Watermelon mosaic virus

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ABSTRACT

Potyviruses have been badly affecting crop yields in most parts of the world, with *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and *Papaya ringspot virus* (PRSV) being of particular economic importance. *Watermelon mosaic virus* (WMV) causes severe economic losses in cucurbitaceous, leguminous, malvaceous and chenopodiaceous plants in temperate and Mediterranean regions. It produces chlorosis, mottling, blisters on leaves and fruits, leaf distortion and stunting in watermelon, muskmelon, squash, pumpkin and cucumber. WMV has been shown to infect experimentally, more than 170 plant species belonging to 27 plant families. The biological variability of WMV has been well-documented. Serologically, it is close to *Soybean mosaic virus* (SMV) and *Papaya ringspot virus* (PRSV), but distantly related to *Potato virus Y* (PVY) and *Bean yellow mosaic virus* (BYMV). The genome of the reported WMV isolates is more than 10kb, flanked by untranslated regions at both the ends. The large open reading frame (ORF) encodes a putative polyprotein of 3217 aa, with a calculated Mr. of 366,904. Sequence analyses of WMV isolates revealed close relationship with the reported isolates of SMV (84.7% to 85.8% aa identity). However, the N-terminal P1 protein encoding region was substantially different, presenting less than 35% identity. SimPlot analysis revealed that WMV arose through an ancestral event of interspecific recombination between SMV and *Bean common mosaic virus* (BCMV)/ *Peanut stripe virus* (PSV)-related potyviruses. Very little genetic material resistant to WMV-2 is available. Cultural practices, crop rotation, cross-protection and genetic resistance have effectively been used against WMV. Coat protein transgenic resistance to WMV has also been reported in squash and cantaloupe.

Keywords: WMV, cucurbit, potyvirus, recombination, sequence

CONTENTS

INTRODUCTION

The genus *Potyvirus* is by far the largest of the known plant virus groups and contains nearly 200 definite and tentative species (Fan *et al*. 2003; Fauquet *et al*. 2005). Viruses in this genus are 680-900 nm in length, 11-13 nm in diameter and encapsidate a genome of about 10 kb comprising multiple copies of a single protein species of 30-47 kDa (Shukla *et al*. 1994). They are transmitted by aphids in a non-persistent manner using helper components. Some of the members are seed-transmitted. Flexuous particles contain (+)-sense ssRNA with a 5′ VPg, 5′ non-coding region, single open reading frame (ORF) encoding a single poly-protein and 3′ untranslated region (UTR). The polyprotein is later processed into 10 functional proteins by virus-encoded proteinases (Shukla *et al*. 1994). On a worldwide basis, potyviruses are badly affecting crop yields, with *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus* (WMV) and *Papaya ringspot virus* (PRSV) being of particular economic importance (Lecoq *et al*. 2001).

WMV, a pathogen of worldwide importance, causes economic losses in *Cucurbitaceous*, *Leguminous*, *Malvaceous* and *Chenopodiaceous* plants (Purcifull *et al*. 1984). The biological variability of WMV is well documented (Purcifull and Hiebert 1979; van Regenmortel 1971). Lindberg *et al*. (1956) carried out the first detailed study of viral isolates from cucurbits and classified the isolates into two groups, namely melon mosaic and squash mosaic groups. Subsequently, WMV isolates were classified as WMV-1 and WMV-2 (Purcifull and Hiebert 1979; Yeh *et al*. 1984). Specifically, isolates that failed to infect non-cucurbitaceous plants were designated WMV-2, while isolates that infected plants outside the *Cucurbitaceae* were designated WMV-1, although the latter is now considered to be a strain of PRSV (Purcifull *et al*. 1984). Purcifull and Heibert (1979) also reported a third isolate that did not react with antisera against either WMV-1 or WMV-2, and which has now been given the status of a distinct potyvirus species, the *Moroccan watermelon mosaic virus* (MWMV). *Soybean mosaic virus* (SMV) and *Blackeye cowpea mosaic virus* (BlCMV) are both closely related to, but distinct from, WMV (Fauquet *et al*. 2005). Although WMV has long been known in many parts of the world, it has not yet been characterized extensively at the molecular level. The complete genome sequences of only three isolates have been reported very recently from France, Pakistan and China.

Received: 13 March, 2007. Accepted: 2 May, 2007.

Invited Mini-Review

ECONOMIC IMPORTANCE

WMV is a virus of world-wide importance in temperate and Mediterranean regions (Lecoq *et al*. 1998). It presents a broader host range than most other potyviruses, causing agronomic problems in important crops, mostly cucurbits, but also peas (Inouye 1964; Schroeder and Provvidenti 1971) and orchards such as *Vanilla fragrans* (Wang *et al*. 1993) and *Habenaria radiata* (Gara *et al*. 1997). Experimentally, WMV has been shown to infect more than 170 plant species belonging to 27 families (Shukla *et al*. 1994). In France, WMV epidemics were observed every year from 1981 to 2002 (Lecoq *et al*. 2003). It has been reported to be most prevalent in melon-growing regions of the Central Valleys of California (Grafton-Cardwell *et al*. 1996), Brazil (Yuki *et al*. 2000) and Spain (Luis-Arteaga *et al*. 1998). Surveys carried out in cucurbit crops from Pakistan (Ali *et al*. 2004), South Carolina (Sammons *et al*. 1989), Israel (Antignus *et al*. 1989), Lebanon (Abou-Jawdah *et al*. 2000) and Turkey (Sevik and Arli-Sokmen 2003) revealed WMV-2 as the dominant virus.

SYMPTOMATOLOGY

WMV presents a broader host range than most potyviruses and produces a variety of symptoms. It induces chlorotic spots on *Chenopodium amaranticolor* and *Chenopodium quinoa* (**Fig. 1A**). Systemic mottling or mosaic symptoms are produced on *Lagenaria siceraria* (**Fig. 1B**), *Cucumis sativus* and *Citrullus lanatus* (Fauquet *et al*. 2005). Dark green vein banding and leaf distortion are observed in *Nicotiana benthamiana*, but the symptoms are not very severe. *N. benthamiana* and *Cucurbita pepo* have been recommended as suitable propagative hosts for virus purification, owing to the high virus titers in these plants. Systemic mottling and necrosis, accompanied by wilting and premature death, occurs in *Pisum sativum.* Systemic mottling is also observed in *Vicia faba*. Severe mosaic symptoms (**Fig. 1C**), puckering and distortion of the leaves and a shoestring appearance, especially at the margin of the leaves, are the prominent symptoms in *C. pepo* (**Fig. 1D**) and *Cucumis melo* var. *flexuosus.* No infection occurs when *Luffa acutangula*, *Vigna unguiculata*, *Nicotiana rustica*, *N. glutinosa* and *N. samsun* are inoculated with WMV (Fauquet *et al*. 2005).

MOLECULAR CHARACTERIZATION

The viral genomes of all reported WMV isolates are greater than 10 kb, flanked at both ends by UTRs (Desbiez and Lecoq 2004; Ali *et al*. 2006). The viral genome encodes a putative polyprotein of 3217 aa with a calculated Mr of 366,904. Nine putative proteinase cleavage sites are present that allow processing of the polyprotein into 10 smaller putative functional proteins by 3 viral-encoded proteinases designated P1, HC-Pro and NIa-Pro. The amino acid sequence contains all the characteristic features of potyviruses (**Table 1**), including the conserved sequence motifs KLSC and PTK in HC-Pro and DAG in CP, which are essential for transmission by aphids. The highly conserved sequence motif KITC, which is required for interactions with stylet canals, has diverged to KLSC, but the lysine residue regarded as crucial for HC-Pro activity remains unchanged. It is interesting to note that most potyviruses closely related to WMV, including *Bean common mosaic virus* (BCMV), *Bean common mosaic necrosis virus* (BCMNV), *Cowpea aphid borne mosaic virus* (CABMV), SMV, ZYMV and *Dasheen mosaic virus* (DMV), have their KITC motif diverged to KLSC. This observation supports previous findings that mutations in the KITC motif have no effect on aphid transmissibility and HC-Pro activity (Atreya and Pirone 1993). It has also been documented that highly basic residues, such Lys or Arg, at this position in HC-Pro play key role in potyviral helper component proteinase activity (Atreya and Pirone 1993).

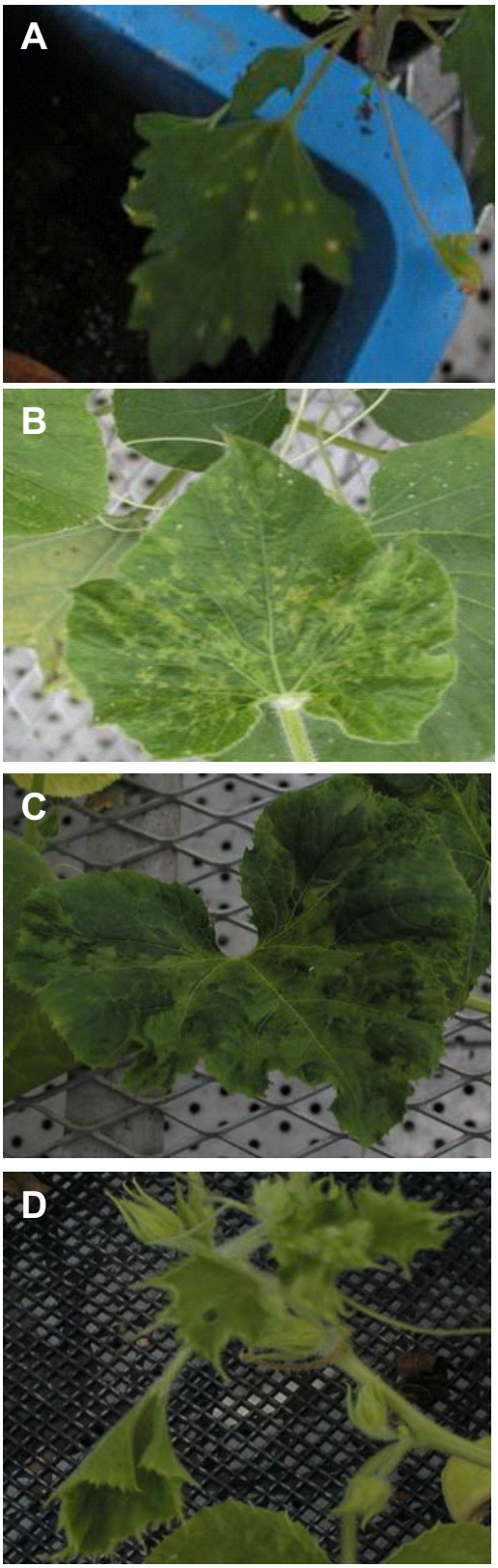

Fig. 1 Symptomology of *Watermelon mosaic virus*. (**A**) Chlorotic spots on *C. amaranticolor*. (**B**) mosaic symptoms induced on *L. siceraria* leaves, (**C**) Leaf of *Cucurbita pepo* showing severe mosaic symptoms infected with WMV and (**D**) *C. pepo* showing dark green enation like symptom, severe puckering and distortion of leaves and shoe string appearance, especially at the margin of the leaves.

The available sequence data, which are mostly confined to partial CP, P1 and CI regions from only a few parts of the world, reveal very little diversity among WMV isolates. Neighbour-joining trees of different regions of the genome (P1, CI and CP) show little variability (Ali *et al*. 2006). The phylogenetic relationship with other members of the family

Table 1 Conserved sequence motifs identified in WMV-Pk polyprotein.

WMV proteins	Motifs	Location	Functions
HC-Pro	**KLSC** [a]	496-499	Aphid transmission.
	PTK	752-754	Aphid transmission
	DPY**ILLMGLI**SP**SI**	930-943	Proteolytic processing
RNA helicase(C1)	GAV**GSGKST**	1384-1392	NTP binding motif (Walker A)
	LE**PTR**PL	1407-1413	NTP binding motif ?
	DEST	2072-2075	ATP hydrolysis (Walker B)
	LKV**SATPP**	1499-1506	RNA binding?
	VATNIIENGVTL	1602-1613	NTP hydrolysis, RNAunwinding
	GERI**QRLGRVGR**	1646-1657	ATPase activity, RNA binding
NIa proteinase	IHM**Y**GVEPENYSML	2047-2060	VPg/RNA linkage (tyrosine 1919)
RDRP (NIb)	SL**KAE**L	2589-2594	RNA polymerase activity
	ADGSQFD	2666-2672	NTP binding
	GNN**SG**QPS**T**VVDN**T**L**M**V	2726-2742	NTP binding
	N**GDDI**	2769-2773	RNA polymerase activity
CP	**DAG**	2946-2948	Aphid transmission

[a] Consensus residues are in bold type

Potyviridae places WMV in the BCMV subgroup (Desbiez and Lecoq 2004). This subgroup is further containing two clusters, one comprising SMV isolates, including WMV, and the other containing the majority of BCMV isolates, BCMNV and CABMV. Although the subgroups have no formal taxonomic status, they make it easier to deal with related viruses and can be useful for diagnosis and plant breeding.

Since there are reports of new data entries in GenBank that were not used in previous analyses, we drew a new phylogenetic tree based on the CP sequence. This resulted in three clusters (**Fig. 2**): the first cluster contains isolates reported from Pakistan, France, Spain, Australia and Israel (showing little divergence); the second cluster comprises isolates from USA, Tonga, New Zealand and Spain (BAD and SG isolates); and the third cluster includes Japanese (Habeneria) and Chinese isolates. The Japanese isolate do not fit well in the cluster and show a little more divergence. The Spanish isolates are distributed in two clusters showing some diversity, and can be divided into two genetic strains, I and II, as reported by Moreno *et al*. (2004). Since the reported sequences of the P1 and CP regions are from different isolates reported at different periods of time, no correlation can be established on the basis of this phylogenetic comparison. Moreno *et al*. (2004) found no evidence of spatial differentiation of the Spanish WMV population. Furthermore, the Spanish WMV population has no metapopulation structure with local extinction and recolonization, and shows different evolutionary dynamics for different regions of the WMV genome. At least 7% of the Spanish isolates are recombinants between genetic strains I and II, presenting two interesting features: (1) crossover points are not detected over the whole genome, they mostly occur between the analyzed regions in the CI and CP cistrons, and not between the P1 and CI cistrons; (2) crossover points are not observed within the analyzed regions encoding the P1, CI and CP proteins (Moreno *et al*. 2004).

Sequence analyses revealed that WMV is very closely related to reported isolates of SMV (84.7-85.8% aa identity), although the N-terminal P1 protein-encoding regions differ substantially (<35% aa identity). SimPlot analyses (Ray 1998) using the WMV-Pk isolate as a query sequence and three other isolates from the BCMV group revealed that WMV arose through an ancestral event of interspecific recombination between SMV and BCMV/*Peanut stripe virus* (PSV)-related potyviruses (Desbiez and Lecoq 2004; Ali *et al*. 2006). High homology in the 5′ coding region (nt 134 to 950) of WMV genome has been observed for PSV and BCMV, after which the trend shifts toward SMV, indicating that only the N-terminal P1 region was subjected to

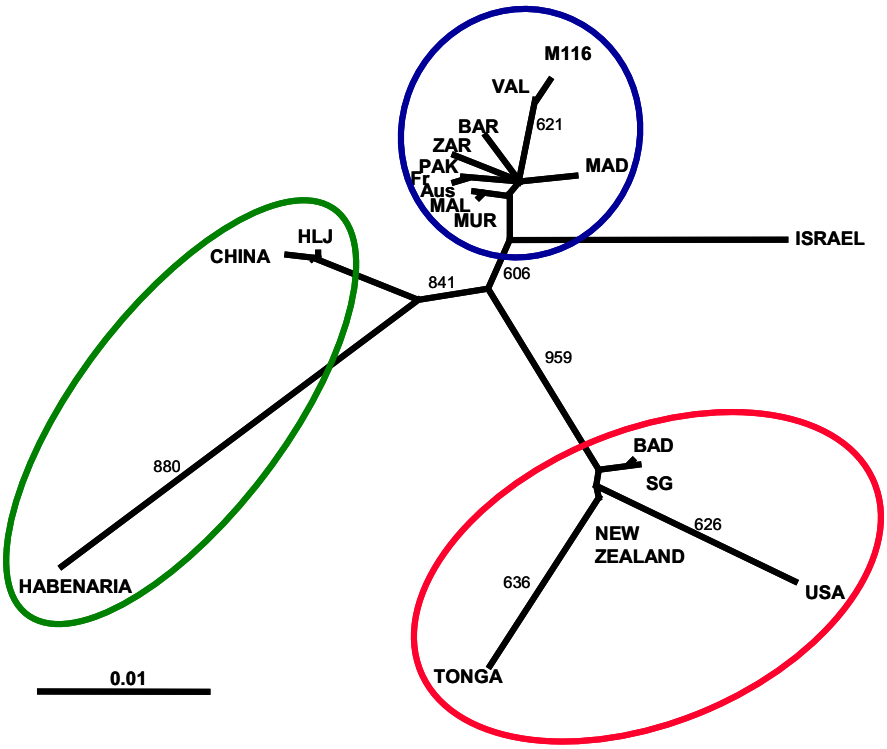

Fig. 2 Phylogenetic comparison among the reported isolates of WMV in the CP region. Neighbour-joining method with a bootstrap value of 1000 was used to calculate the phylogenetic relationship. The accession numbers of the isolates are: SG AJ579481, ZAR AJ579487, BAR AJ579495, VAL AJ579498, MUR AJ579499, BAD AJ579503, MAD AJ579514, MAL AJ579521, Fr. AY437609, HLJ AY464948, NEW ZEALAND AY995215, CHINA DQ399708, USA D13913, TONGA L22907, HABENARIA AB001994, PK A B218280, ISRAEL AF322376 and M116 AF551334.

recombination (Ali *et al*. 2006). Although the available WMV sequence analysis results confirm that this virus is closely related to SMV (Desbiez and Lecoq 2004; Ali *et al*. 2006), there is a discrepancy in its position regarding its molecular and biological properties. According to the criteria proposed by Shukla *et al*. (1994), WMV should be considered as a strain of SMV (Yu *et al*. 1989). However, high variability is observed in the 5′ part of the genome between the two isolates in terms of both the percent identity and genome length. Phylogenetic trees drawn for the N- and C-terminal P1, HC-Pro and 5′ UTR regions clearly indicate that WMV-Pk converges more toward SMV isolates in the 5′ UTR, C-terminal P1 and HC-Pro regions, but is quite distant in the N-terminal P1 region, where the sequence identity is more toward BCMV/PSV isolates, thereby strengthening the assumption that WMV originated as a result of recombination between SMV and BCMV/PSV (Desbiez and Lecoq 2004). The putative recombination spot for the amino acid sequence has been identified (Desbiez and Lecoq 2004). In the 5′ UTR, the nucleotide sequence percent identity is again more closely related to SMV than to PSV/BCMV, but the variability in size and sequence makes it impossible to explore any other recombination events. Nevertheless, the percent identities between WMV and other potyviruses remain below 70%, whereas they are above 90% between strains of SMV (Desbiez and Lecoq 2004). This recombination event may have occurred only once in the emergence of the virus, since the P1 sequences of WMV strains from different geographical origins present the same characteristics with a unique putative recombination point. No WMV strains with SMV-like P1 proteins have been observed to-date. The relatively important divergences between WMV and BCMV or SMV in different parts of the genome indicate that the recombination event took place very early during the evolution and differentiation of potyviruses.

The host range has been widely used in the past to classify WMV strains, but the variability in host range and symptomatology (Dijkstra 1992), cross-reactivity of antisera and ambiguous serological relationships between isolates of the same or different viruses (Shukla *et al*. 1992) make the situation very ambiguous. Putative homologous recombination, which has been recognized for a number of potyviruses, including *Plum pox virus* (PPV) (Glasa and Kudela 2001), *Potato virus Y* (PVY) (Glais *et al*. 2002), BCMV (Revers *et al*. 1996), *Turnip mosaic virus* (TuMV) (Ohshima *et al*. 2002) and *Lettuce mosaic virus* (LMV) (Krause-Sakate *et al*. 2004) adds to the problem of defining reliable criteria for demarcating the taxonomic units. Shukla *et al*. (1994) proposed that species of the same virus should have a 3′ UTR sequence identity of >75%. It has also been proposed that the species demarcation criteria should be <76% nt identity and <82% aa identity, and that the corresponding threshold for individual genes should range from 58% (P1) to 74-78% (other genes). A genus demarcation criterion for the entire ORF of <46% nt identity has been proposed (Adams *et al*. 2005). For CP, 76-77% nt identity was suggested as the optimal species demarcation criterion (Adams *et al*. 2005). According to these criteria, WMV-Pk has a 3′ UTR sequence identity of 81.4% and a CP sequence identity of 86.5% with the SMV-*Svr* strain, which are sufficiently high to consider WMV as a strain of SMV. However, unlike SMV, WMV has a broad host range, which is rare for an individual potyvirus. Even the biological and serological properties of WMV and SMV are different. Although phylogenetic analyses have revealed that WMV clustered with SMV isolates in the BCMV subgroup, its full-length genome is longer than those of reported SMV isolates and the P1 region is closer to BCMV/PSV than to SMV, thereby presenting a very confusing situation. Hence, it has been recommended that WMV should be kept as an independent species in the genus *Potyvirus* rather than categorizing it as a strain of SMV. The CI gene, which most accurately reflects the taxonomic status according to the complete ORF, has been proposed as the best region for diagnostic and taxonomic studies. However, such classification of viruses on the basis of only a fraction of their genomic information requires further reconsideration, and proper weightage should also be given to other taxonomic criteria.

CONTROL

Various strategies for controlling WMV have been used in the past. These stylet-borne non-persistently transmitted viruses are difficult to control through the use of insecticides (Nameth *et al*. 1986). Two other methods used to protect crops from aphids are reflective mulches (Nameth *et al*. 1986; Brown *et al*. 1993; Orozco *et al*. 1994; Summers *et al*. 1995) and oil sprays (Simmons and Zitter 1980). Toscano *et al*. (1979) reported a 90% reduction in viral incidence through the use of aluminum foil mulches and up to 26% reduction using oil sprays. However, aluminum foils are expensive and slow the growth of seedlings, while oil sprays are a potential problem when sprayed under high temperatures and can result in plant injuries. Walters (2003) reported that using row-covers or black or white mulches had greater influences in reducing the incidence of WMV with high total marketable yields. Culture practices, such as weed control and effective rotation, have also been exploited for controlling WMV. Use of resistant varieties is the most effective and inexpensive method of controlling a viral disease. However, very little genetic material resistant to WMV is available. Some tolerance has been reported in the Cantaloupe variety Top-mark (Nameth *et al*. 1986), breeding line 91213 of *C. melo* (Moyer *et al*. 1985), four accessions of *C. colocynthis* from Iran and one from Morocco. High resistance has been reported in Egun PI 164708 from India (Gillaspie and Wright 1993) and PI 494528 and PI 494532 of *C. colocynthis* (Provvidenti 1986). Accessions C-885 and C-769 are potential sources of multiple resistance to PRSV-W, WMV-2 and ZYMV (Diaz *et al*. 2003). Moderate resistance was found in PI 595203 (*C. lanatus* var. *lanatus*), an Egusi type originally collected in Nigeria (Xu *et al*. 2004). The *C. melo* accession TGR-1551 was also resistant (Diaz *et al*. 2003). Cross-protection has been used effectively against WMV (Kosaka and Fukunishi 1997). Transgenic resistance is one of the most effective methods of controlling viral diseases. CP transgenic resistance to WMV has been reported in squash and cantaloupe (Namba *et al*. 1992; Clough and Hamm 1995). The transgenic squash CZW-3 expressing the CP genes of CMV, ZYMV and WMV has also been reported to show high resistance to these three aphid-borne viruses (Fuchs *et al*. 2004).

FUTURE PERSPECTIVES

The biological variability of WMV is well documented (Purcifull and Hiebert 1979; van Regenmortel 1971), but it has not yet been characterized extensively at the molecular level. The complete genome sequences of only three isolates have been reported and we recommend more sequencing of isolates collected from entirely different agro-ecological zones of the world to make the taxonomic status more clear. According to the reported criteria, WMV could easily be considered a strain of SMV. However, unlike SMV, WMV has a broad host range, which is rare for an individual potyvirus. Even the biological and serological properties of WMV and SMV are different. Sequence analysis suggests the event of recombination and the relatively important divergences between WMV and BCMV or SMV in different parts of the genome indicate that this recombination event took place very early during the evolution and differentiation of potyviruses. Based on these confusing results, it has been proposed to place WMV as separate species in the genus, but to support this hypothesis there is a dire need to sequence more and more isolates. No WMV strains with SMV-like P1 proteins have been observed to-date. As WMV presents a broader host range than most other Potyviruses,

experiments must be planed to explore the genomic region, which enabled the virus to have such a large host range. Those viruses which are transmitted by large species of vectors usually have large host range. The interaction of the virus vector also needs to be explored.

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