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# Epitope-based peptide antigen of pepper yellow leaf curl Thailand virus for specific antibody production

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**Abstract** Yellow leaf curl disease (YLCD) is one of the most devastating diseases causing significant yield loss to economic crops, especially peppers in Thailand. One of the reported causal agent is pepper yellow leaf curl Thailand virus (PepYLCTHV) which belongs to *Begomovirus*, Geminiviridae. Anti-PepYLCTHV antibody using peptide conjugate antigen was produced in this research. The peptide sequence was based on the virus coat protein sequences available in the GenBank database. Linear epitope sequences were analyzed using the IEDB program and compared to known, three-dimensional structures of African cassava mosaic virus. The synthesized peptide was conjugated with bovine serum albumin (BSA) and ovalbumin (OVA). Peptide-BSA conjugate was immunized into a New Zealand White rabbit with complete adjuvant in the first week and with incomplete adjuvant in the third week. The antiserum was collected for 12 weeks and the highest titer at 409,600, determined by indirect plate-trapped antigen enzyme-linked immunosorbent assay, was obtained. Characterization of the produced antibody showed specificity to PepYLCTHV and one more begomovirus, tomato leaf curl New Delhi virus, without cross reaction with 31 other viruses tested. Although the antibody had limited sensitivity, development of serological techniques using this antibody demonstrated good results for dot immunobinding assay and tissue print-immunoblotting. This is the first report of antibody production for PepYLCTHV using peptide antigen which can be further applied for virus detection.

**Keywords**: PepYLCTHV, Antibody, Virus detection, Peptide synthesis, Begomovirus

## Introduction

*Begomovirus* belongs to *Geminiviridae* which includes plant-infecting viruses with circular ssDNA genomes encapsidated in non-enveloped, twinned (geminate) particles, about 38 nm in length and 22 nm in diameter (for maize streak virus), comprising 22 pentameric capsomers made of 110 capsid proteins (Hulo *et al*., 2011). They infect dicotyledonous plants and can be transmitted in a persistent manner by whitefly (*Bemisia tabaci*) and grafting (Lawrence *et al*.,

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2014). Geminiviruses are often responsible for serious yield losses in economically important crops including pepper, tomato, maize, cotton, chickpea, and cassava (Brown *et al*., 2012). Pepper yellow leaf curl disease (PYLCD) has become a major problem of pepper growing causing crop damage in many tropical and subtropical regions worldwide (Trisno *et al*., 2009). In the past three decades, a number of virus species have been identified infecting chili pepper as well as a considerable increase in virus disease incidence (Kenyon *et al*., 2014). One of them, pepper yellow leaf curl Thailand virus (PepYLCTHV), was reported to be the causal agent of PYLCD across the country (Srithongchai *et al*., 2010, Chiemsombat *et al*., 2018, Malichan *et al*., 2019, Charoenvilaisiri *et al*., 2020). Initial symptoms include leaf curling and faint chlorotic specks on leaf lamina, which later develop into bright-yellow mosaic patches and distorted leaves (Sakata *et al*., 2008). Early detection based on reliable methods is very helpful in control measures. Begomovirus in infected plants is usually detected using polymerase chain reaction (PCR) analysis, which although sensitive, is not suitable for routine field detection whereas, enzyme-linked immunosorbent assay (ELISA) has become a routine method for detecting plant viruses in several decades (Xie *et al*., 2013). In addition, serological tests based on ELISA enable fast and cost-effective screening of plant materials for virus presence on a large scale. To raise the specific antibody, antigens can be prepared by purifying native virus particles from plant, gene cloning and expression of the virus coat protein or replicase genes, etc. However, the purification process is tedious work and time-consuming. Recombinant viral proteins expressed in bacteria cells such as *E. coli* were favorably used as antigens but the expressed proteins were not glycosylated and can cause a major drawback since many of the immunogenic proteins of viruses such as the envelope glycoproteins, were glycosylated. However, the obvious advantage of recombinant viral antigens is that they are available in unlimited quantities and the production and quality control processes are simple (Prospec Protein Specialist, 2020). Currently, a rapid in-silico informatics-based approach has gained much popularity with the recent advancement in the sequencing of many pathogen genomes and protein sequence databases. Our study was undertaken to design an epitope-based peptide antigen against PepYLCTHV and to develop an antibody-based detection method for this virus.

## Materials and methods

### *Disease sample collection and DNA extraction*

Pepper leaf samples exhibiting virus symptoms were collected from pepper fields in Kanjanaburi, Chaiyaphum, Nakhon Ratchasima, Buri Ram, Surin, Sisaket, and Ubon Ratchathani provinces, Thailand. The presence of the

infecting virus species was detected using indirect plate-trapped antigen ELISA (indirect PTA-ELISA) according to Hongprayoon (2015) using eight antibodies available in our laboratory with specificity to: tobacco mosaic virus (TMV), pepper mild mottle virus (PMMoV), chilli veinal mottle virus (ChiVMV), potato virus Y (PVY), cucumber mosaic virus (CMV), tomato necrotic ring virus (TNRV), watermelon silver mottle virus (WSMoV) and tomato leaf curl New Delhi virus (ToLCNDV).

***DNA amplification and cloning of the begomovirus genome***

Total DNA extracted from the pepper sample CPM 55 using the CTAB method (Doyle and Doyle, 1990) was amplified using the rolling circle amplification (RCA) technique with a TempliPhi Kit (Amersham Biosciences, USA). The DNA product was digested with *Bam*HI, *Hind*III, *Pst*I, and *Sac*I (Fermentas, USA) according to fast digestion by Wang *et al.* (2018). The DNA product was cloned into a pQE 80L expression vector and transformed into *Escherichia coli* strain DH5α by heat shock transformation (Sambrook, 1989), then submitted for nucleotide sequence analysis at the Macrogen Company (Korea).

***Prediction and conservancy analysis of PepYLCTHV surface epitope***

The conservancy of the predicted epitopes of PepYLCTHV CP was analyzed using the web-based tool from IEDB Analysis Resource: BepiPred Linear Epitope Prediction (http://tools.iedb.org/bcell/) (Jespersen *et al*., 2017) and compared with the coat protein amino acid sequences from the GenBank database and the structure of the African cassava mosaic virus (ACMV) (Hipp *et al*., 2017) to select the linear epitopes on the particle surface site. The target peptide sequence was synthesized by Integrated DNA Technologies Company (USA).

***Conjugation of the synthesized peptide and the protein carriers***

The peptide and a carrier protein; bovine serum albumin (BSA) or ovalbumin (OVA) in 0.1 M sodium bicarbonate were dissolved using 1 mL for every 2 mg of carrier protein; then glutaraldehyde solution was added into the peptide-carrier solution to a final concentration of 0.05%. The contents were mixed in a glass tube and stirred with a magnetic stirring bar. The tube was stored at room temperature overnight in the dark. The solution was dialyzed in 1XPBS (Hancock and O'Reilly, 2005). The peptide conjugate was verified

using discontinuous sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in 6% stacking gel and 12% separating gel (Laemmli, 1970).

***Polyclonal antibody production***

A 3-month old New Zealand White rabbit was used for immunization. The rabbit was first injected subcutaneously with the peptide-bovine serum albumin conjugate (peptide-BSA) at 1mg/mL, mixed well with complete Freund adjuvant at a 1:1 ratio, followed by immunization of the same antigen mixed with incomplete adjuvant (1:1) in the third week. Five weeks after the last boost, blood was withdrawn weekly for 2 months. The blood samples were incubated at room temperature for 1 hr and then stored at $4^{o}$C overnight to coagulate and then centrifuged at 8,000 rpm for 10 min. The antiserum was aliquoted and stored at $4^{o}$C until use (Hongprayoon, 2015)**.**

***Antiserum titration using indirect PTA-ELISA***

Each well in a microtiter plate was coated with 50 µL of the peptide-OVA conjugate in coating buffer (5 µg/mL), incubated overnight at $4^{o}$C and nonspecific reaction was blocked with 150 µL of blocking buffer [5% skimmed milk in PBS (NaCl 8 g, $Na_2HPO_4.12H_2O$ 2.9 g, $KH_2PO_4$ 0.2 g, KCl 0.2 g pH 7.4)] for 2 hr at $37^{o}$C. Two-fold serial dilutions of the PAb were prepared ranging from 1:200 to 1: 204,800 in the blocking buffer and 50 µL was added into each well and then incubated for 90 min at $37^{o}$C. Goat anti-rabbit IgG conjugated with alkaline phosphatase (GAR-AP) (Sigma, USA) at a 1:30,000 dilution in blocking buffer was then added (50 µL per well) and incubated for 60 min at $37^{o}$C. Each step was followed by washing the plates three times with 1xPBST (PBS containing 0.05% Tween 20). The reaction was detected by adding *p*-nitrophenyl phosphate at 1 mg/mL in diethanolamine buffer, pH 9.8. After 60 min at room temperature, the absorbance at 405 nm ($A_{405}$) of the chromogenic product was measured using an ELISA reader (Tecan, Switzerland).

***Specificity testing by indirect PTA-ELISA***

Specificity testing for anti-PepYLCTHV PAb was performed using indirect PTA-ELISA with ground plant tissue in coating buffer at a 1:5 ratio (w/v). The commercial positive samples were prepared according to the manufacturer's instructions. Sensitivity testing of the PAb was done by reacting

with diseased plant sap using week 12 antiserum at 1:200 dilution and indirect PTA-ELISA as described in section 6.

Thirty-three plant virus species were tested: genus *Begomovirus*–ACMV, bean golden mosaic virus (BGMV), PepYLCTHV, squash leaf curl virus (SqLCV), ToLCNDV, tomato yellow leaf curl virus (TYLCV), tomato yellow leaf curl Thailand virus (TYLCTHV), and watermelon chlorotic stunt virus (WCSV); genus *Comovirus*–cowpea mosaic virus (CPMV), and squash mosaic virus (SqMV); genus *Cucumovirus*–CMV I, CMV II; genus *Potyvirus*: chilli veinal mottle virus (ChiVMV), pepper mottle virus (PepMoV), papaya ringspot virus (PRSV), pepper veinal mottle virus (PVMV), pepper yellow mosaic virus (PepYMV), tobacco etch virus (TEV), zucchini yellow mosaic virus (ZYMV), and watermelon mosaic virus (WMV); genus *Tobamovirus*: bell pepper mosaic virus (BPeMV), cucumber green mottle mosaic virus (CGMMV), PMMoV, tobacco mosaic virus (TMV), and tomato mosaic virus (ToMV); and genus *Tospovirus*–capsicum chlorosis virus (CaCV), groundnut bud necrosis virus (GBNV), groundnut ringspot virus (GRSV), impatiens necrotic spot virus (INSV), iris yellow spot virus (IYSV), tomato chlorotic spot virus (TCSV), and tomato spotted wilt virus (TSWV) and WSMoV.

***Detection of PepYLCTHV using dot immunobinding assay (DIBA)***

DIBA was performed using the modified method from that described by Hawkes *et al*. (1982). Clarified plant sap samples were prepared by grinding leaf tissue in carbonate buffer at a ratio of 1:5 (w/v). A 3-µL volume of diseased plant sap, peptide-OVA conjugate (a positive control), healthy plant sap and buffer controls were dropped onto a nitrocellulose membrane (NCM) and then air-dried for 10 min. The NCM was incubated in blocking buffer (5 % skimmilk in PBS) for 30 min at 37 $^{o}$C. An optimal dilution of PAb (1:200 in blocking buffer) was added and incubated for 40 min at 37 $^{o}$C. GAR-AP at a 1:30,000 dilution in blocking buffer was added and incubated for 40 min at 37 $^{o}$C. The reaction was detected by immersing the membrane in 5-bromo-4-chloro-3-indolyl phosphate (BCIP), nitro blue tetrazolium (NBT) substrate solution and incubating at room temperature until a purple-violet color developed in the positive control at about 10 min. The reaction was stopped by soaking in tap water for 5 min.

***Detection of PepYLCTHV using tissue-print immunoblotting (TPIB)***

TPIB was carried out by coating leaf samples with celite powder and placing on the NCM. Further steps were performed as described by Kaufmann

*et al.* (1992). The reaction on the printed NCM was developed following the method described in DIBA.

## Results

### *Disease sample collection and DNA extraction*

In total, 299 pepper leaf samples exhibiting virus symptoms were collected from pepper fields in Western and Northeastern Thailand from seven provinces. The result of preliminary screening using ELISA showed virus prevalence varied greatly based on the average frequency of detection, with the highest occurrence for CMV at 43.14% followed by WSMoV, ChiVMV, PMMoV, and ToLCNDV at 10.03, 8.36, 1.67, 1.00, and 0.33%, respectively. However, no PVY and TNRV infection was found among these samples.

### *Begomovirus DNA amplification and cloning of begomovirus genome*

One negative sample with eight antibodies, Hot pepper leaf of CPM 55 (Figure 1) was amplified using RCA. Single digestion of the DNA product showed a DNA band at 2,700 bp from gel electrophoresis with HindIII, *Bam*HI and *Pst*I. This is the size corresponding to DNA-A and DNA-B of the begomovirus genome. Nucleotide sequence analysis revealed that the digested product by *Hind*III was closely related to the PepYLCTHV DNA-A fragment (Accession no. KT322145.1) at 98.36% identity, while the digested product obtained by *Bam*HI and *Pst*I restriction revealed a close genetic relationship to the PepYLCTHV DNA-B fragment (Accession no. KX885225.1) at 97 % identity. Nucleotide sequence data were used for the prediction and conservancy analysis of PepYLCTHV coat protein surface epitopes.

**Figure 1.** Pepper leaf sample, CPM 55, collected from Chaiyaphum province showing yellow leaf curl symptoms

### *Prediction and conservancy analysis of PepYLCTHV surface epitope*

Analysis of the coat protein amino acid sequence belongs to ACMV which used as a structure model in comparison to PepYLCTHV coat protein. It revealed that the amino acids at N131, Q130, and Q156 were located on the exterior of the ACMV coat protein. Therefore, the amino acids located around those positions of the PepYLCTHV coat protein were our target epitope sites (Figure 2). Based on the IEDB Analysis Resource Program, the predicted linear epitopes on the PepYLCTHV coat protein were located at amino acids 126-133 (NIKTKNHT) and 144-155 (RRPYGTPQDFGQ) (Table 1). The peptide synthesis of the PepYLCTHV surface linear epitope was then designed to cover these two amino acid sequences comprising 20 residues as follows: NIKTKNHTRRPYGTPQDFGQ. This design was required because if it is a potent B cell epitope, it must have surface accessibility.

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                    10         20         30         40         50         60
ACMV        -   MSKRPGDIII STPGSKVRRR LNFDSPYRNR ATAPTVHVTN RKRAWVNRPM YRKPTMYRMY
PepYLCTHV   -   MAKRPADIVI STPVSKVRRR LNFDSPYLSR VAAPTVLVTN KKRSWANRPM YRKPRIYRMY

                    70         80         90        100        110        120
ACMV        -   RSPDIPRGCE GPCKVQSFEQ RDDVKHLGIC KVISDVTRGP GLTHRVGKRF CIKSIYILGK
PepYLCTHV   -   KSPDVPRGCE GPCKVQSYEQ RHDVVHVGKV ICVSDVTRGN GLTHRVGKRF CVKSVYVLGK

                   130        140        150        160        170        180
ACMV        -   IWLDETIKKQ NHTNNVIFYL LRDRRPYGNA PQDFGQIFNM FDNEPSTATI KNDLRDRFQV
PepYLCTHV   -   IWMDENIKTK NHTNTVMFFL VRDRRPYGT- PQDFGQVFNM YDNEPSTATV KNDNRDRYQV

                   190        200        210        220        230        240
ACMV        -   LRKFHATVVG GLYCMKEQAL VKRFYRLNHH VTYNHQEAGK YENHTENALL LYMACTHASN
PepYLCTHV   -   LRRFQSTVTG GQYASKEQAI VRKFMKVNNH VTYNHQEAAK YDNHTENALL LYMACTHASN

                   250
ACMV        -   PVYATLKIRI YEYDSIGN
PepYLCTHV   -   PVYATLKIRI YFYDSVQN
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**Figure 2.** Comparison of the amino acid sequences of PepYLCTHV and ACMV, where gray highlighted areas are the target peptide sequences for peptide synthesis comprising 20 amino acid residues

### *Antiserum titration using indirect PTA-ELISA*

The synthesized peptide produced from the company was successfully conjugated with the protein carriers (BSA, OVA), and the peptide-BSA was used for rabbit immunization. Weekly blood samples were taken from week 1 to 12. The titers ranged from 51,200 to 409,600, with the highest titer in week 12 (409,600) followed by week 9-10 (204,800), week 7-8 (102,400), and week 1-6 (51,200); in total, 82 mL of the antiserum was produced.

**Table 1**. Total amino acid sequences of PepYLCTHV coat protein which can potentially be linear epitopes, analyzed using the IEDB Analysis Resource Program

| No. | Start position | End position | Peptide | Peptide length |
|---|---|---|---|---|
| 1 | 1 | 11 | MAKRPADIVIS | 11 |
| 2 | 13 | 16 | PVSK | 4 |
| 3 | 22 | 23 | NF | 2 |
| 4 | 26 | 32 | PYLSRVA | 7 |
| 5 | 36 | 40 | VLVTN | 5 |
| 6 | 42 | 53 | KRSWANRPMYRK | 12 |
| 7 | 60 | 82 | YKSPDVPRGCEGPCKVQSYEQRH | 23 |
| 8 | 96 | 104 | VTRGNGLTH | 9 |
| 9 | 126 | 133 | NIKTKNHT | 8 |
| 10 | 144 | 155 | RRPYGTPQDFGQ | 12 |
| 11 | 159 | 176 | MYDNEPSTATVKNDNRDR | 18 |
| 12 | 185 | 198 | STVTGGQYASKEQA | 14 |
| 13 | 210 | 225 | VTYNHQEAAKYDNHTE | 16 |
| 14 | 236 | 242 | HASNPVY | 7 |

### *Characterization of antibody using indirect PTA-ELISA*

The specificity testing showed a positive reaction with PepYLCTHV and ToLCNDV, both of which belong to *Begomovirus* and found to be pepper-infecting viruses (Table 2). Cross reaction with ToLCNDV resulted to the amino acid sequence at 126-133 of the designed peptide in both viruses had 100 % identity. When the peptide was designed, there was no structural model for this virus available and we had to refer to the ACMV structural model as well as analysis of the linear epitopes using the epitope prediction program described above. As shown in Table 1, there were only two epitopes on the exterior of the virus coat protein. Our preliminary design used only one epitope that was not achieved for the antibody production; therefore, two epitopes at the conserved sequences were combined for this peptide antigen. In this study, the produced antibody provided good reaction with PepYLCTHV and cross-reacted with only one out of seven begomoviruses examined, namely ToLCNDV (Table 2). The antiserum titers were evaluated and result displayed substantially high titers. However, when the antiserum was used in the sensitivity testing by reacting with PepYLCTHV-infected sample, the limit of detection was only at 1:5 dilution.

**Table 2.** Specificity testing of anti-PepYLCTHV polyclonal antibody using antiserum dilution at 1:200 reacting with purchased virus positive samples using indirect PTA-ELISA

| Genus | Virus species[1/] | Antigen/Source | $A_{405}$[2/] | Result |
|---|---|---|---|---|
| *Begomovirus* | ACMV | Positive control/DSMZ | 0.219 | - |
| | BGMV | Positive control/DSMZ | 0.253 | - |
| | PepYLCTHV | Plant sap/pepper | 0.782 | + |
| | SqLCV | Positive control/DSMZ | 0.239 | - |
| | ToLCNDV | Positive control/DSMZ | 0.572 | + |
| | TYLCV | Positive control/DSMZ | 0.258 | - |
| | TYLCTHV | Positive control/DSMZ | 0.256 | - |
| | WCSV | Positive control/DSMZ | 0.219 | - |
| *Comovirus* | CPMV | Positive control/Agdia | 0.194 | - |
| | SqMV | Positive control/Prime | 0.186 | - |
| *Cucumovirus* | CMV I | Positive control/Agdia | 0.278 | - |
| | CMV II | Positive control/Agdia | 0.185 | - |
| *Potyvirus* | ChiVMV | Positive control/DSMZ | 0.184 | - |
| | PepMoV | Positive control/DSMZ | 0.212 | - |
| | PRSV | Positive control/DSMZ | 0.255 | - |
| | PVMV | Positive control/DSMZ | 0.230 | - |
| | PepYMV | Positive control/DSMZ | 0.258 | - |
| | TEV | Positive control/Agdia | 0.166 | - |
| | WMV | Positive control/DSMZ | 0.159 | - |
| | ZYMV | Positive control/DSMZ | 0.158 | - |
| *Tobamovirus* | BPeMV | Positive control/Agdia | 0.225 | - |
| | CGMMV | Positive control/DSMZ | 0.224 | - |
| | PMMoV | Positive control/Agdia | 0.160 | - |
| | TMV | Plant sap/tobacco | 0.221 | - |
| | ToMV | Positive control/Agdia | 0.195 | - |
| *Tospovirus* | CaCV | Positive control/Agdia | 0.175 | - |
| | GBNV | Positive control /Agdia | 0.248 | - |
| | GRSV | Positive control /Agdia | 0.160 | - |
| | INSV | Positive control /Agdia | 0.258 | - |
| | IYSV | Positive control /Agdia | 0.169 | - |
| | TCSV | Positive control /Agdia | 0.160 | - |
| | TSWV | Positive control /Agdia | 0.194 | - |
| | WSMoV | Positive control /Agdia | 0.248 | - |
| Positive control | Peptide-OVA conjugate | Antigen | 2.224 | + |
| Negative control | Pepper | Healthy | 0.180 | - |

[1/] ACMV *African cassava mosaic virus*, BGMV bean golden mosaic virus, PepYLCTHV *pepper* yellow leaf curl Thailand virus, *SqLCV squash leaf curl virus, ToLCNDV* tomato leaf curl New Delhi virus, TYLCV tomato yellow leaf curl virus, TYLCTHV tomato yellow leaf curl Thailand virus, WCSV *watermelon chlorotic stunt virus*, CPMV cowpea mosaic virus, SqMV squash mosaic virus, CMV I cucumber mosaic virus I, CMV II cucumber mosaic virus II, *ChiVMV* chili veinal mottle virus, PepMoV *pepper mottle virus, PRSV papaya ringspot virus,* PVMV *pepper veinal mottle virus,* PepYMV *pepper yellow mosaic virus,* TEV tobacco etch virus, ZYMV *zucchini yellow mosaic virus, WMV watermelon mosaic virus*, BPeMV bell pepper mosaic *virus*, CGMMV *cucumber green mottle mosaic virus*, PMMoV pepper mild mottle virus, TMV tobacco mosaic virus, ToMV tomato mosaic virus, CaCV capsicum chlorosis virus, *GBNV* groundnut bud necrosis virus, *GRSV* groundnut ringspot virus, *INSV impatiens necrotic spot virus,* IYSV iris yellow spot virus, *TCSV* tomato chlorotic spot virus, *TSWV tomato spotted wilt virus, WSMoV watermelon silver mottle virus.* [2/] Values are from duplicates and compared to the negative control which was healthy pepper sap reacting with anti-PepYLCTHV antibody. Cut-off value is twice the average value from the negative control (0.360).

***Detection of PepYLCTHV using dot immunobinding assay and tissue print-immunoblotting techniques***

The reaction of DIBA using diseased pepper sap compared to the healthy one showed that the antibody was specific with diseased pepper sap and peptide-OVA conjugate without any cross reaction with the healthy sap and buffer (Figure 3). A similar result occurred when using TPIB as shown in Figure 4.

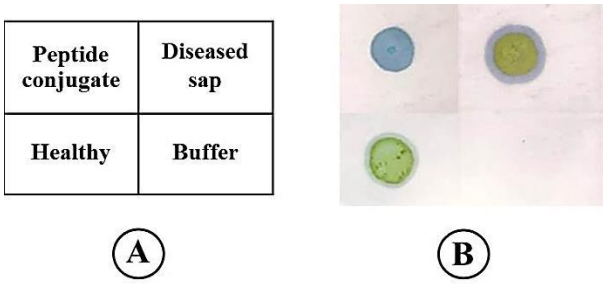


**Figure 3.** Diagram of dot immunobinding assay (A). Samples were allowed to react with anti-PepYLCTHV antiserum from week 12 at 1:200 dilution showing positive reactions on the peptide conjugate and diseased sap (B)

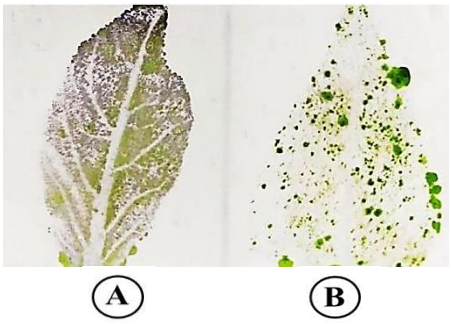


**Figure 4.** Results of tissue print-immunoblotting using anti-PepYLCTHV antiserum from wk 12 at 1:200 dilution showing positive reaction on a diseased pepper leaf (A) as purple areas compared to the negative reaction on a healthy leaf (B)

## Discussion

*Begomovirus* is one of the largest group of plant viruses which has a wide host range and overall they are responsible for a considerable amount of damage to many economic crops. Molecular detection methods based on PCR assay are generally available but the serological methods which are commonly applied for other plant viruses are not readily available for this group due to the difficulty in preparing proper antigen. Our research study providedthe information on antiserum production using the peptide antigen of

PepYLCTHV. The synthesis of the predicted linear epitopes was conducted based on the GenBank database and the IEDB program compared to the known structure of ACMV capsid protein. The target epitope sequences were on the exterior of the virus particle for B cell accessibility and the linear form ensured that the epitope would not denature and affect antigenicity during the antigen preparation process. RCA technique applied in this research provides the amplification of a circular DNA which is the typical character of the begomoviral genome (Khan and Dijkstra, 2002). Antiserum production delivered the highest titer in week 12 at 409,600 when reacting with the OVA-peptide antigen. There is only one previous report about antibody production against YLCD of pepper caused by PepYLCIV-Bogor (Hidayat *et al.*, 2009). They used purified virus as an antigen. The result showed that infection of PepYLCIV-Bogor was detectable in infected plants by the antibody up to dilution 1:16,384 when the sample extract was diluted up to 1:16. No cross reaction was observed with TMV, CMV and ChiVMV tested. However, serological cross reactivity was expected among members of begomoviruses since their amino acid sequences of the coat proteins are highly conserved (Padidam *et al.*, 1995). In this study, the produced antibody provided good reaction with PepYLCTHV and cross-reacted with only one out of seven begomoviruses examined, namely ToLCNDV. Hidayat *et al.* (2009) concluded that DIBA was the method of choice for detection of PepYLCIV because it was cost-effective and reliable practice for mass analyses which agrees with our result. However, this research is the first report in Thailand on making anti-PepYLCTHV antibody as well as using the synthesized peptide antigen of begomovirus infecting peppers. Although the antibody had limited sensitivity, development of ELISA, DIBA, and TPIB were able to differentiate the infected sample from the healthy one and so are potential methods of choice for detection. This antibody may be further applied in immunocapture PCR for trapping both viruses and identify them by each virus-specific primers.

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