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dsRNA Analysis and Sequence of S12 to *Rice dwarf virus* Korean Isolate

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We isolated *Rice dwarf virus* (RDV) from infected plants in rice fields (Korea, Japan, China, the Philippines and Nepal) and analyzed their genomic dsRNAs by polyacrylamide gel eletrophoresis. The genomic dsRNAs of the isolates showed distinct electrophoretic mobility profiles. The S12 coding to nonstructural protein of Korean isolate (RDV-Kr) was further analyzed by sequencing. The S12 of RDV-Kr was 1,066bp long and coded for a protein composed of 312 amino acids including three open reading frames of P12, P12OPa and P12OPb. The sequence identities were 96% and 98.6% with Japanese isolates (H, AN), 94.7% with Nepalese isolate (NEL), 94% with Chinese isolate (CK) and the Philippines isolate (P).

Keywords **:** dsRNA, RDV-Kor, *Rice dwarf virus*, S12 sequence

Rice dwarf virus (RDV), a phytoreovirus, is a member of the family *Reoviridae* (Uyeda and Milne, 1995). It has a genome composed of 12 segmented dsRNAs designated as S1 to S12 with an increasing order of mobility in polyacrylamide gel electrophoresis (PAGE). The virus is persistently transmitted by *Nephotettix* species, and is an agent of dwarf disease of rice plants (Iida, 1972). RDV was first reported in Japan (Iida et al., 1972). The disease has subsequently been reported in Korea, China, Nepal (Iida et al., 1972 ; Dahal et al., 1996) and all in subtropical or temperate zones, and has been reported to be a serious epidemic disease in rice on several occasions. More recently, it has also been isolated in Mindanao, Phillippines (Cabauatan et al., 1993). We isolated RDV from rice fields and analyzed their genomic dsRNAs by using PAGE. Each RDV isolates were isolated from infected plants naturally, listed as in Table 1 and used for the comparison of nucleotide sequence of S12. The analyses showed that genomic variants were common in nature. Although the function of P12 has yet to be established, nucleotide sequence analysis and comparison of amino acid sequence among RDVs, wound tumor virus, and Rice gall dwarf virus

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Table 1. *Rice dwarf virus* (RDV) isolates used and analyzed in this study

Isolate	Origin
RDV-Kr	Milyang, Korea
RDV-CK	China
RDV-NEL	Nepal
RDV-P	Philippines
RDV-N	Niiya, Japan
RDV-AK	Aki-gun, Kitagawa, Japan
RDV-H	Hokkaido, Japan
RDV-AN	Aki-gun, Nahari, Japan

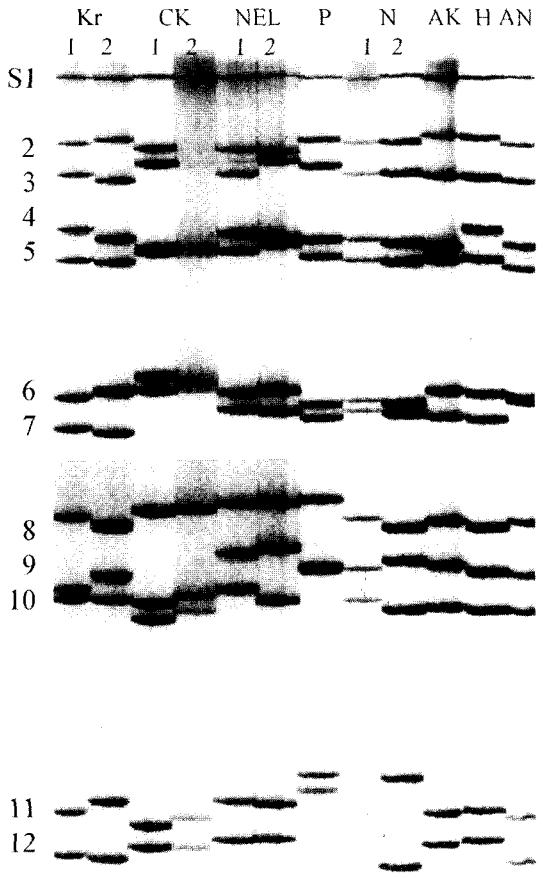

Fig. 1. Genomic dsRNA migration profiles of RDV isolates. The isolates used are indicated above by abbreviations (see Table 1). Segments were numbered on the left.

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1                                                GGTAAATTGAGCAGTATTTCAACATTGTTGTTGGAGTTATA
42   ATGTTCAAGAGCGGGTCCGGTTCTTTGAAGCGACGTTGTTCCATTCCAAGTGTTAAATCATTTTCTGGC
       M  F  K  S  G  S  G  S  L  K  R  R  C  S  I  P  S  V  K  S  F  S  G
111  GATAGTGAAAAAGGACTACCACCTATTTCCCGAGGTAGTGTGTCCATTGCTAGTCAGAACTCTGAACCA
       D  S  E  K  G  L  P  P  I  S  R  G  S  V  S  I  A  S  Q  N  S  E  P
180  TTGATTGTCCCCGCAAGTAGTTCTTCTTTTGCGGCGACTTCTGACTTTGTACCTGAGAAGACAAAGTCC
       L  I  V  P  A  S  S  S  S  F  A  A  T  S  D  F  V  P  E  K  T  K  S
249  GAAGGGAATTTGAAAGATAAGTCTTCCGTCATAACCGGAAACTTTGGGTCCTCTGGACCAATTAATGCT
       E  G  N  L  K  D  K  S  S  V  I  T  G  N  F  G  S  S  G  P  I  N  A
318  CATACCAATCAGAACGCTAATGGCGATCGATTGGCTGAAAATCTTTTGCTTAAGGAATCGTCCAAGGGA
       H  T  N  Q  N  A  N  G  D  R  L  A  E  N  L  L  L  K  E  S  S  K  G
387  CGAGGATCAAGCACATCCGATGCTAGACATACTGCCACAGACTCTCGACTCAGTCAAGAAGTCAAGCAG
       R  G  S  S  T  S  D  A  R  H  T  A  T  D  S  R  L  S  Q  E  V  K  Q
456  TCGTTTCCCGAGGAAAATGCTGGCGGCAACGATCTCAATACTGGAAGAGGAAGTCACGGAACTGGTGAC
       S  F  P  E  E  N  A  G  G  N  D  L  N  T  G  R  G  S  H  G  T  G  D
525  GGAGTTGAACAACACTACAAATTTGACTGCGAAGAAGGAATGTCCGCGTATCACAAACGCGTTGTAGAT
       G  V  E  Q  H  Y  K  F  D  C  E  E  G  M  S  A  Y  H  K  R  V  V  D
594  ACATTCTTTAAATATTTTGAATATTCGGCTGAAGATGGGCATTCCACCCTATATTCGGACGTTATGTTT
       T  F  F  K  Y  F  E  Y  S  A  E  D  G  H  S  T  L  Y  S  D  V  M  F
663  TTATCTGGTCATGGCGATCTTGGTCTACTACTTATGAGTAGATATCAAGAACTCATGACCTTACGGGTG
       L  S  G  H  G  D  L  G  L  L  L  M  S  R  Y  Q  E  L  M  T  L  R  V
732  CGAAGTGCCATCTATGGCATATTTTGTTACCTACAAGCTCTAACCGCCTACCTAACGTATTTTGATGCT
       R  S  A  I  Y  G  I  F  C  Y  L  Q  A  L  T  A  Y  L  T  Y  F  D  A
801  AAAGTAGGTCAAGCTATTATGTTGGATGAGGAGTTGGAGAAATATGAAATCCGACTCGATGTCGCGCAA
       K  V  G  Q  A  I  M  L  D  E  E  L  E  K  Y  E  I  R  L  D  V  A  Q
870  GACGACGATCCAATTGTATTTGAAATCACGACGGGTGTATTCACATCGGGAGTAGCTCACGATCTGCGT
       D  D  D  P  I  V  F  E  I  T  T  G  V  F  T  S  G  V  A  H  D  L  R
939  AAACTTACACAGATACTCGAGGCCTTTTCTCTTGAAAGGTGATATCCAGTCTGGGAGTAATGAACCCAC
       K  L  T  Q  I  L  E  A  F  S  L  E  R  *
1008 GCTGGGGACTCAGTTATATCTCTTCGGGAGTGATATACCGAGTACTGCTCATAACTGAT
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Fig. 2. Nucleotide and deduced amino acid sequence of S12 of RDV-Kr. Initiatin and termination codons of the P12 open reading frame (ORF) are bold. Initiation codons of P12OPa and P12OPb ORFs are underlined and their common termination codon is underlined.

showed that S12 is the most diverse gene within the genus *Phytoreovirus* (Uyeda et al., 1994). First of all, S12 of RDV-Korean isolate (RDV-Kr) was further analyzed by sequencing, because of the diversity.

RDV-Kr isolate was collected from experimental field of National Yeongnam Agricultural Experimental Station. Infected plant was maintained and propagated in greenhouse by insect transmission. Genomic dsRNAs were directly extracted (Murao et al., 1994) from infected leaves, and 200-400 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, the migration patterns differed among each samples, and the isolates could be differentiated by the migration pattern comparison of RDV isolates from different locations. Migration patterns differed even between isolates collected from same location (Kr 1 and 2).

Detection of viral RNA genome was performed using RT-PCR System (Promega Co.) consisting of one step with cDNA synthesis and PCR amplification. S12 specific primers correspond to nucleotide 5'end-3'end. The sequences were 5'ggt aaa ttg agc agt att tca 3' (upstream) and 5'atc agt tat gag cag tac tcg 3' (downstream). PCR products of full-length S12 of RDV were ligated into the pGEM-T vector and insert cDNA was sequenced. S12 was consisted of 1,066 bp long and coded for a protein composed of 312 amino acids including three open reading frames of P12, P12OPa and P12Opb (Fig. 2). The sequence identities were 96%, and 98.6% with Japanese isolates (H, AN), 94.7% with Nepalese isolate (NEL), 94% with Chinese isolate (CK) and the Philippines (P) (Table 2). RDV-Kor isolates could be distinguished from other RDV isolates by PAGE analysis and cDNA sequences of S12.

Table 2. Sequence identity between RDV-Kr and different geographical isolates

Isolates	H	An	NEL	CK	P
Identity (%)	96.0	98.6	94.7	94.0	94.0

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