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Cloning and Sequencing of Coat Protein Gene of the Korean Isolate of *Rice stripe virus*

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The coat protein gene of Korean isolate of *Rice stripe virus* (RSV-Kr) was cloned and its nucleotide sequence was determined. Total RNA was extracted from infected leaves and RSV viral RNA was detected by using RT-PCR with specific primer of coat protein gene. The result of RT-PCR showed a specific band. Purified RT-PCR products of coat protein gene were ligated into the pGEM-T Easy plasmid vector and cloned cDNA was obtained for nucleotide sequence determination. Coat protein gene of RSV-Kr consisted of 969 bp long encoding a protein of 322 amino acids. RSV-Kr showed 94%-99% sequence identities to that of Japanese- and Chinese isolates.

Keywords : Coat protein, *Rice stripe virus*, sequence

Rice stripe virus (RSV) has a broad host range in the *Gramineae* and causes serious damage on rice, particularly the Japonica-type rice varieties (Toriyama et al., 1983). It occurs in temperate and sub-tropical East Asia being found in Japan, South Korea, eastern regions of the ex-USSR and China. RSV, which is transmitted in a persistent manner by the small brown planthopper, *Laodelphax striatellus*, is a type member of *Tenuivirus* group of plant viruses. It has filamentous particles. Four species of ssRNA and four species of dsRNA are found in purified preparations (Toriyama, 1982; Toriyama and Watanabe, 1989; Ishikawa et al., 1989).

Recently the complete nucleotide sequences of RNA3 and RNA4 of RSV have been determined and their ambisense coding strategy identified (Kakutani et al., 1990, 1991; Zhu et al., 1991). In this study, we report for the first time the cloning and sequence analysis of coat protein of RSV Korean isolate (RSV-Kr).

RSV-Kr isolate was collected from experimental field of Yeongnam Agricultural Research Institute. Infected plants were maintained and propagated in greenhouse by insect transmission. Total RNAs were directly extracted from infected leaves (Qiagen Co.). Detection of viral RNA genome was performed by using RT-PCR System (Promega Co.) consisting of one step with cDNA synthesis and PCR amplification.

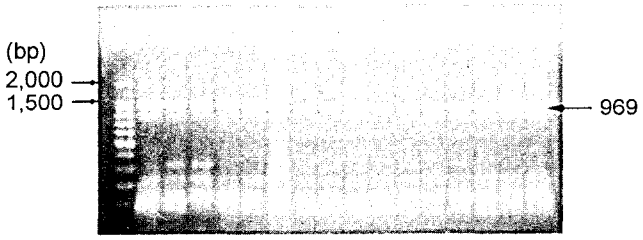

Fig. 1. Agarose gel eletrophoresis of RT-PCR products which are specific to RSV-coat protein gene extracted from infected plants. Lane 1 has molecular size marker of 100 bp ladder. The expected sizes of 969 bp PCR fragment are indicated with arrow.

Coat protein specific primers corresponded to nucleotide 5'end-3'end. The sequencea were 5'atg ggt acc aac cca gcc act c 3'(upstream) and 5' cta gtc atc tgc acc ttc tgc atc a 3'(downstream). PCR products of coat protein of RSV were ligated into the pGEM-T vector and inserted cDNA was sequenced. Coat protein consisted of 969 bp long (Fig. 1) and coded for a protein composing 322 aa, which showed to be encoded by the viral complementary sequence (Fig. 2) The sequence identities were 98%, and 99% with Japanese isolates (DW, HZ), 94% with Chinese isolate (BS), 96% with Chinese isolates (YL), 98% with Chinese isolates (JN, PJ, LY, JD) and 99% with Chinese isolate (SQ) (Table 1).

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1   ATGGGTACCAACAAGCCAGCCACTCTAGCTGATTTGCAGAAGGCAATCAATGACATCTCC
      M  G  T  N  K  P  A  T  L  A  D  L  Q  K  A  I  N  D  I  S
61  AAAGATGCGTTGTCTTACCTGACTGCTCACAAAGCTGATGTTGTGACCTTTGCTGGTCAG
      K  D  A  L  S  Y  L  T  A  H  K  A  D  V  V  T  F  A  G  Q
121 ATAGAGTATGCAGGCTATGATGCTGCAACTCTGATTGGCATATTGAAGGACAAAGGTGGT
      I  E  Y  A  G  Y  D  A  A  T  L  I  G  I  L  K  D  K  G  G
181 GACACACTGGCCAAGGATATGACTATGTGCATCACCATGAGATATGTGAGAGGCACTGGC
      D  T  L  A  K  D  M  T  M  C  I  T  M  R  Y  V  R  G  T  G
241 TTTGTGAGAGATGTCACTAAGAAAGTGAAAGTGGCGGCTGGAAGCACAGAGGCTTCAACC
      F  V  R  D  V  T  K  K  V  K  V  A  A  G  S  T  E  A  S  T
301 TTGGTGTCGAGGTATGGGATAGTGTCCTCAGTGGGGACAAATGCCAATGCTATCACACTT
      L  V  S  R  Y  G  I  V  S  S  V  G  T  N  A  N  A  I  T  L
361 GGAAGGCTGGCTCAGCTATTCCCAAATGTCTCACATGAAGTTGTGAGACAAATTTCTGGT
      G  R  L  A  Q  L  F  P  N  V  S  H  E  V  V  R  Q  I  S  G
421 GTTAAGATGGCTGTGGACTCCTCTGACCTGGGACTAACAGGATGTGACAACTTACTGTGG
      V  K  M  A  V  D  S  S  D  L  G  L  T  G  C  D  N  L  L  W
481 GACTATGTTCCACAATATATCAAACTAGAGAGTGAAACAGCTCCTTACTGCACAACTCAC
      D  Y  V  P  Q  Y  I  K  L  E  S  E  T  A  P  Y  C  T  T  H
541 TCCCTAAGTCACATTTTGTTTGTTGTGCACATCATTCACTCCTTCCAAATAACCAAAAAG
      S  L  S  H  I  L  F  V  V  H  I  I  H  S  F  Q  I  T  K  K
601 ACCATGCCAGAGGGTAAGAAGAAGGAGCGTGGTCTGACAAAAGACATAGACATGATGAAG
      T  M  P  E  G  K  K  K  E  R  G  L  T  K  D  I  D  M  M  K
661 TACACAACTGGTCTCCTGGTCATCACATGCAAGTCAAAGAACCTGGCTGACAAGAAGAAG
      Y  T  T  G  L  L  V  I  T  C  K  S  K  N  L  A  D  K  K  K
721 GAAGATGGCAGAAAGAAGGTCTTAGATGAATTCATCACCAATGGGAAAGTGAAGACCACA
      E  D  G  R  K  K  V  L  D  E  F  I  T  N  G  K  V  K  T  T
781 ATCTTCGATGCGCTGGCTGGTATGTCTGTCAATACTATCAGCACTTATGGGAATCAGACA
      I  F  D  A  L  A  G  M  S  V  N  T  I  S  T  Y  G  N  Q  T
841 AGGCTGTACTTGGCTCAACAGAGCAAACTGATGAAGATCCTTGCTGAGAACACTTCAAAG
      R  L  Y  L  A  Q  Q  S  K  L  M  K  I  L  A  E  N  T  S  K
901 ACAGCATCTGAAGTCAGCGGGTTGGTGAAGGAGTTCTTCGAGGATGAGGCAGAAGCAGGT
      T  A  S  E  V  S  G  L  V  K  E  F  F  E  D  E  A  E  G  A
961 GATGACTAG
      D  D  *
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Fig. 2. Nucleotides and deduced amino acid sequence of coat protein gene of the Korean isolate of RSV. The amino acid sequence, represented as the single-letter amino acid code, is shown below the nucleotide sequence. The asterisk (*) indicates the (UAG) stop codon.

Table 1. Sequence homology of coat protein between RSV-kr and different isolates

Isolates[a]	DW	HZ	BS	YL	JN	PJ	LY	JD	SQ
%	98	99	94	96	98	98	98	98	99

[a]DW, HZ: Japanese isolates; BS, YL, JN, PJ, LY, JD, SQ: Chinese isolates.

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