Plant Pathol. J. 25(4) : 417-421 (2009)

Note

The Plant Pathology Journal

Effects of Recombination on the Pathogenicity and Evolution of *Pepper mottle virus*

Miranda Gilda Jonson[1], Jang-Kyun Seo[1,2], Hong-Soo Choi[3], Jeong-Soo Kim[3] and Kook-Hyung Kim[1,2,*]

[1]*Department of Agricultural Biotechnology and Center for Fungal Pathogenesis, Seoul National University, Seoul 151-921, Korea*

[2]*Institute of Molecular Biology and Genetics, Seoul National University, Seoul 151-747, Korea*

[3]*Department of Agricultural Biology, National Academy of Agricultural Science, RDA, Suwon 441-707, Korea*

(Received on August 17, 2009; Accepted on September 21, 2009)

The analysis of the full length genome of Korean isolates of *Pepper mottle virus* (PepMoV) in previous study showed molecular variations and are found to be related to symptom variation and pathogenicity (Kim et al., 2009, Virus Res. 144:83-88). To fully understand the molecular variation of PepMoV in Korea, we further assessed the role of RNA recombination to biological variation and evolution of PepMoV. Full-length genome of a total of 17 Korean-PepMoV and 2 American (CA and FL) isolates were examined for possible detection of genetic recombination using different recombination detections programs and detected 5 and 8 tentative recombination events using RDP3 and Splits Tree4 programs, respectively. Interestingly, tentative recombinants detected such as isolates 57, 134 and 217 were previously identified as severe isolates and 205135 and 205136 as differentiating isolates (Kim et al., 2009, Virus Res. 144:83-88). In addition, recombination was frequently detected in the Vb isolate, the first PepMoV isolate reported in Korea, suggesting significant involvement in the evolution of PepMoV in Korea. These initial results of our recombination analyses among PepMoV isolates in Korea may serve as clues to further investigate the biological variations and evolution of PepMoV brought about by recombination.

Keywords : genetic structure, molecular and biological variation, PepMoV, recombination

Many studies on sequence analyses of population of various RNA plant viruses provide evidences that recombination may be a major source of biological and evolutionary variation (Garcia-Arenal et al., 2003; Wylie and Jones, 2009). Recombination type of genetic exchange can occur in either segmented or unsegmented viruses when 'donor' nucleotide sequence is introduced into a single, contiguous acceptor RNA molecule to produce a new RNA containing genetic information from more than one source (Worobey and Holmes, 1999).

Pepper (*Capsicum annuum*) is a staple vegetable in Korea and *Pepper mottle virus* (PepMoV) is considered as a threat to pepper production in the country. PepMoV is a non segmented RNA virus belonging to the genus *Potyvirus*, family *Potyviridae* (Shukla, 1994). PepMoV is transmitted by aphids in a non persistent manner and infects not only *Capsicum* sp. but also *Solanum* sp. such as tomato (*S. lycopersicum* L.) (Kim et al., 2008; Verhoeven et al., 2002) and potato (*S. tuberosum*) (Kim et al., 2009). The acquisition of broad host ranges of PepMoV especially potato which is recently identified host for PepMoV in Korea (Kim et al., 2009) is not well understood. Moreover, PepMoV isolates with significant sequence variation in P1 and 6K2 genes manifested symptom differentiations in indicator host plants, *Nicotiana* sp. such as *N. tabacum* cv. Xanthi-nc and *N. benthaminana*, suggesting that these genes might have significant involvement on host specificity and pathogenicity (Kim et al., 2009).

In the advent of advanced molecular tools to detect recombination, many RNA viruses unlocked the key implicated in the RNA genome variability and their evolution (Gagarinova et al., 2008; Posada and Crandall, 2001) that understand the formation of new isolates and evaluate the effectiveness of crop protection strategies. One classic example is the *Soybean mosaic virus* (SMV), another member of *Potyvirus* that provided several evidences of RNA recombination. Gagarinova et al. (2008) first analyzed recombination analysis using RDP3 in SMV full length sequences and found that a number of significant recombination events in the two related but belonging to different pathotypes namely G5 and G7H implying that these two distinct pathotypes can simultaneously infect a host cell and exchange genetic materials through RNA recombination. Furthermore, in recent recombination study of SMV, Seo et al. (2009) have identified three SMV strains prevalent in Korea such as G5, G5H and G7H as recombinants and strongly suggest that recombination plays important roles in

*Corresponding author.
Phone) +82-2-880-4677, FAX) +82-2-873-2317
E-mail) kookkim@snu.ac.kr

resistant-breaking of SMV. In addition, they have indicated that G5H and G7H were found infecting several wild soybean collected from natural fields in Korea. However, these isolates cannot infect resistant soybean cultivar implying that recombination of SMV might occurred in wild soybean infected with more than two SMV populations, possibly resulting to resistant breaking recombinants that may readily infect resistant soybean cultivars.

In our previous study, we have analyzed the genetic diversity and structure of the PepMoV population in Korea and results showed relationship between genetic and biological variation (Kim et al., 2009). In this study, we want to fully understand the molecular variation of PepMoV in Korea, by further assessing the role of RNA recombination in the biological variation and evolution of PepMoV. Here, we have analyzed a total of 19 PepMoV full-length sequences including 13 Korean isolates that were previously analyzed (Kim et al., 2009), 3 new PepMoV isolates (205165, 205187 and 205137), Korean PepMoV-Vb isolate, and 2 American isolates (CA and FL). In addition, included in the analysis is the full-length sequence of *Potato virus Y* (PVY) as an out group. GenBank accession codes for these isolates were shown in Table 1.

All sequences were aligned using Clustal X (Larkin et al., 2007) and subjected to recombination detections software. In this study, we have employed several recombination detection tools such as RDP3 (Martin 2005), PHYLPRO v. 1.1 (Weiller, 1998), and RAT v. 1.0 (by setting window size to 400; Etherington et al., 2005) programs to obtain possible recombination events in different PepMoV sequences. For RDP3 analysis, we have only accepted recombination events that were supported by at least 3 different methods with an associated P value of $<1.0\times10^{-3}$. Next, all likely recombinants identified by the RDP3 were checked again using PHYLPRO v. 1.1 and RAT v. 1.0. Phylogenetic network analysis was performed by Splits Tree v. 4.1 program (Huson and Bryant, 2006).

In RDP3 analysis, we have identified 5 tentative recombination events out of 6 detected recombinants (Table 2 and Fig. 1A). One event (Vb) was not included due to only 2 methods supported recombination event. These tentative recombinants were found in isolates (corresponding parental isolates): 205165 (Vb×743); 205187 (Vb×743), 248 (Vb×743), CA (FL×205187), and 134 (57×Unknown or 205135). Interestingly, RDP3 analysis indicated that Vb isolate may be true recombinants or one of the parents of 205165,

Table 1. Accession numbers of *Pepper mottle virus* isolates used for recombination analyses with their corresponding host and symptoms in different indicator hosts[a]

PepMoV Isolates No.	Host	Symptoms[b]		Accession No.	Reference
		N. tabacum cv. X-nc	*N. benthamiana*		
57	Tomato	SM	N, Y	EU586121	Kim et al., 2009
128	Bell Pepper	SM	SM, Y	EU586122	"
134	Tomato	SM	N, Y	EU586123	"
217	Tomato	SM	N, Y	EU586124	"
248	Bell Pepper	MM	nosi	EU586125	"
731	Potato	–	N, Y	EU586126	"
743	Bell Pepper	MM	nosi	EU586127	"
204040	Bell Pepper	MM	nosi	EU586128	"
205135	Bell Pepper	MM	SM, Y	EU586129	"
205136	Bell Pepper	MM	SM, Y	EU586130	"
205137	Bell Pepper	nt	nt	EU586131	Kim et al., 2009 Unpublished
205165	Bell Pepper	nt	nt	EU586132	"
205187	Bell Pepper	nt	nt	EU586133	"
205197	Bell Pepper	MM	nosi	EU586134	Kim et al., 2009
205205	Bell Pepper	SM	N, Y	EU586135	"
205250	Bell Pepper	MM	nosi	EU586136	"
Vb				AB126033	Yoon et al., 2003
CA				M96425	Vance et al., 1992
FL				AF501591	Warren & Murphy, 2003

[a] Partially adapted from Kim et al. (2009).

[b] Symptom expressions of 16 Korean PepMoV isolates in infecetd *N. tabaccum* cv. X-nc and *N. benthamiana* plants at 4-wks after inoculation. MM, mild mottle; N, necrosis; SM, severe mottle; Y, yellowing; –, no infection; nosi, no symptoms developed but the virus replication was readily detected by RT-PCR; nt, not tested.

Table 2. Recombination in the PepMoV population detected by recombination detection program and PHYLPRO

Recombinant (or true daughter)	Recombination site[a]	Parental isolate[b] (other suspected parent)	RDP3[c] (P value)[d]	PHYLPRO[e]
205165 (Vb)	20-998	Vb×743	**GBMCS** (3.347×10^{-06})	D
205187 (Vb)	165-1958	Vb×743	**GBMCS** (3.347×10^{-06})	ND
248 (Vb)	549-1664	Vb×743	**GBMCS** (3.374×10^{-06})	ND
CA (FL)	526-2177	FL×205187	**MCS** (2.892×10^{-07})	D
134	4221-4890	57×Unknown (205135)	**GBMC** (9.618×10^{-10})	ND

[a] Nucleotide number.
[b] Parental isolate' means the most likely isolate among analyzed isolates; Major parent×minor parent.
[c] RDP3-implemented methods supporting the corresponding recombination sites; G (GENECONV), B (BOOTSCAN), M (MAXCHI), C (CHIMAERA), and S (SISCAN). Supported by 3 or more methods with an associated P value of $\geq1.0\times10^{-02}$ implemented in RDP3.
[d] The reported P-value enclosed is the greatest P-value among the calculated P-values using RDP3-implemented methods and the corresponding method is shown boldface.
[e] PHYLPRO v.1.1 program was used to detect recombination site. D, detected; ND, not detected.

205187, and 248 recombinant isolates. These most likely recombinants were further confirmed by RAT and PHYLPRO (Fig. 1B and 1C). In RAT analysis all of the 5 tentative recombinants were also detected, however, in PHYLPRO analysis, only isolates 205165 and CA isolates were detected as recombinants, which might have been due to the small number of samples analyzed (Table 2 and Fig. 1C). On the other hand, there were 8 tentative recombinants detected by Splits Tree4 (Fig. 2). Apparently, not all of the isolates detected as tentative recombinants (daughters) and sources (recombinants' parents) by RDP3 were also detected by Splits Tree4 (Fig. 2). The isolates that were detected by Splits Tree4 but not by RDP3 were 57, 217, 205135, and 205136. However, isolates that were consistently detected as recombinants by both tools were 134, 205187, Vb, and CA.

The molecular variabilities among Korean-PepMoV isolates have shown relationships in their pathogenicity and host ranges (Kim et al., 2009). For example, the isolates 205135 and 205136 showed symptom differentiations in different *Nicotiana* sp., indicator hosts. In addition, isolates 57 and 134 showed consistent severe symptoms in *Nicotiana* sp. (Table 1). Interestingly, these two groups of isolates formed conspicuous reticular network in Splits Tree4 which further suggest that their genetic and biological variations could be attributed by recombination (Fig. 2). Moreover, isolate 205187 has no biological data yet available but showed positive for recombination in RDP3 and Splits Tree4 (Fig. 1A and Fig. 2, respectively). The reported parents of recombinant isolate 134 were isolates 57 and 205135 which were identified as severe and differentiating isolates, respectively, in previous study (Kim et al., 2009). Moreover, isolate 134 was consistently detected as recombinant by Splits Tree4 (Fig. 2) and RDP3 at the nucleotide 4221-4890 region (recombinant site) and is located within the cytoplasmic inclusion (CI) gene (Fig. 1A). Several studies have indicated that CI may play a role as one of the pathogenic determinants in *Turnip mosaic virus* (Jenner et al., 2000) and SMV (Seo et al., 2009a, 2009b). Therefore, our result suggests that CI together with the previously reported P1 and 6K2 (Kim et al., 2009) may have involvement as pathogenic determinant in the PepMoV pathosystem. However, this would warrant further study.

It has been also demonstrated that recombination is most likely responsible for the emergence of new variants and/or strains that is more virulent than previously existing strain. Choi et al. (2005) reported the first emergence of the resistance breaking (RB) SMV isolates that were capable of overcoming all of the known resistance alleles at the *Rsv1* locus, as well as distinct resistance genes at *Rsv3* and *Rsv4*. Similarly, rapid emergence of RB isolates was reported in *Rice yellow mottle virus* (RYMV) (Fargette et al., 2002). One of the speculations of the emergence of RB isolates in SMV and RYMV in Korean soybean and African rice cultivars, respectively, can most likely be attributed by mutation and/or genetic recombination (García-Arenal et al., 2001; Hajimorad et al., 2003; Harrison, 2002; Roossinck, 1997). In this regard, it is worth mentioning that Seo et al. (2009b) recently identified 19 'clear' recombination events in the SMV population and have shown that several RB strains were identified as recombinants. These results suggesting that recombination might contribute to overcome host resistance in SMV-soybean pathosystem. Similarly, in this study those isolates identified as severe (isolates 57 and 134) and differentiating (isolates 205135 and 205136) isolates were also identified as recombinants. Therefore, these data provide valuable information to breeders for keeping track on those PepMoV strains that will possibly cause breakdown of cultivars' resistances and in the their development of resistant cultivars against PepMoV.

In addition, Korean-Vb isolate appeared several times as either possible daughter or parent of recombinant in RDP3 (Fig. 1A). It also showed noticeable reticular network formation in Splits Tree4 analysis (Fig. 2) which strongly

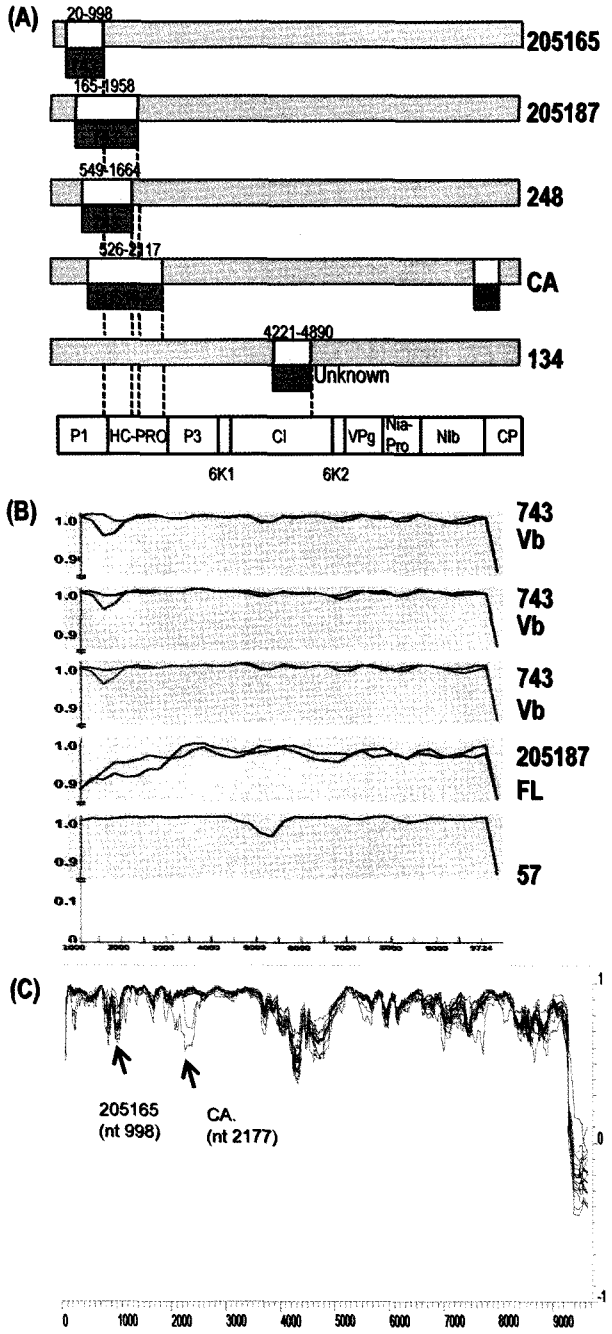

Fig. 1. Recombination events in the PepMoV population by RDP3. Locations of 5 recombination events identified by RDP3. (A) Each full-length genome of a recombinant is represented by a long light gray box and the corresponding isolate name is given to the right of the box. Each recombination event is demarcated by a dark grey box below the recombinant genome. The genome map of PepMoV is shown to scale on the bottom of the figure. (B) Analyses of recombination events using RAT. The outputs for each recombinant in panel A are shown, along with contributing parental sequences (the names are shown on the right of the graph). The *x*-axis and *y*-axis represent the location on the sequence and the genetic distance of each sequence, respectively. (C) Graph obtained analyzed by PHYLPRO method with only two distinct peaks indicates positive for recombination.

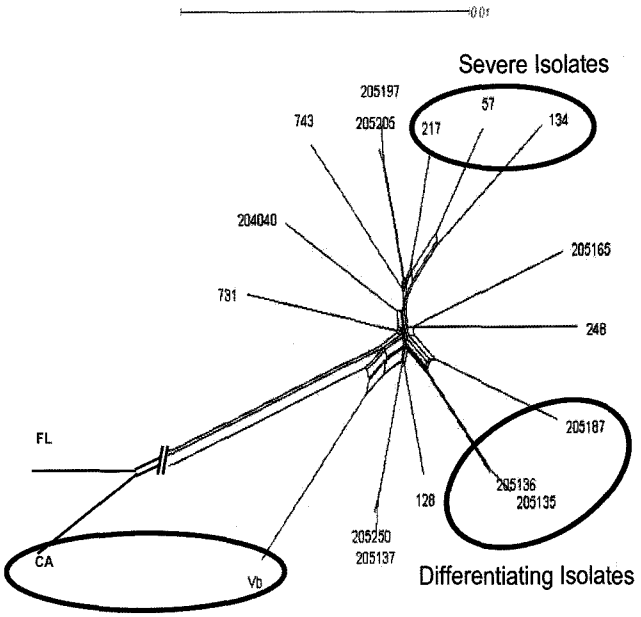

Fig. 2. Phylogenetic evidence for recombination by Split decomposition network of the PepMoV population. Network tree was generated using SplitsTree4. Isolates enclosed showed a reticular network rather than a single bifurcating tree is suggestive of recombination. Isolates with severe and differentiating isolates were identified in the previous study (Kim et al., 2009). The broken branches connecting FL and CA isolates were collapsed 5x.

suggests that Vb isolate may have undergone recombination and might have been also the source of genetic changes to other PepMoV isolates. Although, Korean-Vb isolate was one of the first reported isolate than other Korean isolates analyzed in this study, however, it is still unclear if it had first came out or one of the initial genotypes of PepMoV in Korea. Due to the relative small number (20) of samples included in our analyses, we treated all the recombination events detected in this study as tentative or potential recombinant that will serve as clues for further investigation of recombination in PepMoV evolution.

In our knowledge, this is a first report on PepMoV recombination analysis and we strongly recommend further analyses of recombination events using more diverse PepMoV sequence data. Our present study also reports the occurrence of natural recombination in PepMoV population and similar to SMV recombination studies (Gagarinova et al., 2008; Hajimorad et al., 2003) and it may also give clues for the appearance of the resistance-breaking strains in Korea. For this reason, development of infectious clones especially on those strains that identified as recombinants in this study are needed, to determine cultivar pathogenicity. Thus, this data should be considered in formulating control strategies against PepMoV disease.

Acknowledgments

This research was supported in part by grants from the Rural Development Administration (20090101-030-144-001-05-00), the Agricultural R&D Promotion Center (04601-20090004), and the National Research Foundation of Korea (No. R11-2008-062-02003-0) funded by the Ministry of Education, Science, and Technology (MEST). MGJ and JKS were supported by the post-doctoral and graduate research fellowships from the MEST through the Brain Korea 21 Project, respectively.

References

Choi, B. K., Koo, J. M., Ahn, H. J., Yum, H. J., Choi, C. W., Ryu, K. H., Chen, P. and Tolin, S. A. 2005. Emergence of *Rsv*-resistance breaking *Soybean mosaic virus* isolates from Korean soybean cultivars. *Virus Res.* 112:42-51.

Etherington, G. J., Dicks, J. and Roberts, I. N. 2005. Recombination Analysis Tool (RAT): A program for the high-throughput detection of recombination. *Bioinformatics* 21:278-281.

Fargette, D., Pinel, A., Traoré, O., Ghesquière, A. and Konaté, G. 2002. Emergence of resistance-breaking isolates of *Rice yellow mottle virus* during serial inoculations. *Eur. J. Plant Pathol.* 108:585-591.

Gagarinova, A. G., Babu, M., Strömvik, M. V. and Wang, A. 2008. Recombination analysis of *Soybean mosaic virus* sequences reveals evidence of RNA recombination between distinct pathotypes. *Virol. J.* 5:143.

García-Arenal, F., Fraile, A. and Malpica, J. M. 2003. Variation and evolution of plant virus populations. *Int. Microbiol.* 6:225-232.

Hajimorad, M. R, Eggenberger, A. L. and Hill, J. H. 2003. Evolution of *Soybean mosaic virus*-G7 molecularly cloned genome in *Rsv1*-genotype soybean results in emergence of a mutant capable of evading *Rsv1*-mediated recognition. *Virology* 314: 497-509.

Harrison, B. D. 2002. Virus variation in relation to resistance-breaking in plants. *Euphytica* 124:181-192.

Huson, D. H. and Bryant, D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol. Biol. Evol.* 23:254-267.

Jenner, C. E., Sánchez, F., Nettleship S. B., Foster G. D., Ponz, F. and Walsh J. A. 2000. The cylindrical inclusion gene of *Turnip mosaic virus* encodes a pathogenic determinant to the brassica resistance Gene TuRB01. *Mol. Plant-Microbe Interact.* 13: 1102-1108.

Kim, M.-K. Kwak, H.-R., Han, J.-H., Ko, S.-J., Lee, S.-H., Park, J.-W., Jonson, M.-G., Kim, K.-H., Kim, J.-S., Choi, H.-S. and Cha, B.-J. 2008. Isolation and characterization of *Pepper mottle virus* infecting tomato in Korea. *Plant Pathol. J.* 24:152-158.

Kim, Y.-J., Jonson, M. G., Choi, H.-S., Ko, S.-J. and Kim, K.-H. 2009. Molecular characterization of Korean *Pepper mottle virus* isolates and its relationship to symptom variations. *Virus Res.* 144:83-88.

Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. and Higgins, D. G. 2007. Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947-2948.

Martin, D. P., Williamson, C. and Posada, D. 2005. RDP2: Recombination detection and analysis from sequence alignments. *Bioinformatics* 21:260-262.

Roossinck, M. J. 1997. Mechanisms of plant virus evolution. *Annu. Rev. Phytopathol.* 35:191-209.

Posada, D. and Crandall, K. 2001. Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. *Proc. Natl. Acad. Sci. USA* 98:13757-13762.

Seo, J.-K., Lee, S.-H. and Kim, K.-H. 2009a. Strain-specific cylindrical inclusion protein of *Soybean mosaic virus* elicits extreme resistance and a lethal systemic hypersensitive response in two resistant soybean cultivars. *Mol. Plant-Microbe Interact.* 22:1151-1159.

Seo, J.-K. Ohshima, K., Lee, H.-G., Son, M.-I., Choi, H.-S., Lee, S.-H., Sohn, S.-H. and Kim, K.-H. 2009b. Molecular variability and genetic structure of the population of *Soybean mosaic virus* based on the analysis of complete genome sequences. *Virology* 393:91-103.

Shukla, D. D., Ward, C. W. and Brunt, A. A. 1994. The *Potyviridae*. CAB international, 17 Wallingford, UK.

Vance, V. B., Moore, D., Turpen, T. H., Bracker, A. and Hollowell, V. C. 1992. The complete nucleotide sequence of pepper mottle virus genomic RNA: Comparison of the encoded polyprotein with those of other sequenced potyviruses. *Virology* 191:19-30.

Verhoeven, J. Th., Willemen, T. M. and Roenhorst, J. W. 2002. First report of *Pepper mottle virus* in tomato. *Plant Dis.* 86:186.

Weiller, G. F. 1998. Phylogenetic profiles: A graphical method for detecting genetic recombinations in homologous sequences. *Mol. Biol. Evol.* 15:326-335.

Wylie, S. J. and Jones, R. A. C. 2009. Role of recombination in the evolution of host specialization within *Bean yellow mosaic virus*. *Phytopathology* 99:512-518.

Worobey, M. and Holmes, E. C. 1999. Evolutionary aspects of recombination in RNA viruses. *J. Gen. Virol.* 80:2535-2543.