Development of a simplified RT-PCR without RNA isolation for rapid detection of RNA viruses in a single small brown planthopper (Laodelphax striatellus Fallén)

Qiufang Xu¹, Haoqiu Liu¹, Pingping Yuan¹², Xiaoxia Zhang¹, Qingqing Chen¹, Xuanli Jiang² and Yijun Zhou¹*

Abstract

Background: The small brown planthopper (SBPH), Laodelphax striatellus Fallén (Delphacidae: Hemiptera), is a serious sap-sucking pest of agricultural crops. More importantly, it acts as an insect vector to transmit multiple plant viruses and causes severe yield losses. For example, it transmits reoviruses (RBSDV and maize rough dwarf virus) [1, 2], tenuiviruses (rice stripe virus, RSV) [3], rhabdoviruses (barley yellow striate mosaic virus and northern cereal mosaic virus) [4, 5], and criptoviruses (HiMetobi P virus, HiPV) [6]. Both RSV and RBSDV are transmitted by SBPH in a persistent, circulative and propagative manner. RSV can be transmitted by SBPH from mother to offspring [7], while RBSDV cannot. The rice stripe disease caused by RSV damaged over 957,000 hectares of paddy fields in 2003 and 1,571,000 hectares in 2004, accounting for 80% of the rice fields and a 30–40% yield loss in China [8]. RBSDV not only infects rice plants to cause rice black-streaked dwarf disease, but also causes maize rough dwarf disease in maize [1]. These viral diseases have been economically destructive in the rice- and maize-growing areas in China for decades [9].

Results: We established a simplified RT-PCR method without RNA isolation for RNA virus detection in a single SBPH. This method is achieved by grinding a single SBPH in sterile water and using the crude extract directly as the template for RT-PCR. The crude extract containing the virus RNA can be prepared in approximately two minutes. Rice stripe virus (RSV), rice black streaked dwarf virus (RBSDV) and Himetobi P virus (HiPV) were successfully detected using this simplified method. The detection results were validated by sequencing and dot immunobinding assay, indicating that this simplified method is reliable for detecting different viruses in insects. The evaluation of the sensitivity of this method showed that both RSV and HiPV can be detected when the cDNA from the crude extract was diluted up to 10³ fold. Compared to the traditional RT-PCR with RNA isolation, the simplified RT-PCR method greatly reduces the sample processing time, decreases the detection cost, and improves the efficiency by avoiding RNA isolation.

Conclusions: A simplified RT-PCR method is developed for rapid detection of RNA virus in a single SBPH without the laborious RNA isolation step. It offers a convenient alternative to the traditional RT-PCR method.

Keywords: Small brown planthopper, Virus detection, Simplified RT-PCR

Background

The small brown planthopper (SBPH), Laodelphax striatellus Fallén (Delphacidae: Hemiptera), is a serious sap-sucking pest of agricultural crops. More importantly, it acts as an insect vector to transmit multiple plant viruses and causes severe yield losses. For example, it transmits reoviruses (RBSDV and maize rough dwarf virus) [1, 2], tenuiviruses (rice stripe virus, RSV) [3], rhabdoviruses (barley yellow striate mosaic virus and northern cereal mosaic virus) [4, 5], and criptoviruses (HiMetobi P virus, HiPV) [6]. Both RSV and RBSDV are transmitted by SBPH in a persistent, circulative and propagative manner. RSV can be transmitted by SBPH from mother to offspring [7], while RBSDV cannot. The rice stripe disease caused by RSV damaged over 957,000 hectares of paddy fields in 2003 and 1,571,000 hectares in 2004, accounting for 80% of the rice fields and a 30–40% yield loss in China [8]. RBSDV not only infects rice plants to cause rice black-streaked dwarf disease, but also causes maize rough dwarf disease in maize [1]. These viral diseases have been economically destructive in the rice- and maize-growing areas in China for decades [9].
Rapid diagnosis of virus in vector is important for viral disease forecast and control. Various approaches have been developed for detection of RNA virus in its insect vector, including biological inoculation [10, 11], direct observation using electron microscopes [3, 12], antibody-based serological method [13], and other molecular detection methods. Biological inoculation method is time consuming and labor-intensive; for example, it takes approximately one month for the plants to show disease symptoms after inoculation with RBSDV via viruliferous insects [11]. Electron microscopes are very expensive and require specialty-trained personnel to operate them. In addition, the results of electron microscopy usually need to be confirmed by other methods [12]. Serological methods, such as enzyme-linked immunosorbent assay, are economical for detection of high throughput samples [14], but they are limited by the specificity and availability of antibodies against the virus. Deep sequencing and qRT-PCR and are of high specificity and availability of antibodies against the virus. Deep sequencing and qRT-PCR and are of high throughput samples [14], but they are limited by [18, 19], especially for viruses that do not have antibodies available.

Traditional RT-PCR assays usually require purified RNA for reverse transcription. Isolation of RNA with commercial kits is expensive and time consuming. Besides, as SBPH is a small insect, measuring approximately 2–4 mm long, it is challenging to purify RNA from an individual SBPH. In this study, we developed a simplified RT-PCR assay for RNA virus detection in a single SBPH without RNA isolation. The sensitivity and reliability of this detection method are assessed and compared with those of traditional RT-PCR.

**Methods**

**Preparation of SBPH used for virus detection**

SBPHs free of RSV and RBSDV has been continuously maintained in our lab over ten years. A RSV-viruliferous SBPH population, with a RSV infection rate higher than 80%, was screened and reared on rice seedlings grown in 1 L beakers at 25 °C with a photoperiod of 16 h /8 h (light/dark).

The RBSDV-infected SBPH vectors were prepared as described previously [20]. Non-viruliferous SBPHs were fed with rice black streaked dwarf diseased plants for three days, and then transferred to healthy rice seedlings for two weeks to pass the latent period. The SBPHs were subsequently collected for RBSDV detection. RSV-free and RSV-viruliferous SBPHs in 2nd to 4th instar were mixed and used for HiPV detection and duplex RT-PCR assay.

**Crude extract preparation from a single SBPH for reverse transcription**

The SBPHs reared on rice seedlings were collected and frozen in –20 °C for 5 min. A single SBPH was placed in a 0.2 mL centrifuge tube, washed with 100 μL sterile H2O, and ground with sterile wet toothpicks in 30 μL sterile H2O. After centrifugation at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min, the supernatant at 12,000 g for 1 min. The crude RNA extract was used as template for simplified RT-PCR. The cDNA was synthesized using M-MuLV 1st strand cDNA synthesis kit (Sangon Biotech, P.R. China) according to the manufacturer’s protocol. The procedure is as follows: 11 μL crude sample and 1 μL random primer (Random 6, 0.2 μg/μL) were mixed and incubated at 65 °C for 5 min, the mixture then was transferred onto ice for 30 s immediately. After a short centrifuge, 4 μL 5 x M-MuLV reverse transcriptase buffer, 2 μL dNTP mix (10 mM), 1 μL RNase inhibitor (20U/μL) and 1 μL M-MuLV RT (200 U/μL) were added. The tubes were incubated in 25 °C for 10 min, 42 °C for 1 h, and 70 °C for 10 min. The resulting cDNA could be stored in −20 °C or applied to virus PCR detection directly.

Specific virus primers were used for PCR amplification to detect viruses in a single SBPH (Table 1). PCRs were performed with a final reaction volume of 20 μL, containing 5 μL cDNA, 10 μL 2 × Taq Master Mix (Vazyme Biotech, P.R. China), 0.5 μL each of the primers. The initial denaturation (95 °C, 5 min) was followed by 40 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final extension step at 72 °C for 10 min. A plasmid containing the RSV CP gene was used as PCR positive control for detection of RSV and a plasmid with the RBSDV P10 gene for RBSDV. Crude extracts from non-viruliferous SBPH were used as negative controls. The PCR products were evaluated by agarose gel electrophoresis.

### Table 1 Primers used for RT-PCR or qRT-PCR amplification of RSV, RBSDV and HiPV

| Name          | Sequences (5’→3’)          | Accession number | Amplicon length (bp) |
|---------------|---------------------------|------------------|----------------------|
| RSV CP-F1     | ATGGGTAACAAACAGCCACG      | EF198700          | 936                  |
| RSV CP-R1     | CTAGTCATCTGCACCTTGTG      |                  |                      |
| RBSVD P10-F   | ATGCCGATCATAAGCTTGCA      | NC_053733         | 1677                 |
| RBSVD P10-R   | TCACTCTTGTGTCTGTG         |                  |                      |
| HiPV-F        | CTGGCAGAACATATGATATTAGA   | AB183472          | 678                  |
| HiPV-R        | CTATTCTCCAGTCCAAG         |                  |                      |
| RSV CP-F2     | GCCACCTACGTGTATTGCCA      | EF198700          | 167                  |
| RSV CP-R2     | GTGTCACACCCCTTGTACC      |                  |                      |
Dot immunobinding assay (DIBA)
Crude extracts (1 μL) were dotted individually onto a nitrocellulose membrane (0.2 μm pore size, Pall) and allowed to dry at room temperature. Nonspecific sites were blocked with blocking buffer containing 2% skim milk in PBST (137 mM NaCl, 2 mM KCl, 10 mM Na₂HPO₄, 2 mM KH₂PO₄, pH7.5, 0.05% Tween-20) at 37 °C for 30 min. The membrane was subsequently incubated in blocking buffer containing RSV- or RBSDV-specific monoclonal antibody at 1:5,000 dilution at 37 °C for 1.5 h. After wash for three times (each 5 min) with PBST, membranes were immersed in secondary antibody conjugated with HRP (Sigma-Aldrich, USA) at 1:2,000 dilution with PBST containing 2% skim milk. After another round of washes (3 times), the membranes were developed in a freshly prepared substrate solution containing 6 mg 4-chloro-1-naphtol, 2 mL ethanol, and 7 μL of 30% H₂O₂ in 10 mL PBS. The crude extracts of the individual SBPHs that developed well-defined dots and those with nonspecific sites were washed (3 times), the membranes were developed in a 20 °C, and used as positive and negative controls for the DIBA assay.

Comparison of sensitivity of virus detection with and without RNA isolation
To compare the detection sensitivities of the simplified RT-PCR using crude extract and the traditional RT-PCR using purified RNA, RSV RNA in individual SBPHs was prepared by these two methods and detected using RSV CP primers. Individual SBPHs were ground as described above. An aliquot of 11 μL supernatant was immediately transferred to a new tube for centrifugation and then the crude extract was used for reverse transcription. Another aliquot of 11 μL supernatant was used for Trizol RNA isolation. The reverse transcribed products were evaluated by PCR and qRT-PCR.

As SBPHs cannot be completely ground by toothpick, the tissue in the same volume of the supernatant (11 μL) may not be equal. In order to ensure that the two methods processed the same amount of tissue sample, the RSV-viruliferous SBPHs were treated in another way. Every 10 SBPHs at the third instar stage were collected by toothpick with RSV HiPV was found in SBPH in early 1990s [6, 21] and its presence was confirmed by high-throughput sequencing [17]. We used the RT-PCR method without RNA isolation. The reverse transcribed products were further confirmed to be the RSV CP gene (Additional file 1) by sequencing. The results indicated that RSV was successfully detected by the simplified RT-PCR. To confirm the results, the crude extracts from individual SBPHs were also processed by DIBA assay using RSV monoclonal antibody. As shown in Fig. 1b, the results of DIBA assay are consistent with those of RT-PCR.

The simplified RT-PCR method detected RBSDV in single SBPHs
In addition to detection of RSV in individual SBPHs, we also detected RBSDV using the simplified RT-PCR method without RNA isolation. The specific fragments with expected size of 1,677 bp were obtained in positive samples and confirmed to be the RBSDV P10 gene by sequencing (Additional file 1) by sequencing. The data confirmed that this simplified RT-PCR method was reliable for detecting RNA virus in SBPH.
isolation to analyze whether HiPV existed in our laboratory-reared SBPH. The results showed that the specific bands with expected size of 678 bp were successfully amplified in HiPV-infected SBPHs (Fig. 3). Sequencing results revealed that the PCR products were specific to the corresponding virus (Additional file 1), which validated the specificity and reliability of the assay.

**Duplex RT-PCR simultaneously detected two RNA viruses in single SBPHs**

To test whether the cDNA prepared from crude extract could be used for simultaneous detection of multiple RNA viruses, duplex RT-PCRs were performed to amplify RSV and HiPV in a single SBPH. The primer pairs for the two viruses were mixed and used for virus detection. A 678 bp PCR product corresponding to RSV or a 936 bp corresponding to HiPV was detected in SBPHs infected only by RSV or HiPV; the samples infected with both viruses detected both fragments of 678 bp and 936 bp (Fig. 4).

**Analytical sensitivity of the simplified RT-PCR**

To evaluate the relative sensitivity of this method, a series of 10-fold dilutions of the cDNA obtained from crude extracts were used for PCR analysis. Consistent with the different accumulation in individual SBPHs (shown in Figs. 1, 2 and 3), the detection sensitivity varies in different virus species. RSV and HiPV could be detected up to $10^3$ fold dilution, although the HiPV specific band was not distinct at $10^3$ fold dilution. RBSDV could be detected by the presence of an expected PCR product when the crude extracts were diluted 10 fold (Fig. 5).

**Comparison of the sensitivity of RT-PCR with or without the RNA isolation step**

To compare the detection sensitivity of the simplified RT-PCR and traditional RT-PCR, single RSV-viruliferous SBPHs were ground and RSV in the same volume of the crude suspension was detected by these two methods. The RSV CP gene could be detected in all the five independent samples by both methods (Fig. 6a). RSV abundance was measured by scanning the gray value of the
PCR bands. The ratio of the gray values of PCR product amplified from Trizol extracted RNA and crude samples (Trizol/crude) was close to 1.0 (Fig. 6b), indicating that there are no obviously differences in the detection sensitivities between the simplified RT-PCR and traditional RT-PCR.

To more accurately evaluate the detection sensitivities of these two detection methods, we used qRT-PCR to compare the RSV abundance in the samples prepared by the two methods. The RSV abundance in the samples was evaluated by the CT value of qRT-PCR. The virus abundance in the crude samples was set as the reference, and the relative virus abundance in the samples prepared by traditional Trizol extraction (Trizol/Crude) was calculated by $2^{\Delta CT(Crude-Trizol)}$. The results showed that the viral RNA abundance in samples #6 and #8 extracted using Trizol were higher than those in crude extracts, while sample #7 showed the opposite result (Fig. 6c). We speculated the reason was that the SBPH was not crushed completely by toothpick and thus the tissues could not be divided evenly.

In order to avoid this problem, we further ground 10 SBPHs in nitrogen liquid and distributed equal amount of tissues for further processing by the two different methods; samples #9, #10, and #11 represent this preparation. The qRT-PCR results show that the relative RSV abundance in the samples using Trizol isolation contains an average RSV level seven fold over those prepared using the simplified method (Fig. 6d).

Monitoring the ratio of viruliferous SBPH in the field insect population is important for viral disease forecasting and control. To test whether the simplified RT-PCR could be used for field insect samples, we collected field insect samples from Kaifeng, Henan Province, China for RBSDV detection. Ninety six SBPHs were randomly selected to detect RBSDV using the traditional RT-PCR method and the simplified RT-PCR established in this study. A single SBPH was ground in 30 μL sterile H2O. After centrifugation, 11 μL supernatant was used for detection by the simplified RT-PCR and the rest of the sample by traditional RT-PCR. The results obtained by the two methods are consistent; both methods showed that three samples were RBSDV positive (Additional file 2: Figure S1), suggesting that the simplified RT-PCR is reliable for detecting virus in field insect samples.

Discussion

Plants are infected by a wide range of viruses that cause economic losses and pose threats to certain agricultural industries. Hemipteran insects act as the main vectors for the plant viruses, transmitting as much as 55% of the described plant viruses. Planthoppers in Hemiptera mainly transmit RNA viruses: among the 18 viruses they transmit, 14 are RNA viruses [22]. Analysis of virus infection in the insect vector is important for preventing the potential disease threat. In this study, we developed a simplified RT-PCR method without RNA isolation for RNA virus detection in a single SBPH. The virus RNA preparation can be completed in approximately 2 min and requires no pre-treatments for RNA purification. This simplified method may be expanded for detection of RNA viruses in other insects in Hemiptera with similar insect size, such as aphids, leafhoppers, brown planthopper and whitebacked planthopper.

The traditional RT-PCR for RNA virus detection contains a laborious RNA isolation process [18]. There are several methods to isolate RNA from insects, including using commercial SV total RNA isolation system (Promega) [23], Trizol reagent (Invitrogen) [24], and the RNAiso Reagent (TaKaRa) [15]. The RNA samples isolated by these
methods are of high quality with limited contamination and can produce well-defined amplification products in RT-PCR [25]. However, it is expensive to use these commercial kits. Besides, it is time-consuming and inconvenient to extract RNA from a single SBPH because planthoppers are of small size. Our method offers an effective way to detect viruses in a large number of planthopper samples by eliminating the expensive and laborious RNA isolation process.

The quality of RNA is considered as one of the important factors that affect the accuracy of RT-PCR. RNA samples with their A260/A280 ratios falling between 1.8-2.0 are considered to be of good quality and selected for RT-PCR and subsequent analysis [26]. The A260/A280 ratios of crude extracts of SBPH in our assay are below 1.8 (data not show) and the crude extracts are presumed to contain contaminating components, such as DNA, protein and lipid, which might inhibit RT-PCR reactions. However, the simplified RT-PCR successfully detected the RSV, RBSDV and HiPV in SBPH (Figs. 1, 2 and 3). Moreover, the cDNA prepared by the described protocol can be used for duplex RT-PCR and qRT-PCR for virus detection (Figs. 4, 6c and d). These results suggest that the contaminants in crude extracts do not affect the detection results, which is in agreement with previous studies showing that viruses could be detected specifically in plant samples by RT-PCR using unpurified RNA [27]. One possible explanation is that the contaminants in planthopper extracts that can inhibit RT-PCR reactions are present at very low concentrations, not high enough to cause problems in RT-PCR.

On the other hand, we found that the reverse transcription method affects the result of the simplified RT-PCR. When we first incubated the crude extract at 65 °C with random primer (Random 6) and then proceeded to reverse transcription as described in the Methods, the RNA virus was successfully detected (Figs. 1, 2 and 3). However, when the cDNA was obtained by using the primeScript™ RT Master Mix (Takara, contains every components except the RNA template for reverse transcription) without heat treatment, the RNA virus could not be detected by the simplified RT-PCR. We hypothesize that virus particles in SBPH may require heat disruption at 65 °C to release RNA for cDNA synthesis. Alternatively, contaminating inhibitors may be inactivated by treatment at 65 °C.

The sensitivity of the detection method is another important factor for virus detection. Our results show that the simplified RT-PCR method bears the sensitivity

---

Fig. 5 Detection of RSV, RBSDV and HiPV by simplified RT-PCR in a 10-fold serial dilution.

Fig. 6 Comparison of the sensitivity of simplified RT-PCR (Crude) and traditional RT-PCR (Trizol). a) PCR products amplified from samples prepared by the two methods. b) Relative RSV abundance evaluated by gray value of PCR products. c) Relative RSV abundance evaluated by qRT-PCR in the samples ground by toothpick. d) Relative RSV abundance evaluated by qRT-PCR in the samples ground in nitrogen liquid.
sufficient to reliably detect RSV, RBSDV and HiPV in a single SBPH (Figs. 1, 2 and 3). Both RSV and HiPV were detected when cDNA samples were diluted up to $10^3$ fold (Fig. 5). These results suggest that the simplified method can be practically applied to virus detection using a single SBPH. The relative abundance of RSV analyzed by qRT-PCR using Trizol-isolated RNA was 7 fold higher than that using RNA in crude extract (Fig. 6d). One explanation is that the Trizol reagent disrupts insect tissues more thoroughly to release RNA from tissues whereas some amounts of RNA may be lost together with tissues during centrifugation in the simplified method.

Conclusions
A simplified RT-PCR method was developed for detection of RNA virus in a single SBPH by preparing virus RNA in an easy and fast procedure. Our results demonstrated that crude extracts of SBPHs could be used as the template for RT-PCR. The viral RNA prepared by this method was also suitable for duplex RT-PCR and qRT-PCR detection. This protocol reduces the use of costly reagents, shortens the sample processing time, and improves the efficiency of virus detection. As the method is highly simplified and of sufficient sensitivity, it provides a useful tool to the investigation of epidemics of viral diseases in the early stage by enabling easy detection of viruses within a single insect vector.

Additional file

Additional file 1: Sequencing results of the detected viruses. (DOCX 14 kb)

Additional file 2: Figure S1. Detected RBSDV in individual field-collected SBPH using the simplified RT-PCR and traditional RT-PCR. (A) PCR products of 96 field-collected SBPH detected by simplified RT-PCR. (B) PCR products of SBPHs detected by traditional RT-PCR. (JPG 1439 kb)

Abbreviations
DIMA: Dot immunoabsorbing assay; HiPV: Himetobi P virus; qRT-PCR: Quantitative real time PCR; RBSDV: Rice black streaked dwarf virus; RSV: Rice stripe virus; RT-PCR: Reverse transcription polymerase chain reaction; SBPH: Small brown planthopper

Acknowledgements
We thank Dr. Mawsheng Chern of the University of California, Davis, for the advice and help in English editing of the manuscript.

Funding
This research is supported by the National Natural Science Foundation of China (31471768), the Jiangsu Agriculture Science and Technology Innovation Fund (CX(15)1053), and the Grain High Yield and High Efficiency Technology Innovation Program (2016YFD0300706). These funding bodies had no role in design, collection, and analysis of the study and preparation of the manuscript.

Availability of data and materials
All data generated or analyzed during this study are included in this published article and its additional files.

Authors’ contributions
QX, YZ and XJ participated in experimental design and directed the research. HL, PY, XZ and QC carried out the experiments and analyzed the data. QX, HL, and YZ were involved in the writing of the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References
1. Zhang HM, Chen JP, Lei JL, Adams MJ. Sequence analysis shows that a dwarfing disease on rice, wheat and maize in China is caused by rice black-streaked dwarf virus. Eur J Plant Pathol. 2001;107:563–7.
2. Achon MA, Subira J, Sin E. Seasonal occurrence of Laodelphax striatellus in Spain: Effect on the incidence of Maize rough dwarf virus. Crop Prot. 2013;37:1–5.
3. Deng JH, Li L, Jiang XH, Zhang XY, Yang F, et al. First report of Barley yellow striate mosaic virus on wheat in China. Plant Dis. 2014;98:1450–1.
4. Di DP, Zhang YL, Yan C, Yan T, Zhang AH, Yang F, et al. Detection of RBSDV in rice plants and planthopper vectors. Virol J. 2013;10:1–3.
5. Tanno F, Nakatsu A, Toriyama S, Kojima M. Complete nucleotide sequence of RBSDV: Quantitative real time PCR; RBSDV: Rice black streaked dwarf virus; RSV: Rice stripe virus; RT-PCR: Reverse transcription polymerase chain reaction; SBPH: Small brown planthopper.

Additional file 1: Sequencing results of the detected viruses. (DOCX 14 kb)

Additional file 2: Figure S1. Detected RBSDV in individual field-collected SBPH using the simplified RT-PCR and traditional RT-PCR. (A) PCR products of 96 field-collected SBPH detected by simplified RT-PCR. (B) PCR products of SBPHs detected by traditional RT-PCR. (JPG 1439 kb)
17. Cai LJ, Ma XZ, Kang L, Deng KJ, Zhao SY, Li CB. Detecting Rice stripe virus (RSV) in the small brown planthopper (Laodelphax striatellus) with high specificity by RT-PCR. J Virol Methods. 2003;112:115–20.

18. Cho SY, Jeong RD, Yoon YN, Lee SH, Shin DB, Kang HW, et al. One-step multiplex reverse transcription-polymerase chain reaction for the simultaneous detection of three rice viruses. J Virol Methods. 2013;193:674–8.

19. Xu QF, Ni HP, Zhang JF, Lan Y, Ren CM, Zhou YJ. Whole-genome expression analysis of Rice black-streaked dwarf virus in different plant hosts and small brown planthopper. Gene. 2015;572:169–74.

20. Suzuki Y, Toriyama S, Matsuda I, Kojima M. Detection of a picorna-like virus, himetobi-P virus, in organs and tissues of Laodelphax striatellus by immunogold labelling and enzyme linked immunosorbent assay. J Invertebr Pathol. 1993;62:99–104.

21. Xu Y, Huang LZ, Wang ZC, Fu S, Che J, Qian YJ, et al. Identification of Himetobi P virus in the small brown planthopper by deep sequencing and assembly of virus-derived small interfering RNAs. Virus Res. 2014;179:235–40.

22. Hogenhout SA, Ammar ED, Whitfield AE, Redinbaugh MG. Insect vector interactions with persistently transmitted viruses. Annu Rev Phytopathol. 2008;46:327–59.

23. Li S, Wang X, Xu JX, Li YH, Zhou YJ. A simplified method for simultaneous detection of Rice stripe virus and Rice black-streaked dwarf virus in insect vector. J Virol Methods. 2015;211:32–5.

24. Zhou T, Du L, Fan Y, Zhou Y. Reverse transcription loop-mediated isothermal amplification of RNA for sensitive and rapid detection of southern rice black-streaked dwarf virus. J Virol Methods. 2012;180:91–5.

25. Ridgeway JA, Timm AE. Comparison of RNA isolation methods from insect larvae. J Insect Sci. 2014;14:268.

26. Sambrook JFE, Maniatis T. Molecular cloning: a laboratory manual. 2nd ed. New York: Cold Spring Harbor Laboratory Press; 1989.

27. Suehiro N, Matsuda K, Okuda S, Natsuaki T. A simplified method for obtaining plant viral RNA for RT-PCR. J Virol Methods. 2005;125:67–73.