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Occurrence and Detection of *Rice black-streaked dwarf virus* in Korea

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Until now, occurrence of *Rice black-streaked dwarf virus* (RBSDV) is observed in Gyeongsang provinces, southeastern part of Korea. However, recently, the occurrence of RBSDV is increasing and spreading in Jeonra provinces including Gochang-gun, southwestern part of Korea. RBSDV infected plants showed typical symptoms including stunted, deformed leaves with white waxy or black-streaked swelling along the veins. We extracted viral genomic dsRNA from infected leaves and analyzed dsRNA pattern by polyacrylamide gel electrophoresis. Ten genomic segments with similar sized dsRNAs were observed. We also detected RBSDV by reverse transcription (RT)-PCR using specific primers for S10 from genomic dsRNA and observed amplified DNA fragment specific for RBSDV S10.

Keywords **:** genomic dsRNA, *Rice black-streaked dwarf virus*, S10

Rice black-streaked dwarf virus (RBSDV), a member of the genus *Fijivirus* within the family *Reoviridae*, is propagatively transmitted to rice, maize, barley and wheat in a persistent manner by the planthopper, *Laodelphax striatellus* (Shikata et al., 1977; Wang et al., 2003). The genome composed of 10 segmented dsRNAs designated as S1 to S10 with an increasing order of mobility in polyacrylamide gel electrophoresis (PAGE). To date, complete sequences of fijiviruses with 10 dsRNA segments have been determined only for RBSDV (Fang et al., 2001; Zhang et al., 2001).

RBSDV occurs in Korea, Japan and China. Occurrence of the disease is generally sporadic and limited to small patches in a field, but major outbreaks of the disease were recorded in Japan in 1941 and in Korea, Japan and China during the 1960s (Isogai et al., 2001; Shikata et al., 1977). Until now, occurrence of RBSDV is limited in Gyeongsang provinces, southeastern part of Korea. However, recently, the occurrence of RBSDV is increasing and spreading in Jeonra provinces including Gochang-gun, southwestern part of Korea. It is very difficult to distinguish RBSDV symptoms with chemical injury of rice in field. The symptoms induced on infected plants include stunted, deformed leaves with typical white waxy or black-streaked swelling along the veins (Fig. 1A and B).

We isolated RBSDV from infected plant and analyzed their genomic dsRNAs by using PAGE. Briefly, RBSDV isolates were collected from naturally infected field-grown rice plants. Infected plant was maintained and propagated

Fig. 1. Infected rice plants (**A**) and galls on a rice culms caused by RBSDV (**B**). The symptoms induced of infected plants include stunted, deformed leaves with white waxy or black-streaked swelling along the veins. The prominent dark brown streaks consisting of swollen veins or galls form along the undersides of leaves and on the sheaths and culms at later growth stages.

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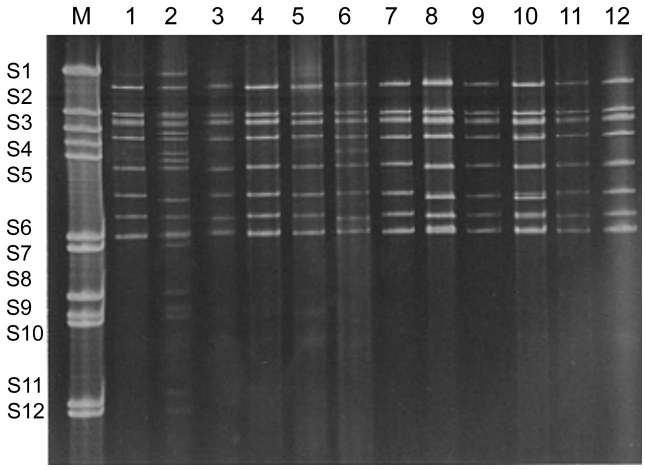

Fig. 2. Genomic dsRNA migration profiles of RBSDV isolates. Lane 1 (M) is genomic dsRNA of Rice dwarf virus (RDV). We used 12 segments dsRNA of RDV by size marker. Segments are numbered on the left. Upper number 1~12 is shows that collected from different locations.

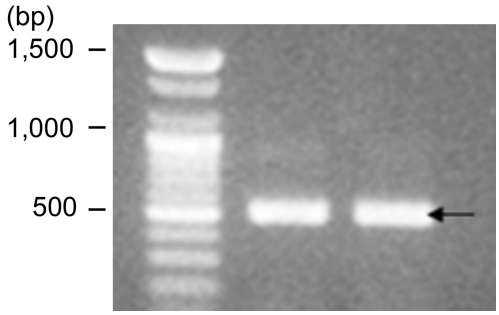

Fig. 3. Agarose gel electrophoresis of RT-PCR products which are specific to including of S10 extracted from dsRNA of infected plants. Lane 1 has molecular size marker of 100 bp ladder. The expected sizes of 501 bp PCR fragment are indicated with arrow.

in greenhouse by insect transmission. Genomic dsRNAs were directly extracted (Uyeda et al., 1994) from infected leaves, and 200 to 400 ng of the genomic dsRNAs were subjected to 9% PAGE at 300 v constant for 40–60 hrs in 40 mM Tris-acetate, 1 mM EDTA buffer (pH 8.0) using a $20 \times 40 \times 0.08$ cm vertical slab gel. When the extracted dsRNAs were compared by PAGE, 10 different segments of genomic dsRNAs were observed in PAGE (Fig. 2). Genomic segments were numbered S1 to S10, from the slowest migrating band to the fastest. The migration patterns of 10 genomic segments was similar, but differed among each samples (Fig. 3). The isolates could be differentiated by comparing the migration pattern of dsRNAs extracted from each RBSDV isolate from different locations. Interestingly, dsRNA migration patterns were differed even between isolates collected from same location indicating the presence of RBSDV dsRNA variations in fields.

To specifically detect RBSDV by reverse transcription (RT)-PCR, we extracted dsRNA from infected leaves and tried to detect RBSDV using specific primers for S10 dsRNA (GenBank AJ297433). Detection of viral RNA genome was performed using RT-PCR System (Promega) consisting of one step with cDNA synthesis and PCR amplification. RBSDV S10 specific primers correspond to 5'end (5'-TGGCTGTACCTTGTTTTGAT-3') and 3'end (5'-GACAATAGCTGAATTTCCCCC-3') of RBSDV dsRNA S10. Expected size (501 bp) of DNA fragment corresponding to RBSDV S10 was observed (Fig. 3). Altogether, dsRNA pattern and RT-PCR analysis appeared to be useful in detecting RBSDV from naturally infected plants.

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