第18卷4期 中国病毒学 18(4):381—386
2003年8月 VIROLOGICA SINIGA August 2003

水稻条纹病毒中国分离物和日本分离物RNA2节段序列比较*

魏太云，林含新，吴祖建，林奇英，谢联辉**

（福建农林大学植物病毒研究所，福建福州，350002）

摘要：测定了来源于我国水稻条纹叶枯病常年流行区的云南楚雄（CX）及病害暴发区的江苏洪泽（HZ）的水稻条纹病毒（RSV）2个分离物RNA2全长序列，其长度分别为3506 bp 和 3514 bp。与已报道的日本T和O分离物RNA2序列进行比较的结果表明，这4个分离物可分为两组，其中，HZ、T和O分离物为一组，组内分离物之间，RNA2的毒义链（vRNA2）及RNA2的毒义互补链（vcRNA2）上的ORF的核苷酸一致性分别为97.2%~98.0%和96.8%~97.1%，5′端和3′端非编码区的序列则完全一致。但HZ分离物与T分离物的亲缘关系更为密切，其基因间隔区（IR）与T和O分离物的等长。另一组为我国CX分离物，组与组之间，vRNA2 及vcRNA2上的ORF的核苷酸一致性分别为95.0%~95.7%和93.9%~94.4%。CX分离物的IR与HZ分离物相比缺失了一段8 nt的片段。5′端非编码区的序列完全一致，但3′末端非编码区有一个碱基的差异。这些结果表明，RSV在自然界的分子变异与其地理分布具有密切的关系。此外，非编码区序列的高度保守性暗示着它们在病毒基因转录和复制的调控方面具有重要的功能。本文还讨论了RSV的分子流行病学。

关键词：水稻条纹病毒；序列分析；亲缘关系

中图分类号：S432.41 **文献识别码**：A **文章编号**：1003-5125 (2003) 04-0381-06

Comparison of the RNA2 Segments Between Chinese Isolates and Japanese Isolates of *Rice Stripe Virus*

WEI Tai-yun, LIN Han-xin, WU Zu-jian, LIN Qi-ying, XIE Lian-hui**

(*Institute of Plant Virology, Fujian Agriculture and Forestry University, Fuzhou* 350002, *China*)

Abstract: The complete nucleotide sequences of the RNA2 of two Chinese isolates of *Rice stripe virus* are determined. One is isolated from endemic sites at Chuxiong (CX), Yunnan Province, the other is isolated from outbreak sites at Hongze (HZ), Jiangsu Province. The total nucleotide sequences of the RNA2 of RSV CX and RSV HZ are 3506 bp and 3514 bp long, respectively. When compared with RNA2 of T and O isolates of Japan, we find that these four isolates could be divided into two groups. HZ, T and O isolates share 97.2%~98.0% and 96.8%~97.1% identities in vORF2 and vcORF2 at the nucleotide level, respectively and form one group. The sequences in 5′ and 3′ terminal non-encoding region are completely identical among these three isolates. In this group, HZ isolate is more closely related to T isolate than to O isolate. The length of intergenic region (IR) of HZ isolate is the same as those of T and O isolates. CX isolate belongs to another group, which shares only 95.0%~95.7% and 93.9%~94.4% sequences identities in vORF2 and vcORF2 between two groups at the nucleotide level, respectively. There is a deletion of 8 nt in length in the IR of CX isolate compared with HZ isolate. Even though no base variation occurred in 5′ terminal non-coding region, there is one base substitution in 3'

Received date: 2003-01-10
收稿日期：2003-01-10，修回日期：2003-05-07
* 基金项目：国家自然科学基金资助项目（30000002、30240017）；高等学校全国优秀博士学位论文作者专项资金资助项目（200150）
作者简介：魏太云（1975-），男，福建省福清人，博士研究生，主要研究水稻病毒的分子生物学。
** 通讯作者。Correspondence author.

terminal non-coding region between CX and HZ isolates. These results show that the isolates are grouped according to their geographical location. Additionally, highly consensus in 5′ and 3′ non-encoding region suggests that these regions play a very important role in transcription and replication of viral genome. Finally, the molecular epidemiology and gene functions of RSV are discussed in this paper.

Key words: *Rice stripe virus*(RSV); Sequences analysis; Evolutionary relationship

CLC number: S432.41 **Document code:** A **Article ID:** 1003-5125(2003)04-0381-06

Rice stripe disease caused by *Rice stripe virus* (RSV) had a trend of higher epidemiology in China in the last two years. This disease broken out in Jiangsu and Henan provinces, and also caused severe damage to rice production in Yunnan, Shandong, Liaoning and Hebei provinces.

RSV is the typical member of *Tenuivirus,* has a broad host range in the Gramineae[1]. RSV is transmitted by the small brown planthopper *Laodelphax striatellus* Fallen. The genome of RSV comprises four ssRNAs segments and the complete nucleotide sequences have been detected [1]. The results suggest - that RNA2, RNA3 and RNA4 segments has ambisense coding strategies except for RNA1 segment's negative nature [2]. The RSV viral-sense RNA2 (vRNA2) encodes a 22.8 kD protein (NS2), which is supposed to be related to virus cell-to-cell movement, and one large open reading frame (ORF) on the viral complementary-sense RNA2 (vcRNA2) encodes a 94.0 kD protein (NSvc2), which is suggested to be closely related to membrane glycoprotein [2~8].

Broad distribution and broad host range of RSV resulted into likely rich virus genetic diversity. Here we analyzed the sequences of RNA2 segment of two isolates of RSV, which were isolated from endemic sites at Chuxiong (CX), Yunnan Province, as well as from outbreak sites at Hongze (HZ), Jiangsu Province, in order to further discuss the molecular variability and evolution mechanisms of RSV.

1 Materials and Methods

1.1 Virus isolates

Diseased rice plants were collected from Chuxiong of Yunnan province in the southwestern China and Hongze of Jiangsu province in the eastern China in the fall of 2002, which are all maintained in a japonica rice variety (Hexi 28) by transmission via the viruliferous smaller brown planthopper, *L. striaterllus.* Infected rice leaves are stored in –70 ℃ and used for purification. The virus isolates are designated as CX and HZ isolates, respectively.

1.2 Total RNA isolation

The viral total RSV is extracted from diseased leaves as described previously [10].

1.3 RT-PCR

According to the sequences of T isolate [2], two pairs of primers are designed and synthesized to amplify CX and HZ isolates RNA2 sequences (Table 1), respectively. The location and orientation of primers are demonstrated in Fig.1.

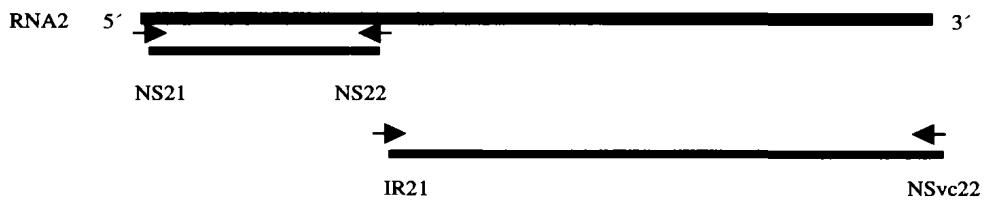

Fig.1 Cloning strategy to cover the complete sequence of RSV RNA2 by RT-PCR

Table 1 Primers used for amplification of RSV RNA2 segment

Primer	Position (nt)	Sequences (5′------ 3′)	T (℃)	Direction
NS21	1-21	5′ ACACAAAGTCCTGGGTAAAAT 3′	50	+
NS22	666-686	5′CCAAATTCACATTAGAATAGG 3′	50	-
IR21	641-660	5′TGTCTTGGTCGGAGCACATG 3′	61	+
NSvc22	3495-3514	5′ACACAAAGTCTGGGTATAAC 3′	61	-

cDNA first strand of RNA2 is synthesized from total RNA with MuLV reverse transcriptase (MBI), respectively. For the PCR, 2 μL of cDNA solution is amplified in a 50 μL reaction containing 5 μL of each primer (10 pmol/μL), 1 μL of each dNTP (10mmol/l), 3 μL MgCl2 (25mmol/l), 1 μL Taq DNA Polymerase (4 U/μL, MBI), 5 μL PCR × Buffer and 28 μL distilled water. the T (℃) of different fragments is indicated in table 1.

1.4 Sequence analysis

PCR products is separated on a 1% agrose gel and desired fragments are recovered by PCR Gel Extraction Kit (Qiagen), ligated into pMD18-T vector (TaKaRa), and then used to transformed *E.coli* DH5α competent cells. Subsequently, inserts are sequenced from both ends by the GeneCore Company (Shanghai, China). Sequence and phylogenetic analysis are performed using DNAMAN software (Lynnon BioSoft, 1994-1998).

2 Results

2.1 Amplification, cloning, and sequencing

Following RT-PCR, the amplification products are consistent with the expected sizes; the 5′ terminal fragment 600 bp, the 3′ terminal fragment 2800 bp, respectively (data not showed). The complete of CX and HZ isolates RNA2 are 3506 bp, 3514 bp long, respectively. The sequences are deposited in GenBank databases, the assigned accession number are listed in table 2.

Table 2 Organization of the corresponding fragments of RSV RNA2 of 4 isolates

	Total size (nts)	5′-UTR (nts)	vORF (nts)	(kD)	IR (nts)	vcORF (nts)	(kD)	3′-UTR (nts)	GenBank accession numbers
HZ	3514	80	600	22.8	299	2505	94.0	30	AY186789
CX	3506	80	600	22.8	291	2505	94.0	30	AY186790
T	3514	80	600	22.8	299	2505	94.0	30	D13176
O	3514	80	600	22.8	299	2505	94.0	30	D13787

2.2 Sequence analysis, phylogenetic relationship analysis

Comparison of the nucleotide and deduced amino acid sequences of RSV RNA2 with those of two Japanese isolates shows that, the most conserved region of RNA2 locates in 5′-untranslated region (UTR), containing 80 nucleotide acid, all four isolates are the same [2]. 3′ UTR terminal sequences of these four isolates are the same except for a base variation at the 26 nt position from the 3′- end of RNA2 of CX isolate. Highly consensus in 5′ and 3′ non-encoding region suggests that these regions playe a very important role in transcription and replication of viral genome.

NS2 genes of four isolates comprise 600 nucleotide acids and encode a protein of 199 amino acids. There are not different in size among all isolates, including T and O isolates of Japan. Bases substitution is the primary ways in NS2 gene variation. Sequence analysis indicates that these four isolates could be divided into two groups according to NS2 genes. HZ, T and O isolates share 97.2%~98.0% identities at the nucleotide level and 99.5%~100% identities at amino acid level, respectively, and form one group. CX isolate belongs to another group. Between two groups, there are only 95.0%~95.7% and 97.5%~98.0% identities at nucleotide and amino acid level, respectively (Table 3).

NSvc2 genes of four isolates comprise 2505 nucleotide acids and encode a protein of 834 amino acids. According to NSvc2 genes, sequence comparison reveals that these four isolates could also fall into two groups. The first group also comprises HZ, T and O isolates, which share 96.8%~97.1%

identities and 98.1%~99.2% identities in nucleotide and amino acid level, respectively. In this group, HZ isolate is more closely related to T isolate than to O isolate. CX isolate belongs to another group. Between two groups, there are only 93.9%~94.4% and 96.0%~97.1% identities in nucleotide and amino acid level, respectively (Table 3). These results show that these four isolates are grouped according to their geographical location.

Most of the nucleotide differences in the coding regions don't result in amino acid substitution. For the 361 nucleotide sites in NSvc2 genes variable between the four sequenced isolates, there are only 41 different in the amino acid sequences, a high proportion of the amino acid differences resides with N-terminal amino acid, and especially in CX isolate. The 63 nucleotides substitutions in NS2 genes between four isolates only result in five changes in the amino acid sequences, which mainly locate in C-terminal in CX isolate. This suggests that the amino acid compositions of NS2 protein are more conservative than those of NSvc2 protein.

The most variable region of the four isolates locates in intergenic region (IR), sequence comparison shows that IR sequences are more conserved (96.6%) among RSV from China, than when compared with those of RSV isolates from Japan (91.7%~94.3%) (Table 3), and there is a 8nt deletion in CX isolates compared to another three isolates. Computer-assisted folding analysis shows that distant hairpin structures could not be formed in RNA2 IR, while these kinds of distant hairpin structures could be formed in RNA3 and RNA4 IR of RSV.

Nucleotide distances (number of nucleotide differences per site) between pairs of sequences using the Kimura 2-parameter method are examined. The results show the nucleotide distance between the first group rangs from 0.020 to 0.028 for NS2 gene and from 0.023 to 0.032 for NSvc2 gene, while the nucleotide distance between two groups are 0.043-0.050 for NS2 gene and 0.056-0.061 for NSvc2 gene (Table 4). This suggests that the closer phylogenetic relationship between HZ and Japan two isolates than between CX and HZ isolates, even though both CX and HZ belong to Chinese isolates.

Two phylogenetic trees of tenuiviruses including RSV, *Rice hoja blanca virus* (RHBV)[6], *Maize stripe virus* (MSpV)[8] and *Rice grassy stunt virus* (RGSV)[7] are performed based on the NS2 and NSvc2 deduced amino acid sequences, respectively. The most likely trees for the two proteins are very similar both in branching pattern and branch lengths (Fig.2). The phylogenetic trees indicat that RGSV is a monophyletic group and apparently apart from the other tenuiviruses, they also appear that RSV and MSpV are evolutionarily more closely related to each other than to RHBV.

Table 3 Percentage identical nucleotides (upper right) and amino acids (in italics) in different genomic regions of RSV RNA2 segment

Isolates	CX	HZ	T	O
(a) NS2 gene				
CX	*	95.0%	95.2%	95.7%
HZ	*97.5%*	*	97.2%	98.0%
T	*98.0%*	*99.5%*	*	97.2%
O	*98.0%*	*99.5%*	*100%*	*
(b) RNA2 IR				
CX	*	96.6%	93.4%	91.7%
HZ		*	94.3%	94.3%
T			*	91.6%
O				*
(c) NSvc2 gene				
CX	*	93.9%	94.2%	94.4%
HZ	*96.0%*	*	97.1%	96.8%
T	*97.1%*	*98.7%*	*	97.1%
O	*97.0%*	*98.1%*	*99.2%*	*

Table 4 Genetic distance between RSV isolates

	HZ	T	O
(a) NS2 gene			
CX	0.050	0.048	0.043
HZ		0.028	0.020
T			0.028
(b)NSvc2 gene			
CX	0.061	0.058	0.056
HZ		0.023	0.032
T			0.029

3 Discussion

Sequence analysis results show that the most conserved regions between the four isolates are the 5′ and 3′ non-coding sequences but the major differences are in the intergenic regions, in two encoding regions, the conserved degree of NS2 protein is slightly higher than NSvc2 protein. The different conservation degree of different regions of RSV RNA2 maybe related to

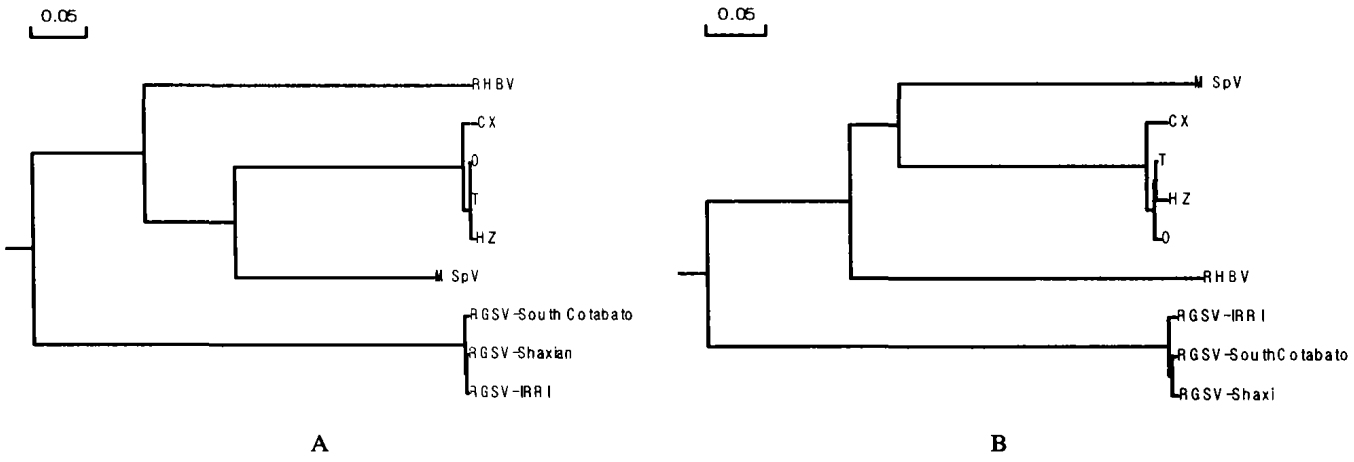

Fig.2 Phylogenetic trees of *Tenuivirus* isolates

A, Nucleotide sequences identities of NS2 gene; B, Nucleotide sequences identities of NSvc4 gene. The scale bar represents a istance of 0.05 per site.

the different function. High consensus in 5′ and 3′ non-encoding region suggests that these regions plays a very important role in transcription and replication of viral genome [10]; IR is rather unstable because of bases variation, which might serve as a transcription terminator during the synthesis of mRNAs from the ambisense segments[2]; NS2 protein is related to virus cell-to-cell movement[3~5], while NSvc2 protein is closely related to membrane glycoprotein, which maybe participate in the interactions between virus and insects vector[2, 6, 7]. These results suggest that different negative selection constraints imposed by different functional regions might have maintained different conserved regions of RSV RNA2.

According to the sequences identities and characteristics of base variation of NS2 and NSvc2 genes, these four isolates could all be divided into two groups. Isolate HZ from Jiangsu province, southeast of China, T and O isolates from Japan share high identities and form group Ⅰ. Another isolate comes from Chuxiong, Yunnan province, locates along southwest of China formed group Ⅱ. These results suggest that there is close relationship between RSV of Japan and RSV of HZ isolate. RSV was already reported in the early 1900 and popularized in Kanto district [11]. However, it was only in 1963 when Zhu *et al* firstly reported in southeast of China, so we suggest that RSV maybe originated in Kanto district of Japan and transmitted to adjacent China. As for how RSV is transmitted from Japan to China, it may be related to Asian monsoon and insect vectors. East China and Japan both belong to temperature and subtropical monsoon zone where prevails southeast wind in summer and northwest wind in winter. Even though the long-distant emigration of *Laodelphax striatellus* was not verified till nowadays, long-distance emigration still recurred to the monsoon according to the fact that a large number of *Laodelphax striatellus* were captured in the East Sea of China [12-13]. Since RSV is not reported in the provinces locating between southeast provinces and Yunnan (southwest of China), together with the fact that group Ⅰ and Ⅱ shows comparatively lower homology of sequences, we suggest that RSV of group Ⅱ was independently originated from Yunnan province.

The results also show that NS2 and NSvc2 proteins could be used to differentiate tenuiviruses at species or genus level. The phylogenetic relationship among three isolates of RGSV also indicates that, Shaxian isolate, from Fujian province, is closer to North Philippines isolate than to South Philippines isolate[14]. RGSV is transmitted by *Nilaparvata lugens*, which has the ability of long-distance migration. Therefore, the high identities between RGSV Shaxian and North Philippines isolates may be caused by long-distance migration of viruliferous insect from

Philippine to southeast coast of China. These data indicate that negative selection caused by NS2 and NSvc2 proteins and founder effects due to vector transmission are proposed to explain RSV and RGSV molecular variation observed.

References

[1] Ramirez BC, Haenni AL. Molecular biology of tenuiviruses, a remarkable group of plant viruses [J]. J GenVirol, 1994, 75 (3): 467~475

[2] Takahashi M, Toriyama S, Hamamatsu C, *et al*. Nucleotide sequence and possible ambisense coding strategy of rice stripe virus RNA segment 2 [J]. J Genl Virol, 1993, 74(4): 769~773

[3] 王晓红, 叶 寅, 王苏燕, 等. Wang X H, Ye Y, Wang S Y, *et al*. Clonging, sequence analysis and expression in prokaryote of vRNA2 ORF segment of rice stripe virus [J]. Chinese Science Bulletin（科学通报）, 1997, 42(4): 438~441 (in Chinese)

[4] Takahashi M, Toriyama S. Detection of the 22.8k protein encoded by RNA2 of rice stripe virus in infected plants [J]. Ann phytopathol Soc Jpn, 1996, 62(3): 340

[5] Takahashi M, Goto C, Matsuda I, *et al*. Expression of rice stripe virus 22.8k protein in insect cells [J]. Ann phytopathol Soc Jpn, 1999, 65(3): 337

[6] de Miranda J R, Hull R, Espinoza A M. Sequence of the pv2 gene of rice hoja blanca tenuivirus RNA-2 [J]. Virus Genes, 1995, 10(3): 205~209

[7] Toriyama S, Kimishima T, Takahashi M, *et al*. The complete nucleotide sequence of the rice grassy stunt virus genome and genomic comparisons with viruses of the genus Tenuivirus [J]. J Gen Virol, 1998, 79 (8): 2051~2058

[8] Estabrook EM, Suyenaga K, Tsai JH, *et al*. Maize stripe tenuivirus RNA2 transcripts in plant and insect hosts and analysis of pvc2, a protein similar to the Phlebovirus virion membrane glycoproteins [J]. Virus Genes, 1996, 12(3): 239~247

[9] 魏太云, 林含新, 吴祖建, 等. Wei T Y, Lin H X, Wu Z J, *et al*. Molecular variability of intergenic regions of Rice stripe virus RNA4 [J]. Chinese Journal of Virology(病毒学报), 2001, 17(2): 144~149 (in Chinese)

[10] Takahashi M, Toriyama S, Kikuchi Y, *et al*. Complementarity between the 5′- and 3′-terminal sequences of rice stripe virus RNAs [J]. J Gen Virol, 1990, 71(12): 2817~2821

[11] Kisimoto R, Yamada Y, Okada M, *et al*. Epidemiology of rice stripe disease [J]. Plant Protection (in Japan), 1985, 39(11): 531~537

[12] Kisimoto R. Synoptic weather conditions induring long-distance immigration of planthoppers, *Sogatella furcifera* Horvath and *Nilaparvata lugens* Stal [J]. Ecol Entomol, 1976, 1: 95

[13] Hoshizaki S. Allozyme polymorphism and geographic variation in the small brown planthopper, *Laodelphax striatellus* (*Homoptera*: *Delphacidae*) [J]. Biochemical Genetics, 1997,35(11-12): 383~393

[14] 林含新. Lin L M. Molecular biology of RNA1-3 of *Rice grassy stunt virus* [D]. Fuzhou: Fujian Agriculture and Forestry University. 2002 (in Chinese)福州：福建农林大学.