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Comparison of the Genetic Organization of Papaya Ringspot Virus with Other Potyviruses

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ABSTRACT

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The complete nucleotide sequence of papaya ringspot virus (PRSV) RNA has recently been determined by our laboratory. The PRSV genome contains 10326 nucleotides excluding the 3'-terminal poly (A) tail. PRSV RNA is larger than those of seven sequenced potyviruses: tobacco etch virus (TEV) RNA is 9496 nucleotides, tobacco vein mottling virus (TVMV) RNA 9472, plum pox virus (PPV) RNA 9741, potato virus Y (PVY) RNA 9704, pea seed-borne mosaic virus (PSbMV) RNA 9924, soybean mosaic virus (SMV) RNA 9588, and pepper mottle virus (PeMV) RNA 9640. The base composition of PRSV RNA shows a high adenine content (31.2%), followed by uracil (27.0%), guanine (23.8%), and cytosine (18.0%), similar to those of the other potyviruses. The genomic RNA of PRSV contains one large open reading frame that starts at nucleotide positions 86 to 88 and ends at position 10118, encoding a polyprotein of 3344 amino acids which is 138-339 residues longer than those of the other potyviruses. The 5' leader sequence contains 85 nucleotides, 46 to 121 nucleotides shorter than those of the other potyviruses. The 3' non-coding region contains 209 nucleotides, which is in common with the other potyviruses (163-331, average 240 nucleotides). The conserved sequence of NAAAUAAAACANNUCAANACAACAUAA at the 5' end of PRSV and those of seven other potyviruses shows 75% similarity, suggesting that this region may play a common important role for potyvirus replication. The genetic organization of PRSV is similar to that of other potyviruses except that the first protein (P1) processed from the N terminus of the polyprotein has an M_r of 63 K, 18-34 K larger than those of the other potyviruses. The P1 protein is the most variable protein among potyviruses and may be considered important for identification of individual potyvirus. The most conserved protein of potyviruses appears to be the NIb protein, the putative polymerase for the replication of potyviral RNA. The proteolytic processing of potyviruses is mediated by three virus-encoded proteinases: the P1 protein liberates its own C terminus, the HC-Pro proteinase also autocatalytically cleaves its C terminus, and the NIa protein which is responsible for *cis* and *trans* proteolytic processing to generate the CI, P5, NIa, NIb, and coat proteins. An internal cleavage site for delimitation of the genome-linked protein (VPg) and proteinase domains in the NIa protein are present in all potyviruses compared. A suboptimal cleavage site upstream the CI protein, recognized by the NIa proteinase and potentially generates a protein of 6 K, is present in several potyviruses. The genetic organization of potyviruses is summarized as VPg-5' end leader-P1 protein-HC Pro protein-P3 protein-CI protein-P5 protein-NIa VPg protein-NIa Pro protein-NIb protein-coat protein-3' non-coding region-poly (A) tract.

Key words: papaya ringspot virus, potyviruses, genetic organization, complete genomic nucleotide sequence, potyviral proteins, polyprotein

INTRODUCTION

The plant potyvirus group contains about 160 members and is the largest and economically important plant virus group (19). A feature shared by all potyviruses is the induction of characteristic pinwheel or scroll-shaped inclusion bodies in the cytoplasm of the infected cells (18). These cylindrical inclusions (CIs) are formed by a virus-encoded protein and considered as the most important phenotypic criterium for assigning

viruses to the potyvirus group (36,53,60). Many potyviruses also induce cytoplasmic amorphous inclusion bodies and some form nuclear inclusions, both are also formed by virus-encoded proteins (8). Most of potyviruses are transmitted by aphids in a non-persistent manner, and some of these viruses are transmitted by mites or possibly by whiteflies. In addition, there are some viruses that share many characteristics with typical potyviruses but have important different properties such as transmission by fungi and encapsidation of bipartite ssRNA genomes in two different filaments. These criteria separate them from potyviruses (28). However, their gene order, the strategy of gene expression, the homology of amino acid sequences and the formation of similar cylindrical inclusions indicate a close relationship with potyviruses. The taxonomy of the potyvirus group based on the mentioned criteria has been proposed (4,52,53,60).

The virus particles are flexous filaments 680–900 nm long and 11 nm wide, containing one molecule of linear positive-sense ssRNA about 10 kb. The complete nucleotide sequences of potyviral genomes have been elucidated from tobacco etch virus (TEV) (1), tobacco vein mottling virus (TVMV) (12), plum pox virus (PPV) (34), potato virus Y (PVY) (50), pea seed-borne mosaic virus (PSbMV) (27), soybean mosaic virus (SMV) (26), papaya ringspot virus (PRSV) (64), and pepper mottle virus (PeMV) (56). The genomic RNAs of the potyviruses encode an unique large open reading frame (ORF) and produce a polyprotein which is processed into 8 or 9 final products via proteolytic processing. Studies on TEV concluded that there are at least three viral proteinases responsible for the processing. The N-terminal protein (NT protein or P1 protein) of the polyprotein autocatalytically cleaves its C-terminus to release the protein from the polyprotein (57,58). The bifunctinonal helper component-proteinase (HC-Pro or P2 protein) adjacent to the P1 protein also autocatalytically cleaves its C-terminus (7). The C-terminus of the P3 protein, the cylindrical inclusion protein (CIP), the 6 K protein, the nuclear inclusion a (NIa) protein, the nuclear inclusion b (NIb) protein, and the coat protein (CP) are processed from the polyprotein by the NIa protein which acts as a proteinase for the *cis* and *trans* cleavages (6,7,14,15,17).

Papaya ringspot virus (PRSV) is a member of potyvirus and has been a major limiting factor for papaya production in Taiwan since 1975 (59). Most of the PRSV isolates belong to one of two major groups, type P and W (42). The type P viruses cause serious problems on papaya throughout the tropical and subtropical areas. Type W isolates, previously described as watermelon mosaic virus 1 (WMV-1), cause serious problems on cucurbits worldwide. Most isolates of the two pathotypes are serologically indistinguishable by their CP and CIP antisera (63). However, the natural hosts of type W limit in Cucurbitaceae only, but the type P viruses infect papaya family in addition (42,63).

An isolate of PRSV HA originated from Hawaii (22) is a typical P type virus and has been well characterized in host range (42,63), serology (63), and gene expression (62). Recently, the complete nucleotide sequence and genetic organization of the RNA genome of PRSV HA has been determined by our laboratory (64). In this report, the genetic organization of PRSV is compared with those of the other sequenced potyviruses. The conserved and the variable regions are analyzed, and the possible functions of each protein are discussed.

COMPARISON OF THE PRIMARY STRUCTURE OF RNAs

The complete nucleotide sequence of PRSV RNA contains 10326 nucleotide residues, excluding the poly (A) tail at the 3' end (64). The potyviral nucleotide sequences used for comparison are TEV from Allison et al. (1), TVMV from Domier et al. (12), PPV from Maiss et al. (34), PVY from Robaglia et al. (50), PSbMV from Johansen et al. (27), PeMV from Vance et al. (56), and SMV from Jayaram et al. (26). The base composition of PRSV HA RNA shows a high adenine content (31.2%), followed by uracil (27.0%), guanine (23.8%) and cytosine (18.0%). This composition is similar to those of the other potyviruses, which have A, U, G, and C contents of 31.0–32.6%, 25.2–27.0%, 22.9–24.4%, and 17.5%–20.4%, respectively (Table 1).

Comparison of the size of the genome, the 5' non-coding region, the 3' non-coding region, and the size of the polyprotein of PRSV with those of seven other reported potyviruses is summarized in Table 2. The genome of PRSV is 402–854 nucleotides longer than those of other seven reported potyviruses. The 5' non-coding region of PRSV contains 85 nucleotides, shorter than those of other potyviruses (131–206 nucleotides, average 160 nucleotides). Comparison of the 5' terminal sequence of PRSV with those of the other potyviruses revealed that the extreme 5' terminus of the RNAs shared a similar structure. A consensus sequence NAAAUAAAACANNUCAACACAACAUAA can be deduced from the first 27 nucleotides at the 5' extreme, showing 75% similarity in eight potyviruses (Fig. 1). This highly conserved sequence at the 5' end suggests a common and important role for potyviral replication, such as the initiation for particle assembly, the binding for the genome-linked protein (Vpg), or the recognition signal for the RNA polymerase. The other part of the 5' non-coding region of PRSV has a high content of A and U, this feature is similar to most of the other potyviruses. The 3' non-coding region contains 209 nucleotides and is in the range of those of the other

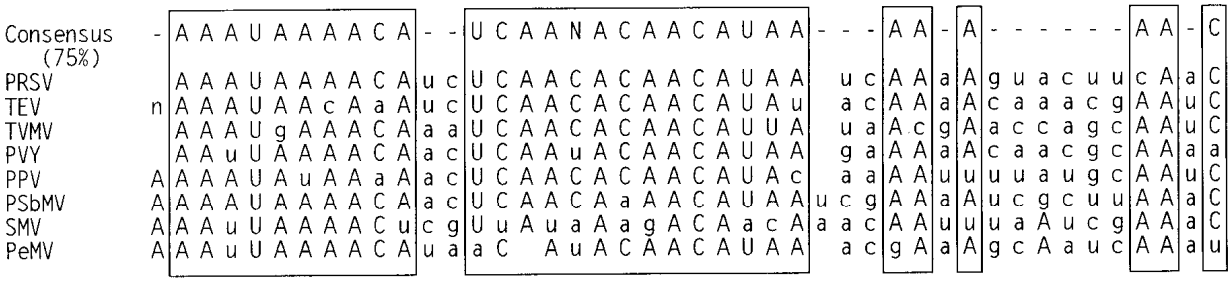

Fig. 1. Alignment of the nucleotide sequences of the 5' termini of the genomes of eight potyviruses. Overall similarity was set at 75%. The consensus nucleotides are boxed.

TABLE 1. Nucleotide composition of the sequenced potyviruses

Viruses	Percentages of			
	Adenine	Uracil	Guanine	Cytosine
PRSV	31.2	27.0	23.8	18.0
TEV	31.3	25.5	24.0	19.1
TVMV	32.0	26.1	22.9	19.0
PVY	31.0	26.9	23.4	18.7
PPV	31.3	25.2	23.0	20.4
PSbMV	32.6	25.6	24.4	17.5
SMV	31.6	26.4	24.1	17.9
PeMV	31.6	27.0	23.2	18.1

TABLE 2. Sizes of the genome, 5'UTL, 3'UTR and polyprotein of potyviruses

Virus	Genome(nt)	5'UTL	3'UTR	Polyprotein(a.a.)
TEV	9496	144	190	3054
TVMV	9472	206	251	3005
PPV	9741	146	220	3125
PVY	9704	184	331	3063
PSbMV	9924	143	163	3206
PeMV	9640	167	265	3068
SMV(G2)	9588	131	259	3066
PRSV	10326	85	209	3344

potyviruses (163–331 nucleotides, average 240 nucleotides).

The genomic RNA of PRSV contains one large open reading frame that starts at nucleotide positions 86–88 and ends at position 10118, encoding a polyprotein of 3344 amino acids which is 138–299 amino acids larger than those of the other potyviruses.

CLEAVAGE SITES OF POLYPROTEIN

Cleavage mediated by the P1 proteinase

The N-terminal sequence of the HC-Pro protein of PRSV was directly determined from the purified HC-Pro protein and the cleavage site between the P1 protein and the HC-Pro protein was placed at AKMDQY/NDVAEKF (64). Localization of the N-terminus of the HC-Pro protein revealed a P1 protein of 63 K which has the same size as the N-terminal protein generated from the *in vitro* translation studies (9,45,61,62).

Verchot et al. (57,58) have reported that the TEV P1 protein (35 K) functions as a serine proteinase cleaving autocatalytically at its C-terminus. The cleavage site for liberating the P1 and the HC-Pro proteins of TEV was located between Phe 256 and Ser 257 of the polyprotein, 24 amino acids downstream from the consensus proteinase functional domain FIVRG (35). The cleavage site for liberating the P1 and HC-Pro proteins of PRSV is also located 24 amino acids downstream from the FI(V)VRG motif (64). It is interesting to learn that from the alignment of the polyproteins of all eight potyviruses, there is a general trend that the cleavage site locates at a similar position downstream the consensus domain (Fig. 2). The structurally similar amino acids Phe and Tyr, 22–24 amino acids away from the FI(V)VRG motif, are found conserved in the P1 position of the cleavage site (Fig. 2).

Cleavage mediated by the HC-Pro proteinase

The P2 protein generated from the potyviral polyproteins is a bifunctional HC-Pro protein, which is the helper component for aphid transmission and also functions as a proteinase to autocatalytically cleave its C-terminus. The C-terminus of the HC-Pro protein of TEV was located at a Gly-Gly dipeptide (7). Similar cleavages for the C-termini of the HC-Pro proteins were found in PRSV (64) and other potyviruses and a consensus sequence Y-VG/G can be deduced for the cleavage (Fig. 3).

Cleavages mediated by the NIa proteinase

Analyses on the other cleavage sites of the C-terminal halves of the potyviral polyprotein revealed that the NIa proteinase cleaves at Q/A, Q/G, Q/S, Q/T, Q/V or Q/E dipeptide sequences, and valine was found in all potyviruses at position P4 of the cleavage

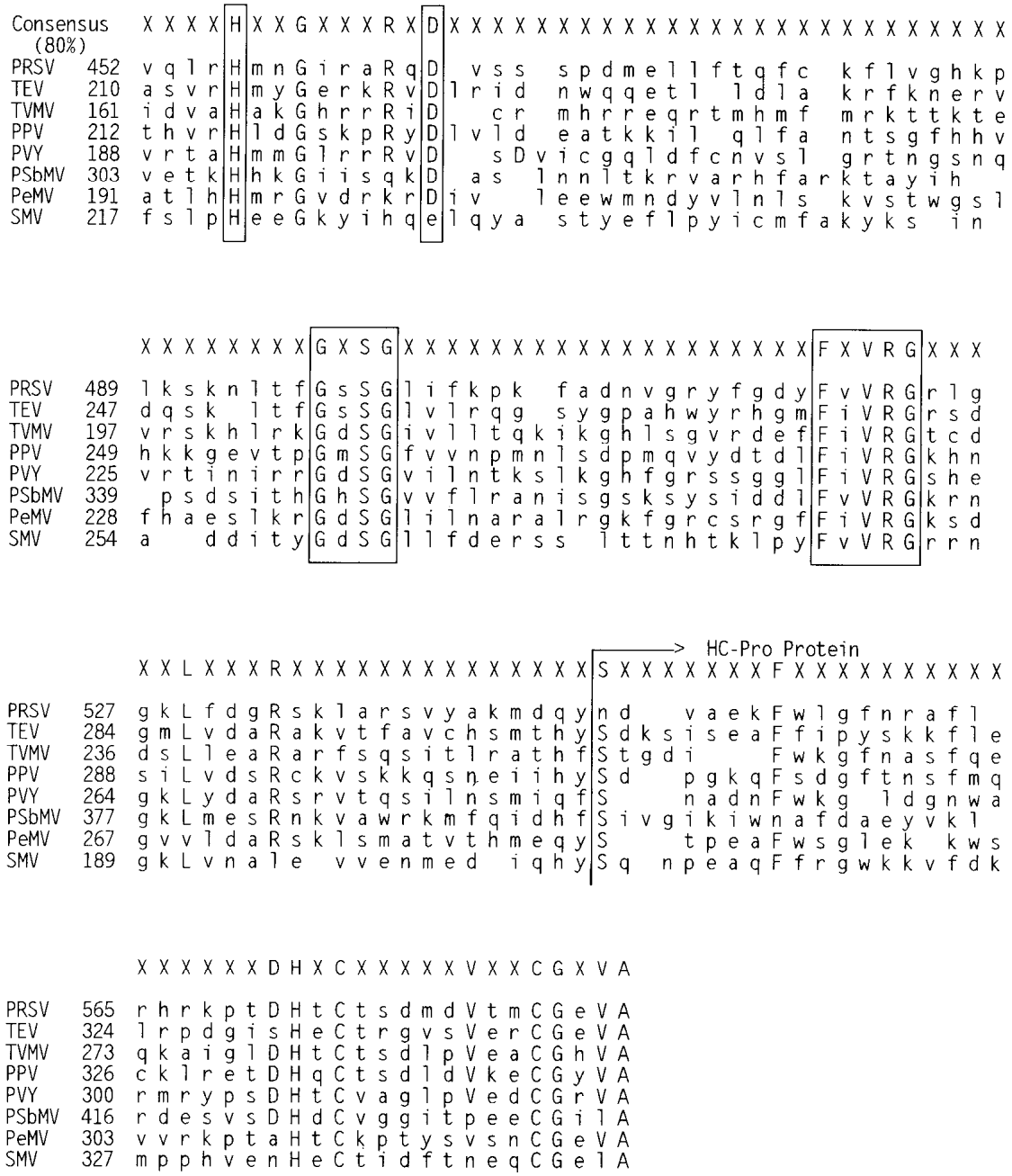

Fig. 2. Alignment of the C-terminal portion of the P1 proteins and the N-terminal portion of the HC-Pro proteins of eight potyviruses. The conserved amino acids essential for the activity of the P1 proteinase (57,58) are boxed. The cleavage sites for generating the P1 and HC-Pro proteins are indicated by an arrow.

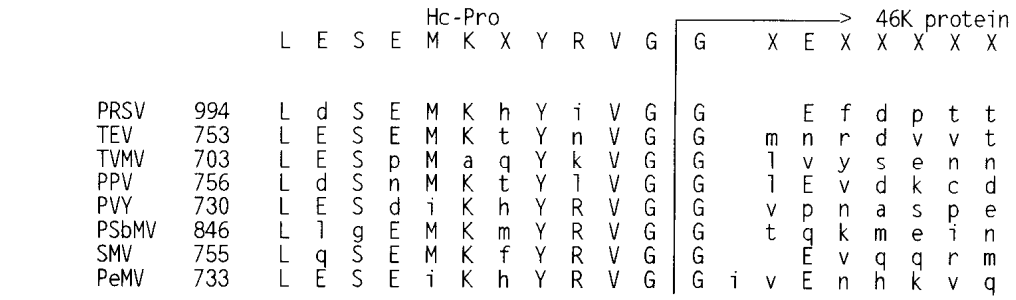

Fig. 3. Consensus cleavage site for the HC-Pro proteins and the 46K (P3) proteins of eight potyviruses.

sites except TEV (10,14,21,27). The consensus sequences for the cleavages are listed in Table 3. The NIa proteinase recognized the specific cleavage sequences in *cis* and *trans* manners and generate P3, CIP, 6 K, NIa, NIb, and coat proteins. The proteolytical processing of these proteins by the NIa proteinase is the major common characteristics of potyviral gene expression. The cleavage sites mediated by the NIa proteinase of PRSV are similar to other potyviruses except that the cleavage site between the NIb and coat proteins was relocated from previously reported VFHQ/SKNE (44) to VYHE/SRGT based on the molecular sizes of the CP and the NIb, and the general cleavage rule for the NIa proteinase (64). However, the new prediction did not rule out the possible cleavage at the previously predicted position. Since both sites fit the general cleave specificity of the NIa proteinase, a heterogenous N-terminus of the PRSV CP may be generated. This may explain why the purified CP of PRSV, either prepared freshly from purified virions or after storage at 4 C, is frequently associated with some degraded forms of CP, 2 K to 5 K smaller than the major 36 K protein (22,43). This phenomenon is unique to PRSV and not reported in other potyviruses. The significance of this heterogeneity remains to be further studied.

TABLE 3. Cleavage sites recognized by the NIa proteinases of eight potyviruses

Viruses	Amino acid positions							
	P6	P5	P4	P3	P2	P1	P1'	P2'
TVMV	—	—	V	R/K	F/T	Q	S/G	—
PSbMV	—	—	V	R/K	—	Q/E	S/G,A	—
PVY	—	—	V	—	H/E	Q	S/G,A	—
PeMV	—	—	V	—	H/E	Q	S/G,A	—
PPV	—	—	V	—	H/T	Q	S/G,A	—
PRSV	—	—	V	Y/F	H/E	Q/E	S/G	—
TEV	E	—	—	Y	—	Q	S/G	—
SMV	E/N	—	V	—	—	Q	S/G	—
Consensus	E/N	—	V	Y/F R/K	H/E,T F/T	Q/E	S/G,A	—

The presence of a potential internal cleavage site in the NIa protein to separate the VPg and the proteinase domains was predicted in PSbMV by Johansen et al. (27) and confirmed later by Dougherty and Parks (16). This cleavage site was also identified in the NIa protein of PRSV (64) and other potyviruses as listed in Fig. 4. All P4 position of the cleavage sites contain Val and the cleavages are conserved in E/G, E/A, or E/S. The P1 Glu of this internal cleavage site is different from the P1 Gln of the other cleavage sites mediated by the NIa proteinase.

A suboptimal cleavage site in front of the CI protein, which generates a 6 K_1 protein has been suggested for PPV (49). This cleavage site is supported by the *in vitro* expression of the P3 protein of TVMV (51), but not detected for TEV (40). The reports for PSbMV, PVY, SMV, PeMV, and PRSV did not include this site (26,27,50,56,64). However, the existence of this site in a modified and suboptimal way can not be ruled out.

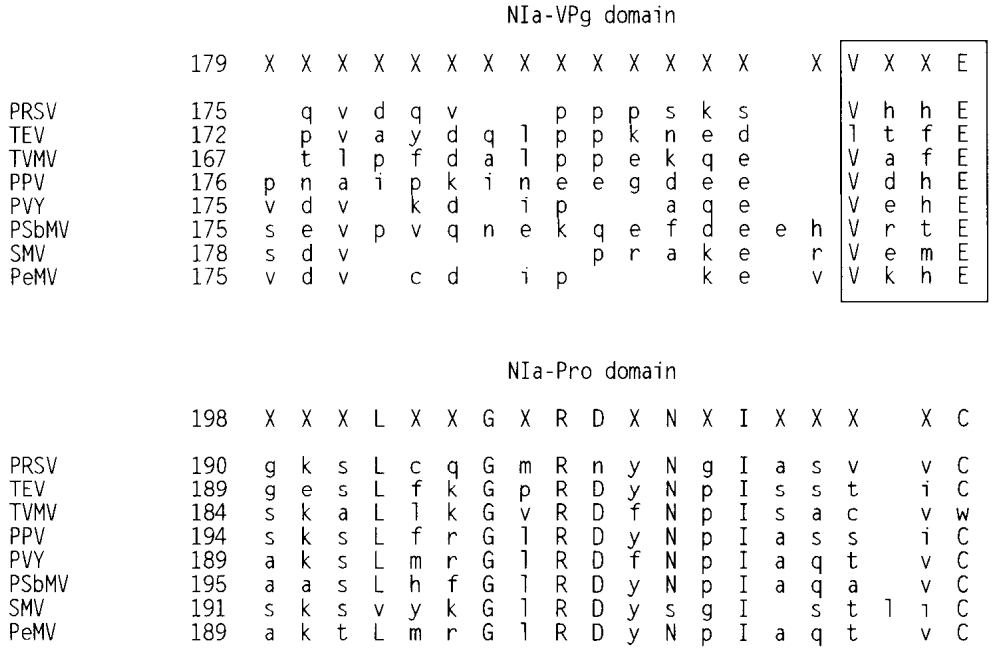

Fig. 4. Internal cleavage site for delimitation of NIa-VPg and NIa-Pro domains in the NIa proteins of eight potyviruses.

TABLE 4. Comparison of proteins of PRSV with those of other potyviruses

Virus	Percentage of Identity								
	NT	HC	46K	CIP	6K	NIa-VPg	NIa-PRo	NIb	CP
TEV	14.3	47.4	32.0	54.3	35.1	45.8	46.9	58.5	56.2
TVMV	10.4	51.6	31.6	50.1	34.5	54.0	43.4	60.3	50.6
PVY	14.7	49.6	30.7	54.2	35.1	45.3	39.8	58.4	56.6
PPV	13.8	51.8	33.0	59.0	33.3	54.4	46.1	60.9	56.2
PSbMV	18.3	45.1	25.7	57.8	31.6	47.5	39.4	59.5	53.2
SMV	14.3	46.0	28.1	55.0	34.5	50.5	47.1	56.2	51.8
PeMV	13.2	49.8	29.3	53.7	42.1	46.6	41.1	56.9	55.3

COMPARISON OF PRSV PROTEINS WITH OTHER POTYVIRAL PROTEINS

The actual and proposed locations of the cleavage sites predict eight or nine proteins for PRSV with calculated M_rs of 63 K (P1 protein), 52 K (HC-Pro protein), 46 K (P3 protein), 72 K (CIP protein), 6 K (P5 protein), 48 K (NIa protein, or 21 K NIa-VPg protein and 27 K NIa-Pro protein), and 35 K (CP) (64). The comparison of the PRSV proteins with those of the other potyviruses is listed in Table 4. The N-termini of the HC proteins of other potyviruses are assigned according to Fig. 2. It appears that PRSV is distinct from other seven potyviruses, because the amino acid identity of each protein of PRSV with those of the other potyviruses is less than 60.9%.

Among the proteins compared, the P1 protein generated from the N terminus of the potyviral polyproteins shows a wide variation in M_r from 29 K to 63 K, and is the most variable potyviral proteins. The P1 63 K protein of PRSV shares very low homology, only 10.4–18.3% identity with those of other potyviruses (Table 4). The high variability and the dramatic changes in size make the protein an important criterium to distinguish and classify different potyviruses.

The NIb protein of PRSV shows 56.2–60.9% identity with those of other potyviruses, and is the most conservative protein of the potyviral proteins (Table 4). The NIb is the putative RNA polymerase of potyvirus (1,12), the relatively higher degree of conservation suggests that the potyviruses share a similar process for RNA replication. The HC-Pro protein, the CIP protein, the NIa-VPg domain, the NIa-Pro domain, and the CP share identities of 45.1–51.8%, 50.1–59.0%, 45.3–54.4%, 39.4–47.1%, and 50.6–56.6%, respectively (Table 4). The range of variation of the HC-Pro protein and the CP are less than 6.7% and 6.0%, respectively, relatively smaller than the other proteins, indicating that the properties of aphid transmission, the formation of pinwheel inclusions, the function of the genome-linked protein, the proteolytic process by the NIa proteinase, and the assembly of the flexous particles are rather conserved among potyviruses.

The P3 (46 K) protein and the P5 (6 K) protein of PRSV share relatively lower identities with those of the other potyviruses, with only 25.7–33.0% and 31.6–42.1%, respectively (Table 4). This suggests that the functions of the two proteins may be more virus-specific.

POSSIBLE FUNCTIONS OF THE PROTEINS

The P1 protein generated from the N-terminus of the polyprotein has been identified as a serine type proteinase which autocatalytically cleaves its C-terminus in a *cis* action (58). The His 214, Asp 223, Ser 256 and the motif Phe-Ile-Val-Arg-Gly 276 to 280 of the TEV 35 K P1 protein are found essential for the function of the proteinase (58). Although the P1 proteins of the potyviruses compared share very limited homology, the essential domains are all conserved among the viruses as shown in Fig. 2. The conserved amino acid residues and motif at the C-terminal region of the P1 protein indicate that it functions as a serine type proteinase to release itself from the polyprotein in all potyviruses compared, although the size of the protein is highly variable among different potyviruses.

The 28 K P1 protein of TVMV shares moderate similarity with the movement proteins of tobamoviruses and tobraviruses, and has been suggested having the similar function for the movement of potyviruses (13). However, this similarity was not present in the P1 protein of other potyviruses including PRSV (27,49,64). The N-terminal portion of the P1 proteins do not share significant similarity, and apparently is not essential for the function of the proteinase since the size of the proteins varies from 29 to 63 K. Whether the function of this part of the protein is responsible for the cell-to-cell movement remains to be investigated. Since the major difference in biological properties of potyviruses is the host specificity and the P1 protein is the most variable protein of potyviruses, one may speculate the relativeness of these two events.

The HC-Pro protein is responsible for the aphid transmissibility of potyviruses and also functions as a cysteine-type proteinase that autocatalytically cleaves its C-terminus (7). The cysteine cluster Cys-X8-Cys-X13-Cys-X4-Cys-X2-Cys in the HC protein is considered to be similar to the zinc fingers of several nucleic-acid binding proteins (50). This consensus sequence for metal-binding sites is found in all HC-Pro proteins compared. The amino acids Gly-Tyr-Cys-Tyr surrounding Cys 649, and His 722 of the TEV HC-Pro protein are essential for the active sites of the proteinase (39). This sequence is also present in the HC-Pro proteins of all potyviruses compared. Thus, the bifunctional role for aphid transmissibility and proteolysis is conserved for all the potyviral HC-Pro proteins.

The P3 protein shares similarity with the 2A protease of picornaviruses (13) and is considered similar to polio P2A (14). However, the consensus sequence PGDCGGXLXCXHG of the 2A protein was not found in the PRSV 46 K P3 protein (64). Since three proteinases, the P1, the HC-Pro, and the NIa proteins, have been found for all cleavage sites of the potyviral polyprotein (5,6,7,57,58), the function of the P3 protein remains vague.

The nucleotide-binding motif GAVGSGKST, which has been identified in CIPs of TEV and TVMV (23), is also present in the CIP of PRSV (64). This motif is found in all the other potyviruses compared. The CIP has been shown having RNA unwinding (helicase) (30) and ATPase activity (29). It may behave as a membrane-bound protein involving in viral RNA replication.

In an early report, a 6 K protein was demonstrated to be associated with TEV RNA (24); and the protein, which has the size similar to the M_rs of VPgs of comoviruses, nepoviruses, and animal picornaviruses, was originally considered as the VPg protein of TEV and PVY (1,50). However, the RNAs of TVMV and PPV are thought to be linked with VPgs of 24 K (54) and 22 K (46), respectively. Recent studies indicated that the TEV VPg is the 24 K N-terminal domain of the 49 K NIa protein (16,38). Thus, the role of the 6 K P5 protein needs to be further investigated.

The NIa protein functions as a proteinase responsible for *cis* and *trans* proteolytic activities for the five cleavages in the C-terminal portion of the polyprotein (6,7,15). The catalytic triad of NIa proteinase, His, Asp and Cys (17), is conserved in all the potyviruses compared.

The NIb proteins contain the consensus motifs YCDADGS, GNNSGQPSTVVDNT(S)LMV and NGDDL-X34-K, responsible for the putative RNA polymerase function (1,12,41) are found conserved in all the potyviruses compared. It is also the most conserved protein among the potyviral proteins (Table 4), indicating that the central events for RNA replication of each potyvirus is rather similar.

The coat protein of the potyvirus is processed from the C-terminal end of the polyprotein. This protein is the only structural protein for the formation of the filamentous particles. The central and C-terminal regions of the protein are highly conserved and are essential for the encapsidation of viral RNA to form particles (53). The C-terminal part of potyvirus CPs share sequence similarity with the CPs of the unrelated filamentous plant viruses potex-, carla-, clostero-, and tobamoviruses (10,31,37). The N and C termini of the coat proteins of potyviruses are surface located and the N terminus of the coat protein is highly variable and contains the major virus-specific epitopes (53). The high variability observed in the surface-exposed N-terminal region of potyvirus CP suggests that it is involved in virus-specific functions or host-vector-virus interactions (48,60). The conserved triplet DAG at the N-terminus of CP (25,31) has been considered involving in virus transmission by aphid (20,34). The hypothesis was confirmed experimentally through the abortion of the aphid transmissibility by mutation of DAG to DAE in the CP gene of TVMV (2,3). In addition to the natural functions, the CP gene has been used to generate genetically engineered CP-mediated resistance against the natural insect transmission (32) or against infection by heterologous potyviruses (33,55).

CONCLUSION

The RNA genome of PRSV contains 10326 nucleotides, 402–854 nucleotides longer than other genomes of seven sequenced potyviruses. The genetic organization of PRSV is similar to the other sequenced potyviruses and is summarized as VPg-5'end leader-P1 (63 K) protein-HC Pro (52 K) protein-P3 (46 K) protein-CI (72 K) protein-P5 (6 K) protein-NIa VPg (21 K) protein-NIa Pro (27 K) protein-NIb (59 K) protein-coat (35 K) protein -3' non-coding region-poly (A) tract. P1 protein of PRSV is 18–34 K larger than those of the other potyviruses and shares very limited homology with other potyviruses. The protein is unique to each individual potyviruses and may be considered as the most important criterium to distinguish and classify distinct potyviruses. The NIb protein is the most conserved potyviral protein, indicating that all potyvirus have a similar replication process, and can be considered one of the important molecular criterium to distinguish the potyvirus group from the other plant virus groups. The high homology in HC-Pro, CI, NIa-Vpg, NIa-Pro and coat proteins indicates that the molecular events for the aphid transmissibility, RNA replication, proteolysis, and RNA encapsidation are quite conserved among potyviruses. The roles of P3 and P5 proteins are still not clear, however, the relatively

lower homology of P3 and P5 proteins suggests that they may be responsible for more variable phenotypes of potyviruses.

The *in vitro* synthesis of biologically functional RNA transcripts from full-length cDNA clones has proved to be a powerful way to overcome difficulties in the study of the molecular biology of plant RNA viruses. The infectious *in vitro* transcripts of three potyviruses have been reported for TVMV (11), PPV (47), and ZYMV (20). The ability to generate virus infection from cloned cDNA of potyviruses opens the possibility of applying genetic engineering techniques for the study of their biology at the molecular level. Aspects of the potyvirus life cycle such as translation, replication, symptom induction, cell-to-cell movement and plant-to-plant spread are now amenable to a new experimental approach, and the functions that have been proposed for potyviral gene products can now be tested *in vivo*. Our current efforts focus on the construction of an *in vitro* infectious transcript from the full-length cDNA clone of PRSV. This will allow us to analyze the particular genomic regions responsible for the pathogenicity of the virus, which will provide clues to modify the virus at the cDNA level. The attenuated virus constructed by the recombinant DNA techniques will be used as a potential protective mutant for control of papaya ringspot disease by cross protection. The other goal of our laboratory is to express the CP gene or other viral products of PRSV in the transgenic papaya to generate genetically engineered resistance in papaya against the severe infection of PRSV. During the past few year, our endeavor was mainly on the elucidation of the complete nucleotide sequence of PRSV. The understanding of the genetic organization of PRSV has provided us a solid base for the future goals. It is therefore easy to predict that the approach to control the notorious PRSV in the next few years will be more fruitful and exciting.

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摘　　要

葉錫東．1994．木瓜輪點病毒基因組成和其他馬鈴薯Y群病毒之比較．植病會刊3:54-64．(臺中市　國立中興大學植病研究所)

木瓜輪點病毒(PRSV)的基因體RNA的全長度核酸序列，業已解出，病毒基因體共含10326個核苷酸(不含3'poly A尾端)，和已解出序列的馬鈴薯Y病毒群(potyviruses)中的菸草蝕刻病毒(TEV，9496核苷酸)、菸草脈斑病毒(TVMV，9472)、李子疹斑病毒(PPV，9741)、馬鈴薯Y病毒(PVY，9740)、碗豆種傳病毒(PSbMV，9924)、大豆嵌紋病毒(SMV，9588)

及番椒斑紋病毒 (PeMV， 9640)相較爲最長者。PRSV RNA的鹽基組成爲 A 31.2%、U 27.0%、G 23.0%、C 18.0%，和其他potyviruses相似。其基因體RNA只含一大轉譯架構，由86至88核苷酸處起始轉譯至第10118止，對映出一含3344個胺基酸的複合蛋白，此複合蛋白比其他potyviruses大138–139個胺基酸。PRSV RNA 3'端非轉譯區含85核苷酸，比其他potyviruses相較顯示，在75%類似度時5'端具有一個共同序列NAAAUAAAACANNUCAANACAACAUAA，此5'端共同區可能和potyviruses病毒複製共通主要功能有關。PRSV的基因組成和其他potyviruses類似，但複合蛋白N端所產生的第一個蛋白(P1)爲63 K，比其他potyviruses大了18–34 K。P1蛋白係所有potyvirus蛋白中期歧異度最大者，可視爲區別不同potyviruses之主要性狀。在所有蛋白中，以NIb蛋白共同性最高，此蛋白被認爲是RNA的複製酶。PRSV蛋白裂解過程由病毒產生的三種裂解酵素執行，其中P1蛋白酶及HC-Pro蛋白酶分別自行切解其C端，而NIa蛋白酶以順反方式切解複合蛋白產生CI、P5、NIa、NIb、及CP等蛋白。所有potyviruses經比較顯示，NIa蛋白中有一內含切位可分解其爲VPg及蛋白酶二蛋白。在有些potyviruses上，CI蛋白上游上有一可能被NIa蛋白酶認識之切位，可產生一6 K之蛋白。Potyviruses的基因體RNA經比較之後可以歸納爲VPg-5'端引導序列-P1蛋白-HC Pro蛋白-P3蛋白-CI蛋白-P5蛋白-NIa VPg蛋白-NIa Pro蛋白-NIb蛋白-鞘蛋白-3'端非轉譯區-Poly A尾端。

關鍵詞：木瓜輪點病毒、馬鈴薯Y群病毒、基因組成、基因體核酸序列、複合蛋白。