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Production of Polyclonal Antibodies for Detection of *Papaya* ringspot virus (PRSV) in Ivy Gourd (*Coccinia grandis* (L.)

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ABSTRACT

The coat protein (CP) gene from the *Papaya* ringspot virus (PRSV) strain Chiang Mai 1 (PRSV-CP) was cloned and expressed in *Escherichic coli* and purified using nickel-nitrilotriacetic acid agarose resin. The recombinant coat protein was used as an antigen to produce polyclonal antibodies for detection of PRSV in ivy gourd. The antibodies were able to detect the recombinant protein and PRSV-infected plant samples. Specificity of the antibodies to PRSV was compared with indirect enzyme-linked immunosorbent assay (ELISA), dot-blot immunobinding assay (DIBA) and tissue blot immunoassay (TBIA). It was found that the antibodies were highly specific and could be used to detect the presence of virus in different parts of ivy gourd. The antibodies were used to detect PRSV infection in ivy gourd using indirect ELISA and DIBA on 211 samples collected from papaya orchards. It was shown that 107 samples (50.71%) were infected by PRSV. In this study, results from germination testing on 195 seedlings from the infected ivy gourd seeds implied that PRSV was not seed transmitted. Furthermore, the results from TIBA revealed that PRSV spread to all parts of the ivy gourd (leaf, flower, flower stalk, stem and root, as well as tendril) even though some of the infected parts did not show any viral symptoms.

Keywords: *Papaya* ringspot virus (PRSV), ivy gourd, antibody, recombinant coat protein

INTRODUCTION

Papaya ringspot virus (PRSV) has been recognized as a crucial problem in papaya and *Cucurbitaceae* orchards in many countries throughout the world, including Thailand (Lecoq *et al*., 2001). PRSV is classified to the genus *Potyvirus*. It has long flexuous particles, about 750–800 nm in length and 12 nm in diameter (Purcifull *et al*., 1984). It has been reported that host plants of PRSV are in *Cucurbitaceae* that is, pumpkin (Srisomchai,1976; Prasartsri *et al*., 1982b; Keeratiyaangul and Deema, 1983) and honeydew and water melon (Prasartsri *et al*., 1982a). In recent years, host plants of PRSV-P have been extended to winter melon, melon, angled gourd, cucumber, wild melon and ivy gourd (Kositratana *et al*., 1990; Bateson *et al*., 2002). Transmission of PRSV is rapid by mechanical methods and it is non-persistently transmitted in 10–30 s by numerous species of aphids such as cotton aphid (*Aphis gossypii*), legume aphid (*Aphis craccivora*) and tobacco aphid (*Myzus persicae*) (Karl and Schmelzer, 1971).

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Ivy gourd (*Coccinia grandis* (L.) Voigt) is a weed, belonging to the family *Cucurbitaceae*, which is wide spread in papaya orchards throughout all regions in Thailand. Since PRSV is capable of infection in papaya and cucurbits including ivy gourd that survive over season through bulbs, PRSV-infected ivy gourd somehow does not show any obvious systems. Hence, it is necessary to develop a serological technique for PRSV detection for the effective management of the disease prior to its outbreak. Both indirect enzyme-linked immunosorbent assay (ELISA) and dot-blot immunobinding assay (DIBA) have been used widely for the detection of plant viruses and diagnosis of plant viral diseases (Clark and Adams, 1977; Mahmoud *et al.*,1996). However, the polyclonal antibodies obtained from rabbit that was immunized with purified virus preparation has been difficult, time-consuming and produced variable background reactions. Thus, the present work produced specific polyclonal antibodies for detecting PRSV with three serological assays. Additionally, the study was useful for orchard PRSV management for the regulation, quarantine and management of PRSV virus in ivy gourd.

MATERIALS AND METHODS

Construction and expression of the recombinant *Papaya* ringspot virus-coated protein in *E. coli*

Coat protein of PRSV strain Chiangmai 1 (DQ085857) was cloned into pGEM-T easy by adding *Bam*HI (5′GGATCCA AACTGAAGCTGCGGATGCTA3′) and *Hind*III (5′AAGCTTTCAATTGCGCATACCCAG3′) on the 5′ and 3′ termini, respectively. The resulting clone contained 861 bp including stop codon (TAG) on the 3′ termini, and was excised by double digestion with *Bam* HI and *Hind* III. Eight hundred sixty-one base pairs were separated on 1% tris-acetate-ethylenediaminetetraacetic acid agarose gel and recovered using a DNA Gel extraction kit (Qiagen; Hilden, Germany). The pQE30 was digested by *Bam* HI and *Hind* III, and then ligated with purified 861 bp by T_4 DNA ligase overnight at 4 °C. Recombinant plasmid was then transformed into *E. coli* DH5∞ using the $CaCl_2$ method, and selected on 2xYT with ampicillin. Plasmid was extracted using the alkaline lysis method (Sambrook *et al.,* 1989) and verified by sequencing. The correct 861 bp insertion coat protein of PRSV was then transformed into *E. coli* strains M15 (Qiagen; Hilden, Germany) and selected on 2xYT supplement with 100 $mg.L^{-1}$ ampicillin.

In this study, recombinant PRSV-coated protein was synthesized in a bacterial cell system as described by Fristch *et al*. (2001): plasmid pQE30 (containing N-terminal 6xHis tag) and PRSV-coated protein gene comprising 864 bp in length was transformed into *Escherichia coli* cells (strains M15) using the $CaCl_2$/heat shock method. A single colony of *E. coli* was selected and grown on 2xYT broth supplemented with 100 $mg.L^{-1}$ of ampicillin, and then the culture was shaken at 180 rpm and 37 ^oC for 2 hr. Next, the culture was induced to synthesize the recombinant PRSV-coated protein by adding isoproply-thio-β-D-thiogalactopyranoside (IPTG) to 1 mM final concentration. The cells were harvested at 1 hr intervals for 6 hr after induction. Expression of the coated protein gene in *E. coli* cells was assayed using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). To assess the purity of the synthesized coat protein, the SDS-PAGE gel was stained with Coomassie Brillant Blue R-250 (weight per volume; w/v), 45% methanol (volume per volume; v/v), 10% acetic acid (v/v), for 15 min, and then destained with 25% methanol (v/v), 7% acetic acid (v/v) for 24 hr or until the protein band appeared. The expected size of the recombinant PRSV-coated protein was estimated by comparing with protein markers (marker# SM0431Lab Aid).

Purification, identification and quantitation of the recombinant *Papaya* ringspot virus-coated protein

E. coli strain M15 containing pQE30 with the recombinant PRSV-coated protein gene was cultured under standard conditions for which the highest yield of the recombinant PRSV-coated protein was obtained. The cells were harvested from 1 L of culture using centrifugation at 10,000 rpm and 4 ^{o}C for 20 min, and the cell pellet was initially lysed with lysozyme and re-suspended with buffer B (8M urea, 10 mM Tris-Cl, 100 mM NaH_2PO_4, pH 8.0). The flask containing the cell suspension was shaken at room temperature for 30 min. Subsequently, the lysed cells were sheared 10 times using an ultrasonicator with 30 s on ice or until clear, runny lysate was obtained. The lysate was subjected to centrifugation at 10,864 g and 4 ^{o}C for 20 min. The collected supernatant was purified using a nickel-nitrilotriacetic acid agarose column. The purification step was carried out according to the manufacturer's protocol (Qiagen; Hilden, Germany). Finally, each flow through was eluted and the size of the recombinant PRSV-coated protein was determined using SDS-PAGE. Each fraction was subsequently dialyzed in 1XPBS, pH 7.4 at 4 ^{o}C. Dialysis was repeated four times for 4 hr each time. The concentration of purified recombinant PRSV-coated protein was determined using a Bradford protein assay employing bovine serum albumin as a standard (Marion *et al.*, 1976). Absorbance was read at 595 nm.

Production of polyclonal antibodies

Immunization was performed by injecting 2 mth-old, female, New Zealand white rabbits. The first subcutaneous injection was performed by injecting 2 mL of antigen (containing PRSV-coated protein 1 mg.mL^{-1}) and complete Freund's adjuvant at the ratio of 1:1 (v/v), three subsequent injections were given of 1 mg.mL^{-1} of the concentrate antigen mixed with 1 mL of incomplete Freund's adjuvant at the ratio of 1:1 (v/v). Blood was collected weekly for 10 wk starting from 1 wk after the last injection.

Purification of immunoglobulin (IgG) was done using a protein extraction kit (HiTrap Protein A HP, GE) and SDS-PAGE determination was used for testing the purity of the IgG. The concentration of IgG was adjusted to a desirable value and the extinction coefficient of IgG was employed for neutral pH adjustment. Ultimately, the concentration of the IgG was calculated as: O.D.280/1.4 = x mg.mL^{-1} (Clark and Adams, 1977).

Detection of *Papaya* ringspot virus in ivy gourd using indirect enzyme-linked immunosorbent assay and dot-blot immunobinding assay

To test the sensitivity levels of polyclonal antibodies for detection of PRSV, comparative analysis was carried out using indirect ELISA and DIBA.

Two hundred eleven samples of ivy gourd were collected from papaya orchards in different geographic regions of Thailand: Khonkaen, Ma Ha Sarakham, Sakon Nakhon, Nong Khai, Mukdahan, Chonburi, Rayong, Chantaburi, Lobburi, Chiang Rai, Bangkok and Nakhon Prathom. For indirect ELISA assay, ivy gourd extracts were prepared by grinding plant tissues in carbonate buffer (pH 9.6) at a sample-to-buffer ratio of 1:10 (w/v), while for the DIBA assay, the sample extraction was prepared in a buffer of phosphate buffered saline (PBS) at the same ratio. For the indirect ELISA assay, the ELISA plate wells were coated with 100 µL of sample extracts and incubated at 4 ^{o}C overnight. After incubation, five washings with 200 µL per well of PBST (PBS containing 0.05% Tween-20) were performed for 3 min each. Subsequently, the wells were blocked by adding100 µL per well of PBST containing 2% skimmed milk, for 60 min at 37 ^{o}C. Blocking was done five times for 3 min each. Next, the plate was loaded with 100 µL per well of the IgG of PRSV polyclonal antibodies (1:1,000) and incubated at 37 ^{o}C for 60 min. After being washed five times

with PBST, each well was added with 100 µL of goat anti-rabbit IgG conjugated with alkaline phosphatase at 1:10,000. The plate was incubated at 37 °C for 60 min and washed with PBST as described previously. Finally, 100 µL of substrate solution (1 $mg.mL^{-1}$ of p-nitrophenyl phosphate in diethanolamine buffer, pH 9.8) were added and the plate was incubated at 37 °C for 30 min under dark and moist conditions. The reactions were stopped by adding 100 µL of 3 N NaOH and the absorbance was read at 405 nm using an ELISA microplate reader. A positive threshold was set for each plate at twice the value of a healthy plant.

For DIBA, 20 µL of each sample extract were dotted on nitrocellulose membranes which were then allowed to dry at room temperature. The nitrocellulose membrane was soaked in blocking buffer containing 2% skimmed milk in tris-buffer slaine (TBS) and 0.4% Triton X-100 for 60 min. The membrane was then incubated with the antibody solution in TBS (1:1,000 dilution) for 60 min. After washing three times with TBST (0.05% Tween20 in tris-buffer saline), the nitrocellulose membrane was incubated with goat anti-rabbit IgG conjugated with alkaline phosphatase 1:10,000 diluted in blocking buffer with gentle shaking and washed with TBST three times for 5 min each. Finally, the membrane was incubated with substrate solution containing Napthol AS-MX phosphate (0.25 $mg.mL^{-1}$) and fast red TR salt (5-chloro-2-toluidincdiazonium chloride, hemizenecholoride; 0.5 $mg.mL^{-1}$) in the dark for 10 min. The reaction was stopped by discarding the substrate solution and washing the nitrocellulose membrane with distilled water. The membrane was left to dry and the final color visualization reading was carried out using a camera.

Inoculation test of *Papaya* ringspot virus on host plants

PRSV-infected ivy gourds were selected and the sap extracted from the infected plants was used to inoculate healthy seedlings of ivy gourd, squash, and pumpkin. Two grams of the PRSV infected ivy gourd tissues were ground in 0.1 M PBS, pH 7.4 supplemented with 0.2% Na_2SO_3 (1:10 w/v) using a cold mortar and a pestle. The extracted sap was mixed with Celite®, and then was spread on leaves of the healthy seedling and the seedlings were left for 10 min. After the inoculation, the leaves were washed with sterile distilled water. Subsequently, the plants were grown under greenhouse conditions until symptoms developed. Once the symptoms were obvious, both inoculated and un-inoculated plants were examined by indirect ELISA.

Examination of *Papaya* ringspot virus seed transmission

195 seeds of PRSV-infected ivy gourd were collected and germinated in the greenhouse. One month after germination, 2–3 leaves of each ivy gourd seedling were examined for PRSV using indirect ELISA and compared with healthy seedlings.

Examination of *Papaya* ringspot virus distribution in different plant parts using tissue blot immunoassay and indirect enzyme-linked immunosorbent assay

PRSV distribution in different plant parts (leaf, flower, flower stalk, stem, root and tendril) was examined using tissue blot immunoassay (TBIA) as described by Martin *et al*. (2002) with some modifications. Some parts of ivy gourd were chosen in this study. Each PRSV-infected parts was dissected and pressed on each nitrocellulose membrane for 5–10 s and the membrane was left to dry. Subsequently, the nitrocellulose membranes were treated according to the standard conditions and procedures as described in the DIBA assay.

RESULTS AND DISCUSSION

Construction and expression of the recombinant *Papaya* ringspot virus-coated protein in *E. coli*

The *E. coli* cells (strain M15) harboring

the recombinant plasmid (pQE30-PRSV-coated protein gene) were cultured in 2xYT broth for various incubation times to determine when the highest yield of the coat protein was produced. The results revealed that the highest yield of the target protein was obtained after 5 hr incubation, and after that the expressed protein yield was steady as shown in Figure 1a. Purification and characterization of the coat protein were carried out under the experimentally determined conditions.

Purification, identification and quantitation of the recombinant *Papaya* ringspot virus-coated protein

The recombinant PRSV-coated protein was expressed in *E. coli* strain M15. Purification and identification of the recombinant PRSV was visualized using SDS-PAGE. High-purity protein was found in fractions of buffer E. A single target protein band of about 34 kDa appeared in buffer E (Figure 1b) which indicated that the pH had an effect on protein purification. The molecular weight of the coat protein was calculated from 861 bp using http://web.expasy.org/cgi-bin/translate/DNA and http://www.sincegateway.org/tools/protein resulting in 292 (with 6 His) amino acid and a value of 33.43 kDa was obtained. Since 6xHis contains an aromatic ring containing nitrogen atoms, it has two free electrons forming an ionic bond at high pH (8.0) with N^{++} attached NTA resin. By changing the pH to acid (pH 3.9) the ionic bond between N-Ni was dissociated by the excess H^+, resulting in bound 6x-His PRSV-coated protein being released from the column. The recombinant PRSV-coated protein was measured by Bradford protein assay, using a standard curve generated with bovine serum albumin at concentrations from 1 to 10 $mg.mL^{-1}$. The estimated protein concentration was 5.24 $mg.mL^{-1}$. The concentration of protein was finally adjusted to 1 $mg.mL^{-1}$ for use in immunization.

Production of polyclonal antibodies

Production of polyclonal antibodies against PRSV using recombinant PRSV-coated protein has been well recognized since it is capable of producing a high yield and high purity of the antibody. It also resolves the difficulties of increasing purified virus particles from infected host plant (Ling *et al.*, 2000; Abouzid *et al*., 2002; Hema *et al*., 2003; Hourani and Abou-Jawdah, 2003). The production of polyclonal antibodies against PRSV-coated protein was successfully prepared in rabbit. Blood was withdrawn every week for 10 wk. The total antiserum was 73 mL with a titer value between 1;3,200 and 1:409,600. Low titer values were observed in the first, second and third collections and were elevated in later collections. Among the collections, the eighth collection gave the highest titer value of

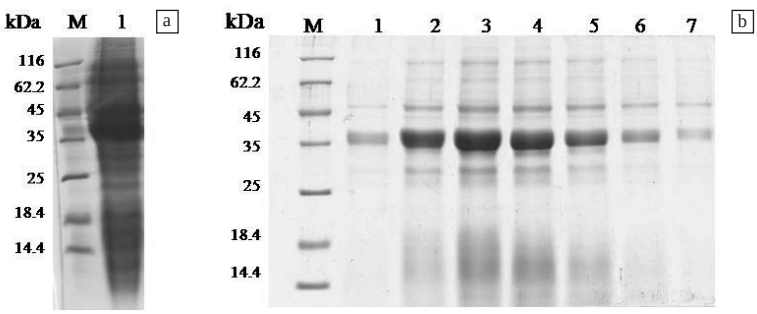

Figure 1 SDS-PAGE analysis of expressed and purified recombinant *Papaya* ringspot virus-coated protein: (a) M = Protein marker, lane 1 = Recombinant PRSV-coated protein after 1 mM IPTG induction for 5 hr cultivation; (b) M = Protein markers, lanes 1–7 = Eluted fractions after column washing with buffer B, pH 4.5.

approximately 1:409,600. Therefore, it should be noted that the eighth collection should be used for the IgG purification. The IgG from the antiserum was purified through a Hi Trap Protein A HP column (GE; Helsinki, Finland) and had high purity with heavy and light chains of 55 and 25 kDa, respectively, which was equivalent to 17.63 $mg.mL^{-1}$. Consequently, the IgG was further investigated for its reactivity and specificity against PRSV in ivy gourd. The results from the ELISA assay indicated that the IgG was specific for PRSV in ivy gourd and papaya (Table 1 and Figure 2) Both the isolates had similar serological features as determined by direct antigen coated-enzyme-linked immunosorbent assay with polyclonal antisera (Gourgopal *et al.*, 1999) and a possible reason might have been that PRSV-coated protein in papaya and cucurbits has a similar percentage of amino acids—about 98.7–99.0% (Baker *et al.*, 1991). Moreover, production of antibodies against either PRSV type-P or type-W could be available for PRSV detection in the same host plants (Yeh *et al*., 1992)

Detection of *Papaya* ringspot virus in ivy gourd by indirect enzyme-linked immunosorbent assay and dot-blot immunobinding assay

PRSV-infected ivy gourd samples were collected from papaya orchards and the sap was extracted from the PRSV-infected plants and used as inoculum in healthy ivy gourd. Analysis revealed that plants receiving PRSV inoculation showed no disease. Nonetheless, it was confirmed that all inoculated plants had been infected by PRSV. Two hundred eleven samples were detected for PRSV by indirect ELISA and DIBA using self-made antibody and 107 samples were found to be positive for PRSV, which was equivalent

Table 1 Detection of *Papaya* ringspot virus (PRSV) in different parts of ivy gourd using indirect enzyme-linked immunosorbent assay (ELISA) and dot-blot immunobinding assay (DIBA). Polyclonal antibodies against PRSV at dilution of 1:1,000. Healthy ivy gourd and extraction buffer were used as controls.

Part of plant	Number of samples	PRSV infection detection		Disease percentage (%)
		Indirect ELISA	DIBA	
Leaf	154	98	98	46.44
Fruit	13	8	8	3.79
Root	10	1	1	0.47
Seedling	34	0	0	0.00
Total	211	107	107	50.71

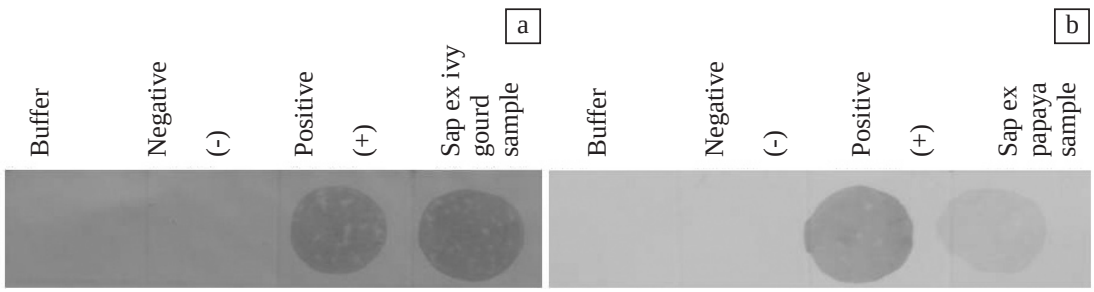

Figure 2 Comparison of polyclonal antibodies specified for *Papaya* ringspot virus detection between: (a) Ivy gourd; (b) Papaya. Both samples were collected from papaya orchards. Each sample was measured after 5 min substrate addition.

to 50.71% infection. In addition, different plant parts were also investigated. It was shown that the leaves were the predominant location where the PRSV existed (Table 1). The results of the DIBA test were similar to those from indirect ELISA (Figure 2). Papaya orchards are places where aphids (the PRSV transmission vector) are predominant (Figure 3).

Consequently, it could be stated that ivy gourd is a source of inoculum and distribution since ivy gourd is a perennial vine and survives for several years. In addition to wounded or mechanical transmissions, PRSV can be also transmitted *via* an insect vector within 15–60 s and then rapidly spread to other locations. Gonsalves *et al*. (2010) recorded that PRSV seed transmission was mostly found in *Cucurbitaceae* rather than papaya trees. Since no symptoms caused by PRSV were noticed, and the owner of the papaya orchard was not aware of ivy gourd growing in the orchard, no protective measures had been undertaken resulting in the rapid and coincidental distribution of PRSV.

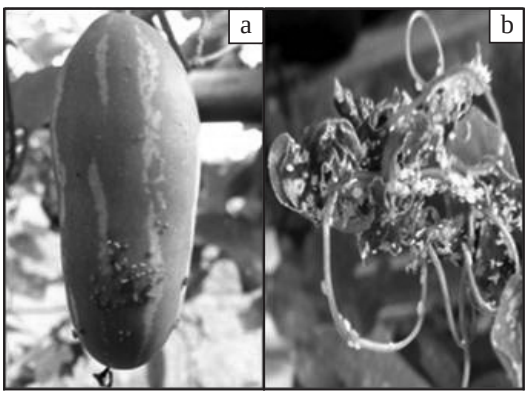

Figure 3 Symptoms of infection by aphids as *Papaya* ringspot virus transmission vector in ivy gourd grown in papaya orchards: (a) Papaya fruit; (b) Leaf, leaf stalk and tendril.

Inoculation test of *Papaya* ringspot virus on plants

Three months after inoculation, ivy gourd leaves showed no mosaic distortion as found in squash (*Cucurbita pepo* L.) or pumpkin leaves (*C. moschata* (Duch) Pior) after receiving PRSV re-inoculation (Figure 4). Pathogenesis is a complicated process involving many factors. For example, the appearance of disease symptoms in each host plant varies with crop variety, viral

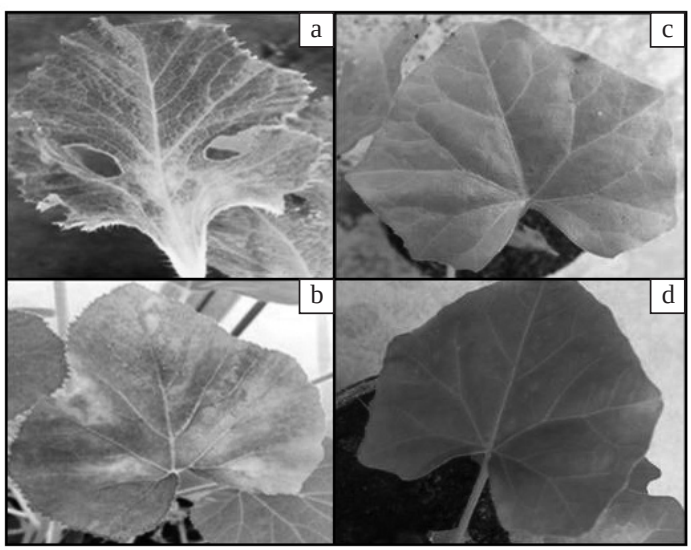

Figure 4 Development of yellow mosaic symptoms caused by *Papaya* ringspot virus (PRSV) on: (a) Squash leaf, (b) Pumpkin; (c) Un-inoculated ivy gourd; (d) No symptoms from PRSV-inoculated ivy gourd 1 mth after re-inoculation.

strains, temperature and infection time (Matthews, 1970; Gibbs and Harrison, 1976). Interestingly, alternative host plants for PRSV other than ivy gourd have not been reported to date. Thus, due to a lack of knowledge, owners do not strictly eradicate PRSV host plants from their papaya orchards, resulting in the rapid spread of the problem and difficulties in protection due to the existence of the virus both above and below ground.

Examination of *Papaya* ringspot virus seed transmission

This study was carried out by germinating seeds derived from 195 infected ivy gourd samples using polyclonal antibodies against PRSV at a dilution up to 1:1,000 using the TBIA method. No color appeared after 30 min of substrate addition which indicated that PRSV was not present in the seedling. This result implied that PRSV was not transmitted *via* ivy gourd seeds. Bayot *et al.* (1990) reported that PRSV could be detected in 0.15% of the papaya seedlings grown from infected seed. The disease symptoms of 6 wk-old seedlings appeared to be mosaic, mottled and distorted leaves. This was consistent with the finding of Alma *et al*. (2012) who recorded that PRSV was seed transmissible in black locust (*Robinia pseudoacacia* L.) up to 48%. Taken together this indicated that PRSV was seed transmissible although low viral particles were detectable in plant tissues. Furthermore, papaya is also a dominant host plant and a global outbreak of PRSV might result from its transmission. Although the results from the current study were not in accordance with Bayot *et al.* (1990) and Alma *et al*. (2012), it is recommended that further investigation on ivy gourd seed transmission should be undertaken with many more samples and more frequent sampling. Since the incubation time of PRSV was not long enough, PRSV could not be detected in the ivy gourd. In addition, many birds feed on ivy gourd fruits and could provide a vector to distribute seeds more effectively worldwide. The findings that PRSV can be seed transmissible could account for it becoming a pandemic. Ivy gourd is also propagated through harvesting since most Asians consume it as a vegetable, which could provide over-seasonal inocula.

Detection of *Papaya* ringspot virus distribution in different parts of ivy gourd by tissue blot immunoassay and indirect enzyme-linked immunosorbent assay approaches

Investigation of PRSV present in ivy gourd was undertaken using TBIA. It was found that PRSV was replicated and spread throughout the whole plant as detected by TBIA. After infection to the host plant, it was deduced that PRSV would move inside the phloem cells and later inside other parts. Finally, PRSV would distribute throughout the plant cells. Virus particles could be detected in the phloem and cytoplasm using transmission electron microscopy (Purcifull *et al*., 1984), demonstrating that PRSV was distributed through all plant parts including the phloem and xylem. Phloem and young leaves showed a high accumulation of the virus which is one reason why PRSV was detected in every part of ivy gourd (Figure 5 and Table 2). The result was also confirmed by indirect ELISA. Infected plants gave readings of A_{405} nm ranging from 0.431 to 1.893, whereas healthy plants show values ranging from 0.113 to 0.183. Both approaches provided strong supporting data for the findings on the accumulation of PRSV in ivy gourd and that it was significantly distributed throughout the whole plant.

CONCLUISIONS AND FUTURE PROSPECTS

The PRSV-coated protein gene was efficiently expressed in a bacterial cells system. Hence, the recombinant PRSV-coated protein had an estimated MW of about 34 kDa. The highest quantity of the protein was obtained after 5 hr induction with 1 mM IPTG. The purified protein

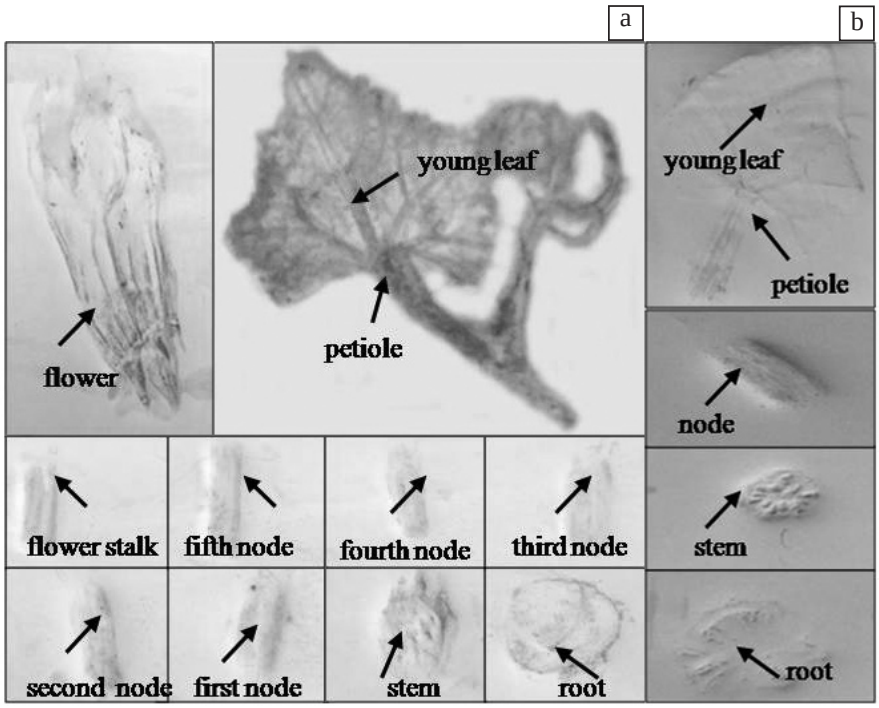

Figure 5 Detection of *Papaya* ringspot virus (PRSV) in different parts of ivy gourd using tissue blot immunoassay: (a) Different parts of ivy gourd infected by PRSV; (b) Healthy ivy gourd.

Table 2 Comparison of the sensitivity for *Papaya* ringspot virus detection using indirect enzyme-linked immunosorbent assay (ELISA) and tissue blot immunoassay (TBIA).

Fraction	$OD_{405\ nm}$ (indirect ELISA)[a]	Color limit (TBIA)[b]
Flower	0.435	+
Young leaf	1.893	+++
Petiole	1.785	+++
Tendrils	1.441	+++
Flower stalk	0.705	++
Fifth node	0.773	++
Fourth node	0.431	+
Third node	0.763	++
Second node	0.553	++
First node	0.625	++
Stem	1.123	+++
Root	0.585	++
Negative control	0.113–0.183	-

[a] = Absorbance value at 405 nm measured by indirect ELISA.

[b] = Level of intensity of red stain detected by TBIA.

yielded 5.24 mg.mL^{-1}, which was adequate for use in rabbit-injected polyclonal antibody production. The resulting polyclonal antibodies against PRSV were yieldable for PRSV detection in ivy gourd using serological assays such as indirect ELISA, DIBA and TBIA with high accuracy at a dilution of 1:1,000. These techniques produced the same finding that ivy gourd is a host plant of PRSV without noticeable disease symptoms. In addition, PRSV was distributed throughout the whole plant and PRSV was not seed transmissible in ivy gourd. However, further studies need to be carried out.

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