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# Development of a Dot-Elisa Assay for Diagnosis of Southern Rice Black-Streaked Dwarf Disease in the Field

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## Abstract

Outbreaks of the Southern rice black-streaked dwarf virus (SRBSDV) have caused significant losses in many rice-growing areas in Vietnam, especially in both North and Central Vietnam in recent years. To detect the virus, traditional reverse transcription polymerase chain reaction (RT-PCR) methodology and immunoassays are currently employed. RT-PCR is accurate but requires expensive chemicals and instruments, as well as complex procedures that limit its applicability for field tests. To develop a cheaper, simpler, and reliable SRBSDV diagnosis assay based on the dot-enzyme-linked immunosorbent assay (dot-ELISA) method, anti-SRBSDV polyclonal antibodies were produced by using the antigens derived from the P10 coat protein of SRBSDV, which was achieved from a previous study. The IgG antibody purified from the antiserum of recombinant P10-immunized mice by protein A-agarose affinity chromatography could specifically detect both the target protein and SRBSDV at a dilution of 1:100000. In the trial test of SRBSDV diagnosis, the dot-ELISA assay using the obtained anti-SRBSDV antibody showed an accuracy rate of 90.9% in comparison with the standard RT-PCR assay. These results are important premises for the large-scale application of dot-ELISA assay for SRBSDV diagnosis in order to protect rice crops against viral disease damage.

## Keywords

Antibody, diagnosis, dot-ELISA, P10 protein, SRBSDV

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## Introduction

Southern rice black-streaked dwarf virus (SRBSDV) was first observed in Guangdong province in 2001, identified in 2008, and

caused major losses in rice production on thousands of hectares in North and Central Vietnam in 2009. The disease continued to cause extensive crop damage in 2010 and 2011 with more than 60,000 ha of paddy fields in 29 provinces becoming infected (Zhou *et al.*, 2013), and then reemerged strongly in 2017 with about 2,000 ha of infected paddy fields in 7 provinces (Plant Protection Department – Vietnam's Ministry of Agriculture and Rural Development, 2017).

SRBSDV is transmitted by white-backed planthoppers (WBPHs) (Zhou *et al.*, 2018), and the main mode of transmission is feeding rather than spawning (Wang *et al.*, 2010). For SRBSDV, it is nearly impossible to control once SRBSDV disease-symptomatic rice plants have been observed in the field. The detection of the viral content in plant samples (symptomatic/asymptomatic) does not bring much of a significant difference to disease control either. However, early detection of viruliferous WBPHs in the field is tremendously valuable for controlling southern rice black-streaked dwarf disease (SRBSDD) as it allows farmers to actively eliminate WBPHs and helps to minimize the risk of SRBSDD emergence.

The current laboratory diagnosis of SRBSDV disease employs both reverse transcription polymerase chain reaction (RT-PCR) and immunoassay to detect viral RNA in disease plant samples (Zhou *et al.*, 2010a; Ji *et al.*, 2011). RT-PCR diagnosis of SRBSDV can achieve results with high accuracy, but it comes at a high price, and requires professional laboratory technicians, making it unsuitable for application on a large scale. Meanwhile, immunoassays also provide reliable results but at a reasonable price, which fits perfectly with the economic situation in Vietnam.

Dot-enzyme-linked immunosorbent assay (dot-ELISA) is a highly versatile solid-phase immunoassay for antibody or antigen detection, and is also a simple, rapid, and scalable procedure for screening a large number of samples at one time (Manoharan *et al.*, 2004). Optimization and standardization of reagents and test procedures for serodiagnosis of viral diseases are vital for assay validity, reproducibility, and quality control (Michael *et al.*, 1984). In previous studies, we produced anti-SRBSDV antibodies by immunizing mice with either recombinant protein or synthetic peptides that were derived from the P10 coat protein of SRBSDV (Hanh *et al.*, 2015; Phuong *et al.*, 2020; Hanh *et al.*, 2021a; Hanh *et al.*, 2021b) but the titers of these antibodies were not high enough to apply in a practical virus diagnostic assay. This may have been the result of the immunization method with an inadequate amount of injected antigens.

In the present study, our goal was to produce anti-SRBSDV antibodies with high sensitivity and antigenic specificity in order to develop an SRBSDV diagnostic dot-ELISA assay for further large-scale application in Vietnam, and for laboratories around the world that are not well equipped.

## Materials and Methods

### Materials

*E. coli* Rosetta strains (DE3) transformed either pET28a or pET28a/P10 (Tam *et al.*, 2013) and were provided by the Molecular Pathology Department (Agricultural Genetics Institute).

Four antigens, namely purified P10 protein and P10.1, P10.2, and P10.3 synthetic peptides obtained from previous studies (Hanh *et al.*, 2015; Phuong *et al.*, 2020; Hanh *et al.*, 2021a; Hanh *et al.*, 2021b) were supplied by the Molecular Pathology Department (Agricultural Genetics Institute).

SRBSDV-, rice ragged stunt virus (RRSV-), and rice grassy stunt virus (RGSV-) infected rice plants, and WBPH were prepared as previously described (Li *et al.*, 2017); the virus-free sample was obtained from rice plants growing in a greenhouse. These inoculated and uninoculated samples were supplied by the Research Center for Tropical Plant Pathology in Gia Lam district, Vietnam. Suspected SRBSDV rice samples collected from the 2020 summer crop in northern and north central provinces were supplied by the Northern Plant Protection Center and the Zone IV Plant Protection Center.

## Methods

*Preparation of plant and WBPH test samples*

Five grams of a rice shoot sample or five individuals of a WBPH sample were ground in liquid nitrogen. One-half of the ground sample was used for the total RNA extraction by Trizol according to Invitrogen's procedures and subsequently applied in the RT-PCR assay. The rest of the ground sample was homogenized in PBS buffer for the immunoassay, including the dot-ELISA method and the quick-stick method.

*Purification of anti-SRBSDV IgG antibodies*

Anti-SRBSDV antisera were produced using P10 protein and P10.1, P10.2, and P10.3 peptides as antigens for immunization in mice as previously described (Hanh *et al.*, 2015; Phuong *et al.*, 2020; Hanh *et al.*, 2021a; Hanh *et al.*, 2021b) with several minor modifications for the P10 antigen. The four achieved antisera were diluted at serial concentrations from 1:5000 to 1:500000 and used for the dot-ELISA assay to detect the purified recombinant SRBSDV P10 protein (50 ng/dot) (Tam *et al.*, 2013). IgG antibodies were purified from the anti-SRBSDV antiserum that showed the highest titer using a protein A-agarose affinity chromatography column (Sambrook & Russell, 2001).

*Western blotting and dot-ELISA assay*

The target proteins in the rice samples and extracts of the *E. coli* Rosetta strain (DE3) transformed either the pET28/P10 or pET28a vectors (Tam *et al.*, 2013) and were detected via Western blotting assay (Sambrook & Russell, 2001). The protein samples, after being separated by SDS-PAGE, were then transferred to a PVDF membrane in Tris-glycine buffer with 20% methanol. The membrane was immobilized by gently shaking (for 30-60 min) in 1% BSA solution and incubated alternately with primary and secondary antibodies for 1 hour. The targeted protein was colored by incubation in p-nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) solution as a substrate.

For the dot-ELISA method, the PVDF membrane was activated by soaking it in methanol for 10 seconds and used for the assay (Sambrook & Russell, 2001) with some optimizations. Briefly, 1.0g of rice shoot tissue was ground in liquid nitrogen with a mortar and pestle, and homogenized in 3mL of 1 X TBS buffer, pH 7.5. WBPHs were individually crushed with toothpicks in 1.5mL centrifuge tubes containing 20µL of 1 X TBS buffer, pH 7.5. Three µL of crude extract was blotted individually onto the PVDF membranes. The membrane blocking, antibody incubating, and color developing steps were similar to the above Western blotting assay (Sambrook & Russell, 2001).

*SRBSDV detection by RT-PCR*

SRBSDV in the rice and WBPH samples was detected using RT-PCR as described previously (Zhou *et al.*, 2010b). The RT-PCR reactions contained 0.5µL of the RNA template (50ng), 0.6µL of each primer (10 pmol/µL), 1.5µL of 10 X *Taq* DNA polymerase buffer, 1.5µL of 2 mM dNTPs, 0.2µL of *Taq* DNA polymerase (5 U/µL), 0.75µL of reverse transcriptase (5 U/µL), 0.75µL of RiboLock RNase, and 8.6µL of deionized, sterilized $H_2O$. The RT-PCR reactions were performed at 42$^o$C for 10min, then 94$^o$C for 5min, followed by 35 cycles of 94$^o$C for 30s, 56$^o$C for 20s, and 72$^o$C for 1min 30s, and ended with 72$^o$C for 7min. RT-PCR products were analyzed by gel electrophoresis. *Actin1* was used as the internal control gene.

*Evaluating the accuracy of the SRBSDV diagnostic immunological assay*

The rice and WBPH samples were tested simultaneously by the dot-ELISA method and the quick-stick method. The RT-PCR results were used as the standard for evaluating the accuracy of the two immunological assays by following the formula: (Number of immunological tests having the same result as the standard RT-PCR tests/Total number of immunological tests) x 100%.

*Statistical analysis*

All experiments were repeated three times with the indicated factors. The investigator was

not blinded during the experiment or when assessing the outcome. Distribution was tested using the modified Shapiro-Wilks method. When parameters followed Gaussian distribution, the Student's t-test was used for the two groups' analyses and a one-way analysis of variance (ANOVA) ($P < 0.05$) was used for comparing more than two groups to evaluate the statistical significance. When parameters did not follow Gaussian distribution, the Kruskal-Wallis H test was used for comparing more than two groups to evaluate the statistical significance. When homogeneity of variance was violated, Welch's ANOVA Test was used instead of one-way ANOVA. All statistical analyses were carried out using Microsoft Excel version 2013 software.

## Results and Discussion

### Assessment of anti-SRBSDV anti-sera

Four antisera were collected from mice immunized with two types of antigens: recombinant SRBSDV P10 protein (called anti-P10 antiserum) or one of three synthetic SRBSDV P10-derived peptides localized at the N-terminus, middle, and C-terminus of the SRBSDV P10 protein (called anti-P10.1, anti-P10.2, and anti-P10.3 antiserum, respectively). Titers of the antisera were evaluated via dot-ELISA to select the antiserum with the highest anti-P10 antibody content. The results showed that only the anti-P10 antiserum could detect P10 protein at diluted concentrations over 1:500000; while those from synthetic peptide-immunized mice had lower titers, ranging from 1:25000 to 1:100000 (**Figure 1**). Anti-P10.3 antiserum had a relatively good titer result, ranging from 1:50000 to 1:100000. Based on the test results, anti-P10 antiserum was chosen to produce the anti-SRBSDV antibody for the virus diagnostic assay.

### Titer and specificity of the anti-P10 antibody

In this study, the IgG antibody purified from anti-P10 antiserum was titered using the dot-ELISA method with purified recombinant P10 protein and SRBSDV-infected rice (**Figure 2**). The titer analysis showed that the purified antibody at a dilution of 1:100000 gave positive diagnostic results in both the P10 protein and SRBSDV-infected rice tests (**Figure 2**, P10 and IR columns). This demonstrated that the IgG antibody maintained a high titer (> 100000) even after purification. The redundant components in the anti-serum were eliminated during purification, which improved the specificity of the antigen-antibody interaction later on.

Specificity analysis of the purified IgG antibody was carried out using Western blot for rice plants carrying different viruses (SRBSDV, RRSV, RGSV), and *E. coli* Rosetta (DE3) harboring either the pET28a/P10 or pET28a vector (**Figure 3**). After color development, wells loaded with SRBSDV-infected rice extracts and raw extract of P10-expressing bacterial cells each showed a band of 66 kDa, corresponding to the size of the P10 protein while the others did not show this band. This result indicated that our antibody at a 1:100000 diluted concentration could specifically detect the P10 protein. Thus, the anti-SRBSDV IgG antibody was successfully purified from anti-P10 antiserum, which reached a titer of over 1:100000, and was able to bind specifically to P10 in the extracts of viruliferous plants and P10 expressing *E. coli* cells, which met all the requirements for SRBSDV dot-ELISA diagnosis.

Producing specific polyclonal antibodies using antigenic peptides has been previously studied to detect plant viruses through immunoassay (Gallo *et al.*, 2013; Ahmed *et al.*, 2019). The peptides chosen to use as antigens were designed based on virus coat protein sequences with different lengths and could be situated on the N-terminus or C-terminus depending on the structural characteristics of the corresponding proteins, and specific research purposes (Wang *et al.*, 2012; Gallo *et al.*, 2013; Ahmed *et al.*, 2019; Miyoshi *et al.*, 2020). For rice, Wang *et al.* (2012) produced specific polyclonal antibodies for SRBSDV from three peptides of 20 amino acids each, located at the positions 20-39, 140-159, and 319-339 in the P10 protein sequence. All antibodies/anti-sera produced from these studies achieved a titer value greater than 1:500000 in ELISA analysis

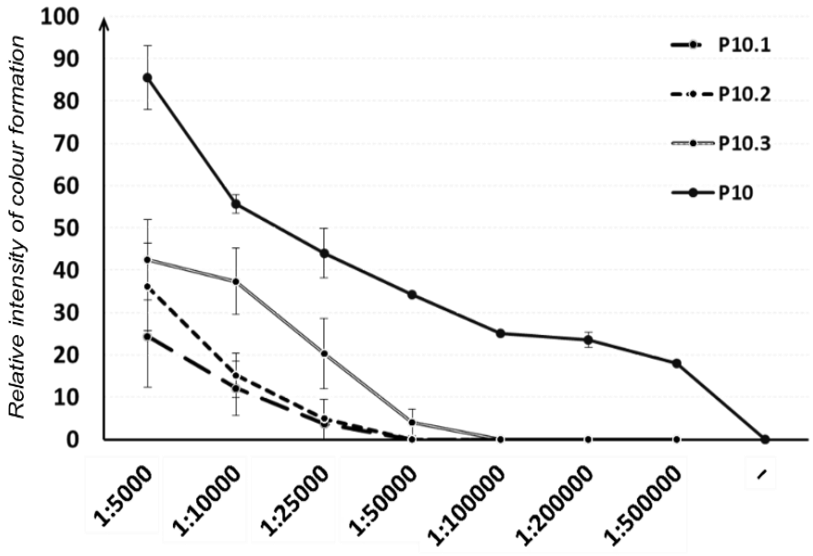


**Figure 1. Titers of the anti-SRBSDV antisera.** P10, P10.1, P10.2, and P10.3 represent the anti-P10, anti-P10.1, anti-P10.2, and anti-P10.3 antisera, respectively. The dot-ELISA results were analyzed by ImageJ 1.48v software. The results are shown as the mean ± standard deviation (SD) from three biological replicates.

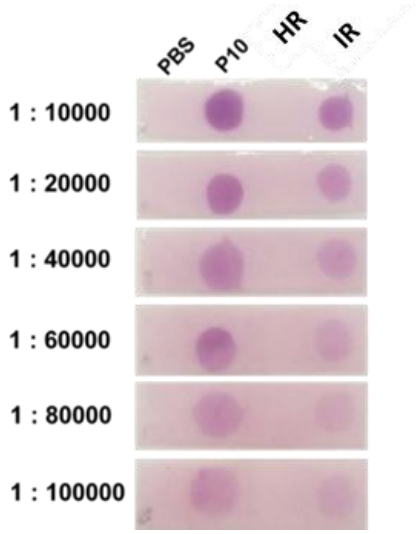


**Figure 2. Titers of the anti-P10 antibody using dot-ELISA.** Purified P10 protein (P10), SRBSDV-infected rice (IR), and healthy rice (HR) extracts were blotted on a PVDF membrane, and incubated with purified anti-P10 IgG antibody at dilutions of 1:10000, 1:20000, 1:40000, 1:60000, 1:80000, and 1:100000, respectively. (PBS) blank sample. The experiment was biologically replicated two times.

(Wang *et al.*, 2012). We have previously produced anti-SRBSDV specific IgG with a titer of 1:5000 by using the recombinant P10 protein (557 amino acids in length) for immunization of mice (Hanh *et al.*, 2015). In other recent studies, we have succeeded in the production of anti-SRBSDV antisera from three synthetic peptides derived from P10 protein, which showed a higher titer than that of the previous study (Phuong *et al.*, 2020; Hanh *et al.*, 2020; Hanh *et al.*, 2021). In this study, using directly purified P10 solution instead of P10 protein bands extracted from SDS-PAGE gel, as described by Hanh *et al.* (2015), for immunization significantly increased the content of anti-SRBSDV IgG antibody in the blood of immunized mice with a titer of over 1:100000. In comparison with the results of Wang *et al.* (2012), our antibody showed lower titer, which may be the result from differences in the tittering assay (DAP ELISA assay in the

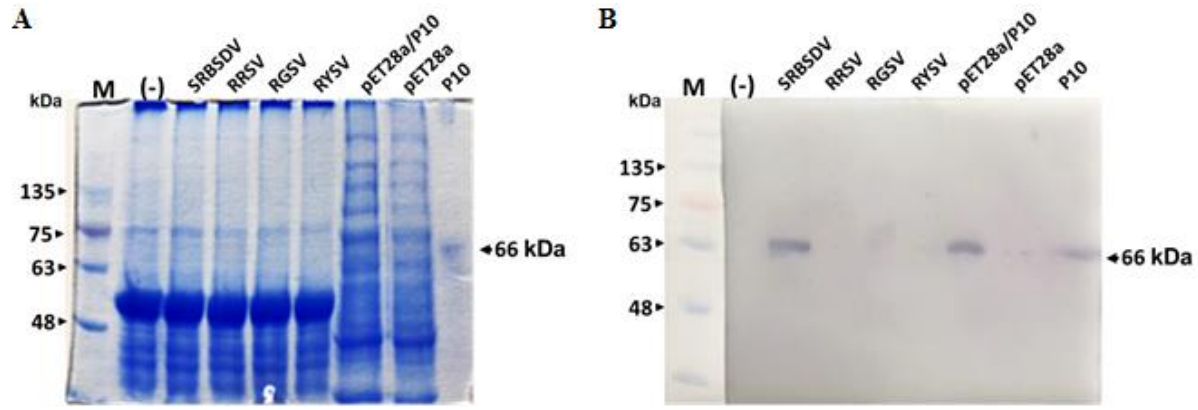


**Figure 3. Specificity of the anti-P10 antibody. (**A) Protein samples were separated on 12% polyacrylamide gel (SDS-PAGE). (**B**) P10 protein was identified in samples through Western blot. Crude extracts from the SRBSDV-, RRSV-, RGSV-, and RYSV-infected rice plants, respectively, and *E. coli* Rosetta (DE3) strains harboring the pET28a/P10 (pET28a/P10) and pET28a (pET28a) vectors, respectively, were blotted onto PVDF membranes for dot-ELISA using the anti-P10 PAb at a dilution of 1:100000. (M) prestained protein ladder (iNtRON); (-) negative control (virus-free rice plant sample); (P10) positive control (purified recombinant P10 protein).

study of Wang *et al.* (2012) vs. dot-ELISA assay in our study). It is a fact that the presence of chlorophyll in rice samples can affect the results of ELISA analysis. Therefore, in the studies of Wang *et al.* (2012) and Ahmed *et al.* (2019), the antibody was used at dilutions of 1:1500 and 1:250, respectively, for the SRBSDV diagnosis ELISA assays; while our purified anti-SRBSDV IgG antibody was diluted at 100000 and was able to distinguish samples with and without the virus in the Western blot experiment. This result has a significant impact on the application of anti-SRBSDV antibody for dot-ELISA diagnostic kits to detect early SRBSDV in the field.

### SRBSDV diagnosis with dot-ELISA assay in local laboratories

SRBSDV diagnosis based on the dot-ELISA method using archived anti-SRBSDV IgG antibody were independently tested by two local plant protection units, namely the Northern Plant Protection Center and the Zone IV Plant Protection Center. All samples were tested by RT-PCR assay (**Figure 4**) before being used for comparing results between the dot-ELISA method and the quick-stick method (**Table 1**).

The test results obtained from the Northern Plant Protection Center (**Table 1**) showed that the SRBSDV diagnostic procedure based on dot-ELISA with anti-P10 IgG antibody had an accuracy rate of 91.9% and 90.0% on average for the rice and WBPH samples, respectively. Meanwhile, the quick-stick test used as the control had an accuracy rate of 89.7% and 87.5% on average for the rice and WBPH samples, respectively. Likewise, the report from the Central Plant Protection Center (**Table 1**) indicated that the developed SRBSDV dot-ELISA diagnostic assay showed either higher or similar accuracy rates in comparison with the quick-stick method (94.4% vs. 94.1% for rice samples and 87.5% vs. 82.5% for WBPH samples).

Therefore, according to the reports from the two different testing units, the dot-ELISA assay using anti-SRBSDV polyclonal antibody exhibited an acceptable accuracy (91.9%, on average) compared to the RT-PCR assay for SRBSDV diagnosis, while excelling at cost efficiency, saving time, and work requirements. This dot-ELISA assay was fully capable of being applied as a SRBSDV diagnostic kit, and could be used in practical conditions in Vietnam.

## Conclusions

The anti-SRBSDV purified polyclonal IgG antibody was produced by using the recombinant SRBSDV P10 protein as the antigen source, which had a titer of 1:100000 and bound specifically to the target protein. The dot-ELISA assay was successfully developed from the archived antibody for SRBSDV diagnosis and showed an accuracy rate of 90.9% on average in

**Table 1. SRBSDV** test results from two Plant Protection Centers (PPCs)

| Samples | Northern PPC | | | Zone IV PPC | | |
|---|---|---|---|---|---|---|
| | No. of samples | Accuracy rate (%) | | No. of samples | Accuracy rate (%) | |
| | | DE test[1] | QS test[2] | | DE test[1] | QS test[2] |
| Symptomatic inoculated rice plants | 55 | 92.7 | 94.5 | 70 | 91.4 | 94.3 |
| Asymptomatic inoculated rice plants | 70 | 81.4 | 74.3 | 50 | 88.0 | 84.0 |
| Healthy rice plants | 45 | 100 | 100 | 50 | 100 | 100 |
| Field-collected rice plants | 30 | 93.3 | 90 | 50 | 98.0 | 98.0 |
| Healthy female WBPHs | 10 | 100 | 90 | 10 | 100 | 80 |
| Healthy male WBPHs | 10 | 100 | 90 | 10 | 100 | 100 |
| Inoculated female WBPHs | 10 | 80 | 80 | 10 | 70.0 | 70.0 |
| Inoculated male WBPHs | 10 | 80 | 90 | 10 | 80.0 | 80.0 |

*Note: Accuracy rate was calculated by the following formula: (Number of immunological tests having the same result with the standard RT-PCR tests/Total number of immunological tests) x 100%*
*DE test: dot-ELISA test using purified anti-SRBSDV IgG antibody.*
*QS test: quick-stick test.*

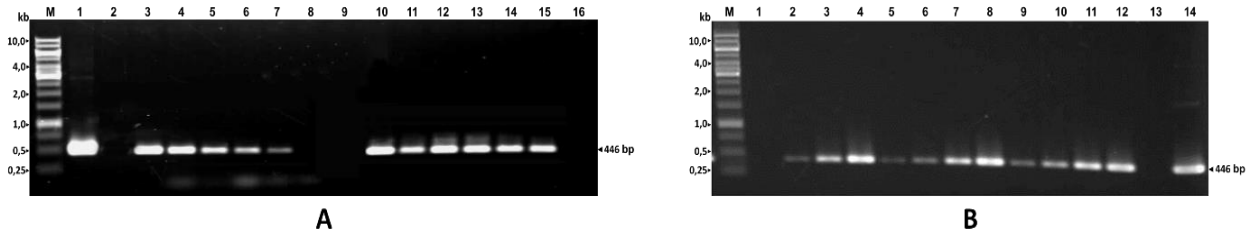


**Figure 4. SRBSDV diagnosis with the RT-PCR method.** (A) Diagnostic detection of SRBSDV in rice plants; well 1: positive control; well 2: negative control (virus-free rice); wells 3-16: rice plant samples. (B) Diagnostic detection of SRBSDV in WBPH; well 1: negative control (virus-free WBPH); wells 2-13: WBPH samples; well 14: positive control. Well M: 1.0 kb DNA ladder (Thermo Scientific).

comparison to the standard RT-PCR assay in thetrial tests. These results are the premise for the large-scale application of dot-ELISA assay SRBSDV diagnosis in Vietnam.

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## References

Ahmed M. S., Khalid A. A., Adel A. R., Hossam S. E. B. & Sherif M. E. G. (2019). Production of polyclonal antibodies to some potato viruses using synthetic peptides. Fresenius Environmental Bulletin. 28: 4870-4877.

Plant Protection Department - Vietnam's Ministry of Agriculture and Rural Development (2017). Announcement of pest situation for the third week of September. Retrieved from https://www.ppd.gov.vn/thong-bao-tinh-hinh-svgh-7-ngay/thong-bao-tinh-hinh-dich-hai-tuan-3-thang-

9x002f;2017.html on September 25, 2020 (in Vietnamese).

Gallo Y., Gutiérrez P. A. & Marín M. (2013). Detection of PMTV using polyclonal antibodies raised against a capsid-specific peptide antigen. Medellin Journal National School of Agronomy, 66(2): 6999-7008.

Hanh T. D., Hang T. P., Thanh Ha N., Ha T. T. N., Huong P. T. T, Xuan Hoi P. & Phuong N. D (2020). Production of peptide-specific antibody against protein P10 of Southern rice black-streaked dwarf virus. Journal of Biotechnology. 19(3): 1-11.

Hanh T. D., Minh N. A., Cuu N. V., Huong P. T. T, Xuan Hoi P. & Phuong N. D. (2021a). Generation of synthetic peptide-specific antibody for development of southern rice black-streaked dwarf virus diagnostic test. Vietnam Journal of Agricultural Sciences. 4(3): 1176-1184.

Hanh T. D., Hang P. T., Ha N. T., Ha N. T. T., Huong P. T. T., Xuan Hoi P. & Phuong N. D (2021b). Production of peptide-specific antibody against protein P10 of Southern rice black-striked dwarf virus. Journal of Biotechnology. 19(4): 687-693.

Hanh T. D., Van T. P. & Xuan Hoi P. (2015). Production of polyclonal anti-southern-rice-black-streaked-dwarf-virus antibody using recombinant protein. Vietnam Journal of Agricultural Sciences. 23: 38-43.

Ji Y. H., Gao R. Z., Zhang Y., Cheng Z. B., Zhou T., Fan Y. J. & Zhou Y. J. (2011). A simplified method for quick detection of rice black-streaked dwarf virus and Southern rice black-streaked dwarf virus. Chinese Journal of Rice Science. 25: 91-94.

Li S., Zhang T., Zhu Y., Zhou G. (2017). Co-infection of two reoviruses increases both viruses accumulation in rice by up-regulating of viroplasm components and movement proteins bilaterally and RNA silencing suppressor unilaterally. Virology Journal. 14: 150.

Manoharan S., Parthiban M., Prabhakar T. G., Ravikumar G., Koteeswaran A., Chandran N. D. J. & Rajavelu G. (2004). Rapid serological profiling by an immunocomb-based dot-enzyme-linked immunosorbent test for three major poultry diseases. Veterinary Research Communications. 28: 339 -346.

Michael G. P., Ruta H. & Wayne T. H. (1984). Standardization of the dot enzyme-linked immunosorbent assay (dot-ELISA) for human visceral leishmaniasis. American Journal of Tropical Medicine and Hygiene. 33: 1105-1111.

Miyoshi S., Tokunaga S., Ozawa T., Takeda H., Aono M., Miyoshi T., Kishi H., Muraguchi A., Shimizu S., Nozawa A. & Sawasaki T. (2020). Production of a rabbit monoclonal antibody for highly sensitive detection of citrus mosaic virus and related viruses. PLoS ONE. 15(4): e0229196.

Phuong N. D., Hang T. P., Huong P. T. T, Quyen L. C., Van T. P., Xuan Hoi P. & Hanh T. D. (2020). Production of polyclonal anti-southern-rice-black-streaked-dwarf-virus antibody using recombinant peptide. Vietnam Journal of Agricultural Sciences. 11: 12-19.

Sambrook J. & Russell D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed). Cold Spring Harbor Laboratory. Cold Spring Harbor, NY.

Tam T. P., Van T. P., Quang H. N., Phuong N. D. & Xuan Hoi P. (2013). Expression and purification of P10 coat protein of southern rice black-streaked dwarf virus in *E. coli*. Vietnam Journal of Agricultural Sciences. 24: 35-40.

Wang K., Zheng J. J., Zhang S. G. & Zhou G. H. (2010). Seedling test confirms Southern rice black-streaked dwarf virus cannot be transmitted via rice seed. Guangdong Agricultural Sciences. 7: 95-96.

Wang Z. C., Yu D. D., Li X. Y., Zeng M. J., Chen Z., Bi L. A., Liu J. J, Jin L. H., Hu D. Y., Yang S. & Song B. A. (2012). The development and application of a dot-ELISA assay for diagnosis of Southern rice black-streaked dwarf disease in the field. *Viruses*. 4: 167-183.

Zhou G. H., Wen J. J. & Cai D. J. (2008). Southern rice black-streaked dwarf virus: A new proposed *Fijivirus* species in the family Reoviridae. Chinese Science Bulletin. 53: 3677-3685.

Zhou G. H., Zhang S. G., Zou S. F., Xu Z. W. & Zhou Z. Q. (2010a). Occurrence and damage analysis of a new rice dwarf disease caused by Southern rice black-streaked dwarf virus. Plant Protection. 36: 144-146.

Zhou Q., Zhu J. Z., Liang J. G., Chen X. Y. & Gao B. D. (2010b). Rapid detection of southern rice black-streaked dwarf virus. Genomics Applied Biotechnology. 29: 1009-1012.

Zhou G., Xu D., Xu D. & Zhang M. (2013). Southern rice black-streaked dwarf virus: a white-backed planthopper-transmitted fijivirus threatening rice production in Asia. Frontiers in Microbiology. 4: 270.