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Short communication

One-step multiplex reverse transcription-polymerase chain reaction for the simultaneous detection of three rice viruses

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A B S T R A C T

Rice stripe virus (RSV), *Rice black-streaked dwarf virus* (RBSDV), and *Rice dwarf virus* (RDV) are major rice-infecting viruses in Korea that can cause serious crop losses. A one-step multiplex reverse transcription-polymerase chain reaction (mRT-PCR) was developed for the simultaneous detection of these rice viruses. Three sets of specific primers targeted to the capsid protein coding genes of RSV, RBSDV, and RDV were used to amplify fragments that were 703 bp, 485 bp, and 252 bp, respectively. The one-step mRT-PCR assay proved to be a sensitive and rapid method for detecting the three rice viruses. This method could be used to facilitate better control of rice viruses.

Rice (*Oryza sativa*, *Oryza* spp.) is one of the most economically important and widely consumed cereal grain staple foods worldwide, of which more than 90% is produced in Asian countries (Kuenzer and Knauer, 2013; Welch et al., 2010). Rice virus disease, known as rice cancer, is technically difficult to control and affects seriously rice production. Although fungal disease has been responsible for the majority of rice diseases in Korea, viral disease has recently been documented in western coastal regions, spreading rapidly and causing huge losses in rice yield due to overseas migratory insects from China (Kim et al., 2011; Otuka et al., 2012). Future widespread pest outbreaks due to long-distance insect migration are likely to intensify due to climate change. Climate change could alter the development of pathogens, modify host resistance, and result in response changes in host–pathogen interactions (Wang et al., 2008). In Korea, only three rice viruses have been reported: *Rice stripe virus* (RSV), *Rice black-streaked dwarf virus* (RBSDV), and *Rice dwarf virus* (RDV) (Chung, 1974; Shikata and Kitagawa, 1977) (Fig. 1). RSV (genus *Tenuivirus*) is the most devastating rice virus in Korea, China, and Japan (Wei et al., 2009). In 2007, it affected 4457 ha in the western region of Korea, causing partial and whole plant death (Kim et al., 2008). RBSDV (genus *Fijivirus*) caused serious rice and maize yield losses in Japan during the 1940s (Shikata and Kitagawa, 1977), and in Korea and China in the 1970s (Lee et al., 1977; Zhang et al., 2001). Although it was rarely found during the ensuing 40 years, the disease reoccurred in the 2000s. RDV (genus *Phytoreovirus*) infects naturally rice and some grass weeds. The epidemic in Japan that started around 1895 has affected continuously rice production (Fukushi et al., 1962). The three rice viruses are transmitted mostly by planthoppers and leafhoppers such as *Laodelphax striatellus*, *Nephotettix cincticeps*, and *Nilaparvata lugens* (Hibino, 1996; Omura and Mertens, 2005; Shikata and Kitagawa, 1977). These insect vectors are distributed widely in Asian countries and usually migrate over long distances. Virus-infected rice plants show unique symptoms. However, such symptoms are often overlooked because similar symptoms can be caused by multiple viruses and abiotic stresses in the field. These symptom similarities underlie the importance for developing a rapid method that detects accurately rice viruses in the field. Multiplex reverse transcription-polymerase chain reaction (mRT-PCR) has been developed to detection several viruses in a single reaction, thus providing a reliable, fast, and inexpensive method for routine detection of plant viruses in the field. Even so, designing compatible primers can present technical challenges (Chamberlain et al., 1988; Nie and Singh, 2002). This paper presents the development of a one-step mRT-PCR

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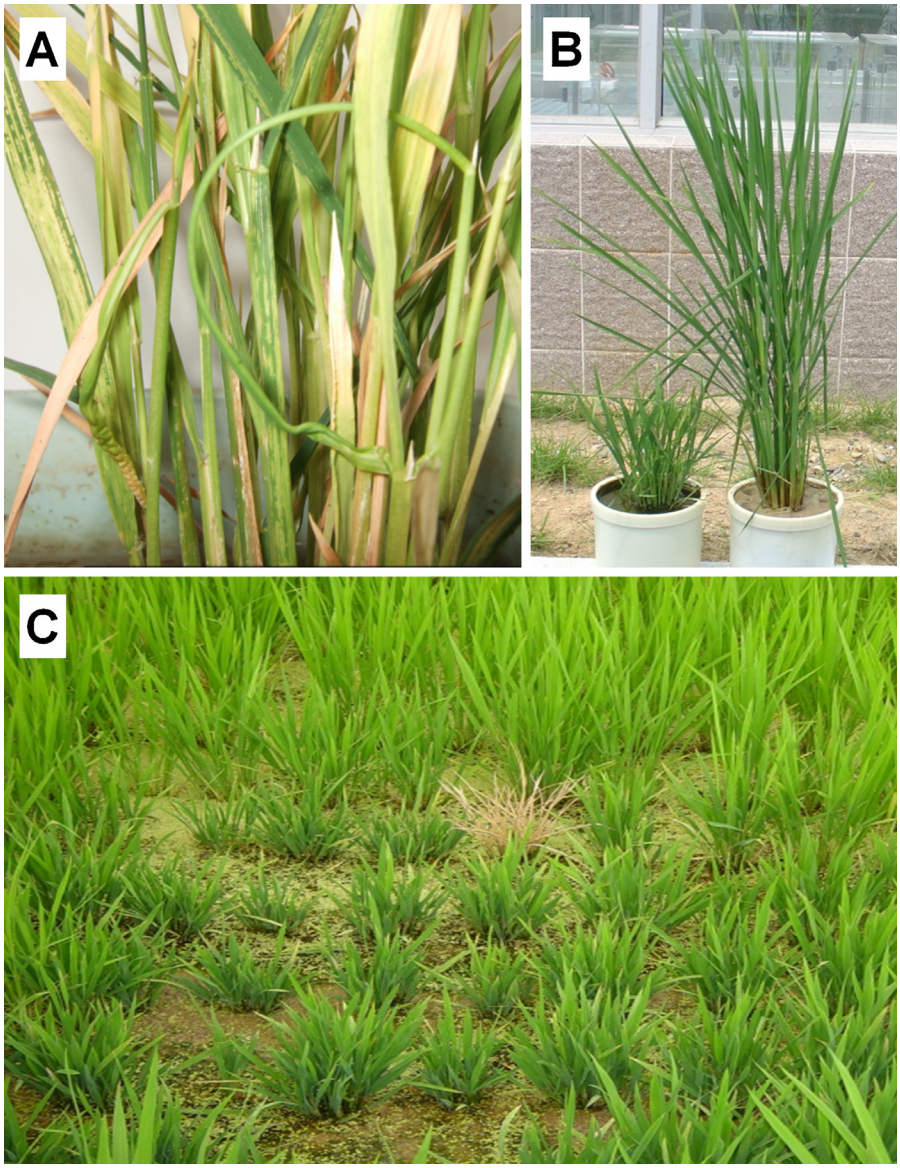

Fig. 1. Leaf symptoms on rice plants infected with (A) *Rice stripe virus* (RSV), (B) *Rice dwarf virus* (RDV), and (C) *Rice black-streaked dwarf virus* (RBSDV).

method for the simultaneous detection of RSV, RBSDV, and RDV.

Virus-infected rice leaves were collected from artificially infected or naturally infected rice in several provinces of Korea. Infected rice plants with typical rice virus symptoms were collected from several locations in Korea during June–July 2012. Three viruses were propagated in rice plants using insect vectors. Briefly, the insect vectors were fed leaves from virus-infected rice for 1 day to allow them to acquire the virus. Next, uninfected plants were exposed to the insects, and the rice leaves were collected 14 days post-inoculation for total RNA extraction. Total RNA were extracted from 100 mg of leaf tissue from infected plants. Total RNA extraction was carried out using RNeasy plant mini kit (QIAGEN, Hilden, Germany) as directed by the manufacturer's procedure. The concentration and purity of the extracted total RNA were determined by measuring the absorbance ratio of 260:280 nm using a NanoDrop ND-1000 spectrophotometer (Thermo Scientific, Wilmington, USA). Primers were designed from conserved regions of the capsid protein (CP) gene of viruses and nucleotide sequence variation in Korean isolates of viruses was not so high (96–99% homology). The Genbank accession numbers for the CP coding genes are as follows: GU230170 (RSV RNA3), U36565 (RDV segment 10 RNA), and JX994211 (RBSDV segment 10 RNA). Of the six primers designed in this study (Table 1), three primer sets (RSV RNA3 703 F/R, RBSDV S10 485 F/R, and RDV S10 252 F/R) amplified the expected targets for RSV, RBSDV, and RDV, respectively. RT-PCR was performed using One-Step RT-PCR kit (QIAGEN, Hilden, Germany) and following the manufacturer's procedure with some modifications. Briefly, one-step mRT-PCR was performed in a 20 μl volume, in which the reaction mixture contained three sets of specific primers, RNA template (5 ng μl^{-1}), 5× buffer, dNTP mix, and enzyme mix. RT-PCR reactions were performed under the following conditions: RT for 30 min at 50 °C, for 15 min at 95 °C followed by 35 cycles of denaturation for 1 min at 95 °C, annealing for 1 min at 58 °C, and extension for 40 s at 72 °C. The final extension at 72 °C was allowed to proceed for 10 min. Amplified products were detected on a 1.2% Tris–acetate–EDTA agarose gel with loading star (DYNE BIO, Seongnam, Korea). The specific primer sets was investigated by one-step mRT-PCR reactions using total RNA from virus-infected and uninfected rice leaves. The three virus primer sets was investigated initially using 10 pmol primer concentrations in PCR reactions with total RNA from virus-infected and uninfected