (Sulandari 2004). Multiple infections of Begomovirus and *Chilli veinal mottle virus* add to the severity of Begomovirus disease (Trisno et al. 2021). Polston and Anderson (1997) have reported that the presence of new virus strains, cultivars, plant age at infection, the activity of insect vectors, and different environmental conditions (temperature, humidity, and topography) can cause variations in symptoms. Some of the symptoms of Begomovirus in chili plants have been previously reported in various countries. For example, light green-yellow mottled leaves in mainland Southeast Sulawesi (Taufik et al. 2018), mosaic and bright yellow leaves in Java and Sumatra (Fadhila et al. 2022), curly and clustered leaves in India (Shingote et al. 2022), yellow leaves and thickened leaves in Uganda (Mollel et al. 2020) and narrowed leaves and stunted plants in Myanmar (Kwak et al. 2022). Zerbini et al. (2017) reported yellow mosaic, gold mosaic, and leaf curl symptoms in host plants.

The incidence and severity of Begomovirus disease in six areas on the two islands are classified as severe. The disease incidence and severity of Begomovirus are highest in Bau-Bau City, Buton Island, at 73% and 49.5%, respectively. In Muna, the incidence and severity of Begomovirus disease in chili plants is moderate; the highest was in West Muna, e.i. 73.78% and 53.5% (Table 1). The trend for high levels of Begomovirus infection in the two regions is due to farmers' insufficient knowledge and skills about the geminivirus. The interviews with farmers revealed they did not know the types of diseases, host plants, or vectors that could transmit Begomovirus. Farmers' chili seedlings are still on open land and have not been treated. This method can accelerate the early infection of Begomovirus disease in chili plants from the nursery. The expansion of farmers' chili planting areas without any control efforts, such as destroying infected plants, rotating varieties, and controlling insect vectors, triggers the development and spread of Begomovirus disease on the two islands. Several previous reports have described the high severity of the Begomovirus disease. Ningrum et al. (2008) reported that the severity of disease in Top and TM 999 was 90% in Cangkringan and Pakem, Yogyakarta (DIY), Apollo and TM 999 varieties in Borobudur and Congkriangan, Central Java, 55.5% and 90%, respectively. Even the severity of Begomovirus disease in cayenne pepper in Jawa and Sumatera can reach 100% (Fadhila et al. 2022). In Thailand and Germany, the severity of Begomovirus disease in cayenne pepper is 95% and 100% (Chiemsombat et al. 2018: Thakur et al. 2018), disease incidence of Begomovirus disease in cayenne pepper in Myanmar 69.6% (Kwak et al. 2022). Begomovirus attacks on chili plants in India can reduce production by 42.81% (Shingote et al. 2022).

Chili cultivation habit by farmers on Buton and Muna islands has also become one of the triggers for the development of Begomovirus disease on these two islands. In general, chili cultivation is still planted simultaneously (intercropped) with other plants such as tomato

(*Lycopersicon esculentum*), eggplant (*Solanum melongena*), cucumber (*Cucumis sativus*), luffa or oyong (*Luffa acutangula*), and other plants. Meanwhile, these commodities are the mainstays in this region. Abidin (2018) uses the abbreviations *Location Quotient* (LQ) and *Dynamic Location Quotient* (DLQ) to determine the base commodity to produce tubers as the base commodity in this region. Moreover, these cultivated plants can act as alternative hosts for Begomovirus disease. Farmers have not understood this fact in the six areas on the island of Buton and Muna observed. Various weeds, such as babadotan (*Ageratum conyzoides*), ceplukan (*Physalis* sp.*)*, and others around the chili cultivations, must be considered by farmers as host plants on the two islands. Moreover, it is known that 600 species of Begomovirus host plants include cultivated plants such as tubers and weeds. The sweet potato plant has been identified as a new host for Begomovirus in Malang, Indonesia (Damayanti et al. 2019), *Tomato leaf curl New Delhi virus* (ToLCNDV) di India (Simón et al. 2018), *Sweet potato and cassava* di Sudan (Misaka et al. 2020) and *Pepper golden mosaic virus* (PepGMV) di America (Barboza et al. 2019). *Leguminosae* and *Solanaceae* are the most common Begomovirus hosts (Wilisiani et al. 2014; Wang et al. 2020; Kwak et al. 2022). Weed babadotan (*Ageratum conyzoides*) (Kandito et al. 2019), (*Physalis* sp) (Lopez-lopez 2014) and *Verbesina encelioides*, *Calotropis procera*, *Mimosa pudica* is the most common alternative host in chili cultivation (Prajapat et al. 2014).

The existence of whitefly insect populations (Hemiptera: Aleyrodidae) also indicates the spread of Begomovirus disease symptoms in chili plants on the two islands. The whitefly vector insect population in chili cultivations on Buton Island is relatively high, and the highest whitefly insect population is found in Bau-Bau City, namely, 24%. On the island of Muna, the population level of whitefly insects is moderate, namely 15.5% (moderate) (Table 3). The abundance of whitefly insects in chili cultivations on the islands of Buton and Muna is also one of the triggers for the spread of Begomovirus disease on the two islands. The number of whiteflies can accelerate the spread of the virus in the crop. One whitefly could transmit Begomovirus from sick chili plants to healthy plants (Sulandari et al. 2001; Hadiat et al. 2022). In the field, Begomovirus (PepYLCIV) is only transmitted by whitefly insects (*Bemisia tabaci)* (Ghosh et al. 2019; Pinheiro-Lima et al. 2020). The higher the population of whitefly vector insects, the higher the incidence of Begomovirus disease (Narendra et al. 2017). The population of whitefly vector insects will continuously grow if the availability of host plants supports it, even at all times. The data shows that the area of chili planted on the two islands is increasing from time to time with a crop pattern that is not synchronized so that the availability of hosts is also continuously available. This phenomenon can be observed in the various ages of chili plants. Gambley et al. (2022) state that food availability affects insect vectors' activity. Based on observations of symptoms, incidence, and severity of disease in the two islands, whitefly populations are always found in chili cultivations. Therefore, an appropriate control strategy is needed to prevent the increased incidence of Begomovirus disease on the island.

Molecular identification of symptomatic chili leaf samples in six areas of Buton and Muna islands was observed using PCR with a pair of Begomovirus universal primers. That resulted in a positive Begomovirus infection. The positive sample showed a 580 bp DNA fragment, except that the Begomovirus isolates from West Muna could not be amplified. However, the typical symptom of yellow curly leaves was found in these plant samples (Figure 3). The DNA fragments in the two islands are similar to the Begomovirus DNA in chili plants in Yogyakarta and Central Java (580 bp) (Kandito et al. 2019). The size of DNA fragments from chili Begomovirus PCR results in several areas in Indonesia has been previously reported, such as in Java and Sumatra, at 400 bp and in Bali at 912 bp (Fadhila et al. 2022; Selangga et al. 2019). Several countries have also previously reported the size of DNA fragments due to molecular identification of Begomovirus in chili plants by PCR. Overseas Begomovirus has also been reported in Thailand at 258 bp (Chiemsombat et al. 2018), Myanmar at 2759 bp (Kwak et al. 2022), Arab Saudi at 290 bp (Rezk 2016), Mesir at 530 bp (Noha et al. 2014) and Israel at 438 bp (Dombrovsky et al. 2010).

The sequencing analysis of Begomovirus DNA in chili cultivations on Buton and Muna islands, Southeast Sulawesi, observed by Sanger sequencing dideoxy nucleotide chain termination method. Another observation by an ABI Prims 3500-Avant Genetic Analyzer (Applied Biosystem 3500 Genetic Analyzer 2500). Those observation results yielded the species Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV) shown in Figure 4. The PepYLCIV (B) and PepYLCIV (MN) species on the two islands have over 96% genetic similarity with the PepYLCIV group, which has been previously reported in the Genk Bank database, such as MZ605913 PepYLCIV Pepper Indonesia. The observed PepYLCIV group is similar to MN738463 PepYLCIV Chili *pepper* Indonesia, MN094866 PepYLCIV *Capsicum annum* Indonesia, AB267838 PepYLCIV *Ageratum* Indonesia, DQ083765 PepYLCIV Thai Pepper, and KX900491 PepYLCIV Thai Pepper. Paradisa et al. (2022) explained that the PepYLCIV group also has over 93% genetic similarity (homology) with PepYLCIV in Congkrang, Muntilan, Central Java, in North Sumatra-Chile LC051112, in East Java Chile MN738463, in Bali-Pseuderanthemum MN094868, and Bogor-Chile DQ083764. It was further explained that Thai Pepper DQ083765 PepYLCIV has 100% genetic similarity with PepYLCIV AB267834, PepYLCIV NC008283, and PepYLCIV DQ083764 (Sinha et al. 2013). Therefore, it concluded that the chili plants on the islands of Buton and Muna had been infected by PepYLCIV disease.

It can be concluded that PepYLCIV diseases have infected chili plants in Buton and Muna islands, Southeast Sulawesi. Those conclusions based on the symptoms of PepYLCIV, the presence of whitefly vector insect populations around chili cultivations, the DNA fragment size of 580 bp from PCR results, and the DNA genetic

homology with the species group Pepper Yellow Leaf Curl Indonesia Virus (PepYLCIV) found in GenBank. These results are the first information on the presence of PepYLCIV in chili plants in Buton and Muna Islands, Southeast Sulawesi.

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