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RESEARCH PAPER

Recombinant antibody production by cloning of *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) coat protein gene

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ABSTRACT

Pepper yellow leaf curl Indonesia virus (PepYLCIV) is an important pathogen on chili cultivation and is transmitted through the seed. Serological tests are sensitive, accurate, efficient and it has been widely used for the detection of seed-transmitted plant viruses. This study aimed to produce PepYLCIV recombinant protein as a material to produce recombinant antibodies PepYLCIV. DNA was extracted from infected chili leaves collected from Congkrang, Muntilan, Central Java verified using primer PepYLCIV-*Bam*HI and PepYLCIV-*Eco*RI and produced an amplicon at 840 bp. The amplified fragments were cloned into the pET32a then transformed to *Escherichia coli* BL21. The percentage of nucleotide sequence identity and sequence of amino acid, PepYLCIV CK-6 isolates had the highest similarity of nucleotide and amino acid sequences to of chili isolates from Bandung. The expressed recombinant protein was obtained with IPTG concentration 0.5 mM and harvested at 6 hours after IPTG induction. SDS PAGE analysis of the recombinant plasmid Begomovirus CK-6 showed that the coat protein size was about 29 kDa. Immunization was carried out on rabbits by injecting 150 µg of recombinant protein 4 times with an interval of 1 week to produce crude antiserum and pure antiserum capable of detecting PepYLCIV in chili and *Ageratum conyzoides* using I-ELISA and DIBA tests.

Key words: Begomovirus, chili, polyclonal antibody, recombinant protein

INTRODUCTION

Chili is an important horticultural product that has high economic value. In Indonesia, chili has been widely used as a spice and one of the raw materials in the food and pharmaceutical industries (Munandar et al., 2017). During 2010 to 2019, the chili cultivating area increased, resulting in an increase in chili production and export volume (PUSDATIN, 2020). Between the years 2000 to 2019, the export volume of chili was boosted by 7.42% (PUSDATIN, 2020). Recently, however, improvements in chili production are currently hampered by problems of plant pests and diseases, one of which is a viral disease. Infection of viral disease in the field was very high, up to 100% (Sudiono et al., 2005). Several viruses were also found in chili, including *Cucumber mosaic virus*, *Chilli veinal mottle virus* (Veniari et al., 2015), *Tobacco mosaic virus* (Kumar et al., 2011), and the other viruses from the genus of Begomovirus (Laprom et al., 2019).

One of the viruses that cause major losses to chili plants is *Pepper yellow leaf curl Indonesia virus* (PepYLCIV) (Fadhila et al., 2020; Neriya et al., 2020). Chili plants infected with PepYLCIV have yellow mosaic, mottle, and yellowing symptoms (Selangga & Listihani, 2021). PepYLCIV was caused by Begomovirus already spread out in various locations in Indonesia like Java (Sulandari, 2004; Fadhila et al., 2020), Bali (Neriya et al., 2020; Selangga & Listihani, 2021) and also found in Sumatra (Jamsari & Pedri, 2013; Koeda et al., 2018). PepYLCV belongs to Family Geminivirus and Genus Begomovirus which infect dicotyledonous plants. This virus can be transmitted by

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whitefly (Inoue-Nagata et al., 2007), grafting (Koeda et al., 2018), and injection (Jamsari et al., 2015). Besides that, PepYLCIV is a seed-transmissible virus in chili pepper plants. According to Fadhila et al. (2020), 25–67% of PepYLCIV DNA-A and 50–100% of DNA-B were detected from infected chili pepper seeds.

Seed is an important component in increasing agricultural production. Seedborne pathogens have a very potential role because they can suppress growth and reduce crop yields because the virus inhibits plant growth from the beginning of growth (Nordenstedt et al., 2017). Seedborne pathogens can also cause a loss in germination and vigor, discoloration and shriveling, biochemical changes in seeds, and alteration in the physical properties of seeds (Gaur et al., 2020). Besides that, farmers generally in the field have difficulty in identifying viral diseases due to symptoms variations such as distortion, leaf streaking, vein clearing, dwarf, mosaic, and mottle which were similar to symptoms caused by abiotic stress, phytotoxic caused by pesticides application and variations in nutrient levels (Jones, 2014; Islam, 2017).

Virus detection in seeds and plants can be done by biological, physical, serological, and molecular methods (Sastry, 2013). Currently, the most widely used detection method is the detection method based on properties because it is sensitive, accurate, and more efficient in the use of time and energy (Anggraini & Hidayat, 2014). But serological tests have been widely used to detect and identify plant viruses, especially seed-transmitted plant viruses. Serological tests can be applied for routine detection, cheap, easy, and can be used for virus testing in large numbers (Boonham et al., 2014). Serological tests in detecting seed-transmitted plant viruses depend on the antibodies used to detect specific antigens of plant pathogens (Kumar et al., 2020).

The main challenge of using serological tests for the detection of PepYLCIV is the availability of antibodies which is one of the main materials. Begomovirus has been reported to be found restricted in the phloem and cells of the vascular system with limited population (Morra & Petty, 2000). Begomoviruses were always a problem due to the extreme low concentration of virus and difficulty producing good quality DNA (Malathi, 2017). These factors make PepYLCIV difficult to purify, thus affecting pure virus as raw material for antibody production.

The use of antigens directly obtained from the host in serological tests can cause a decrease in the sensitivity of the reaction and often gives variable background reactions due to contamination from the host plant (Viswanathan et al., 2011). Molecular techniques through cloning of coat protein using expression vectors can be one alternative to solve the problem. The technique of cloning the coat protein gene has been carried out on several plant viruses such as *Sugarcane streak mosaic virus* (SCSMV) (Hamdayanty et al., 2016; Astuti et al., 2019), Pepper vein yellows virus (Apindiati et al., 2015), and *Bean golden mosaic virus*, *Cabbage leaf curl virus*, *Tomato yellow leaf curl virus*, and *Tomato mottle virus* (Abouzid et al., 2002). This study aimed to produce recombinant antibodies PepYLCIV from Congkrang isolates. The antibodies will be used in serological tests for early detection on the presence of PepYLCIV.

MATERIALS AND METHODS

Research Site. The research was carried out at the Microbiology Laboratory, Department of Fisheries, and the Laboratory of Plant Diseases, Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

Source of PepYLCIV. The source of virus was chili plants showing symptoms of yellowing, leaf curl, and stunt collected from Congkrang, Muntilan, Central Java, Indonesia (here after Congkrang isolates: CK).

DNA extraction and PCR Amplification. Total DNA was extracted from part of chili plants with symptoms of yellowing and leaf curl that had been dried with CaCl2. Extraction was carried out using CTAB method Dellaporta et al. (1983) with modification. The modification we made was precipitation which was only done once using 5M KOAc and isopropanol without the addition of NaOAC. The supernatant was discarded and the pellet was air-dried. After drying, the pellets were suspended with 100 µL of distilled water and stored at -20 ºC. All the centrifugation step was performed using H-9R Cooled high-speed centrifuge (Kokusan, Japan).

PCR was conducted in total volume 25 µL with a composition of 12.5 µL GoTaq® Green Master Mix 2X (Promega, USA), 7.5 µL Nuclease-Free Water, 1.5 µL forward primer, 1.5 µL reverse primer 10 µM, dan 2 µL DNA Template 100 ng/µL. PepYLCIV-*Bam*HI forward primer (5'-TGTAATGGATCCATGCCGAAGCGTTCCATCGA-3') and PepYLCIV-*Eco*RI reverse primer 10 µM (5'-CTCGACGAATTCTGGGATGTACTTGAACA-3'), inserting restriction sites *Eco*RI and *Bam*HI. PepYLCIV primers were used to amplify the PepYLCIV-CP gene with the expected size of amplicon DNA ±840 bp. This primer was designed based

on several nucleotide sequences of *Pepper yellow leaf curl Indonesia virus*, complete genome (Accession number DQ083765, DQ083764, and NC_008283). PepYLCIV primer does not contain additional histidine sequences.

PCR amplification was carried out by predenaturation (94 ºC for 2 min) and followed by 35 cycles: denaturation (94 ºC; 1 min), annealing (51 ºC; 1 min) and elongation (72 ºC; 1 min), followed by a final extension of 72 ºC for 5 min. The PCR results were analyzed by polyacrylamide (PAGE 6%) and electrophoresed in tris-boric acid EDTA (TBE) buffer. The results of the electrophoresis were stained with ethidium bromide and visualized on a UV transilluminator (Bio-Rad, USA).

Construction of Coat Protein in Expression Vector Plasmids. The PCR products were purified using a DNA Purification Kit (Roche Diagnostics, Germany) according to the manufacturer's instructions. The purified DNA was digested by using restriction enzymes *Eco*RI (Toyobo, Japan) and *Bam*HI (Toyobo, Japan) and then ligated to the pET32a expression vector which had been digested with the same enzyme. Ligation was performed by mixing the insert DNA and the DNA vector (1:3) using T4 DNA ligase (Roche Diagnostics, Germany) at 16 ºC for 24 hours, then cloned into expression bacteria *E.coli* BL 21 (Sambrook et al., 1989). The transformants were cultured into Luria-bertani Agar containing 50 mg/mL of Ampicillin and incubated for 24 hours at 37 ºC. Plasmid DNA from selected colonies was isolated according to the instructions (Sambrook & Russell, 2001a). Selected Plasmid DNA was verified by PCR using a pair of primers PepYLCIV-*Eco*RI and PepYLCIV-*Bam*HI, digested with restriction enzymes *Eco*RI and *Bam*HI and sequencing with ABI 3100-Avant Genetic Analyzer.

The results of PCR and digested with restriction enzymes were analyzed by Agarose 1% and electrophoresed in tris-boric acid EDTA (TBE) buffer. The results of the electrophoresis were stained with ethidium bromide and visualized on a UV transilluminator (Bio-Rad, USA). DNA sequencing was carried out at PT Wilmar Benih Indonesia using PepYLCIV-*Eco*RI and PepYLCIV-*Bam*HI primers. Similarities of DNA and amino acid sequences were compared with corresponding sequences in the GenBank using Blast analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Expression and Purification of Coat Protein. One recombinant PepYLCIV-CK-6 clone containing coat protein (CP) gene approximately 840 bp was cultured for 8 hours at 37 °C in Luria-Bertani (LB) medium at 50 mg/mL Ampicillin. Then induced by the addition of *Isopropyl-ß-D-thiogalactopyranoside* (IPTG) to a final concentration of 0.1–2 mM. Six hours after induction, bacterial cells were harvested by centrifugation (5,000 g/10 min) and stored at -80 °C. Total protein extract was obtained by re-suspension in lysis buffer (50 mM Tris-HCl pH 8.0, 100 mM NaCl, 2 mM EDTA,), followed by lysozyme treatment and sonication as described by Noueiry et al. (1994). The CP extract, resuspended in 1 mL 100 mM NaHCO3, pH 9.0, added 0.5% SDS (w/v) was purified by affinity chromatography in a Ni-NTA column (Qiagen, Germany), according to the manufacturer's instructions. The expressed recombinant protein was evaluated by SDS-PAGE and spectrophotometer analysis.

Immunization. Approximately 150 ng of recombinant protein was injected intravenously and intramuscularly into white New Zealand rabbits, with 1 injection (1-week injection interval). The first injection was performed with complete Freund's adjuvant (1:1 v/v), the second and third injections with incomplete Freund's adjuvant (1:1 v/v). The final injection as a booster was carried out with purified recombinant protein. One week after the last injection, rabbit blood was taken. Blood samples were allowed to freeze for 1 hour at 37 ºC or 30 min at 4 ºC, then centrifuged at 3,000 g/10 min. The supernatant (antiserum) was stored at -20 ºC.

Recombinant Antibody Purification. The immunoglobulin (IgG) fraction of the antiserum was purified by Direct Ammonium Sulfate Precipitation and Dialysis using the method described by Grodzki & Berenstein (2010). Dialyze with stirring at 4 °C against four to five changes of PBS (*Phosphate Buffer Saline*, 0.5× pH 7.4) for a minimum of 4 hours each.

Serological Test. The serological detection of different sources of begomovirus species was carried out using crude antiserum and IgG from these recombinant antibodies with *Indirect Enzyme-Linked Immunosorbent Assay*/I-ELISA (Koenig, 1981) dan *Dot Immunobinding Assay*/DIBA (Hibi & Saito, 1985) according to the instructions.

RESULTS AND DISCUSSION

PepYLCIV Congkrang Isolate. Plants that were infected with PepYLCIV which showed yellow curling symptoms on their leaves and dwarf chili plants were collected from Congkrang, Muntilan, Central Java

(Figure 1). Infected plants had bright yellow leaves, smaller leaves, and curled leaf shoots. This phenomenon was the same as described by Lestari et al. (2018). The chili plants infected with PepYLCIV had typical symptoms such as yellow mosaic, leaf curling, and stunting. PepYLCIV also caused shorter fruit length (Ganefianti et al., 2017). PepYLCIV is a member of the begomovirus (Brown et al., 2012). Begomoviruses have either bipartite or monopartite genomes, whose bipartite genome consists of two circular single-stranded DNA (2.5–2.7 kb) referred to as DNA A or DNA B (Brown et al., 2012). Open Reading Frame (ORF) AV1 is located in the DNA A encoding coat protein gene (Malathi, 2017). Amplification of the DNA of infected plants using a specific primer that encodes PepYLCIV coat protein produced an amplicon at 840 bp (Figure 2). Based on the sequence used to design PepYLCIV specific primer (Accession number DQ083765, DQ083764, and NC 008283), it is known that the primer contained 780 bp sequence encodes PepYLCIV coat protein and and the 60 bp sequence is part of the sequence encoding C3 protein. The coat protein is an important part of the begomovirus which plays a role in ssDNA encapsulation, viral particle formation, cell-to-cell spread, systemic spread, viral DNA accumulation, and insect transmission (Roshan et al., 2017). AC3/C3 replication enhancer protein (REn) has a function to stimulate replication (Guerrero et al., 2020).

Recombinant Plasmid Verification. Verification of recombinant plasmids is a very important step in the gene cloning process. In this study, the presence of recombinant plasmids was verified by PCR, digesting the plasmid recombinant DNA using restriction enzymes and DNA sequencing. The result showed that one of the CK-6 plasmids carried the gene encoding the coat

Figure 1. Pepper yellow leaf curl symptoms of Congkrang isolate (CK) infecting Cayenne pepper.

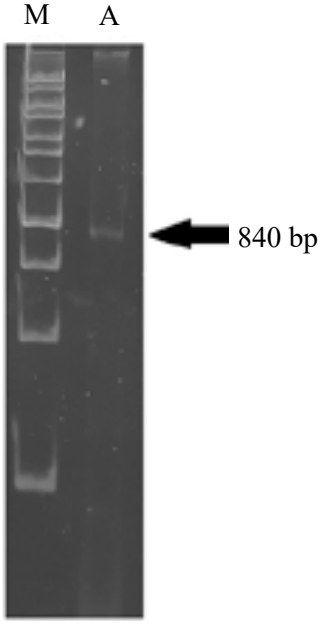

Figure 2. Electrophoresis of PCR product of PepYLCIV-CK6 isolate (A) and DNA Marker 1 kb (M) (promega, USA) in PAGE 6%.

protein of PepYLCIV isolate Congkrang.

PepYLCIV DNA (CP) that had been purified from the PCR product was ligated to the pET32A vector then transformed into *E. coli* BL21. Several colonies of *E. coli* suspected as recombinant carrying PepYLCIV DNA (CP) were validated by PCR testing. The results showed that one of the recombinants showed a DNA band measuring about 840 bp (data not shown). The size of the DNA was the same as the PepYLCIV DNA which is used as a DNA insert for gene cloning. The recombinant was named CK6.

The restriction enzymes *Eco*R1 and *Bam*HI were able to cut CK-6 plasmid DNA, indicating that the plasmid contains a gene encoding the PepYLCIV coat protein Congkrang isolate. Restriction enzymes, also called restriction endonucleases, are enzymes that cut DNA sequences specifically at recognized sites (Buckhout-white et al., 2018). *Bam*H1 and *Eco*R1 were restriction enzymes that have been widely used during ligation to determine the presence of the inserted DNA. In this study, these enzymes were able to cut the recombinant plasmid into two parts, namely vector DNA and PepYLCIV DNA which were used as inserts (Figure 3).

In this study, sequencing was carried out only to determine whether the recombinant plasmid carried the inserted PepYLCIV CP. Therefore, sequencing was only performed using PepYLCIV-*Eco*RI and PepYLCIV-*Bam*HI primers. PepYLCIV primer has a target DNA about 840 bp. However, only 744 nucleotide sequences could be read (Figure 4). The type of Taq polymerase enzyme and the PCR cycle used in the PCR step of library preparation before sequencing had a very important impact on the proportion of correct reads after sequencing, but the cycle has less impact than the enzyme (Brandariz-Fontes et al., 2015). Errors and bias may be introduced at nucleic acid extraction, reverse transcription, PCR amplification of sequence targets or during library preparation and sequencing and at many steps in the bioinformatics pipeline (Bartkus, 2016).

According to the percentage of nucleotide sequence identity and sequence of amino acids, PepYLCIV CK-6 isolates had the highest similarity of nucleotide and amino acid sequences to corresponding sequences of chili isolates from Bandung. Whereas, PepYLCIV CK-6 isolate against *Pseuderanthemum*, chili, tomato, Ageratum from Bali, North Sumatera, Bogor, East Java, and Bandung had a similarity of nucleotide sequences ranging from 97–93% and had a similarity of amino acid sequences ranging from 95–96% (Table 1). Viruses are the same as other viral species if the amino acid sequence encodes the envelope protein (CP) between viruses and other viruses is more than 90% (Selangga & Listihani, 2021).

Recombinant Protein Expression. The recombinant CK-6 plasmid carrying the PepYLCIV coat protein gene was cultured in LB medium containing 50 g/mL Ampicillin. Furthermore, the recombinant protein was

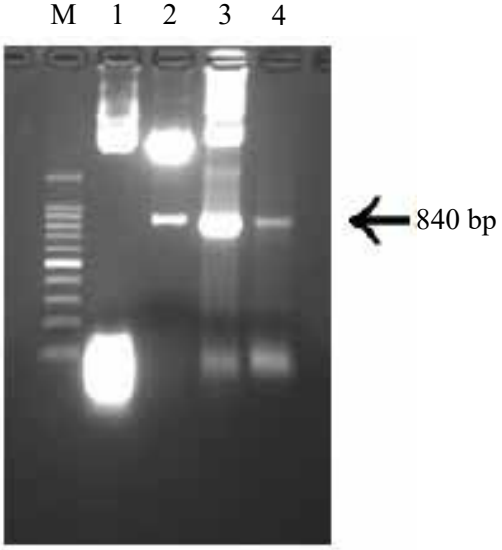

Figure 3. Verification of plasmid recombinant using restriction enzyme digestion Using *Eco* RI and *Bam*HI in Agarose 1%. M= Marker 100 bp promega, USA; 1= Recombinant DNA plasmid; 2= Recombinant DNA plasmid digested by restriction enzymes *Bam*HI dan *Eco*R1; 3= PCR amplification using template of recombinant DNA plasmid and PepYLCIV primer; (4) Positive control (PCR amplication using template of DNA PepYLCIV Congkrang isolate as an DNA insert).

induced with IPTG and purified using Ni-NTA. Observations using a spectrophotometer showed that the presence of protein was observed at the absorbance peak at 280 nm. The result showed that the concentration of recombinant protein produced was still low (Table 2). The establishment of recombinant protein was also validated using SDS-PAGE analysis. The results revealed that the recombinant plasmid PepYLCIV CK-6 isolates could produce a recombinant protein of about around 29 kDa (Figure 5). According to Malathi (2017), Coat protein Begomovirus has predicted molecular weight around 29.8 kDa. PepYLCIV belongs to the genus Begomovirus, so it is possible that PepYLCIV have the same molecular weight.

Although the result of SDS-PAGE showed that the expressed recombinant protein had an identical size with PepYLCIV protein, the spectrophotometer results indicated that the recombinant coat protein had a relatively low concentration. This was probably caused by the low concentration of *E. coli* BL 21 hosts as a

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1    GTGTCGTCGTTTGCAAGGTCTTTAACACGCCGGAAACTAAACTACGCGAGTCAGTA-   60
     CAGT
     V  S  S  F  A  R  S  L  T  R  R  K  L  N  Y  A  S  Q  Y  S
61   CTCCCTGCTGCTGCCCCCACTGCCCCAGGCATGTCTTACAAACGAAGAGCATGG-   120
     TAAAT
      L  P  A  A  A  P  T  A  P  G  M  S  Y  K  R  R  A  W  V  N
121  CGGCCTATGAATCGGAAACCCAGATTCTACAGGGGTCGAAGGACCAGTGATGTTC-  180
     CACGG
     R  P  M  N  R  K  P  R  F  Y  R  G  R  R  T  S  D  V  P  R
181  GGTTGTGAAGGACCTTGTAAGGTCCAATCCTTTGAACAGAGACATGACGTAACACAT-  240
     ACT
     G  C  E  G  P  C  K  V  Q  S  F  E  Q  R  H  D  V  T  H  T
241  GGGAAGGTCCTTTGCGTTTCCGATGTCACTAGAGGTAATGGTATTACGCATAGAGTAG-  300
     GG
     G  K  V  L  C  V  S  D  V  T  R  G  N  G  I  T  H  R  V  G
301  AAAAGATTCTGTGTGAAATCTGTATATATTATTGGCAAAGTATGGATGGACGAGAA-   360
     CATC
     K  R  F  C  V  K  S  V  Y  I  I  G  K  V  W  M  D  E  N  I
361  AAGTCGAAGAACCACACTAATAACGTCATGTTTTGGCTTGTTCGTGACCGGCGAC-   420
     CAGTT
     K  S  K  N  H  T  N  N  V  M  F  W  L  V  R  D  R  R  P  V
421  ACAACTCCTTATGGCTTCGGCGAGTTGTTCAACATGTATGATAATGAACCCAGCACAG-  480
     CA
     T  T  P  Y  G  F  G  E  L  F  N  M  Y  D  N  E  P  S  T  A
481  ACTATCAAGAACGATCTGCGTGATCGTGTTCAGGTGTTACATCGTTTCTCAGCCACG-   540
     GTG
     T  I  K  N  D  L  R  D  R  V  Q  V  L  H  R  F  S  A  T  V
541  ACAGGTGGTCAGTATGCAAGCAAAGAACAAGCAATCGTGAAGAGATTTTTTAGAGT-   600
     TAAC
     T  G  G  Q  Y  A  S  K  E  Q  A  I  V  K  R  F  F  R  V  N
601  AACTATGTTGTGTACAATCATCAGGAAGCAGCAAAATATGAAAATCACAC-         660
     CGAAAATGCA
     N  Y  V  V  Y  N  H  Q  E  A  A  K  Y  E  N  H  T  E  N  A
661  TTGCTGTTGTATATGGCATGTACCCATGCATCTAATCCTGTGTATGCGACATGGAAAGTT  720
     L  L  L  Y  M  A  C  T  H  A  S  N  P  V  Y  A  T  W  K  V
721  CGTATATACGTCTACGACAATGTA                                    744
     R  I  Y  V  Y  D  N  V
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Figure 4. Nucleotides (the first line) and amino acids (the second line) sequence from Coat Protein PepYLCIV isolate congkrang.

place to produce recombinant protein. The advantages of using *E. coli* as the host organism are it has unparalleled fast growth kinetics, high cell density cultures are easily achieved, rich complex media can be made from readily available and inexpensive components, and transformation with exogenous DNA is fast and easy (Rosano & Ceccarelli, 2014).

The less time for sonication process and incomplete lysis process caused the concentration of protein which was produced was not optimal, although IPTG was also added during the culture of recombinant plasmids in LB media. IPTG is an effective inducer and cannot be metabolized (Donovan et al., 1996; Li, 2018). IPTG is widely used for heterogeneous gene expression in *E. coli* (Li, 2018). In this study, IPTG was added until the final concentration reached 0.5 mM. However, according to Sambrook & Russell (2001b), the expression level of the cloned may be low due to inefficient initiation of translation, protein instability, RNA instability, and inappropriate termination of expression steps.

The low expression of recombinant protein was

Table 1. The percentage of nucleotide sequence identity and sequence of amino acid of PepYLCIV Congkrang isolate with another isolate of *Pepper yellow leaf curl Indonesia virus* PepYLCIV

Isolat	Host	Accession Number	% Homology	
			Nucleotide	Amino acid
PepYLCIV-Ck6*	Chili	-	-	-
Bandung: 2003	Chili	AB267834.1	98	97
Bali: 2018	Pseuderanthemum	MN094868.1	97	96
North Sumatera: 2012:BA_C1-1	Chili	LC051113.1	95	95
Bogor : 2000	Tomato	DQ083765.1	94	96
East Java : 2018: EJavaCP1	Chili	MN738463.1	94	96
Bogor: 2000	Chili	DQ083764.1	94	96
North Sumatera- 2012:BA_A6-1	Chili	LC051112.1	94	96
East Java: 2018: EJavaT1	Tomato	MN738466.1	94	95
Bandung: 2003	Ageratum	AB267838.1	93	95

*isolate used in this study.

Table 2. Absorbance, ratio A260/280, and concentration from protein recombinant, crude antiserum, and purified antiserum from PepYLCIV Ck6

Sample	Absorbance		A260/A280	Concentration (mg/mL)
	A260	A280		
Protein Rekombinan Ck 6	1.116	0.732	1.525	0.286
CK6 recombinant crude antiserum	0.512	0.555	0.923	0.397
CK6 recombinant purified antiserum	0.322	0.328	0.982	0.234

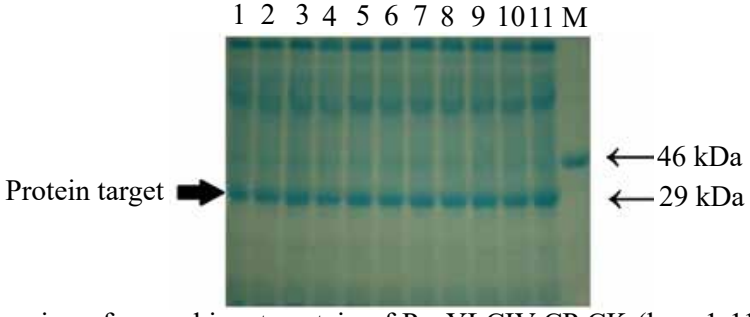

Figure 5. The expression of recombinant protein of PepYLCIV-CP CK (lane 1-11) induced by IPTG with concentration 0.5 mM at 6 hours after induction. M= VNN protein marker (collection of Dr. Murwantoko, M.Sc).

also suspected to have occurred. It can be seen on the low concentration of protein bound to the Ni-NTA column. At the time of SDS-PAGE analysis, proteins that had been filtered with Ni-NTA had thinner protein bands than before screening (data not shown). Low protein binding may be caused by inappropriate binding conditions, such as lower pH values and higher imidazole concentrations (Liu & Yang, 2012). In this research, pET32a was used as a vector PepYLCIV CP gene and it was confirmed the recombinant could express the recombinant protein. According to Liu & Yang (2012), pET-32a vector for protein expression and purification in *E. coli* is fast, inexpensive and scalable.

Recombinant Antibody Production. Recombinant PepYLCIV CP was used as antigens to produce recombinant antibodies. Immunization in rabbits has been carried out by injection using a dose of 150 mg in a volume of 100 L with an interval of a week which was repeated four times. The expected dose would produce better antibodies compared to the same process with more immunization doses. The dose of immunogen could affect the quantity and quality of antibodies. According to Stills (2012), low doses of immunogens might not be sufficient to stimulate antibody production although low doses could lead to the production of B cells memory which could be stimulated by secondary exposure to immunogens. Meanwhile, excessive doses of immunogen could produce low-affinity antibodies or the development of tolerance without antibody production.

This study used rabbits to produce recombinant proteins. Rabbits were generally used for the production of polyclonal antibodies. Rabbit antibodies can recognize epitopes on human antigens that are not immunogenic in rodents (Weber et al., 2017). Besides that Rabbits were easy to handle, quickly breed, cheaper, and need less skill than monoclonal antibody production, produce a highly specific antibody and produce high concentrations of antibody even using serum in small quantities (Stapleton et al., 2005). In addition, the use of rabbits technically allowed them to collect large amounts of blood samples due to the availability of the marginal ear vein and central auric artery. Rabbits also have excellent responsiveness to a wide variety of antigens, the presence of a single primary Immunoglobulin G isotype as well as the availability of information on its production and purification of its immunoglobulin (Stills, 2012).

After the antiserum was taken from the rabbits, it was analyzed using a spectrophotometer. The results showed an absorbance band that peaked at a wavelength of 280 nm, indicating that the immunized antigen was able to produce a protein (Table 2). The produced protein was antiserum which could be used for serological diagnosis. A titer is the highest dilution of a reactant (antibody or antigen) in a liquid that still shows its reactivity using a certain method. Based on the results of antibody titer testing (data not shown), the crude antiserum produced had a titer of 1:100,000, and an antibody had a titer of 1:100 after purification. The crude and purified antibody contains Gamma globulin (IgG) with a concentration of about 0.397 and 0.234 mg/mL, respectively.

Begomovirus Serology Detection using Recombinant Antibodies. Serological test can detect the presence of PepYLCIV in the host even thought the titer produced is low. The results of the PepYLCIV serological test by the I-ELISA test using crude antiserum can be seen in Table 3. The I-ELISA test using purified antiserum can be seen in Table 4. Serological detection using IgG isolated from crude antiserum (not pure) was still in doubt because the absorbance ratio between infected plants and healthy plants had not 2 times fold yet. However, there was a tendency that it was able to detect the presence of PepYLCIV in infected chili plants and Babadotan weeds (*Ageratum conyzoides*) (Table 2). The high level of absorbance in healthy plants might be due to crude antiserum. Apart from IgG, the crude antiserum also contains other proteins that play a role as paratypes. Paratypes might react with proteins found in healthy plants so that cross-reactions will occur.

DIBA (Dot Immunobinding Assay) test shows the same results as I-ELISA. The results of the DIBA test using crude antiserum (not pure) is shown in Figure 6. Using the DIBA test, the virus concentration in the sample is indicated by the intensity of the colour change. The stronger the color displayed, means the better antibody. According to Anggraini & Hidayat (2014), the sensitivity limit for DIBA is higher than the I-ELISA method, so it requires slightly lower antibodies than I-ELISA for testing with the same sample. A negative result might be due to the low quality of purified antiserum. Antibody reactivity was still not optimal due to the low quality of antiserum and the low immunogenic power of Begomovirus (Wyatt & Brown, 1999). PepYLCIV belongs to the genus Begomovirus, so PepYLCIV may have the same low immunogenic power.

The low antibody titer level was also due to incomplete recombinant protein expression, resulting in

low concentration of recombinant protein (as an immunogen). Low expression in *E. coli* may be due to protein may be toxic before induction, protein may be toxic after induction, and codon bias (Rosano & Ceccarelli, 2014). Recombinant protein expression was also influenced by the concentration of IPTG inducer and duration of induction (Pratiwi, 2019). According to Hamdayanty et al. (2016), optimal protein expression of SCSMV-CP recombinant was obtained at 25 °C with IPTG concentration 0.25–1.00 mM and harvested at 9–12 hours after IPTG induction in *E. coli* BL21(DE3), and at 30 °C with IPTG concentration 0.25–1.00 mM and harvested 3–12 hours after IPTG induction in *E. coli* Rosetta(DE3)pLysS. In this research, protein expression was done with IPTG concentration 0,5 mM and harvested at 6 hours after IPTG induction in *E. coli* BL21. In the future, it is necessary to re-optimize PepYLCIV protein expression by treating various concentration ranges, time, temperature, and bacterial host.

Recombinant antibodies can detect PepYLCIV in infected chili and babadotan weeds. Serological tests could be further developed as a detection tool to reveal the presence of PepYLCIV in various types of plants. Although the PCR method has several positive points, several negative points were also found, such as: (1) the availability of PCR machines, (2) material of reagents were very expensive, and (3) needing expert technicians. The success of producing recombinant antibodies can be used as an alternative detection method to cover limitations on PCR. The use of cloned antibodies had several advantages over conventional methods. The antigens can be provided continuously at any time, without any limitations of quantity, with good quality of antigens.

CONCLUSION

The recombinant plasmid carrying the coat protein gene from the PepYLCIV isolate congkrang (CK-6) was able to express a recombinant protein with a size of about 29 kDa. Recombinant protein injection in rabbits could produce crude antiserum and pure antiserum which was able to detect begomovirus, the causal agent of main viral disease on chili and *Ageratum conyzoides*, using I-ELISA and DIBA tests.

Table 3. Detection of PepYLCIV by I-ELISA using crude antiserum (1:1000 dilution)

The samples	A405nm			Result
	1	2	Mean	
Infected pepper leaves	1.259	1.125	1.192	+/-
Infected weed leaves	0.998	1.018	1.008	+/-
Healthy pepper leaves	0.832	0.813	0.8225	-

(-) Begomovirus absence
(+) Begomovirus presence

Table 4. Detection of PepYLCIV by I-ELISA test using purified Antibodi (dilution 1:100)

The samples	A405nm			Result
	1	2	Mean	
Infected pepper leaves	0.503	0.493	0.498	+
Infected weed leaves	0.445	0.439	0.442	+
Healthy pepper leaves	0.225	0.217	0.221	-

(-) Negative reaction
(+) Positive reaction

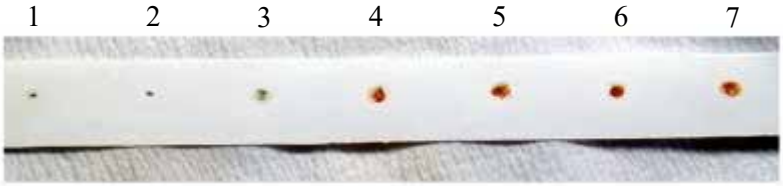

Figure 6. Visualiazation of DIBA test using crude antisera (1:1000). (1) Buffer; (2–3) Healthy pepper; (4–5) PepYLCIV infecting chili; (6–7) PepYLCIV infecting Babandotan weeds.

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AUTHORS' CONTRIBUTIONS

YBP and SS are the main contributors. SH, SM, MW, DWKS, and CRH are the member contributors. YBP and SS considered the experiment, planned the experiment, performed recombinant antibody purification, performed recombinant antibody purification, performed data analysis, and prepared the manuscript. SM, SH, and MW have collected samples, identified infected plants, and performed PCR analysis. YBP, SS, DWKS, CRH have done construction of coat protein in expression vector plasmids. YBP, SH, SH performed expression and purification of the coat protein. YBP, SS, and SM performed immunization and serological test. The authors provided responses and comments on the research flow, data analysis, and interpretation as well as the shape of the manuscript. All the authors have read and approved the final manuscript.

COMPETING INTERESTS

Authors declares that there is no competing interest regarding to the publication of this manuscript.

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