Section 5. Advances in Research

Chapter 3. Molecular genetics of major virus resistance in rice

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1. Introduction

Plant viruses that cause diseases in rice are often severe resulting in substantial reduction of plant vigor, yield, and product quality and they are difficult to control. Development of resistant cultivars is one of the most efficient strategies available to mitigate yield losses due to viruses. Traditional or conventional breeding techniques are effective, economical, and safe for humans, animals, and the environment. However, with the arrival of modern biotechnology, genetic engineering, which allows the transfer of useful genes into desirable cultivars to obtain transgenic lines for virus resistance, has surpassed conventional breeding in enhancing disease management and crop improvement.

The critical prerequisite to developing genetically engineered resistant plants is having knowledge about the genes to be utilized from either the pathogen, host, or vector sources. In pathogen-derived gene resistance, a full understanding of the functions of genes that confer the virus resistance and/or restrict feeding by the insect vector is very necessary. Recently, Takahide et al (2014) reviewed transgenic strategies specifically employing RNA interference (RNAi) and its recent progress as a tool to confer resistance against tenuiviruses and reoviruses infecting rice plants. On the other hand, due to the small genome sizes of the viruses and the low number of genes they encode, they rely heavily on host factors to perform their cycle, especially in different steps of viral infection that include replication, translation, and virus trafficking in the cells. For this reason, host-derived resistance is equally promising to naturally defend plants from virus attack.

Hibino (1996) described 15 viruses affecting rice. In this chapter, we cover 10 notable rice viruses due to their economic impact namely: Rice tungro bacilliform virus (RTBV); Rice tungro spherical virus (RTSV); Rice stripe virus (RSV); Rice hoja blanca virus (RHBV); Rice grassy stunt virus (RGSV); Rice dwarf virus (RDV); Rice black-streaked dwarf virus (RBSDV); Southern rice black-streaked dwarf virus (SRBSDV**);** Rice ragged stunt virus (RRSV); Rice yellow mottle virus (RYMV).

Here, we deal with the viruses' molecular genetics specifically their genome organization and currently reported evidence on the functions of specific viral genes. Also, this chapter focuses on genetics of virus resistance in rice drawn from current research efforts to genetically engineer rice to increase the crop's resistance to major rice viral diseases.

2. Rice tungro disease (RTD)

Rice tungro disease (RTD) is the most destructive viral disease in Southeast Asia. Two different viruses are associated with tungro disease: Rice tungro bacilliform virus (RTBV) and Rice tungro spherical virus (RTSV) (Hull 1996). RTBV is a plant pararetrovirus or DNA reverse-transcribing virus formerly under the genus *Badnavirus* and later reassigned to a new genus *Tungrovirus* that placed RTBV as a type species in the Caulimoviridae family. RTSV is a plant picornavirus that belongs to the genus *Sequivirus* originally in the Sequiviridae family; now it is classified in the Secoviridae family (Thompson 2014). RTSV assists in the semi-persistent transmission of RTBV, which causes symptoms (Hibino and

Cabauatan 1987). The genome of each virus is entirely different from one another. The nonenveloped bacilliform RTBV particles contain a circular double-stranded DNA genome and RTSV has a linear RNA genome (Jones et al 1991). The RTBV and RTSV genomes and biological functions of their specific genes were reviewed by Hull (1996).

What follows is a review of recent research contributions done for the further identification of gene functions and their involvement in the resistance studies.

2.1. Rice tungro bacilliform virus (RTBV)

2.1.1. Genome organization and function of RTBV-encoded proteins. MG Figure 1 and MG Table 1 show the RTBV genome structure and functions of RTBV-encoded proteins, respectively. The RTBV genome contains a single molecule of noncovalently closed circular double-stranded DNA of about 8.0 kbp with sites of two discontinuities, D1 and D2, on each strand of the genome resulting from the replication process by reverse transcription. The genome has four open reading frames (ORFs); ORFs 1, 2, 3, and 4 that encode proteins for P24, P12, P194, and P46, respectively. ORFs 1, 2, and 3 are closely packed while ORF4 is separated from ORF3 by a short intergenic region-noncoding region while there is a large intergenic region between ORFs 4 and 1. The large intergenic region has three components: pregenomic RNA promoter, polyadenylation signal, and a minus-strand primer-binding siteORFs 1 and 2 and ORFs 2 and 3 have overlapping stop codons of the ATGA type with ATG being the start codon of the downstream ORF and TGA being the stop codon of the downstream ORF.

ORF1 has a unique characteristic of not having an AUG start codon; instead translation of the ORF1 initiates at an ATT codon. This start codon is located downstream of a 690-nucleotide long leader sequence and is accessed by ribosomes in a very precise shunt mechanism that bypasses the ATG-rich central part of the leader and also allows a more efficient recognition of the ATT start codon than would occur with a normal scanning mechanism (Fűtterer et al 1996).

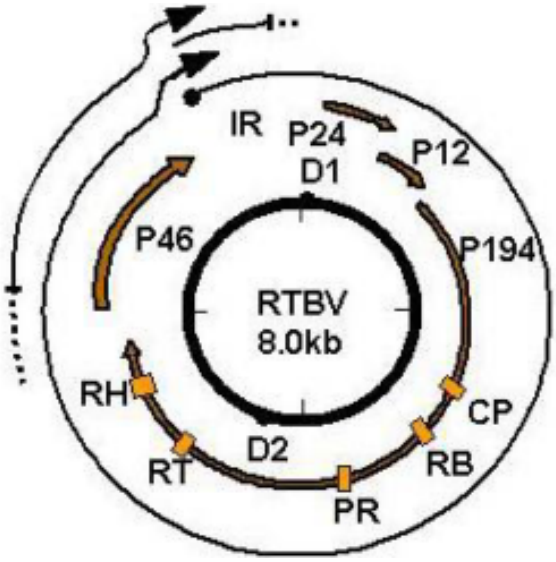
ORF2 is a virion-associated protein and suggests that P2 could participate in RTBV capsid assembly (Herzog et al 2000).

ORF3 contains a putative movement protein (MP), coat protein (CP), RNA binding site (RB), aspartate protease (PR), reverse transcriptase (RT), and Ribonuclease H (RH) activity. The C-terminal portion of the ORF3 encodes a polyprotein and carries reverse-transcriptase-RNAse H activity, a common feature of all pararetroviruses. The CP coded in this ORF is reported to have a variety of CP molecules of different sizes. Qu et al (1991) detected two major proteins with molecular masses of 37 and 33 kDa, although only the 37-kDa protein was detected in the infected rice tissues. Also, Kano et al (1992) reported 32 kDa CP in the predicted translational product. Hull (1996) suggested having two CPs of about 37 kDa and 33 kDa molecular mass. However, Marmey et al (1999) showed that RTBV virions contain a single protein of 37 kDa using matrix-assisted laser desorption/ ionization (MALDI) mass spectrometry. The single 37 kDa has been mapped from nucleotides 2427-3372 within ORF3. The second peptide (34 kDa), however, probably is a degradation product of the 37-kDa protein generated during virus purification. The processing of the CP on the other hand was completed by protease and further proved that the 37-kDa CP is dependent on the presence of the protease in the constructs (Marmey et al 2005). A polyprotein containing PR and RT was also encoded in ORF3 as mentioned by Qu et al (1991) in their study; however, it was Laco et al (1994, 1995) who characterized the PR/RT proteins of RTBV. They have determined the enzymatic activities of 87kDa that was processed to yield P62 and P55, which shared common amino termini and corresponds in molecular mass to that predicted PR/RT DNA sequence. The putative

PR and RT domains were expressed using recombinant baculovirus in insect cells. Results proved that there is an accumulation of polypeptides in processing RT, DNA polymerase, and RNAse H activities.

ORF4 is translated from subgenomic mRNA that is generated by splicing from pregenomic RNA; the splice donor is located at RNA position +100 and the splice acceptor is located at RNA position +6571. Hull (1996) in his review indicated that RTBV ORF4 has a sequence motif characteristic of a leucine zipper that, however, lacks the basic DNA-binding region usually associated with classical leucine zipper transcription factors. This ORF is found to have a similar genome position as ORF6 of CaMV that is known to have involvement as a translational activator, suggesting that P46 might be also be involved in some manner in the control of the expression of the RTBV genome.

Translations of RTBV ORFs were studied continuously by Fűtterer et al (1997) and strongly suggest that the translation of ORFs 1and 4 are synthesized by specialized mechanisms known as leaky scanning from pregenomic RTBV RNA, which is used as the template for viral replication and also serves as polycistronic mRNA. The region at the 5' end of the RTBV leader sequences, i.e, downstream of the transcription initiation site, was shown to have dramatic effect on reported gene expression in the protoplast. The core promoter contained there was identified within the 100 bases upstream of the transcription site (Chen et al 1994) and this region was further characterized and it was found that the sequence made up of the first 90 nt of the transcribed region (downstream promoter sequence) is sufficient to enhance RTBV upstream promoter activity (Chen et al 1996). Rice transcription factors RF2a and RF2b bind to Box II, a cis element adjacent to the TATA box and control gene expression from the promoter. Dai et al (2006) made mutations in the promoter to delete or mutate Box II and the mutated promoters were fused to a reporter gene. Results indicated that Box II and its interactions with cognate rice transcription factors, including RF2a and RF2b, are essential to the activity of the RTBV promoter and are probably involved in expression of the RTBV genome during virus replication.



MG Fig. 1. RTBV genome organization. The inner circle represents the double-stranded DNA genome of RTBV (8.0 kb) with sites of two discontinuities (D1 and D2). The arc shows positions of the ORFs with sizes of the proteins they encode in kDa. The outer circle and arc show the more than full length and spliced transcripts, respectively. Illustration source: Azzam and Chancellor (2002).

MG Table 1. Biological functions of RTBV encoded proteins.

ORF	Length (bp)	Protein (kDa)	Function	References
1	666	24	Associated with particle assembly supports nucleic acid binding	
2	332	12	Interacts with the coat protein (CP) domain of the viral gene III polyprotein; participates in RTBV capsid assembly	Herzog et al (2000)
3	5,027	194	Cell-to-cell movement protein (MP), coat proteins (CP), aspartate protease, and, most importantly, the replicase comprising reverse transcriptase and RNaseH activities	Hull (1996)
4	1,169	46	Involved in the control of RTBV expression	Hull (1996), Jacquot et al (1997)

2.1.2. Sources of resistance and molecular biology of RTBV and RTSV. Sta Cruz et al (2008) reviewed the natural and transgenic host resistance against tungro. Efforts to identify the sources of resistance to the two tungro viruses at the International Rice Research Institute (IRRI) can be utilized in the development of tungro resistance by using conventional breeding. Most of the identified sources of resistance were vector resistant (Khush 1989, Koganezawa 1998, Azzam and Chancellor 2002). The development of agroinoculation with an infectious RTBV clone enabled the assessment of resistance to the virus (Sta. Cruz 1999). Evaluated cultivars with tolerance to RTBV by agroincoulation were ARC11554, Balimau Putih UtriMerah, and Utri Rajapan. Utri Merah, an Indonesian cultivar, showed resistance to tungro (Azzam et al 2001, Cabunagan et al 1999). RTBV can still multiply from these tolerant cultivars but no symptoms are manifested. To understand the nature of RTD resistance, Encabo et al (2009) evaluated the reactions of near-isogenic lines developed from Utri Merah and susceptible Taichung Native 1 (TN1) against RTBV and RTSV. Results indicated that the RTD resistance of Utri Merah involves suppression of both RTSV and RTBV symptoms and further indicated that suppression is inherited separately. Using cultivars with resistance to both viruses is the ideal strategy to attain stable resistance to the disease.

There have been substantial research efforts to study the molecular biology of both RTBV and RTSV (Hull 1996) and to genetically engineer tungro resistance in rice. Azzam et al (1999) evaluated 71 transformed IR64, TN1 Taipei 309, and Kinuhikari rice plants with viral genes of RTBV and the coat protein 3 for their resistance against RTBV and RTSV by insect inoculation assay at the transgenic CL4 greenhouse facility at IRRI. These transgenic rice plants were developed at the laboratories of the Institute of Plant Sciences, ETH, Zurich, Switzerland and from the John Innes Centre (JIC), Norwich, England. Results of evaluation from the T1 and T2 generations showed that none of the 71 transgenic lines tested provided protection against tungro infection (Azzam et al 1999). Moreover, Sta Cruz et al. (2008) developed another set of transgenic lines at JIC using different viral genes to confer resistance. One of the genes that was found promising is the mutant RTBV RT and results showed tungro protection at varying levels.

2.1.3. Development of cultivar resistance against RTBV. Promising results on transgenic resistance against RTBV was obtained by transformation and expression of the coat protein (CP) gene of an Indian isolate of RTBV in indica rice cultivar Pusa Basmati-1. Rice plants containing the transgene integrated in low copy numbers were obtained in

which the CP was shown to accumulate in the leaf tissue. The progenies representing three independent transformation events were challenged with Indian isolates of RTBV using viruliferous green leafhoppers, and the viral titers in the inoculated plants were monitored using DNA dot-blot hybridization. As compared to nontransgenic controls, two independent transgenic lines showed significantly low levels of RTBV DNA, especially towards later stages of infection and a concomitant reduction of tungro symptoms (Ganesan et al 2009).

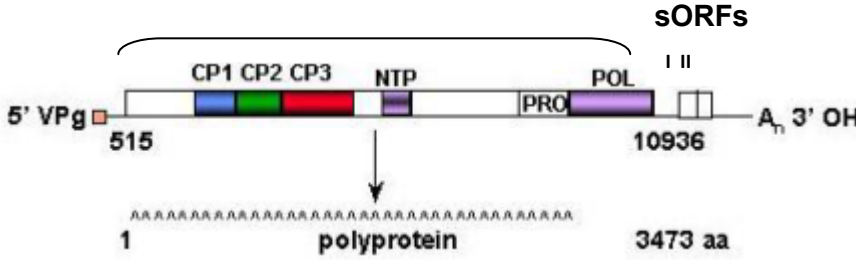
Tyagi et al (2008) conducted another strategy in obtaining transgenic resistance to RTBV through RNA-interference (RNAi). They did an expression of DNA encoding ORF4 of RTBV, both in sense and antisense orientation under the CaMV 35S promoter, resulting in the formation of double-stranded (ds) RNA. Two transgenic lines were generated, RTBV-O-Ds1 and RTBV-O-Ds2, which expressed ds-RNA, with different resistance responses against RTBV. RTBV-O-Ds1 showed an initial rapid buildup of RTBV levels following inoculation comparable to that of untransformed controls, followed by a sharp reduction, resulting in approximately 50-fold lower viral titers, whereas the untransformed controls maintained high levels of the virus until 40 days post-inoculation (dpi). In RTBV-O-Ds2, RTBV DNA levels gradually rose from an initial low to almost a 60% level of the control by 40 dpi. RTBV-O-Ds1 showed symptoms of tungro similar to the untransformed control lines whereas RTBV-O-Ds2 showed extremely mild symptoms.

Roy et al (2012) used RTBV-O-Ds2 as the resistance donor to two popular high-yielding, but tungro-susceptible rice cultivars from West Bengal, IET4094 (cvar Khitish) and IET4786 (cvar Satabdi), by backcross breeding. Progenies at BC_2F_1 showed mild tungro symptoms and near homozygosity was achieved until the BC_2F_3 stage, which suggests that the lines can be used as valuable resistance sources for further rice breeding against RTD (Roy et al 2012).

2.2. Rice tungro spherical virus (RTSV)

2.2.1. Genome and functions of RTSV-encoded proteins. The RTSV genome is composed of a monopartite positive sense single-stranded RNA genome with a size of about 12 kb. It contains one large ORF that encodes a large polyprotein (3,473 amino acid that starts after the 515 nucleotides leader sequence and has a suggested covalently protein (Vpg) at its 5' end and a poly (A) sequence at its 3'end. The polyprotein is processed by at least one virus-encoded protease (PRO) located adjacent to the C-terminal putative RNA polymerase (POL), which shows sequence similarity to viral serine-like proteases. The precursor polyprotein is presumed to be processed by a virus-encoded protease(s) to give several products including the three coat proteins (CPs) and putative proteins such as an NTP-binding protein, 3C-like protease and a polymerase. MG Figure 2 shows the positions of the three coat proteins (CP1, CP2, and CP3) and of the motifs for the nucleotide triphosphate-binding domain (NTP), protease (PRO), polymerase (POL), and at the extreme of the 3' end possibly two more short open-reading frames (sORFs). However, these sORFs have not been confirmed, yet although have been reviewed by Hull (1996) and Choi (2010**).** Thole and Hull (1998) studied the RTSV CPs from infected plants and demonstrated the proteolytic activity in the C-terminal region of the polyprotein. This protease rapidly cleaved its polyprotein precursors in vitro. Mutating a potential cleavage site located on the N-terminal to the protease domain, Gln2526-Asp2527, diminished processing. The transversion mutation at the putative C-terminal cleavage site of the protease, at Gln2852-Ala2853, led to a delayed and partial processing. Thole and Hull (2002) further demonstrated the proteolytic activities indicated that the substitution mutation of Asp2735 of the RTSV polyprotein had no effect on proteolysis. However, His 2680, Glu2717, Cys2811, and

His2830 proved to be essential for catalytic activity and could constitute the catalytic centre and/or substrate-binding pocket of the RTSV 3C-like protease. Sekiguchi et al (2005**)** speculated that a major internal cleavage site was in the C-terminal half. A point mutation was introduced at a potential major self-cleavage site (C2763). The mutation abolished the catalytic activity, suggesting that the mutation site is important for the recognition of the protease.



MG Fig. 2. The single-strand RNA genome of RTSV (12.2 kb) is shown as single-line coding regions as colored boxes. Illustration source: Azzam and Chancellor (2002).

2.2.2. RTSV genes for resistance. Sebastian et al (1996) first reported the GLH (green leafhopper) resistance gene. They indicated that a dominant gene(s) conferring resistance to both GLH and RTSV in rice cultivar ARC11554 is tightly linked and controlled by a dominant gene located within 5.5 cM of RFLP marker RZ262 on the short arm of rice chromosome 4. Also, Romero et al (2008**)**, showed significant association of the RTSV resistance flanked by markers RM8213 (4.44Mb) and RM3471 (6.31Mb) on chromosome 4, which had been consistent with the findings of Sebastian et al (1996). However, in contrast with these previous results, recent fine mapping of the GLH resistance locus in ARC11554 and the association of the ***tsv1*** gene on chromosome 7 with the RTSV was examined by Dela Cruz (2013). Results of this study showed that the two types of RTD resistance genes are distinguishable and were localized on different rice chromosomes, i.e., the single dominant GLH resistance gene reported in ARC11554, named ***Glh14***, was mapped with SSR markers within 4.04–4.45 Mb region of chromosome 4. On the other hand, the RTSV resistance was clearly associated with the ***tsv1*** gene on chromosome 7. The ***eIF4G^{tsv1}*** gene was also shown to be a promising candidate gene in finding rice plant's resistance to RTSV.

The F_3 and F_4 progenies carrying at least the ***tsv1*** gene demonstrated complete protection against RTSV, unlike the F_3 plants carrying GLH-resistance alone. Further mapping studies were done by Lee et al (2010) using rice cv. Utri Merah. They showed that resistance to RTSV in Utri Merah was controlled by a single recessive gene (***tsv1***) mapped within an approximately 200-kb region between 22.05 and 22.25 Mb of chromosome 7. On comparing the allele types of the putative translation initiation factor 4G (*eIF4G*) situated within the ***tsv1*** gene among various resistant and susceptible plants, they further found the association of RTSV resistance with one of the single nucleotide polymorphism (SNP) sites in the exon 9 of the gene. Examination of the SNP site in the *eIF4G^{tsv1}* gene among various rice plants resistant and susceptible to RTSV corroborated the association of SNP or deletions in codons for Val1060-1061 of the predicted *eIF4G^{tsv1}* with RTSV resistance in rice.

2.2.3. RTSV transgenic plants. Transgenic plants resistant to RTSV utilizing the viral component responsible for transmission of the tungro virus complex by GLH have been reported. Sivami et al (1999) reported the first study on pathogen-derived resistance to infection by RTSV. Transgenic plants were produced with CP genes *CP1*, *CP2,* and

CP3 of RTSV being incorporated and introduced individually or together to indica and/or japonica rice cells by particle bombardment. Moderate levels of protection to RTSV infection was achieved, ranging from 17 to 73% of the seedlings that escaped infection and a significant delay of virus replication under greenhouse conditions in plant lines that expressed the RTSV-*CP1, CP2,* and *CP3* genes singly or together. There was no additive effect on resistance when more than one CP gene was expressed.

Also, Huet et al (1999) developed transgenic japonica rice plants containing the RTSV replicase (Rep) gene in the sense or antisense orientation. Plants producing antisense sequences exhibited significant but moderate resistance to RTSV (60%). Accumulation of antisense RNA was substantial, indicating that the protection was not of the homology-dependent type. Plants expressing the full-length Rep gene, as well as a truncated Rep gene, in the (+)-sense orientation were 100% resistant to RTSV even when challenged with a high level of inoculum. Accumulation of viral RNA was low; leading to the conclusion that RTSV Rep-mediated resistance is not protein-mediated but is of the cosuppression type.

Verma et al (2012) developed another transgenic plant resistant to RTSV. Indica rice plants were transformed using DNA constructs designed to express an untranslatable sense or antisense RTSV RNA. Progeny of primary transformants showing low copies of the integrated transgenes and accumulating the corresponding transcripts at low levels were challenged with viruliferous GLH. Three out of four transgenic plant lines expressing untranslatable RTSV RNA in the sense orientation and two out of the four lines expressing an RTSV gene in the anti-sense orientation showed delayed buildup of RTSV RNA over time. All of these reported transgenic plants against RTSV were unable to assist transmission of RTBV, thus transmission of RTBV from the above lines was reduced significantly.

3. Rice diseases caused by tenuiviruses

There are two economically important rice viruses in Southeast and East Asia that belong to the ***Tenuivirus*** genus: rice stripe virus (RSV) and rice grassy stunt virus (RGSV). Another member of the same genus, which is also important is rice hoja blanca virus (RHBV) in the Western Hemisphere. RSV is predominant in temperate regions such as China, Korea, and Japan. Major outbreaks of RGSV have been recorded in tropical regions of the Philippines, Thailand, Indonesia, and Vietnam. RHBV epidemics have continuously occurred in Colombia and other regions in South and Central America. These viruses have single-stranded negative sense RNA (ssRNA) and are all known for their segmented genome with ambisense coding strategies. There is no assigned family yet to this genus but are tentatively grouped under *Bunyaviridae* family. *MG Table 2* shows the difference in sizes of the filamentous virion, transmitted by different species ofbrown planthopper and varying number of ssRNA segments in each virus.

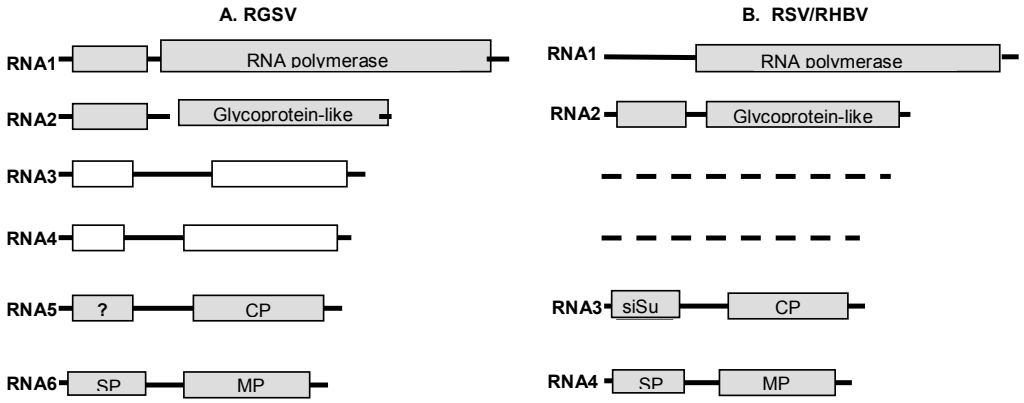
The type species of tenuiviruses is RSV while RHBV and RGSV are members along with others such as maize stripe virus and echinoclohoa blanca virus. The term

MG Table 2. Rice diseases caused by tenuiviruses.

Virus	Size (nm)	Brown planthopper vector	No. of segments
Rice stripe	100-1,200 nm in length	*Laodelphax striatellus*	4 ss RNAs
Rice hoja blanca	3 nm in width	*Sogatodes orizicola or Tagosodes orizicolus*	4 ssRNAs
Rice grassy stunt	200-2400 nm in length	*Nilaparvata lugens*	6 ssRNAs

"tenuivirus" is derived from the Latin word "tenui" meaning thread-like filaments. The major characteristics of the tenuiviruses are: (1) having circular-thin filamentous morphology of the virus particles; (2) transmission by insects; (3) abundant production of needle-like inclusions known as noncapsid protein (NCP) or disease-specific proteins (SP); and (4) composed of a segmented genome with ambisence coding strategy.

RSV consists of four ssRNA segments similar to RHBV but RGSV has six segments (MG Figure 3). The RSV-RNAs 3 and 4 are equivalent to RNAs 5 and 6 of RGSV, respectively. RNAs 3 and 4 of RGSV are considered unique segments among the tenuiviruses (Toriyama et al 1997, 1998). All six negative ssRNA segments are ambisense wherein each segment encodes two proteins at proximal 5' terminal ends of viral (v or p) and viral complimentary (vc or pC) strands. The intergenic noncoding region is highly variable among regions separating the ORFs of two proteins in the RGSV genome (Miranda et al 2000).



MG Fig. 3. Genome organizations of tenuiviruses: (A) RGSV; (B) RSV and RHBV. Each RNA segment in RGSV (shaded rectangle) has its corresponding RNA segment in RSV/RHBV. RGSV-RNA3 and 4 segments (unshaded rectangles) have no corresponding segments in RSV/RHBV presented by broken lines. RGSV-RNA segments 5 and 6 correspond to RNA segments 3 and 4 in RSV/RHBV. The question mark (?) in p5 of RGSV-RNA5 as to function as silencing suppressor similar to RSV has not yet been studied. CP: coat protein; siSu: silencing suppressor; SP: disease-specific protein; MP:-movement protein. The MP function of pC4 for RSV has been proven but not yet for RHBV.

3.1. Rice stripe virus (RSV)

3.1.1. Genome organization and gene functions. RSV is the prototype member of the genus ***Tenuivirus.*** It has a 17-Kb genome with a multicomponent RNA virus comprising four single-stranded RNA segments, designated as RNA1-4 in decreasing size (Toriyama and Watanabe 1989). For the four RNA segments of RSV, the RNA1 segment is the only one that does not follow an ambisense coding strategy (MG Figure 3B). MG Table 3 shows RSV-RNA segments and function(s) of each encoded protein. The 8.9-kb RNA1 is completely negative sense and contains a single ORF in the viral-complementary (vc) sense encoding the 336.8-kDa protein, the putative RNA-dependent RNA polymerase (RdRP) (Toriyama et al 1994). RNAs2-4 segments are all ambisense. The ambisense 3.5-kb RNA2 encodes a 22.8-kDa nonstructural protein (NS2) in the viral (v) sense and the 94-kDa NSvc2 protein in the viral complementary sense (Takahashi et al 1993). The 2.7-kb RNA3 encodes a 23.9-kDa nonstructural protein (NS3) in the v sense and was reported as suppressors of post-transcriptional gene silencing (PTGS; Hemmes et al 2007) while a 35.1-kDa CP encoded in the vc sense (Kakutani et al 1991). The 2.1-kb RNA4 encodes two nonstructural proteins, in-

cluding a 20.5-kDa disease-specific protein (SP) in the v sense (Kakutani et al 1990, Zhu et al 1992) and a 32.4-kDa NSvc4 in the vc sense, which encodes a movement protein (Xiong et al 2008).

MG Table 3. RSV RNA segments and function(s) of each encoded protein and their corresponding degree of resistance against RSV.

Segment/ Protein	dsRNA(bp)/ length (aa)	Protein size (kDa)	Function	Resistance[1]	Reference
S1/pC1	8970	337	RNA polymerase	Strong	Kakutani et al (1990)
S2/p2	3514	22.8	Unknown	Moderate	
S2/pC2		94	Glycoprotein-like	Absent	Zhu et al (1991)
S3/p3	2504	23.9	Silencing suppressor	Moderate	Xiong et al (2009)
S3pC3		35	Nucleocapsid protein	Immune	Takahashi et al (1993)
S4/p4	2157	21.5	Crystalline inclusion	Absent	Toriyama et al (1994)
S4/pC4		32.5	Movement protein	Immune	Xiong et al (2008)

[1] Degree of resistance against RSV infection in transgenic rice plants induced by different RNAi-targets of RSV genes. Immune, no symptoms developed, and no virus was detected by ELISA in inoculated rice plants through harvest; Strong, weak symptoms developed but were delayed for 2-4 weeks, but growth was almost the same as for mock-inoculated rice plants; Moderate, typical symptoms developed but were delayed 2-4 weeks, and growth was slightly stunted after RSV infection; Absent, typical symptoms developed, as severe as those of RSV-infected non-transgenic rice plants (Sasaya et al 2014).

3.1.2. Studies on the functions of RSV proteins. NS3 or p3 encoded by RSV segment 3 acts as a suppressor of RNA silencing in planta, possibly through sequestering siRNA molecules generated in cells that are undergoing gene silencing (Xiong et al 2009). As a suppressor, p3 can reverse the GFP silencing, can prevent long distance spread of silencing, and can significantly reduce the levels of small interfering RNAs (siRNAs) in silencing cells. Also, p3 was found to bind 21-nucleotide ss-siRNA, siRNA duplex and long ssRNA but not long double-stranded (ds)-RNA. Both the N and C terminals of the p3 protein are critical for silencing suppression and mutation of the putative nuclear localization signal decreases its local silencing suppression efficiency and blocks its systemic silencing suppression. NS3 was proven to be a pathogenicity determinant in the PVX heterogenous system.

NSvc4 or the pC4 protein of RSV functions as a cell-to-cell movement protein, long-distance movement and symptomatology. Using transcomplementation experiments and biolistic bombardment, Xiong et al (2008) first described pC4 (NSvc4) as being involved in cell-to-cell movement. This protein supports the intercellular trafficking of a movement-deficient potato virus X in leaves of *Nicotiana benthamiana* (Xiong et al 2008). It also enhanced green fluorescent protein (eGFP) fusion proteins that localize predominantly near or within the walls of onion and tobacco epidermal cells. In addition, the NSvc4:eGFP fusion protein can move from initially bombarded cells to neighboring cells in *Nicotiana benthamiana* leaves. Immuno-cytochemistry using tissue sections from RSV-infected rice leaves and an RSV NSvc4-specific antibody showed that the NSvc4 protein accumulated in walls of RSV-infected leaf cells.

Further studies were conducted simultaneously by two groups of researchers on mechanisms of RSV movement into the cell. Xu and Zhou (2012) provided evidence that the NSvc4 moves on the actin filament and endoplasmic reticulum network, but not the microtubules, to reach cell wall plasmodesmata (PD). Disruption of cytoskeleton using different inhibitors alters NSvc4 localization to the PD, thus impeding RSV infection of

Nicotiana benthamiana. Sequence analyses and the deletion mutagenesis experiment revealed that the N-terminal 125 amino acids (AAs) of the NSvc4 determine PD targeting and that a transmembrane domain spanning AAs 106–125 is critical for PD localization.

The pC4 proteins were also found in chloroplasts of infected cells. Analyses using deletion mutants revealed that the N-terminal 73 AAs are essential for chloroplast localization. Whereas, Zhang et al (2012) cloned the *pC4* gene into a movement-deficient tobacco mosaic virus (TMV) and showed that, aside from spreading cell to cell in *N. tabacum*, the hybrid TMV-pc4 also moved systemically and induced foliar necrosis. They also reported participation of *pC4* during RSV infection as demonstrated by systematic alanine-scanning mutagenesis study in its experimental host *N. benthamiana*. The *pC4* gene is substantially associated with cell-to-cell movement in the K122–D258 region. However, those mutants with replacements of KGR122–124, D135, ED170–171, ER201–202, EFE218–220, or ELD256–258 with ala-nine(s) no longer moved cell to cell. KGR122–124 was the only amino acid group linked with long-distance movement. The D17–K33 region was recognized as a crucial domain for leaf necrosis response and mutagenesis of DD17–18 or RK32–33 greatly attenuated necrosis.

3.1.3. Use of transgenic plants against RSV. RNAi is one of the effective strategies to control viral infection by suppressing viral genes. Zhou et al (2012) have prepared an RNAi construct containing coat protein (CP) gene and disease specific protein (SP) gene sequences encoded in pC3 and p4, respectively from RSV. The RNAi construct transformed the two japonica varieties, Suyunuo and Guanglingxiangjing, from susceptible to resistant against rice stripe disease. The homozygous progeny of rice plants in the T5 and T7 generations containing RNAi constructs, after self-fertilization, were strongly resistant to viral infection. RT-PCR indicated that viral replication of SP and CP in the transgenic plants was significantly inhibited. Shimizu et al (2011) also examined other coding genes in the RSV genome such as pC2, pC3, pC4, and p4 using transgenic rice plants that expressed a set of inverted-repeat (IR) constructs. Transgenic plants that harbored IR constructs specific for the pC3 and pC4 genes were immune to infection by RSV but not for pC2 and p4. Sasaya et al (2013) further supported these results. They also obtained a degree of immunity on those transgenic plants with RSV genes for pC3 and pC4, however, in their study they make use of RNAi trigger plasmids that would be transcribed into dsRNAs of the 5′-proximal regions of each gene in the RSV genome (MG Table 3). Near immunity of the transgenic plants with the RNAi trigger plasmids of RSV genes for pC3 and pC4 is due to the virus not being amplified in the transgenic rice plants through to harvest (around 4 months). Furthermore, morphology and growth, based on plant height, number of tillers, and rice grain yields, apparently did not differ between the transgenic and mock-inoculated nontransgenic rice plants. In contrast, the transgenic plants with the introduced pC2 or p4 gene did not have any resistance against RSV infection; symptoms developed at the same rate and severity as in the susceptible nontransgenic control plants. Transgenic rice plants with the p2 and p3 constructs exhibited moderate resistance against RSV infection; typical symptoms were induced but their appearance was delayed for 2 to 4 weeks and plant growth was moderately stunted by RSV infection (MG Table 3).

3.2. Rice hoja blanca virus (RHBV)

3.2.1. Genome organization and functions of RHBV-encoded proteins. This virus has an 18 Kb-genome comprising four species of RNA (Ramírez et al 1993, Ramirez and Haenni 1994). Similar to RSV, RHBV RNAs (MG Figure 3B) have different sizes totaling seven ORFs (Ramírez and Haenni 1994). The 9-kb RNA1 is generally of negative polarity, whereas the remaining RNAs 2 to 4 visible ssRNA species have an ambisense translation strategy.

Sequence analysis of RHBV RNAs has revealed a viral RNA polymerase ORF in RNA1 similar to RSV RNA1 (Toriyama et al 1994). Two ORFs encoding putative membrane-glycoproteins of unknown function are found in the ambisense RNA2 (de Miranda et al 1995, 1996). The ambisense RNA3, which encodes the viral nucleocapsid protein, has a size of 34 kDa and is encoded by the 5'-proximal region of the virion-complementary sense strand of RNA3 (de Miranda et al 1996). The nonstructural protein (NS3 or p3) was found accumulated in the cytoplasm together with the viral nucleocapsid protein but not in the viral inclusion bodies, vacuoles, or chloroplasts of RHBV-infected plants (Munoz et al 2004).

Recently, it was reported to function as a silencing suppressor protein (Hemmes et al 2007, 2009; Schnettler et al 2008; Yang et al 2011) similar to RSV. The virion-sense RNA4 molecule encodes a major nonstructural protein (also known as NCP or SP) of 17.5 kDa, which accumulates in RHB-infected rice plants whose function is still unclear. The function of other ORFs located in the ambisense RNA4 encode nonstructural protein (Ramírez et al 1993) still not studied. However, for RSV this protein was known to function as movement protein (Xiong et al 2008).

3.2.2. Studies on the functions of RHBV proteins. NS3 or p3 coded in a viral strand of RNA4 is also proven to function as an RNA silencing suppressor similar to RSV-p3 by a number of scientific groups (Hemes et al 2007, 2009; Schnettler et al 2008, 2009**;** Yang et al 2011). Each group of scientists worked on the NS3 protein and studied thoroughly the suppressing RNA silencing capacity in both plants and insect cells. Hemmes et al (2007) conducted biochemical analyses of p3 on its binding efficiency to siRNA as well as miRNA molecules. Binding of NS3 is greatly influenced by the size of small RNA molecules, as 21 nucleotide (nt) siRNA molecules are bound > 100 times more efficiently than 26 nt species. Competition assays suggest that the activity of NS3 is based on binding to siRNAs prior to strand separation during the assembly of the RNA-induced silencing complex. In addition, NS3 has a high affinity for miRNA/miRNA duplexes, indicating that its activity might also interfere with miRNA-regulated gene expression in both insects and plants. On the other hand, Schnettler et al (2008) showed activity of NS3 in mammalian cells using a firefly luciferase-based silencing assay. This activity is independent of the inducer molecule used. Using either synthetic siRNAs or a short hairpin RNA construct, NS3 was able to significantly suppress the RNAi-mediated silencing of luciferase expression in both monkey (Vero) and human (HEK293) cells. These results support the proposed mode of action of NS3 to act by sequestering siRNAs, the key molecules of the RNAi pathway conserved in all eukaryotes.

The controversy behind RNAi as a natural defense mechanism in mammalian cells was addressed in the study by Schnettler et al (2009). The miRNAs might also regulate components of the antiviral interferon (IFN) pathway and thus provide a possible link between the RNAi and IFN pathways. Also, a number of mammalian viruses have been show to encode an RNA silencing suppressor (RSS) protein. Thus in their study, Schnettler et al (2009) have shown that plant viral RSS lacks IFN antagonistic activity, yet it is able to substitute the RSS function of the Tat protein of human immunodeficiency virus type 1. An NS3 mutant that is deficient in RNA binding and its associated RSS activity is inactive in this complementation assay. This cross-kingdom suppression of RNAi in mammalian cells by a plant viral RSS indicates the significance of the antiviral RNAi response in mammalian cells and the usefulness of well-defined RSS proteins.

Further study by Hemmes et al (2009) in determining whether this siRNA binding property is the critical determinant for the suppressor activity of NS3. NS3 was altered by alanine point mutations and the resulting mutant proteins were tested for both siRNA binding ability and RNAi suppressor activity in plants. Alanine substitutions of lysine residues at positions 173–175 resulted in mutant proteins that lost both their affinity for

siRNAs and their RNAi suppressor activity in planta. This indicates that siRNA binding of NS3 is indeed essential for the suppressor function of NS3 and that residues at positions 173–175 are involved in the siRNA binding and suppressor activities.

Another strategy to confirm activity of NS3 as a suppressor protein was done by Yang et al (2011) using the crystal structure of the N-terminal domain of RHBV NS3 (residues 21–114) at 2.0 A˚. The RHBV NS3 N-terminal domain forms a dimer by two pairs of α-helices in an anti-parallel mode, with one surface harboring a shallow groove at the dimension of 20 A x 30 A˚ for putative dsRNA binding. In vitro RNA binding assay and RNA silencing suppression assay have demonstrated that the structural conserved residues located along this shallow groove, such as Arg50, His51, Lys77, and His85, participate in dsRNA binding and RNA silencing suppression. These results provide the initial structural implications in understanding the RNAi suppression mechanism by RHBV NS3.

3.2.3. Transgenic plants against RHBV. Lentini et al (2003) first reported the transgenic resistance to RHBV and the transformation of an indica rice variety from Latin America. Rice transformed with the RHBV nucleocapsid (N) protein gene had a significant reduction in disease development characterized by the production of local lesions like a hypersensitive reaction or a recovery phenotype with the emergence of symptomless new leaves. Several reactions were observed that ranged from susceptible to completely resistant plants (immunity). The resistant reactions were characterized by the production of local lesions like a hypersensitive reaction or a recovery phenotype with the emergence of symptomless new leaves. These transgenic RHBV-resistant rice lines expressed the N gene RNA at low levels that were below the detection limit by Northern blots and only resolved by RT-PCR. The nucleocapsid protein could not be detected in any of the transgenic plants either by Western or ELISA tests.

These results suggest that the resistance encoded by the N gene in these plants appears to be mediated by RNA. When challenged with RHBV, the resistant transgenic lines showed a significant increased performance for important agronomic traits including the number of tillers, the number of grains per plant and the yield as compared to the susceptible control. Furthermore, upon inoculation some of the most-resistant transgenic lines showed agronomic traits similar to the uninoculated nontransgenic Cica 8 control. Using both agronomic traits and disease severity as criteria, several of the most resistant lines were followed through the R_4 generation and demonstrated that the N gene and RHBV resistance was inherited in a stable manner.

3.3. Rice grassy stunt virus (RGSV)

3.3.1. Genome organization and functions of RGSV-encoded proteins. RGSV has a 25-Kb genome consisting of six ambisense segments (MG Figure 3A) and 12 proteins (MG Table 4). RNA1 (9.8kb) encodes p1 at vRNA1 with an unknown function and pC1 an RNA dependent RNA polymerase (RdRp,) encoded at vcRNA1; RNA2 (4.1Kb) has p2 with an unknown function and pC2 a membrane protein (Estabrook et al 1996). The function of proteins encoded in both RNA3 (3.1kb) and RNA4 (2.9kb) is still unknown; p5 encoded in RNA5 (2.7 kb) is similar to p3 of RSV (Xiong et al 2009) and RHBV (Bucher et al 2003), which functions as a silencing suppressor, but no direct evidence yet for RGSV. Another protein codes for pC5, a nucleocapsid (N) protein (Toriyama et al 1997). The two proteins encoded in RNA6 (2.6 kb), p6 and pC6—p6 being a noncapsid protein (NCP) that is abundant in the host plants (Miranda et al 1995) and with an unknown function and pC6 for cell-to-cell movement (Hiraguri et al 2011, Shimizu et al 2013)—are similar to RSV pC4 (Xiong et al 2008**)**. Moreover, pC6, a nonstructural protein encoded by RGSV-RNA6, is thought to

correspond functionally to the nonstructural protein pC4 of RSV-RNA4, which can support viral cell-to-cell movement (Hiraguri et al 2011).

3.3.2. Transgenic plants against RGSV. One of the most promising methods to confer resistance to RGSV is the use of RNA interference (RNAi). Shimizu et al (2013) transformed rice plants by introducing an RNAi construct of the RGSV genes for the nucelocapsid protein pC5 or movement protein pC6 (MG Table 4). All progenies from self-fertilized transgenic plants had strong resistance to RGSV infection and did not allow the proliferation of RGSV. Similar findings obtained from previous RNAi experiment with RSV, another tenuivirus (Shimizu et al 2011), revealed that the genes for nucleocapsid and movement proteins were appropriate targets for RNAi to confer resistance against RSV. Thus, the use of the RNA interference (RNAi) strategy to target genes for nucleocapsid and movement proteins for conferring viral resistance might be applicable to the plant viruses in the genus ***Tenuivirus***.

MG Table 4. RGSV RNA segments and function(s) of each encoded protein and their corresponding degree of resistance against RGSV.1

Segment/ protein	dsRNA (bp)	Protein size (kDa)	Protein-function	Resistance[2]	Function reference
S1/p1	9760	19			
S1/pC1		339	RNA polymerase		Toriyama (1987)
S2/p2	4069	23	Unknown		
S2/pC2		94	Glycoprotein-like		Toriyama et al (1998), Estabrook (1996)
S3/p3	3127	22	Unknown		
S3pC3		31	Unknown		
S4/p4	2909	19	Unknown		
S4/pC4		60	Unknown		
S5/p5	2704	22	Unknown		
S5/pC5		36	Nucleocapsid protein	Strong	
S6/p6	2590	21	Crystalline inclusion		Miranda et al (1995)
S6/pC6		36	Movement protein	Strong	Hiraguri et al (2011)

[1] South Cotabato-Philippine isolate, Miranda et al (2000).

[2] Shimizu et al (2013).

4. Rice virus diseases associated with the Reoviridae family

There are three known viruses belonging to three genera that infect rice are members of the *Reoviridae* family, namely *Phytoreovirus, Fijivirus*, and *Oryzavirus* (Attoui et al 2012). These genera are different on the basis of virion properties, genome organization, antigenic properties, and insect vector specificity (*MG Table 5*). In genus *Phytoreovirus*, three rice viruses have been identified: Rice dwarf virus (RDV), Rice gall dwarf virus (RGDV), and rice bunchy top virus (RBSV) and all contain 12 dsRNA genome segments. Two rice viruses of the *Fijivirus* genus, Rice black-streaked dwarf virus (RBSDV) and RBSDV2 or southern rice black-streaked dwarf virus (SRBSDV). Genus *Oryzavirus* includes rice ragged stunt virus (RRSV). Both *Fijivirus* and *Oryzavirus* contain 10 dsRNA genome segments (MG Table 5).

MG Table 5. Rice viruses in Family Reoviridiae.

Genera	Member	Isometric virus size diameter (nm)	Vector	Genome segments
Phytoreovirus	Rice dwarf virus	70	*Nephotettix cincticeps*	12
	Rice gall dwarf virus	65	*N. cincticeps, N. nigropictus, Recilia dorsalis*	12
	Rice bunchy stunt virus	60	*Nephotettix cincticeps N. virescens*	12
Fijivirus	Rice black-streaked dwarf virus (RBSDV)	80	*Laodelphax striatellus*	10
	RBSDV2 / Southern Rice black-streaked dwarf virus		*Sogatella furcifera*	
Oryzavirus	Rice ragged stunt virus	50	*Nilaparvata lugens*	10

4.1. Genus *Phytoreovirus*: Rice dwarf virus (RDV)

4.1.1. Genome organization and gene functions. The RDV genome consists of 12 dsRNA segments designated as S1 through S12. RDV encodes seven structural proteins, P1, P2, P3, P5, P7, P8, and P9 encoded in S1, S2, S3, S5, S7, S8, and S9, respectively, and five nonstructural proteins, Pns4, Pns 6, Pns10, Pns11, and Pns12 encoded in S4, S6, S10, S11, and S12 (MG Table 6). Among the structural proteins, P2, P8, and P9 are associated in the outer shell of RDV capsid while the P1, P5, and P7 proteins are enclosed by the P3 core capsid protein. The P3 core and P8 major outer capsid proteins have the ability to form double-shelled particles both in vivo and in vitro, thus constitute the major framework of the architecture of RDV. P7, a nucleic acid-binding protein, expresses in a similar manner and is incorporated into the virus-like particles that also binds to P3 and is an important contributor to the conformation of RDV particles (Ueda and Uyeda 1997**,** Hagiwara et al 2003)**.**

The information of RDV gene products and their potential functions (MG Table 6) has been obtained from their sequence, cell suspension transfection, in vitro studies, and using antisera to expressed proteins. Although the detailed functions of most of the gene products are not known, accumulating information should lead to understanding them.

4.1.2. Evidence on the functions of RDV proteins. The P2 protein is one of the proteins essential for infection by the virus of vector cells and, thus, it influences transmissibility by vector insects (Uyeda et al 1994, Yan et al 1996). This was further elucidated by Tomaru et al (1997) using two RDV isolates: TC (transmission-competent) and TD (transmission-defective) where P2 is found to be present and absent, respectively. Transmission studies show that TD lacks the ability to infect cells. Genome segment S2 and its transcript were detected in both isolates, however, sequence analysis of the S2 segment of the TD isolate revealed the presence of a termination codon due to a point mutation in the open reading frame, which might explain the absence of P2 in the TD isolate. This study was further supported by Omura et al (1998) where they let insects acquire RDV particles that lack the P2 protein through membrane feeding and found that P2 is unable to attach and infect cells. However, when intact virus particles were physically introduced into the hemolymph of insects by injection via a glass capillary tube, they successfully infected the insects, which became able to transmit the virus. This ability of intact particles of RDV to be adsorbed and to enter monolayer-cultured cells and further

MG Table 6. RDV RNA segments and function(s) of each encoded protein and their corresponding degree of resistance against RDV.

Segment/ protein	dsRNA (bp)	Protein aa/kDa	Function	Resistance[1]	Reference(s)
S1/P1	4423	1444/164	RNA Polymerase	Strong	Xiao et al (1998) Uyeda et al (1994), Yan et al (1996), Omura et al (1998), Tomaru et al (1997)
S2/P2	3512	1116/123	Minor outer capsid required for adsorption of the virus to host insect cells essential for RDV infection of the insect cell; interact with ent-kaurene oxidases for biosynthesis of plant growth hormones gibberellins in infected plants	Absent	Zhu et al (2005)
S3/P3	3195	1019/114	Major inner core protein involve in stabilizing the core particles.	Moderate	Hagiwara et al (2004)
S4/Pns4	2468	727/83	Nonstructural formed bundles of minitubules, might be involved in the process of assembly of the RDV virion	Strong	Suzuki (1993) Wei et al (2006c)
S5/P5	2570	801/91	Guanyltransferase /NTP binding (capping enzyme)	Absent	Suzuki et al (1996)
S6/Pns6	1699	509/56	Component protein of viroplasm Viral cell to cell movement protein	Immune	Suzuki (1993), Li et al (2004)
S7/P7	1696	506/58	Nucleic acid binding protein	Absent	Suzuki N (1993)
S8/P8	1427	421/46	Major outer capsid protein; interact with glycolate oxidase (GOX) important roles in RDV targeting into the replication site of host cells.	Immune	-Suzuki and Sugawara (1991), Zhou et al (2007a)
S9/Pns9	1305	351/49	Nonstructural/outer capsid protein	Absent	Suzuki (1993), Zhong et al (2003)
S10/Pns10	1321	353/35	Nonstructural exhibits RNA silencing suppressor Formation of tubular structures	Absent	Suzuki (1993), Cao et al (2005) Wei et al (2006a)

MG Table 6. Continued.

Segment/ protein	dsRNA (bp)	Protein aa/kDa	Function	Resistance[1]	Reference(s)
S11/Pns11	1067	181	Nonstructural/ nucleic acid binding protein	Strong	Xu et al (1998)
S12/Pns12	1066	312	Nonstructural Cytoplasmic inclusion (viroplasm associated protein) involved in virion assembly Potential target for RNAi	Immune	Suzuki et al (1992) Wei et al (2006b) Shimizu et al (2009)

[1] Degree of resistance against RDV in transgenic rice plants induced by different RNAi-targets of RDV genes (Sasaya et al 2014). Immune, no symptoms developed, and no virus was detected by ELISA in inoculated rice plants through harvest; Strong, weak symptoms developed but were delayed for 2–4 weeks, but growth was almost the same as for mock-inoculated rice plants; Moderate, typical symptoms developed but were delayed 2–4 weeks, and growth was slightly stunted after RDV infection; Absent, typical symptoms developed, as severe as those of RDV-infected nontransgenic rice plants.

multiply within the cells of the insect vector *Nephotettix cincticeps* indicates that P2 is essential for RDV infection of insects and thus influences the transmission of RDV by the insect vector**.**

Zhou et al (2007a) further investigated the role of P2 as a plant viral protein that has membrane fusion-inducing activities. They indicated that the N-terminal and HR regions were critical for inducing membrane fusion. P2 caused syncytium formation when the N-terminal hydrophobic peptide, two heptad repeat (HR) sequences, and a transmembrane region, features of P2 (type-I fusion proteins of enveloped viruses), are ectopically expressed and displayed on the surface of insect cells. These findings establish that RDV P2 is essential in studying the mechanisms of cell entry by nonenveloped viruses and further in comparative studies on the common and distinct mechanisms that the enveloped and nonenveloped viruses have evolved to enter host cells for multiplication.

Moreover, P2 was found to have an effect on expression of ent-kaurene oxidases in the rice host during RDV (Zhu et al 2005). The enzyme ent-kaurene oxidase is required during the biosynthesis of gibberellic acid (GA). The major active GA in rice vegetative tissues was significantly reduced in rice plants infected with RDV resulting to dwarf symptoms. However when supplying infected plants with GA3, the dwarf phenotype in infected rice plants was partially restored. These findings suggest that the P2 protein interacts in vivo with an enzyme in the GA biosynthesis pathway, leading to diminished accumulation of GA and to the dwarf phenotype exhibited by RDV-infected rice plants.

P3 is one of the outer capsid proteins that constitutes the major framework of the RDV architecture. Serial deletions of the amino-terminal sequence region, expressed in a baculovirus system, formed particles with gradually decreasing stability. These results demonstrated that insertion of the amino-terminal arm of one P3 protein into another appears to play an important role in stabilizing the core particles (Hagiwara et al 2004). P3 outer capsid proteins of phytoreoviruses were also suggested to possibly play a role in the transmembrane transport of viral particles in insect-vector cells.

Pns4 was detected at the periphery of the viroplasm and it was then observed on amorphous or fibrillar inclusions, which were identified as bundles of minitubules at later stages of infection (Wei et al 2006c). The Pns4-specific RNAi construct was introduced into rice plants. The progeny of these plants with Pns4-specific RNAi constructs showed strong

resistance. A delay of symptoms appeared in some plants of each line (Shimizu et al 2009, Sasaya et al 2014).

The Pns6 protein is associated with cell-to-cell movement. Li et al (2004) showed this when they cobombarded plasmids containing an infectious clone of Potato virus X (PVX) defective in cell-to-cell movement and expressing either β-glucuronidase or green fluorescent protein (GFP) with plasmids containing ORFs from RDV gene segments S1 through S12 onto leaves of *Nicotiana benthamiana*. Results showed that movement-defective PVX was restored by cobombardment with a plasmid containing S6 and in the absence of S6, movement was not supported. Furthermore, a mutant S6 lacking the translation start codon did not complement the cell-to-cell movement of the movement-defective PVX. By immunocytochemistry, Pns6-enhanced GFP fusion was observed near or within the cell walls of epidermal cells from *N. tabacum* while unfused Pns6 was localized to plasmodesmata in rice leaves infected with RDV. This indicates that P6 is a movement protein (MP) and therefore is likely required for the cell-to-cell movement of RDV. On the other hand, Ji et al (2011) reported that Pns6 binds both dsRNAs and ssRNAs but Pn6 shows preference for ssRNA sequences derived from the conserved genomic 59- and 39-terminal consensus sequences of RDV. Furthermore, Pns6 exhibits magnesium-dependent ATPase activities. Mutagenesis identified the RNA binding and ATPase activity sites of Pns6 at the N- and C-termini, respectively. This finding will help establish a biochemical basis to enable further studies on the mechanisms of dsRNA viral MP functions.

P7 core protein binds specifically and with high affinity to all 12 genomic RDV dsRNAs. P1, P5, and P7 are encapsidated within the virion and also bind viral transcripts based upon in vitro binding assays. P1, P5, P7, and genomic dsRNAs were lacking empty particles purified from infected tissues that also yielded fractions containing intact, infectious particles. In addition, P7 forms complexes with P1 and P3, a core capsid protein, in viral particles. These results indicate the possibility that core proteins and dsRNAs interact as one unit, which suggests a mechanism for an assortment of viral RNAs and subsequent packaging into core particles (Boxiong et al 2004). Moreover, Nakagawa et al (2003) further support this assembly with a model consisting of P3 inner capsid proteins, P8 outer capsid proteins, and fragments of P7, the nucleic acid-binding protein. They obtained the atomic structure of RDV, determined at 3.5 A˚ resolution by x-ray crystallography. The atomic structure suggests a self-assembly mechanism for both homologous and heterologous capsid proteins.

P8, identified as an outer-capsid, and P3, identified as an inner-capsid, were together involved in packaging of the viral genome and the enzymes required for its transcription. P8 proteins were also found to mediate the secretion of assembled virus-like particles from S. *frugiperda* insect cells and, therefore, most probably also from insect-vector cells (Miyazaki et al 2010). Suzuki and Sugawara (1991**)** also found P8 to interact with host factors, such as GOX, 40 kDa, by immunoprecipitation assays and confocal immunofluorescence microscopy (Zhou et al 2007b). Further investigations indicated that P8 is colocalized with GOX in the peroxisomes of insect cells. GOX may target P8 into peroxisomes when GOX was coexpressed with P8. This interaction between P8 and GOX may play an important role in RDV targeting into the replication site of host cells. In addition, P8 was found to interact with P3 and that these two proteins provide the structural integrity required for the formation of VLPs in rice cells, independent of other structural proteins and nonstructural or viral genomic double-stranded RNAs (Zheng et al 2000).

Cao et al (2005) reported Pns10 to have RNA silencing suppressor (RSS) activity exhibited in coinfiltration assays with the reporter green fluorescent protein (GFP) in transgenic *Nicotiana benthamiana* line 16c carrying GFP. They indicated that Pns10

suppressed local and systemic silencing induced by sense RNA but did not interfere with local and systemic silencing induced by dsRNA. Also, expression of Pns10 increased the expression of β-glucuronidase in transient assays and enhanced ***Potato virus X*** pathogenicity in *N. benthamiana*. This further suggests that Pns10 targets an upstream step of dsRNA formation in the RNA silencing pathway. Another function of Pns10 was reported by Wei el al (2006a) involving in cell-to-cell movement but in another mechanism by formation of tubular structures approximately 85 nm in diameter. These tubular structures were found to be composed of the nonstructural viral protein Pns10 and contained viral particles. Moreover, these tubular structures, when associated with actin-based filopodia, were able to protrude from the surface of cells and to penetrate neighboring cells.

Xu et al (1998) suggested that Pns11 has a binding activity that is essential in virus replication and/or genome assortment. They showed that Pns11 interacts with single- and double-stranded forms of DNA and RNA in a sequence-nonspecific manner. The truncated derivative, which contains both the zinc-finger and the C-terminal basic regions, has the same binding properties as the full-length Pns11. However, removal of either of these domains prevents the binding activity. The binding activity of Pns11 was drastically reduced when the blots were treated with a high concentration of EDTA. Moreover, Pns11 extracted from infected rice also binds to single-stranded RNA.

Pns12 has the intrinsic ability to form viral inclusions-like structures or viroplasm (putative site of viral replication) when expressed in a baculovirus system and no similar inclusion in nonhost insect cells upon expression of Pns12 (Wei et al 2006b). However, when expression of this protein was inhibited by introducing RNAi constructs targeting Pns12 into rice plants, there was strong resistance to viral infection. These results suggest that interference with the expression of a protein that is critical for viral replication, such as the viroplasm matrix protein Pns12, might be a practical and effective way to control viral infection in crop plants (Shimizu et al 2009).

4.1.3. Transgenic plants against RDV. Several studies on the development of transgenic rice plants were conducted in efforts to control RDV. Having ideas on some of the functions of RDV genes (MG Table 6), Sasaya et al (2014) analyzed each RDV-coding gene as a potential target sequence for RNAi constructs. They reported that the transgenic plants that harbored the RNAi constructs, which targeted the genes for Pns6, P8, or Pns12, were completely resistant (immune) against the RDV infection. This suggests that these proteins are key components at the early stages of viral proliferation. On the other hand, transgenic plants, which harbored the RNAi construct specific for the genes for P2 and Pns10, were susceptible to RDV (MG Table 4). This further supports the study of Pu et al (2011), in which RDV is maintained in rice plants for a long period, that nonsense mutations gradually accumulate in RDV segments 2 and 10, resulting in a decrease in the expression of these proteins and complete loss of insect-transmissibility. They further indicated that these proteins might not be essential for viral proliferation in rice plants but instead function in the insect vectors. Thus, transgenic plants with the introduced RNAi constructs, targeting the RDV genes for P2 and Pns10, seem not to have induced resistance against RDV infection. In addition, Sasaya et al (2014) concluded that not all RNAi constructs against RDV genes are equally effective in preventing viral infection. The genes for the major outer capsid (P8), viroplasm associated proteins (Pns12), and viral movement protein (P6) can be considered the "Achilles' heel" of the rice-infecting reoviruses, which can be targeted for a RNAi attack for engineering resistance in plants (MG Table 6).

4.1.4. Natural resistance to RDV. Yoshii et al (2009) investigated the host factors involved in the multiplication of RDV. They screened Tos17 insertion mutant lines of rice for

mutants with reduced susceptibility to RDV and identified one mutant, designated rim1-1, which did not show typical disease symptoms upon infection with RDV. The accumulation of RDV capsid proteins was also drastically reduced in inoculated rim1-1 mutant plants. Cosegregation and complementation analyses revealed that the rim1-1 mutation had been caused by insertion of Tos17 in an intron of a novel NAC gene. The rim1-1 mutant remained susceptible to the two other viruses tested, one of which is also transmitted by leafhoppers, suggesting that the multiplication, rather than transmission of RDV, is specifically impaired in this mutant. They have further proposed that RIM1 functions as a host factor that is required for multiplication of RDV in rice.

4.2. Genus *Fijivirus*: Rice black-streaked dwarf virus (RBSDV) and Southern rice black-streaked dwarf virus (SRBSDV)

RBSDV occurs mainly in China, Japan, and Korea. It is a member of the genus *Fijivirus* within the family *Reoviridae*. The virus is transmitted naturally to rice, maize, barley, and wheat in a persistent propagative manner by three different species of planthopper, ***L****aodelphax striatellus, Unkanodes sapporona,* and *U. albifascia*.

SRBSDV is a novel species in the genus *Fijivirus* (family *Reoviridae*) first recognized in 2008. Rice plants infected with this virus exhibit symptoms similar to those caused by RBSDV. Since 2009, the virus has rapidly spread and caused serious rice losses in East and Southeast Asia (Zhou et al 2013). SRBDV can be transmitted efficiently by the white-backed planthopper, WBPH (*Sogatella furcifera*, Hemiptera: *Delphacidae*).

Both RBSDV and SRBSDV have a similar genome structure with an approximate total genome of 29 kb containing 10 segments of dsRNA that encoded at least six putative structural proteins (P1, P2, P3, P4, P8, and P10) and five putative nonstructural proteins (P6, P7-1, P7-2, P9-1, and P9-2) (Zhang et al 2013, Xue et al 2013). Most genome segments contain one ORF, but S5, S7, and S9 each contain two ORFs or a total of 13 genes were encoded in the entire genome (MG Table 7). Nucleotide and amino acid sequences variations between RBSDV and SRBSDV genome segments range from 71- 79% and 62-89%, respectively. The highest sequence variation was obtained in segment 6 and has an important role in pathogenicity while segments' S1 and S10 are the most conserved segments (Wang et al 2010; MG Table 7).

4.2.1. Functions of RBSDV and SRBSDV encoded proteins. **MG Table 8** shows the summary of RBSDV and SRBSDV RNA segments and function(s) of each encoded protein. Studies revealed similar functions were reported to both viruses by different workers. Reiterating the functions of these proteins, P1 encodes for polymerase; P2 is a major core capsid protein; Pns6 functions as a viral movement protein; and Pns7-1 and Pns9-1 are component proteins of the viroplasm.

4.2.2. Putative functions of SRBSDV proteins. P5-1 and P6 interaction may be involved in the formation of viroplasms (Li et al 2013). The truncated mutant of P5-1 and P6 at N-terminal region (amino acids 9-231) and N-terminal fragment (amino acids 1-93), respectively, showed interaction in YTH analysis. They formed granules positioned at the cell periphery in *N.benthamiana* leaves; P6 was present in both the cytoplasm and the nucleus and formed punctate bodies associated with the cell periphery. Immunogold labeling showed that both P6 and P5-1 localized within viroplasms in infected cells of rice plants.

Pns9-1 was detected in the viroplasm together with viral RNA, outer-capsid proteins, and viral particles in virus-infected cultured insect vector cells, as revealed by transmission electron and confocal microscopy (Jia et al 2012). The matrix of viroplasms observed in virus-infected cells was composed basically of P9-1. However upon knockdown of P9-1 expression, due to RNA interference (RNAi) induced by synthesized double-stranded RNA

MG Table 7. Percent nucleotide comparisons between the segments of the RBSDV and SRBSDV.

Genome segment	Total size (bp)		Percent divergence		Reference
	RBSDV1	SRBSDV	nt	aa	
S1	4501	4500	79.0	86	Wang et al (2010)
S2	3812	3815	79.0	89.0	Wang et al (2010)
S3	3572	3618	73	73	Wang et al (2010)
S4	3617	3571	77	84	Wang et al (2010)
S5-1	3164	3167	71	69	Wang et al (2010)
S5-2				62	
S6	2465	2651	70.6	63.1	Wang et al (2010)
S7-1	2193	2176	73	80	Zhang et al (2008)
S7-2				60	
S8	1936	1928	72	67.7	Zhang et al (2008)
S9-1	1900	1899	74.0	77.5	Zhang et al (2008)
S9-2				72	Zhou et al (2008)
S10	1801	1797	78.0	83.1	Zhang et al (2008), Zhou et al (2008)

[1] RBSDV Chinese isolate (Zhang et al 2001).

(dsRNA) from the P9-1 gene, strongly inhibited viroplasm formation and viral infection. This further indicates that P9-1 was essential for viroplasm formation and viral replication.

P3, P7-1, P9-2. Across the SRBSDV genome, the mRNA expression levels of these proteins in rice plants and WBPH insect hosts were highest, and the levels of P6 and P10 were lowest, indicating that SRBSDV uses the same infection strategy in plant and insect hosts. Therefore, they are likely to be genes with the most crucial function and could be used as sensitive molecular detection targets for SRBSDV (He et al 2013). SRBSDV P7-1 was found to have an important role in viral infection. The relative expression level of the SRBSDV *S7-1* gene in infected rice suspension cells was assayed by real-time quantitative polymerase chain reaction to evaluate the antiviral activities of various drugs. Results showed that dufulin displayed the highest inhibitory activity against SRBSDV S7-1 expression. Furthermore, the validity of this approach was confirmed in an in vivo experiment in which dufulin was found to effectively inhibit SRBSDV (Yu et al 2013).

P5, P6, P9-1. These proteins are collectively required for the genesis and maturation of the filamentous and granular viroplasm matrix induced by SRBSDV infection as demonstrated in the continuous cell culture of WBPH vector (Mao et al 2013). They examined the viroplasm by electron and confocal microscopy and found that viral RNAs P6 and P9-1 were accumulated in the granular region while P5 and P6 together with viral RNAs, progeny cores, and viral particles were accumulated in filamentous region of viroplasm. Thus, P5 formed filamentous inclusions also thought to be the site for the assembly of progeny virions and P9-1 formed granular inclusions in the absence of viral infection, suggesting that the filamentous and granular viroplasm matrices were formed primarily by P5 and P9-1, respectively. P6 was apparently recruited in the whole viroplasm matrix by direct interaction with P9-1 and P5. Thus, the present results suggested that P5, P6, and P9-1 are collectively required for the genesis and maturation of the filamentous and granular viroplasm matrix induced by SRBSDV infection.

MG Table 8. Summary of RBSDV and SRBSDV RNA segments and function(s) of each encoded protein.

Protein name	Protein (kDa)	RBSDV		SRBSDV	
		Function	Reference	Function	Reference
P1 (Pol)	169	Polymerase	Zhang et al (2001)	RNA polymerase	Wang et al (2010)
P2	142	Major core structural	Zhang et al (2001)	Core capsid	Wang et al (2010)
P3	132	?			
P4	136	B-spike	Zhang et al (2001)		
Pns5-1	107	?		May be involved in the formation of viroplasms	Li et al (2013)
				Associated only with filamentous matrix	Mao et al (2013)
Pns5-2					
Pns6		Silencing suppressor	Zhang et al (2005)	suppressor -may be involved in the formation of viroplasms, virus morphogenesis, and virus pathogenicity	Li et al (2013)
				-involved in the formation of both the filamentous and granular matrices of viroplasm	Mao et al (2013)
Pns7-1	41	Reacted with tubular structures and viroplasm	Isogai et al (1998)	Necessary for the formation of tubules probably associated to movement protein	Liu et al (2011)
Pns7-2	36				
P8	68	-NTP binding; - core capsid -Acts transcriptional regulatory proteins	Nibert and Kim (2004) Isogai et al (1998), Liu et al (2007)	Core capsid	Wang et al (2010)
Pns9-1	40	Reacted with tubular structures and viroplasm	Isogai et al (1998)	essential for viroplasm formation and viral replication	Jia et al (2012)
				major component of the granular matrix of viroplasm	Mao et al (2013)
Pns9-2	24	?			
Pns10	63	Major outer capsid	Isogai et al (1998), Liu et al (2007)	plays an important role in virus patho-genicity and vector transmission,	Zhang et al (2008); Zhou et al (2008)

4.3. Genus *Oryzavirus*: **Rice ragged stunt virus (RRSV)**

RRSV is another virus disease infecting rice that is transmitted by the brown planthopper in a persistent propagative manner and is the type species in the genus *Oryzavirus* in the Reoviridae family. RRSV is one of the economically important virus diseases in several Southeast and East Asia. RRSV outbreaks have been reported, especially in Indonesia, the Philippines, and Thailand during 1977-90. An RRSV outbreak in 2005 was reported in Vietnam (Cabauatan et al 2009) and found to be coinfecting with RGSV, resulting in a more severe yellowing syndrome causing significant yield loss (Du et al 2007)**.** Another virus that was reported coinfecting RRSV is SRBSDV resulting in more aggravated disease symptoms. Wang et al (2014) conducted field investigations on insect interactions and found that WBPH and BPH transmitted SRBSDV and RRSV, respectively. They found that the virus-free WBPHs significantly preferred to feed on infected rice plants rather than on healthy plants and virus-free BPHs significantly preferred healthy plants. However, the RRSV-carrying BPHs preferred SRBSDV-infected rice plants. With these results, they have indicated that SRBSDV might assist RRSV transmission and infection.

4.3.1. RRSV genome and protein functions. The RRSV genome is composed of 10 double-stranded RNA segments (MG Table 9) each having the genus-specific conserved terminal nucleotide sequences of 5'-GAUAAA---GUGC-3' (Yan et al 1992). Each RNA segment encodes a single protein or at least 10 proteins encoded for the entire genome. Among the proteins, at least seven are structural proteins namely P1, P2, P3, P4A, P5, P8B, and P9 and three nonstructural proteins, Pns6, Pns7, and Pns10 (Boccardo and Milne 1984; Hagiwara et al 1986; Upadhyaya et al 1996, 1997, 1998).

4.3.2. Reported evidences on the functions of RRSV proteins. The P4A structural protein functions as a putative RNA-dependent RNA polymerase (RDRP). The P1 protein of plant Reoviruses such as RDV, RBSDV, and SRBSDV encode for RDRP, however, these conserved RDRP motifs, including the GDD motif protein, are contained in RRSV P4a (Upadhyaya et al 1998).

Pns6 accumulated predominately in the cytoplasmic membrane fraction. Shao et al (2004) demonstrated the interaction of Pns6 with single- and double-stranded forms of DNA and RNA, by both competition and displacement assays. They indicated that the basic region from amino acid 201 to 273 of Pns6 was the unique nucleic acid binding domain by gel mobility shift assays. Another function, however, was reported by Wu et al (2010) where Pns6 could complement the cell-to-cell movement of the movement-deficient TMV in *Nicotiana tabacum* Xanthi nc and *N. benthamiana* plants. Both the N- and C-terminal 50 amino acids of Pns6 were essential for the cell-to-cell movement.

The Pns9 protein proved to be the viral spike protein or one of the protein components involved in forming the viral spike in previous reports on vector-mediated viral infection (Lu et al 1987, 1999; Hagiwara et al 1986).

The Pns10 nonstructural protein was concentrated at viroplasms that formed in the midgut epithelium, visceral muscles, and salivary glands of infected insects by immunofluorescence microscopy. Since the sites of virus replication and assembly of progeny virions occurred in viroplasms, these results show that Pns10 of RRSV is essential for viroplasm formation and virus replication in the vector insect (Jia et al 2012).

4.3.3.Transgenic plants against RRSV. Transformation of rice with gene constructs derived from several different RRSV genome segments, such as segments' 5,7,8, 9, and 10 (MG Table 9) were reported by Upadhyaya et al (1998**,** 2001). These constructs gave resistance ranges from low to high but among them, segment 9 (S9) progeny plants of T1 transgenic lines of an Australian rice cv. Jarrah, showed strong resistance against RRSV infection (Upadhyaya et al 2001). Similarly, Matsumura and Tabayashi (1995) transformed rice with gene constructs derived from RRSV S9. They expressed an mRNA of

MG Table 9. RRSV RNA segments and function(s) of each encoded protein.[1]

Segment/ protein	dsRNA(bp)/ length (aa)	Protein (kDa)	Function	Resistance[2]	Reference
S1/P1	3849/ 1237	137.7	Core, B spike protein		
S2/P2	3808/1192	133.1	Structural protein Capsid shell protein		
S3/P3	3699/1173	130.8			
S4/P4A	3823/1255	141.4	RNA-dependent RNA polymerase		Upadhyaya et al (1998)
S4/P4B	/327	36.9			
S5/P5	2682/808	91.4	-capping enzyme/ guanyl transferase	Medium to high	Li et al (1996)
S6/Pns6	2157/592	65.6-71	-Nucleic acid binding -Cell to cell movement		Shao et al (2004), Wu et al (2010)
S7/Pns7	1938/608	68		Low to medium	
S8/P8	1802/562				
1914	62				
67.3	major outer capsid protein	Medium	Nakashima et al (1994)		
S8/P8A	1814/596	67.3	Self-cleaving protease		Lu et al (2002)
	/224	25.6			Upadhyaya (1996)
	/558	41.7			
S9/P9	1132/338	38.6	spike	Low to high	Uyeda et al (1995); Upadhyaya et al (1995)
S10/Pns10	1162/297	32.3	Viroplasm formation and virus replication in the insect	Medium	Jia et al (2012)

[1] Thai isolate; 2 Ranges of resistance (low, 50-75; medium, 25-50; high, 0-25% infection) from transgenic rice lines containing different promoter-driven RRSV gene construct (Upadhyaya et al 2001).

appropriate size but the protein was not apparently expressed and therefore did not show any resistance when inoculated with RRSV. Instead, symptom expression was intensified. Furthermore, another recent study (Chaogang et al 2003) used transgenic rice lines, containing rice Actin1 promoter-driven RRSV S9 construct. Their results showed that the insect vector, ***N. lugens***, fed on transgenic rice plants expressing the 39 kDa protein prior to feeding RRSV-infected plants, which were significantly protected against RRSV infection.

4.3.4. Natural resistance to RRSV. Songbai et al (2013) identified and characterized a rice protein that is homologous to eukaryotic translation elongation factor 1A (eEF-1A), designated OseEF-1A, and its interactions with viroplasm-associated proteins of RRSV and SRBSDV. OseEF-1A interacted with RRSV Pns6 and Pns10 in yeast two-hybrid screening and was confirmed by bimolecular fluorescence complementation. RRSV-Pns6

and -Pns10 function in cell-to-cell movement and viroplasm formation, respectively. When expressed together, OseEF-1A colocalized with RRSV Pns10 in the epidermal cells of *Nicotiana benthamiana*. In addition, they also reported that OseEF-1A interacted with RBSDV-Pns6. However SRBSDV-Pns6 interacted with itself and colocalized with Pns9-1 in *N. benthamiana*. In the presence of Pns6, OseEF-1A colocalized with Pns9, an aputative viroplasm matrix protein of SRBSDV. They further indicated that the translation inhibition by RRSV and SRBSDV may also be host-specific, as this may help viral mRNAs compete for the cellular translation machinery. However, the inhibition might also be used by viruses for other purposes. For example, primary transcripts of reoviruses function as both mRNAs for protein translation and as templates for genome replication. Replication must be coupled with translation. Therefore, besides the inhibition of host protein synthesis, the inactivation of OseEF-1A might be a mechanism used by the two viruses to switch their primary transcripts from translation to replication.

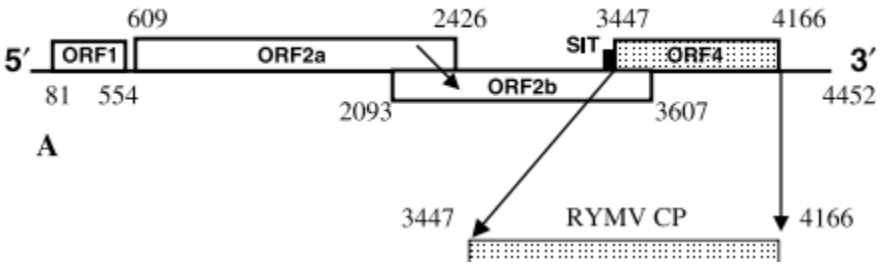
4.4. *Sobemovirus* genus: Rice yellow mottle virus (RYMV)

Rice yellow mottle is a major viral disease causing substantial economic losses that mainly affect rice production in Africa (Kouassi et al 2005). RYMV is a member of the ***Sobemovirus*** genus that is characterized by isometric particles of 25 to 28 nm in diameter. The virus is transmitted by mechanical transmission and by species of chrysomelid beetle, commonly Sesselia ***pusilla*** and ***Chaetocnema pulla***. RYMV-infected rice plants showed typical symptoms including yellow to orange discolored leaves, reduced tillering, stunted growth, and sterile flowers.

4.4.1. Genome and gene function. RYMV has a single-stranded positive-sense RNA having a compact genome with a size of 4,450 nucleotides (nts) with only 330 nts being in noncoding regions. The genome has a 5'VPg but lacks a 3' poly(A) sequence (Hull 1988, Yassi et al 1994) and the entire genome consists of four ORFs encoding four proteins and most of these predicted ORFs overlap each other (MG Figure 4; MG Table 10).

ORF1 from nucleotides 81 to 554 encodes the P1 protein initiated at nucleotide 80 and ends at nucleotide 553 with 157 amino acids and a size of 17.8 K. It has been said to play an important part in the virus cell-to-cell movement and acts as suppressor of gene silencing involved in the propagation of RYMV in the rice plant (Bonneau et al 1998). They also expressed P1 with mutation at the initiation codon in transgenic rice plants and produced systemic infection. Results further indicate that the P1 of RYMV is dispensable for virus replication and is required for virus spread in plants. This study was further supported by Siré et al (2008). They identified the conserved amino acids C95 and C64 amino acids in P1 gene that were directly involved in cell-to-cell movement and the strength of suppression, respectively.

ORF2, which is from nt 608 to nt 3607, codes for a polyprotein with 999 amino acids and a size of 110.7 K. Within ORF2, there are two overlapping ORFs. ORF2a (nucleotides



MG Fig. 4. RYMV genomic organization. Source: Kouassi et al (2006).

MG Table 10. RYMV gene products and functions.

ORF/ protein	Nucleotide location[1]	Number aa	size (kDa)	Function	Reference
I/P1	80-553	157	17.8	Virus movement Gene-silencing suppressor	Bonneau et al (1998) Siré et al (2008)
II	608-3607	999	110.7	Polyoprotein	Yassi et al (1994)
2a	609-2426			Serine protease and Genome-linked protein (VPg)	Yassi et al (1994) Fragette et al (2004)
2b	2093-3607	134		RdRp	Fragette et al (2004)
III	within ORF2	126	13.7	Polypeptide	Yassi et al (1994)
IV	3447-4166		26	Coat protein	Yassi et al (1994)

[1] Genome size and nucleotide positions may slightly vary depending on isolate (Kouassi et al 2005).

609-2426, N terminal part) encodes serine protease and a viral genome-linked protein. ORF2b (nucleotides 2093-3607, C terminal half part), where the first 134 amino acids of this polyprotein would correspond to the VPg protein, followed by the virus protease, the helicase, which is further translated through an a-1 ribosomal frameshift mechanism as a fusion protein, encodes the RNA-dependent RNA polymerase (Fargette et al 2004). The VPg protein, also known to be involved in virulence towards rymv1–2-mediated resistant rice, reported earlier at position 374 in P2a (Hebrard et al 2006), is located at position 48 within the VPg.

ORF3 is embedded within ORF2 and is located from nt 2092 to nt 2467. It codes for a 126-amino acid polypeptide of 13.7 K whose function is not known.

ORF4 is located from nt 3447 to nt 4166, thus overlapping the 3' end of ORF2. It codes for a 26K protein, which corresponds to the RYMV coat protein (MG Figure 4).

4.4.2. plants with RYMV resistance. Studies on the use of viral genes, such as CP and viral genome-linked protein (VPg), have demonstrated resistance to RYMV. Ndjiondjop et al (2001) monitored the accumulation of coat protein (CP) and viral RNA in different varieties carrying different reactions of resistance against RYMV by serological and Northern blot analysis. Their results showed that there was high CP and RNA accumulation at 5 dpi in whole plants in susceptible plants (IR64, Tog5673, and ***Ac***. 2428), suggesting that cell-to-cell and vascular movements occurred before 5 dpi in inoculated leaves. There was a 2-day delay of viral accumulation in inoculated leaves of the moderately resistant cultivar Azucena. However, CP and viral RNA were not detected in the resistant cultivars, Tog5681 and Gigante. The comparison of viral accumulation in protoplasts and plants suggests that resistance of the highly resistant cultivars is not due to the inhibition of virus replication but rather to the failure of cell-to- cell movement.

Results were further supported by Kouassi et al (2006) when they studied pathogen-derived resistance against RYMV utilizing the CP gene. Eighty percent of the independent transgenic lines that they analyzed contained CP gene sequences. Two types of reactions were produced on those transgenic lines that were challenged with RYMV. Transgenic plants expressing antisense sequences of the CP and untranslatable CP mRNA mostly showed a delay in virus accumulation of up to a week and the level of virus accumulation was reduced by ELISA, Southern, and Western analyses compared with nontransgenic TP309 plants. The transgenic plants expressing RYMV wild-type CP (wt.CP) and deleted CP (ΔNLS.CP) accumulated the highest levels of virus particles. These results suggest that antisense CP and untranslatable CP mRNA induced moderate resistance,

whereas transgenic CP enhanced virus infection. Mutagenesis in the genome-linked viral protein (VPg) gene was demonstrated by Hebrard et al (2006). They did single substitution G1729T as introduced by a nonresistance breaking (nRB) infectious clone and found it to be sufficient to induce symptoms in uninoculated leaves of the cultivar Gigante. This is the first evidence that VPg is a virulence factor in plants with recessive resistance against viruses.

4.4.3. Natural resistance against RYMV. Eukaryotic translation initiation factors, such as eIF4E and eIF4G and its isoforms, are also required for infection by some potyviruses. These initiation factors were found to interact with the viral genome-linked protein (VPg) of potyviruses wherein this protein has been found to be associated in resistance breaking. Thus, these translation initiation factors and their isoforms were further recognized as naturally occurring resistance in plant/virus interaction in some potyivruses (Leonard et al 2000). These findings supported the studies done in RYMV in identifying natural resistance in RYMV. The resistance to the yellow mottle virus (***Rymv***1) locus was found to be associated with recessive resistance of RYMV-resistant cultivars such as *O. sativa* cultivar (cv) Gigante and *O. glaberrima* cv Tog5681 (Ndjiondjop et al 1999, 2001).

This *Rymv*1 locus was isolated and mapped to a 160-kb interval and found to contain a gene from the eIF(iso)4G family (Albar et al 2006). They developed transgenic resistant lines carrying this eIF(iso)4G gene derived from a susceptible variety and resulted in the loss of resistance. This suggests an important role of genes eIF(iso)4G identified in rice resistant to RYMV. Hebrard et al (2008, 2010) demonstrated molecular mechanisms involved in the resistance and virulence factors of eukaryotic translation initiation factor eIF(iso)4G1 and the VPg, respectively. They did find a direct interaction between the RYMV-VPg and the central domain of rice eIF(iso)4G1 both in vitro, using recombinant proteins, and in vivo, using a yeast two-hybrid assay. The insertion of the E309K mutation in eIF(iso)4G1, conferring resistance in planta, strongly diminished the interaction with avirulent VPg.

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