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Short communication

A simplified method for simultaneous detection of Rice stripe virus and Rice black-streaked dwarf virus in insect vector

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A B S T R A C T

Rice stripe virus (RSV) and Rice black-streaked dwarf virus (RBSDV) are transmitted by their common vector small brown planthopper (SBPH) that cause serious crop losses in China. A simple reverse transcription-PCR method was developed for the simultaneous detection of RSV and RBSDV in single SBPH. Three primers targeted to RSV-RNA4 and RBSDV-S2 segments were designed to amplify respectively 1114-bp and 414-bp fragments in a reaction. The method is reliable, rapid and inexpensive for detecting the two viruses in vector, which could facilitate better forecasting and control of the virus diseases. Using this method, it was found that SBPH could carry RSV and RBSDV simultaneously.

Rice (*Oryza sativa*, *Oryza* spp.) is an important cereal grain food worldwide, and more than 90% rice is produced in Asian countries (Welch et al., 2010). Rice virus disease, known as rice cancer, is technically difficult to control and affects seriously rice production (Hibino, 1996). Currently, Rice stripe virus (RSV) and Rice black-streaked dwarf virus (RBSDV) are the two most important viruses in rice production in East and North China. RSV is the type member of the genus *Tenuivirus*, and has been reported to cause severe losses in rice fields in temperate regions of East Asian including China, Japan and Korea (Hibino, 1996; Wei et al., 2009; Cho et al., 2013). For example, in 2004, it affected dramatically about 1.57 million hectares rice cultivation in Jiangsu Province in China, which was more than 70% of local rice cultivated area (Zhou et al., 2012). RBSDV, a member of the genus *Fijivirus* in the family *Reoviridae*, not only infects rice plant to cause serious rice black-streaked dwarf disease, but also cause maize rough dwarf disease in maize (Wang et al., 2003). In recent years, outbreaks of RBSDV occurred on rice in Jiangsu and on maize in maize-growing areas in North China, resulting in severe losses in rice and maize production. In 2009, an outbreak of RBSDV inflicted heavy losses on 0.43 million hectares rice cultivation in Jiangsu (Zhu, 2012). The two viruses are transmitted mainly by their common vector small brown planthopper (*Laodelphax striatellus* Fallén, SBPH) (*Hemiptera*: *Delphacidae*) in a persistent, circulative-propagative manner (Toriyama, 1986; Falk and Tsai, 1998). Somewhat differently, RSV can be transmitted from female adults to their progeny via eggs, while RBSDV is not transovarial transmission (Suzuki et al., 1992; Deng et al., 2013). The epidemics and outbreak of RSV and RBSDV generally have close relationships with a high density of vector, a high proportion of viruliferous SBPH in overwintering populations, and changes in cultivation practices and climate (Hibino, 1996; Zhou et al., 2012; Otuka, 2013). Transovarial (vertical) transmission of RSV and lack of RBSDV-resistant rice cultivars also increase difficulty of disease control. In addition, latest research showed that SBPH (from East China) could also transmit rice stripe disease to overseas rice fields through long-distance migration in East Asian countries (Otuka et al., 2012; Otuka, 2013).

In agricultural production, if the effective preventive measures are not taken in advance, when the disease symptoms appear and extensive infection occurs, it will be too late to recover damages. Therefore, the information about the two viruliferous rates of SBPH field populations (especially overwintering and 1st generation insect) is very important for forecasting and preventing the potential disease threaten (Kisimoto, 1993). A rapid and reliable diagnosis for RSV and RBSDV is essential for the development of efficient forecasting and viral disease control. Western-blot, ELISA and Real time PCR techniques are also suited for large-scale virus detection and have been used to forecast epidemics of rice stripe disease (Wang et al., 2004; Zhang et al., 2008), but detection of RBSDV is just too immature, that immunoassay is limited by the supply and quality of RBSDV-specific antibody, and quantitative PCR is expensive. A multiplex reverse transcription-PCR (RT-PCR)

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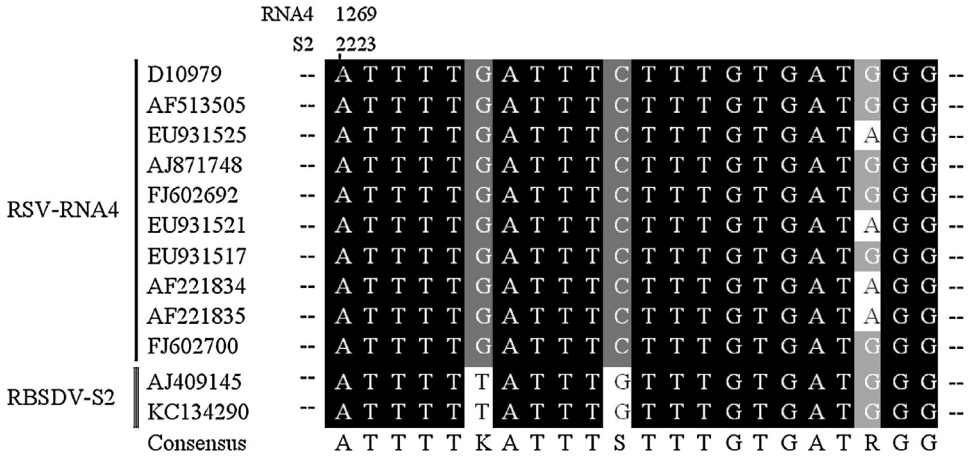

Fig. 1. The high identity regions between RSV-RNA4 and RBSDV-S2 segments.

has been developed to detect three viruses (RSV, RBSDV and Rice dwarf virus) from infected rice plants (Cho et al., 2013), but this reaction needs 6 specific primers, which is not very convenient. In addition, infected rice plants by RSV and RBSDV show unique symptoms respectively and are visually distinguishable, so diagnosis of the two viruses is more meaningful in vector SBPH than in rice plants. This paper presents a simple RT-PCR method for the simultaneous detection of RSV and RBSDV in single SBPH, and the reaction only needs 3 primers, thus providing a reliable, fast, and inexpensive method for detection of the two viruses in insect vector.

RSV is a single stranded RNA virus with 4 segmented genomes which contain 7 ORFs (Hamamatsu et al., 1993; Toriyama et al., 1994), and RBSDV genome contains 10 segments of double-stranded RNA (S1-S10) which are numbered in decreasing order of size (Wang et al., 2003). After sequence alignment between the two viruses via DNAstar-MegAlign software with Clustal V method, it was unexpectedly found that two section nucleotide sequences (from RSV-RNA4 and RBSDV-S2 segments respectively) shared high identities (Fig. 1), which facilitates accurate identification of the two viruses by designing a common primer. Three specific primers (RS-F, RB-F and RSRB-R) were designed according to the sequence alignment results of RSV-RNA4 against RBSDV-S2 (refer GenBank No. D10979, AF513505, EU931525, AJ871748, FJ602692, EU931521, EU931517, AF221834, AF221835, FJ602700, AJ409145 and KC134290). Primer RSRB-R (5′-CCYATCACAAASAAATMAAAAT-3′) was complementary to nucleotide 1269–1290 of RNA4 and 2223–2244 of S2, respectively. Primer RS-F (5′-AGATCCAGAGAGAGTCACGGAAG-3′) corresponded to nucleotide 177–199 of RNA4, which could pair with RSRB-R to amplify a specific 1114-bp fragment for detection of RSV. Primer RB-F (5′-GTTCAAAGACAATACACTCAAAA-3′, corresponding to nucleotide 1831–1853 of S2) and RSRB-R were used to amplify a 414-bp fragment, which was specific for RBSDV.

SBPH used in this study was collected from Haian, Jiangsu Province, China (32.57° N, 120.45° E with an elevation of 5 m a.s.l.), and has been maintained as stock populations in the laboratory for nearly 10 years. The viruliferous (RSV) and non-viruliferous strains were screened and reared respectively, as described previously (Li et al., 2011). RBSDV-infected rice plants showing typical stunting or dwarf symptoms were collected and identified from diseased field in Jiangsu, which was used for virus acquisition experiment of SBPH. Approximately 400 SBPH nymphs (2nd instar) from viruliferous (RSV) populations were pre-starved for 3 h and then released onto RBSDV-infected rice plants. After a 72 h acquisition-feeding period, the surviving nymphs were transferred to healthy rice seedlings for SBPH passing the latent period. After 15 days

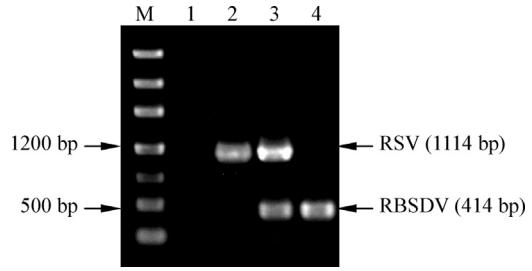

Fig. 2. Detection of RSV and RBSDV in SBPH by multiplex RT-PCR using three primers. Lane M: DNA markers; lane 1: non-viruliferous SBPH as negative control; lane 2: RSV-infected SBPH; lane 3: RSV and RBSDV doubly infected SBPH; lane 4: RBSDV-infected SBPH.

feeding on healthy rice, the insects were collected and stored at −20 °C for subsequent virus detection.

Total RNA from individual SBPH was extracted using SV total RNA isolation system (Promega, Madison, USA) following the manufacturer's instruction. The concentration and quality of each RNA sample was determined with a NanoDrop 2000C spectrophotometer (Thermo Scientific, Wilmington, USA). Only the RNA samples with an A_{260}/A_{280} ratio (an indication of protein contamination) of 1.9–2.1 and an A_{260}/A_{230} ratio (an indication of reagent contamination) greater than 2.0 were used for the analysis. Average 1.324 μg RNA was extracted from each insect. For the simultaneous detection of RSV and RBSDV in single SBPH, a multiplex RT-PCR was developed. First strand cDNA was synthesized with random hexamers by using PrimeScript™ 1st strand cDNA Synthesis Kit (TaKaRa, Dalian, China) according to the manufacturer's protocols. The subsequent PCR reaction was performed in a 25-μl mixture, which

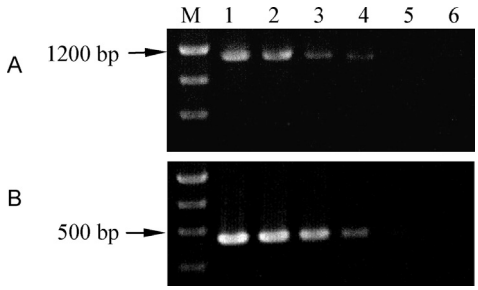

Fig. 3. Sensitivity of RT-PCR in amplification of serially diluted insect cDNA for the detection of RSV (A) and RBSDV (B). Lane M: DNA markers; lanes 1–6: serially diluted (A: 500 ng, 100 ng, 50 ng, 10 ng, 1 ng and 0.1 ng of cDNA; B: 500 ng, 100 ng, 10 ng, 1 ng 0.1 ng and 0.01 ng of cDNA) cDNA samples from virus-infected SBPH.