Detection and diagnosis of rice-infecting viruses

Tamaki Uehara-Ichiki1, Takuya Shibita1, Keiichiro Matsukura1, Takanori Ueno1, Masahiro Hira1 and Takahide Sasaya3

1Classification and Evaluation Research Unit, Genetic Resources Center, National Institute of Agrobiological Sciences, Tsukuba, Ibaraki, Japan
2Plant Protection Division, Agricultural Research Center, National Agriculture and Food Research Organization, Tsukuba, Ibaraki, Japan
3Kyushu-Okinawa Agricultural Research Center, National Agriculture and Food Research Organization, Kikusui, Kumamoto, Japan
4Food and Agricultural Materials Inspection Center, Kodaira, Tokyo, Japan

Correspondence:
Takahide Sasaya3
e-mail: tuehara@affrc.go.jp
305-8602, Japan
Kannondai, Tsukuba, Ibaraki

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INTRODUCTION

Rice, second only to corn in worldwide crop production, is produced in all Asian countries, in most of South and Central America, and in some of central and eastern Africa. More than 80% of the world's rice is grown in South, East and Southeast Asian areas where the hot, humid climate favors not only rice but also the viruses and their vectors and about 30 rice viruses have been reported (Abo and Sy, 1998).

In the early years of plant virus research, detection and identification of viruses were based on symptom development on infected plants or biological indexing. However, the use of symptoms for diagnosis is not reliable because symptoms vary depending on the virus strain, the presence of any mixed viral infections, the virus strain, the presence of any mixed viral infections, the cultivar and growth stage, growing environment, and sometimes, the resemblance of viral symptoms to those induced by environmental injury. Biological assays are one of the most widely used methods among the many diagnostic techniques for plant viruses because the assays are simple and do not require special knowledge or skill (Jones, 1993; Naidu and Hughes, 2001). However, the majority of rice viruses are transmissible by vectors, and their host range is limited to graminaceous plants (Abo and Ali Fadala, 2001). Therefore, experimental transmission of rice viruses, e.g., with mechanical or graft inoculation of indicator plants, is inconvenient and not applicable to detect or diagnose rice viruses. In addition, since almost all rice viruses are vector-borne, detection methods for rice viruses in their vectors, which show no noticeable symptom, have been needed to forecast and counter disease outbreaks. For these reasons, the development of serological methods based on an antigen–antibody reaction have been active areas of research, and highly sensitive and specific methods (RT-PCR, RT-LAMP, real-time RT-PCR) based on molecular or nucleic-acid techniques have recently become available. This review introduces major methods to detect target rice viruses from the 1980s and summarizes the potential of the current technologies in contributing to diagnosis of rice diseases.

BASIC PROPERTIES OF RICE VIRUSES

The major rice viruses in Asian areas are transmitted by sucking insects. Eight of these are transmitted by planthoppers or leafhoppers in a persistent manner (Hashimoto, 1996; Zhou et al., 2008): Rice stripe virus (RSV, a member of the genus Tenusivirus, negative sense ssRNA virus), Rice dwarf virus (RDV, member of Reoviridae, dsRNA virus), Rice gall dwarf virus (RGDV, a member of the genus Reoviridae, dsRNA virus), Rice ragged stunt virus (RRSV, a member of the genus Reoviridae, dsRNA virus), Rice grassy stunt virus (RGGN, a member of the genus Tenusivirus, negative sense ssRNA virus), Rice transitory yellowing virus (RTYV, same species as Rice yellow stunt virus (RYSV), a member of the genus Reoviridae, dsRNA virus), Rice grisea brown spot virus (RGSV, a member of the genus Tenusivirus, negative sense ssRNA virus), Rice black spot virus (RBVD, a member of the genus Reoviridae, dsRNA virus) and Southern rice black streaked dwarf virus (SRBSDV, may be a member of the genus Reoviridae, dsRNA virus). Detection of these viruses in insect vectors may be easier than in rice plants, since these viruses propagate in insect bodies and the entire insect can be tested in the procedure. The tungro-disease-associated viruses, Rice tungro bacilliform virus (RTBV, a member of the genus Cucumoviridae, ssRNA) and Rice tungro spherical virus (RTSV, a member of the genus Secoviridae, positive sense ssRNA virus) are non-propagative
and transmitted in a semipersistent manner by leafhoppers, therefore, it is difficult to detect these two viruses in the insect vector using serological methods, but more sensitive methods such as real-time RT-PCR and RT-LAMP can be used (Le et al., 2010; Malathi and Mangrauthia, 2013). Detection of viruses in the insect vectors is very important to forecast outbreaks and monitor disease spread because major outbreaks of insect-borne viruses are generally associated with high densities of their respective vectors. For example, we routinely detect and diagnosis RSV in its insect vector, small brown planthopper, using a commercial DAS-ELISA detection kit and a polyclonal antibody (Japan Plant Protection Association), as described later in detail.

In the Americas, Rice hoja blanca virus (RHBV, a member of the genus Bromiviruses, positive sense ssRNA virus) is transmissible by mechanical inoculation (RYMV, a member of the genus Mottle virus, ssRNA virus) are leafhoppers (Monjane et al., 2011). Rice yellow mosaic virus (RYMV, a member of the genus Sobemoviruses, positive sense ssRNA virus) is transmissible by mechanical inoculation and insect vectors leaf beetles (Aho and Sy, 1998; Nwilene, 1999; Baznawa et al., 2001).

After the viruses are initially transmitted to rice plants by their vectors, the rice viruses spread from the infection foci to the entire host plant through the vascular system. RSV, RDV, and RGSV propagate in the vascular tissue and mesophyll cells, but RBSDV, RGYDV and RRSV are localized in the phloem and gall tissues. RTYV and RTSV propagate in the phloem tissues, and RTBV is localized in the vascular bundles (Hibino, 1996). The localization of SRBSDV, which sometimes induces confusingly similar symptoms to those caused by RBSDV infection (Zhang et al., 2008; Zhou et al., 2008), is presumably similar to that of RBSDV in host plants.

As is the case with many plant viruses, rice viruses are known to be distributed unevenly within the host plant. In addition, as the host plants grow and tiller, some of the tillers from one individual may be infected while others may be virus-free. Therefore, samples should be taken from several parts of the entire plant body to be certain of the diagnosis.

### SEROLOGICAL DIAGNOSIS

The overview of detection methods for viruses is shown in Table 1. Serological methods can generally be subdivided into liquid and solid phase tests. The liquid-phase tests are the precipitin test, latex agglutination reaction (LAR), and passive hemagglutination (PHA), and solid-phase examples include the enzyme-linked immunosorbent assay (ELISA), dot-immunobinding assay (DIBA), Gel-based assay (double immunodiffusion gel assay, DIGA) have been also reported. These serological methods are used widely to detect rice viruses at pest control stations, plant protection stations, and agricultural experiment stations in Asia. Once the serological detection system is established, highly sensitive testing of a large number of samples is simpler and cheaper even though the methods are classical and

| Virus/family | Vectors/transmission mode | Technique |
|--------------|---------------------------|-----------|
| RBSDV/Reoviridae | Planthoppers/semipersistent | ELISA (Wang et al., 2006; Wu et al., 2013a), RT-PCR (Hong et al., 2008; Cho et al., 2013; Wu et al., 2013b), RT-LAMP (Le et al., 2010) |
| RDV/Reoviridae | Leafhoppers/semipersistent | ELISA, PHA, LAR (Kimura et al., 1984), RT-PCR (Cho et al., 2013), RT-LAMP (Le et al., 2010) |
| RGDV/Reoviridae | Leafhoppers/semipersistent | DIBA (Kimura et al., 1990), RT-LAMP (Le et al., 2010) |
| RRSV/Reoviridae | Planthoppers/semipersistent | ELISA (Wang et al., 2006; Wu et al., 2013a), RT-PCR (Hong et al., 2008; Cho et al., 2013), RT-LAMP (Le et al., 2010) |
| SRBSDV/Reoviridae | Planthoppers/semipersistent | RT-PCR (Hoang et al., 2011; Wu et al., 2013a), RT-LAMP (Zhou et al., 2012), real-time RT-PCR (Matsukura et al., 2013; Zhang et al., 2013), DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RSV/Tenuivirus | Planthoppers/semipersistent | ELISA (Wang et al., 2006; Wu et al., 2013a), RT-PCR (Hong et al., 2008; Cho et al., 2013), RT-LAMP (Le et al., 2010) |
| RGGV/Tenuivirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RRVS/Tenuivirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RRSV/Tenuivirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RHBV/Tenuivirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RWSV/Tenuivirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RSVM/Benyvirus | Planthoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RTBV/Sobemoviridae | Leaf beetles/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RTSV/Sequiviridae | Leafhoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |
| RTBV/Caulimoviridae | Leafhoppers/semipersistent | DIBA (Chen et al., 2012a), ELISA (Wang et al., 2012) |

In Africa, a few viruses have been reported to infect rice plants. Rice stripe necrosis virus (RSNV, May be a member of the genus Benyvirus, positive sense ssRNA virus), is transmitted by the soil-inhabiting fungal pathogen Polymyxa graminis. The major vectors of Maize streak virus strain A (a member of genus Mastrevirus, ssDNA virus) are leafhoppers (Monjane et al., 2011). Rice yellow mottle virus (RYMV, a member of the genus Sobemovirus, positive sense ssRNA virus) is transmissible by mechanical inoculation and insect vectors leaf beetles (Aho and Sy, 1998; Nwilene, 1999; Baznawa et al., 2001).

In Asia, a few viruses have been reported to infect rice plants. The major vectors are leafhoppers, leaf beetles, and planthoppers. The overview of detection methods for viruses in rice plants and in insect vectors is shown in Table 1.
Western blot analysis, the sensitivity of antiserum against the dis- et al., 1991) more sensitive, yields clear results and is inexpensive (Takahashi et al., 1991). To reduce these undesirable non-specific or cross-reactions, antiseria against a viral protein or an Escherichia coli-expressed viral coat protein are now being used (Chen et al., 2012a).

Antiseria that recognize linear epitopes instead of conformational epitopes are chosen for immunodetection of denatured proteins. For example, target proteins that are boiled or treated with β-mercaptopethanol, can be separated by SDS-PAGE and detected in a Western blot by using antiseria that recognize linear epitopes. In contrast, antiseria that recognize conformational epitopes are preferred for ELISA and other methods that target proteins whose structure is preserved. In an ELISA to detect RDV in rice plants, an antiserum against intact RDV particles was more sensitive than that against RDV dissociated by SDS. But for Western blot analysis, the sensitivity of antiserum against the disassociated RDV was higher than that against intact RDV particles (Chen et al., 2012).

LATEX AGGLUTINATION REACTION
Latex agglutination is a classical technique for immunchemical reactions in which the antigen or the antibody is attached to the surface of red blood cells or to carrier particles, e.g., latex. The virus or antibody is simply attached to the latex particles by adsorption. In FIA, virus particles or antibodies are coupled to erythrocytes by various chemical treatments. In Japan, LAR was once the most commonly used method to detect RSV in SRBPs because it is more sensitive than other precipitin assays and requires little time and minimal facilities (Figure 1A). But the double antibody sandwich (DAS)-ELISA in commercially available kits has started to overtake the LAR because this technique is more sensitive, yields clear results and is inexpensive (Takahashi et al., 1991).

ELISA
Enzyme-linked immunosorbent assay is a solid-phase assay that uses antibodies labeled with enzymes that react with a substrate to yield a color change, thereby identifying the presence of a substance. We find that DAS-ELISA is easy to use for our routine diagnostic detection of RSV in insect vectors; monitoring the rate of viruliferous vector insects in early summer before rice planting season is very important to forecast rice stripe disease, an economically devastating disease; in the coming year. As shown in Figure 1B, up to 96 insect samples can be processed at the same time with plastic multi-sticks; for a few insect samples, we use one stick. This method is simple, and the results are robust and easy to interpret regardless of the age or sex of the insect (Uehara-Ichiki et al., 2013). Other rice viruses can also be detected with DAS-ELISA, not only in vectors but also in rice plants, and a few steps of DAS-ELISA can be skipped by grinding samples with the antibody-enzyme conjugate (Takahashi et al., 1987, 1988).

The DBRA or tissue immunoblotting assay are similar to ELISA, but in these methods, the sample extracts are spotted onto a membrane as a solid support matrix. Although false positive or false negative results are often obtained, methods to reduce the interference of chlorophyll have been reported (Srinivasan and Tolin, 1992; Chen et al., 2012a).

NUCLEIC-ACID-BASED TECHNIQUES
Detection based on viral nucleic acids is more sensitive and specific than serological methods, and any region of a viral genome can be targeted. Rice viruses, except for RTBV, are RNA viruses, and synthesis of cDNA of the viral genome by reverse transcription (RT) is necessary before the target DNA sequence is amplified. Commercial kits to extract RNA and to synthesis cDNA from several companies such as Life Technologies, QIAGEN, Takara Biotecnologies, BIO-RAD and Promega are available. The variations of RT-PCR (e.g., multiplex RT-PCR, real-time RT-PCR) and RT-loop-mediated isothermal amplification (LAMP) have been applied to detect rice viruses from plants or insects. The commercial kits for RT-PCR are sold by the companies mentioned, and the RT-LAMP kit is sold by Eiken Chemical.

MULTIPLEX RT-PCR
Multiplex RT-PCR uses multiple gene-specific primer sets within a single PCR mixture and can simultaneously detect two or more products in a single reaction. Therefore, the method is cost effective when two or more viruses are present in a single host plant (Lopez et al., 2009). Figure 1C outlines the procedure and shows the resulting bands in the agarose gel after electrophoretic separation of the DNA fragments amplified by multiplex RT-PCR with the gene-specific primer sets (1 and II), designed from coding sequences for the rice viral capsid or capsid-like proteins using the program FastPCR 6.0 (Institute of Biotechnology, University of Helsinki). Primer set I comprises two specific primers for rice actin as an internal control and 10 specific primers for RDV, RSV, RBSDV, RTYV, and RTSV, which have been reported in north-eastern and eastern Asia. Primer set II comprises two specific primers for rice actin as an internal control and 10 specific primers for SRBSDV, RGDV, RRSV, RTBV, and RGSV, which have been reported in southeastern and southern Asia. Since the RT-PCR products derived from each virus differs in size, two viruses such as RDV/RSV, which are major rice viruses in Japan can be detected in a single reaction (Figure 1C-c). In rice fields of some countries where mixed infections with RRSV and RGSV, RBSDV, and RSV and RBSDV, RSV and RBSDV, and RDV have been reported (Du et al., 2007; Cho et al., 2013; Wu et al., 2013b), multiplex RT-PCR is useful to rapidly and simultaneously identify the viruses.

RT-LAMP
The LAMP technique, developed by Notomi et al. (2000), is one of the most sensitive detection methods. This method has distinct advantages because it is highly specific for the target sequence and can be done quickly without special equipment. The specificity of LAMP is due to the recognition of six distinct
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FIGURE 1 | Outline of procedure, required time, cost comparison of reagent kits per diagnosis, and results of five techniques. (A) Latex agglutination reaction (LAR) to detect RSV in insect vectors. RT, room temperature. (a) Materials for LAR and DAS-ELISA: (1) Microtiter plate, (2) multi-sticks, (3) single stick. (b) Results of LAR. +, positive, −, negative. (B) DAS-ELISA to detect RSV in insect vectors. (a) The frozen insects in each well. (b) Homogenization of insects with multi-sticks. (c) Visual assessment. (Yellow; positive). (C) Multiplex RT-PCR to detect and distinguish 10 rice viruses. (a) Agarose gel electrophoresis of RT-PCR products from healthy or infected rice plants using primer set I. Lane 1, infected with RDV (1106 bp); lane 2, infected with RSV (917 bp); lane 3, infected with RBSDV (734 bp); lane 4, infected with RTYV (631 bp); lane 5, infected with RTSV (504 bp); lane 6, healthy plant (339 bp). (b) Agarose gel electrophoresis of RT-PCR products from healthy or infected rice plants using primer set II. Lane 1, infected with SRBSDV (1097 bp); lane 2, infected with RGSV (934 bp); lane 3, infected with RRSV (934 bp); lane 4, infected with RTBV (899 bp); lane 5, infected with RGDV (834 bp); lane 6, healthy plant (419 bp). (d) Agarose gel electrophoresis of RT-PCR products from rice plants infected with RDV and RSV. (D) RT-LAMP to detect and distinguish between SRBSDV and RBSDV. (a) Rice plants infected with SRBSDV. (b) Rice plants infected with RBSDV. (c) Healthy rice plants. Left, SRBSDV primer sets. Right, RBSDV primer sets. (E) Real-time RT-PCR to quantify SRBSDV in rice and insect vectors. (a) Samples with a higher density of virus yield an earlier rise in the fluorescence amplification curve, which corresponds to the density of RT-PCR products. The threshold cycle (Ct), calculated from the amplification curve, is used as an indicator of virus titer in the sample (higher virus titer results in lower Ct). (b) Virus titer is usually given as the relative density of virus titer to expression of a housekeeping gene (e.g., actin and ubiquitin) from the host or vector.
sequences by four specifically designed primers, which partly alleviates the general problem of the background associated with all nucleic acid amplification methods. The LAMP reaction occurs at 60–65°C for 60 min in a water bath or heat block. We established a RT-LAMP detection system for nine rice viruses in Asia (Le et al., 2010). After SRBSDV was reported (Zhang et al., 2008a), we designed new primers derived using the S10 sequence of the SRBSDV genome for RT-LAMP using PrimerExplorer V4 (http://primerexplorer.jp/elamp4.0.0/index.html) and confirmed that our RT-LAMP system could distinguish between SRBSDV and RBSDV in infected plants (Figure 1D). Meanwhile, Zhou et al. (2012) detected and distinguished between SRBSDV and RBSDV in host plants and insect vectors with RT-LAMP targeting S9 of the SRBSDV genome.

**REAL-TIME RT-PCR**

In real-time RT-PCR, amplification of DNA is monitored by the detection and quantitation of a fluorescent reporter signal, which increases in direct proportion to the amount of PCR product in the reaction. This technique, combined with RT, can be used to quantify target RNA. As noted already, RT-LAMP and conventional RT-PCR techniques offer rapid, sensitive, and accurate diagnosis, but they do not provide quantitative information like real-time PCR can. The starting amount of the target nucleic acid is quantitated, and the reaction can be monitored while in progress.

Real-time RT-PCR usually consists of four steps. After the first step, RT from RNA to cDNA, PCR is run using a specific primer set for the target region in the second step (usually 25–40 cycles). During this second step, fluorescence intensity, which corresponds to the amount of PCR product, is monitored for each cycle (Figure 1E-a). A cycle threshold (Ct), calculated from an amplification curve of fluorescence, is regarded as the virus titer in the sample (note that samples containing a higher density of virus have lower Ct values). In the third step, reaction reagents are incubated sequentially from 65 to 97°C at increments of ca. 0.1°C/s to obtain a melting curve for checking specific amplification of target region. The last step is cooling. Because virus titer is usually shown as the density of the virus relative to expression of a housekeeping gene in the same sample (Figure 1E-b), expression of the housekeeping gene (i.e., RNA titer transcribed from these genes in the sample) also should be determined by real-time RT-PCR to quantify target RNA. As noted already, RT-LAMP and conventional RT-PCR techniques offer rapid, sensitive, and accurate diagnosis, but they do not provide quantitative information like real-time PCR can. The starting amount of the target nucleic acid is quantitated, and the reaction can be monitored while in progress.

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Conventional detection methods have only provided infer- mation on the presence of target pathogens. But progress in the development of technologies to diagnose plant dis- eases may not only contribute to the control of rice viral diseases, but also open opportunities to analyze potential pathogens/candidate pathogens and to develop a comprehen- sive understanding of the ecology of microorganisms in rice fields.

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Malathi, P., and Mangraurthi, S. K. (2013). Descripting the multi- ple manifestation of rice yellow banded virus by absolute quantitation through real-time PCR. Arch. Phytopathol. Plant Protect. doi: 10.1007/s00325-2013-1794-5

Marx, E., and Carballis, O. (2007). Development of a diagnostic tool for the rice “hojiblanca” virus in Venezuela. Interciencia 32, 262–265.

Matsumura, K., Tawara, T., Saka, J. L., Omak, O., Okada, M., and Matsunuma, M. (2013). Dynamics of southern rice black-streaked dwarf virus in rice and implication for virus acquisiton. Phytopathol. 105, 509–512. doi: 10.1017/PFPT.2012.248

Montane, A. L., Hinkins, G. W., Martin, D. P., Lamer, P., Lefebvre, P., Shepherd, D. N., et al. (2011). Reconstructing the history of maize streak virus strain A disposed to reveal diversification hot spots and its origin in southern Africa. J. Virol. 85, 9625–9636. doi: 10.1128/JVI.00640-11

Morales, E. J., Ward, E., Canuto, M., Arevalo, J. E. A., Louroso, I., and Adams, M. J. (1998). Emer-gence and partial characterization of rice stripe mosaic virus and its fun- nigen vector in South America. Plant Dis. 82, 645–650. doi: 10.1094/PDIS.1998.82.7.645

Mullin, K. B., and Falabella, F. A. (1987). Specific synthesis of DNA in rice via a polymerase-catalyzed chain reaction. Methods Enzymol. 135, 335–356. doi: 10.1016/0076-6879(87)93055-0

Naidu, R. A., and Hughes, J. A. (2001). “Methods for the detection of plant virus diseases,” in Plant Virology in Sub-Saharan Africa. Proceedings of a Conference Organized by IITA eds J. d’A. Hughes and B. O. Odu (Ibadan: International Institute of Tropical Agriculture), 253–260.

Notomi, T., Okayama, H., Kinksu-ichi, H., Yonekura, T., Watabe, K., Amano, N., et al. (2000). Loop- mediated isothermal amplification of DNA, Nucl. Acid. Res. 28, 2653–2657. doi: 10.1093/oxfordjournals.100829.6

Novella, F. (1999). Current status and management of insect vectors of Rice yellow virus (RYMV) in Africa.
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Omura, T., Hibino, H., Ueno, H., Morioka, T., Tsuchizaki, T., et al. (2013). Detection of rice viruses in plants and individual insect vectors by latex flocculation test. Plant Dis. 97, 197–205.

Sharma, S., and Dasgupta, I. (2012). Real-time PCR assays for quantitative detection of Rice tungro bacilliform virus and Rice tungro spherical virus. J. Virol. Methods 181, 86–92. doi: 10.1016/j.jviromet.2012.01.038

Ishimaru, I., and Ishimaru, S. (1982). Detection of three viruses of cotton by direct tissue immunofluorescence. Phytopathology 82, 721.

Takahashi, Y., Omura, T., Harauchi, T., Shokara, K., and Tsuchizaki, T. (1998). Detection of rice transitory yellowing virus (RTYV) in infected rice plants and insect vectors by simplified ELISA. Ann. Phytopathol. Soc. Jpn. 54, 217–219. doi: 10.5860/phytopath.54.217

Takahashi, Y., Omura, T., Kenichiro, S., and Tsuchizaki, T. (1997). Rapid and simplified ELISA for routine field inspection of Rice stripe virus. Ann. Phytopathol. Soc. Jpn. 53, 254–257. doi: 10.5860/phytopath.53.254

Takahashi, Y., Omura, T., Shokara, K., and Tsuchizaki, T. (1991). Comparison of four serological methods for practical detection of ten viruses of rice in plants and insects. Plant Dis. 75, 436–441. doi: 10.1094/PD-75-0408

Ushara-Lekhi, T., Shiba, T., Ueno, T., Sasaoka, T., Hayash, Y., and Higuchi, H. (2013). Identification of the small brown planthopper (Laodelphax striatellus Fallen). J. Phytopathol. 161, 113–116. doi: 10.1139/jphytopath-2013-0115

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