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Complete genomic sequence and recombination analysis of *wheat yellow mosaic virus* isolate from Zhouzhi in China

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Wheat yellow mosaic virus (WYMV) is the causal agent of wheat yellow mosaic disease in China. WYMV was detected in wheat sample collected from Zhouzhi of Shanxi province. The nearly complete genomic sequence of Zhouzhi isolate (WYMV-ZZ) was determined; it was compared with six complete sequences of WYMV isolates (five Chinese isolates and one Japanese isolate). WYMV-ZZ and the other six different WYMV isolates shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2, respectively; at the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2, respectively, with the other six isolates. Phylogenetic analysis showed that the NIa-VPg region can separate the Chinese isolates from Japanese isolate. Based on the recombinant analysis, there were three possible recombination events; one event was located in RNA1 CI region of WYMV-ZZ with a RDP *P*-value of 8.526×10^{-06}. This work advanced our understanding of the WYMV molecular variation and was helpful to study the disease spread.

Key words: Sequence comparison, phylogenetic analysis, NIa-VPg, recombinant event.

INTRODUCTION

Wheat yellow mosaic virus (WYMV), is the causal agent of wheat yellow mosaic disease of wheat in China and Japan, belongs to the genus *Bymovirus* within the family Potyviridae and is a soil-borne pathogen, it is transmitted by the fungus-like organism *Polymyxa graminis* (Sawada, 1927). In China, the disease was found in Sichuan province in the 1960s (Tao et al., 1980) and spread gradually to the middle and lower valleys of the Yangtze and Huai Rivers (Li et al., 1997; Chen, 1999). Wheat yellow mosaic virus causes typical symptoms including mosaic, yellowing, dwarfing, stunting or excessive tillering, and subsequently decreasing yield. Under low-temperature conditions in the field, WYMV infects wheat. When spring comes, the infected wheat shows light green, oval- or spindle-shape spots; the temperature back to 10°C, the infected leaves show yellow mosaic symptom with the disease spots expand and emerge. Finally, WYMV causes serious damage as a result of

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yield losses (Wang et al., 2015).

The genome of WYMV is composed of two (+) single-stranded RNAs, RNA1 encodes for P3, pretty interesting Potyviridae ORF (PIPO), 7K, cytoplasmic inclusion protein (CI), 14K, nuclear inclusion protein a (NIa) which contains viral genome-linked protein (VPg) and C-terminal protein, nuclear inclusion protein b (NIb) and coat protein (CP); RNA2 encodes for a polyprotein that contains 28- and 72-kDa proteins (Chen et al., 1999; Clover and Henry, 1999; Yu et al., 1999).

Full-length of five Chinese WYMV isolates and one Japanese WYMV isolate have been detected including isolate that came from Huangchuan, Henan province (WYMV-HC) (Yu et al., 1999); from Yangzhou, Jiangsu province (WYMV-YZ) (Chen et al., 2000); from Ya'an, Sichuan province (WYMV-YA) (Chen et al., 2000); from Japan (WYMV-JPN) (Namba et al., 1998); from Zhumadian, Henan province (WYMV-ZMD) (Zhang et al., 2010); and from Xiaqiao, Jiangsu province (WYMV-XQ with GenBank accession numbers FJ361764 and FJ361767). In this study, WYMV was detected in wheat leaves collected from Zhouzhi, Shanxi province where WYMV has not been reported before. The complete sequence of Zhouzhi isolate (WYMV-ZZ) was cloned, sequenced and compared with the other six complete sequences. Phylogenetic and recombination analyses were performed among these seven isolates.

The analysis of virus sequence and identification of virus type are helpful for breeding wheat resistant varieties (Jin et al., 2016). Full-length of five Chinese WYMV isolates and one Japanese WYMV isolate have been retrieved from GenBank database (Table 2) including isolate that came from Huangchuan, Henan province (WYMV-HC) (Yu et al., 1999); from Yangzhou, Jiangsu province (WYMV-YZ) (Chen et al., 2000); from Ya'an, Sichuan province (WYMV-YA) (Chen et al., 2000); from Zhumadian, Henan province (WYMV-ZMD) (Zhang et al., 2010); from Xiaqiao, Jiangsu province (WYMV-XQ); and from Japan (WYMV-JPN) (Namba et al., 1998).

MATERIALS AND METHODS

Sample

Wheat sample was collected from Zhouzhi, Shanxi province of China in 2008.

Total RNA extraction, reverse transcription-polymerase chain reaction (RT-PCR) and sequencing

Total RNA from wheat leaves was extracted by LiCl precipitating method (Zhang et al., 2011). Primer VP-1M was used for reverse transcription (RT) reaction and primer pair VP-1P/VP-1M was used to amplify a 704 bp fragment which was the VPg region in RNA1; primer ut-1M was used for RT reaction and primer pair ut-1P/ut-1M was used to amplify a 880 bp fragment which was the 3-termino-UTR in RNA2 (Ohto and Sakai, 2005). The purified bands were cloned into pMD18-T vector and 2-3 clones were sequenced by companies (Introvigen and BiMad). The sequence fragments were combined together by DNAMAN 7.0.

Western blotting

The wheat leaves were grinded by liquid nitrogen, added 2×SDS protein buffer, blended and incubated at 100°C for 5 min, then put the samples on the ice for 5 min, and centrifuged 12,000 rpm for 10 min; the supernatant was carried onto the SDS polyacrylamide gel electrophoresis. After electrophoresis, the sample was transferred to Hybond-C membrane, and the membrane was incubated with TBST buffer (20 mM Tris, 137 mM NaCl, 0.3% Tween20, pH 7.6) containing 5% skimmed milk powder for 2 h at 37°C and added antiserum of WYMV-CP which was prepared by Yan-hong Han storing in my lab to incubate for 1 h at 37°C. Blot was rinsed by TBST for 3 times and incubated for 1 h with anti-goat IgG diluted 1:10,000. After washing in TBST, blot was visualized by NBT and BCIP (Han et al., 2002).

Phylogenetic analysis

To better understand the relationship of WYMV-ZZ and other six WYMV isolates, the full-length sequence alignments and phylogenetic analysis of nucleotide and amino acid were conducted. Phylogenetic trees were constructed for by the neighbor-joining method and visualized using MEGA X (Molecular Evolutionary Genetics Analysis version X) with 1000 bootstraps replicate (https://www.megasoftware.net) (Kumar et al., 2018).

Recombination analysis

Recombination of seven WYMV isolates was constructed by RDP4.97 (Recombination Detection Program version 4.97) (Martin et al., 2015). Various recombination detection methods were used to analyze putative recombinants and recombination breakpoints, including the programs RDP, GENECONV, BOOTSCAN, MAXCHI, SISCAN and 3SEQ. The recombination events which were surveyed by at least five different methods could be received (Zhou et al., 2012).

RESULTS

Detection of WYMV-ZZ by RT-PCR and Western blotting

WYMV-ZZ was detected from wheat sample of Zhouzhi, Shanxi province using RT-PCR and Western blotting (Figure 1).

Complete genomic sequence of WYMV-ZZ and its comparison with other six isolates

The genomic RNA sequence of WYMV-ZZ was obtained from the wheat sample by amplification of four overlapping cDNA fragments for RNA1 and two overlapping cDNA fragments for RNA2 using the primer pairs WY1001F/WY11920R, WY11858F/WY13832R, WY13578F/WY15446R, WY15378F/HC511-BHR for RNA1, and WY2001F/WY22012R, WY21927F/HC511-

Table 1. Primers used for RT-PCR and determining full-length sequences.

Primer	Sequence (5′-3′)	Genomic position
WY1001F	AAA AATAAATAACACAGACCAAAACCATCAAAACG (+)	RNA1, 1-36 nt
WY11920R	TGATACAAGCCTGGATCCGTTGC (-)	RNA1, 1920-1898 nt
WY11858F	CACGCAATGGATCCAGGCTTCATA (+)	RNA1, 1858-1881 nt
WY13832R	CTGGCTCTGCGCGGTCTGATATCTT	RNA1, 3832-3808 nt
WY13578F	TATTGAAGATGACTCCAGCGATG (+)	RNA1, 3578-3600 nt
WY15446R	AACTTCCTGCTCGCTGAGATGTGC	RNA1, 5446-5423 nt
WY15378F	ACTTCCGCCGGACCAAGCTACCAG (+)	RNA1, 5378-5401 nt
WY2001F	AAAATAAAACCACCACAAACAAAAC (+)	RNA2, 1-26nt
WY22012R	CTGAATTGTTGCTGGTGAGACATCAT	RNA2, 2102-2077 nt
WY21927F	GAAATCTCCAAGAGCTTCAAGCAGTCA (+)	RNA2, 1927-1953 nt
HC511-BHR	GGATATCTGCAGGATCCAAGC (-)	universal primer
OligdT	GGATATCTGCAGGATCCAAGCTTTTTTTTTTTTTTTTT (-)	universal primer
VPg-1P	TGAAGATGACTCCAGCGATG (+)	RNA1, 3578-3587 nt
VPg-1M	GACCTGGGATAGGAGAATTC (-)	RNA1, 4281-4262 nt
ut-1P	CTTAAGAGGTGGAGCACGGA (+)	RNA2, 2736-2755 nt
ut-1M	GACGATCGACAGGTGCATTG (-)	RNA2, 3595-3576 nt

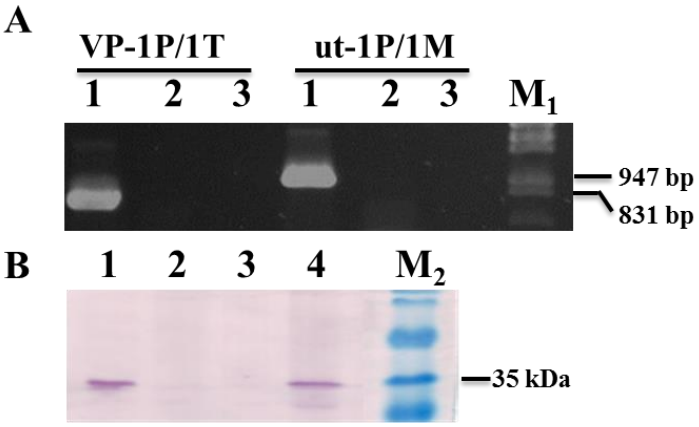

Figure 1. Result of RT-PCR (A) and western blotting (B). Lane 1: Wheat sample from Zhouzhi; Lane 2: Healthy control; Lane 3: Mock; Lane 4: Positve control; M_1: λ DNA digested by *Hin*d III and *Eco*R I; M_2: Protein marker (SM 0671, NEB).

BHR for RNA2 (Table 1). These primers were derived from the conserved region of six known isolates. A nearly complete nucleotide sequence of WYMV was determined, apart from short regions where the primers annealed at the 5'- and 3'-terminus. The full-length sequence of WYMV was submitted to GenBank with accession number FJ261765 for RNA1 and FJ361768 for RNA2 (Table 2).

Based on the full-length nucleotide comparison, WYMV-ZZ shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2, respectively, with the other six isolates (WYMV-HC, WYMV-YA, WYMV-YZ, WYMV-XQ, WYMV-ZMD, WYMV-JPN). At the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2, respectively, with other six isolates; the identity for genes of RNA1 and RNA2 was >90% between WYMV-ZZ and six other isolates (Table 3). The nucleotide sequence identities for the individual ORFs and UTR of RNA1 between WYMV-ZZ and six other isolates were 98.0 to 99.0% for P3, 96.5 to 98.5% for 7K, 95.5 to 97.7% for CI, 93.5 to 96.7% for 14K, 96.4 to 98.9% for NIa-VPg, 96.1 to 97.1% for NIa-Pro, 96.9 to 97.5% for CP, 96.6 to 97.7 for 5′ UTR and 96.5 to 98.1% for 3′UTR; for the individual ORFs and UTR of RNA2 the nucleotide sequence identities between WYMV-ZZ and six other isolates were

Table 2. GenBank accession numbers of seven WYMV isolates.

Isolate	Place	RNA1	RNA2
WYMV-HC	Huangchuan, Henan Province	AF067124	AF041041
WYMV-YZ	Yangzhou, Jiangsu Province	AJ131981	AJ131982
WYMV-YA	Yaan,Sichuan Province	AJ239039	AJ242490
WYMV-ZMD	Zhumadian, Henan Province	FJ361766	FJ361769
WYMV-XQ	Xiaqiao, Jiangsu Province	FJ361764	FJ361767
WYMV-ZZ	Zhouzhi, Shanxi Province	FJ361765	FJ361768
WYMV-JPN	Japan	D86634	D866350

94.9 to 98.3% for P1, 93.8 to 98.3% for P2, 91.1 to 98.2% for 5′ UTR, and 98.2 to 99.0% for 3′ UTR. At the amino acid level, the identities for the individual ORFs of RNA1 between WYMV-ZZ and six other isolates were 96.6 to 98.8% for P3, 95.5 to 98.5% for 7K, 93.9 to 97.6% for CI, 91.9 to 97.6% for 14K, 93.0 to 98.9% for NIa-VPg, 90.0 to 97.3% for NIa-Pro, and 98.0 to 98.6% for CP; for RNA2 the identities were 94.5 to 98.8% for P1 and 94.0 to 98.0% for P2.

Phylogenetic analysis of seven different isolates

To better understand the relationship between WYMV-ZZ and six other isolates, the phylogenetic analysis of seven different isolates was constructed. Phylogenetic trees were constructed for P3, 7K, CI, 14K, NIa-VPg, NIa-Pro, CP, and full-length of RNA1, and for P1, P2, and full-length of RNA2 by the neighbor-joining method and visualized using MEGA (version X) with 1000 bootstrap replicates. The results showed that P3, CI, 14K, NIa-VPg, NIa-Pro, NIb and CP of WYMV-ZZ were more closely related to WYMV-YA, 7K of WYMV-ZZ was close to WYMV-XQ; and that P1 and P2 of WYMV-ZZ were close to WYMV-ZMD (data not shown). The full-length of WYMV-ZZ was close to WYMV-YA for RNA1 and was close to WYMV-ZMD for RNA2 (Figure 2A and B). The phylogenetic trees generated based on the NIa-VPg region of RNA2 showed that this region clustered together with the other five Chinese isolates, while WYMV-JPN formed a distinct branch (Figure 2C and D).

Recombination analysis

The seven sequences of WYMV isolates were processed and examined for recombination at the same time. The major parent, minor parent, the event and the corresponding *P*-value of four recombination events are as shown in Figure 3 and Table 4. The most possible one recombination event of WYMV-ZZ, which is located in 2,598 to 4,019 nt of RNA1 CI region, may recombined with unknown major parent (WYMV-JPN) and minor parent (WYMV-HC) with a RDP *P*-value of 8.526×10^{-06}. The second recombination event of WYMV-HC, which is located in 564 to 894 nt of RNA2 P1, may recombined with major parent (WYMV-ZZ) and minor parent (WYMV-YA) with a RDP *P*-value of 5.444×10^{-05}. The third recombination event of WYMV-XQ, which is located in 1698 to 3196 nt of RNA P2 and 3′ UTR regions, may recombined with major parent (WYMV-ZMD) and minor parent (WYMV-YA) with a RDP *P*-value of 3.147×10^{-05}.

In this study, WYMV was detected in wheat leaves which is collected from Zhouzhi, Shanxi province of China in 2008. The wheat samples were infected with WYMV confirming by both RT-PCR and western blotting. For RT-PCR, targeting the VPg region in RNA1, primer VP-1M was used for reverse transcription reaction and primer pair VP-1P/VP-1M was used to amplify a 704 bp fragment; targeting the 3-termino-UTR in RNA2, primer ut-1M was used for RT reaction and primer pair ut-1P/ut-1M was used to amplify the 880 bp fragment. Using antiserum of WYMV-CP, Western blotting was carried out with the 32 kD positive band (Figure 1B).

Apart from short regions where the primers annealed at the 5'- and 3'-terminus, a nearly complete nucleotide sequence of WYMV-ZZ was determined and given the GenBank accession number FJ361765 for RNA1 and FJ361768 for RNA2, respectively (Table 2).

Based on the nucleotide sequence comparison, WYMV-ZZ shared 96.6 to 97.7% and 95.1 to 98.2% nucleotide sequence identity for RNA1 and RNA2 with the other six isolates (WYMV-HC, WYMV-YA, WYMV-YZ, WYMV-XQ, WYMV-ZMD, WYMV-JPN) infecting wheat in different parts of the world. At the amino acid level, WYMV-ZZ had 94.1 to 98.2% identity for RNA1 and 94.1 to 96.7% identity for RNA2 with other six isolates. The identity for genes of RNA1 and RNA2 were >90% between WYMV-ZZ and six other isolates (Table 3).

To better understand the relationship between WYMV-ZZ and other six isolates, the phylogenetic analysis were carried out using MEGA (version X) with 1000 bootstrap replicates. The results showed that P3, CI, 14K, NIa-VPg, NIa-Pro, NIb and CP of WYMV-ZZ were more closely related to WYMV-YA, 7K of WYMV-ZZ was close to WYMV-XQ; and that P1 and P2 of WYMV-ZZ were close

Table 3. Sequence identity comparison of WYMV-ZZ with other six isolates.

WYMV-ZZ	WYMV-HC		WYMV-YZ		WYMV-YA		WYMV-XQ		WYMV-ZMD		WYMV-JPN	
	nt (%)	aa (%)	nt (%)	aa (%)	nt (%)	nt (%)	aa (%)	aa (%)	nt (%)	aa (%)	nt (%)	aa (%)
A for full length												
RNA1	97.0	94.4	97.3	96.8	97.2	96.6	95.8	95.1	97.1	96.1	97.7	96.6
RNA2	96.9	96.7	97.3	96.5	95.1	95.9	94.1	96.1	97.0	96.5	98.4	98.2
B for coding region												
P3	98.9	98.5	99.0	98.5	98.3	98.0	96.6	98.8	98.8	98.2	99.0	98.2
7K	96.5	95.5	98.0	97.0	98.5	97.5	97.0	98.5	98.0	98.5	97.5	98.5
CI	96.4	96.1	96.7	95.6	96.8	95.5	93.9	97.6	96.7	95.8	97.7	97.1
14K	93.5	91.9	95.2	95.2	96.2	94.6	94.4	96.8	94.4	93.5	96.2	97.6
NIa-VPg	98.2	96.8	98.9	98.4	98.2	96.4	93.0	98.4	98.2	98.9	98.4	97.9
NIa-Pro	96.8	90.0	96.7	95.0	97.1	96.5	95.5	97.3	96.1	94.5	96.7	95.0
NIb	97.7	98.7	97.7	98.5	97.2	97.3	98.5	99.1	97.5	98.5	98.0	98.7
CP	97.2	98.0	97.4	98.3	97.3	97.5	98.6	98.6	96.9	98.0	97.5	98.6
5′UTR	97.0	-	97.3	-	97.2	96.6	-	-	97.1	-	97.7	-
3′UTR	98.1	-	97.3	-	98.1	96.5	-	-	97.7	-	97.7	-
P1	94.9	94.5	97.1	97.2	95.4	95.5	94.5	98.0	97.4	98.4	98.3	98.8
P2	97.0	97.5	96.8	96.1	93.8	95.1	94.0	95.4	96.2	95.7	98.3	98.0
5′ UTR	97.1	-	97.1	-	91.1	95.8	-	-	97.1	-	98.2	-
3′ UTR	98.7	-	98.7	-	99.0	98.2	-	-	98.7	-	98.8	-

to WYMV-YA for RNA1 and was close to WYMV-ZMD for RNA2 (Figure 2A and B). The phylogenetic trees generated based on the NIa-VPg region of RNA2 showed that this region clustered together with the other five Chinese isolates, while WYMV-JPN formed a distinct branch (Figure 2C and D).

Recombination of seven WYMV isolates was analyzed by RDP4.97. Six recombination detection methods were used to analyse putative recombinants and recombination breakpoints. The programs used were RDP, GENECONV, BOOTSCAN, MAXCHI, SISCAN and 3SEQ.

Detected by at least five different methods, three recombination events were received (Zhou et al., 2012). The recombination event detected in RNA1 CI region of WYMV-ZZ, which is located in 2,598-3,344 nt, may be recombined with unknown major parent (WYMV-YZ) and minor parent (WYMV-HC) with a RDP *P*-value of 8.526×10^{-06}. There was no recombination event RNA2 of WYMV-ZZ. Consistent nucleotide and amino acid, and close phylogenetic relationships may imply the molecular evolution of WYMV is a genetic stability progress.

DISCUSSION

The genomic RNA sequence of WYMV-ZZ was determined. Sequence comparison, phylogenetic tree and recombination analysis were performed among WYMV-ZZ and other six known WYMV isolates. Consistent nucleotide and amino acid, and close phylogenetic relationships may imply the molecular evolution of WYMV is a genetic stability progress.

VPg is a multiple function protein, which participates in the genomic replication and interacts with 3-terminal poly-A to achieve similar function with 5-terminal cap structure (Gallie et al., 1995; MURPHY et al., 1996; Ohshima et al., 2007). So far, there was little research about WYMV-VPg, the VPg of Potato virus Y exists in different forms to exercise different functions

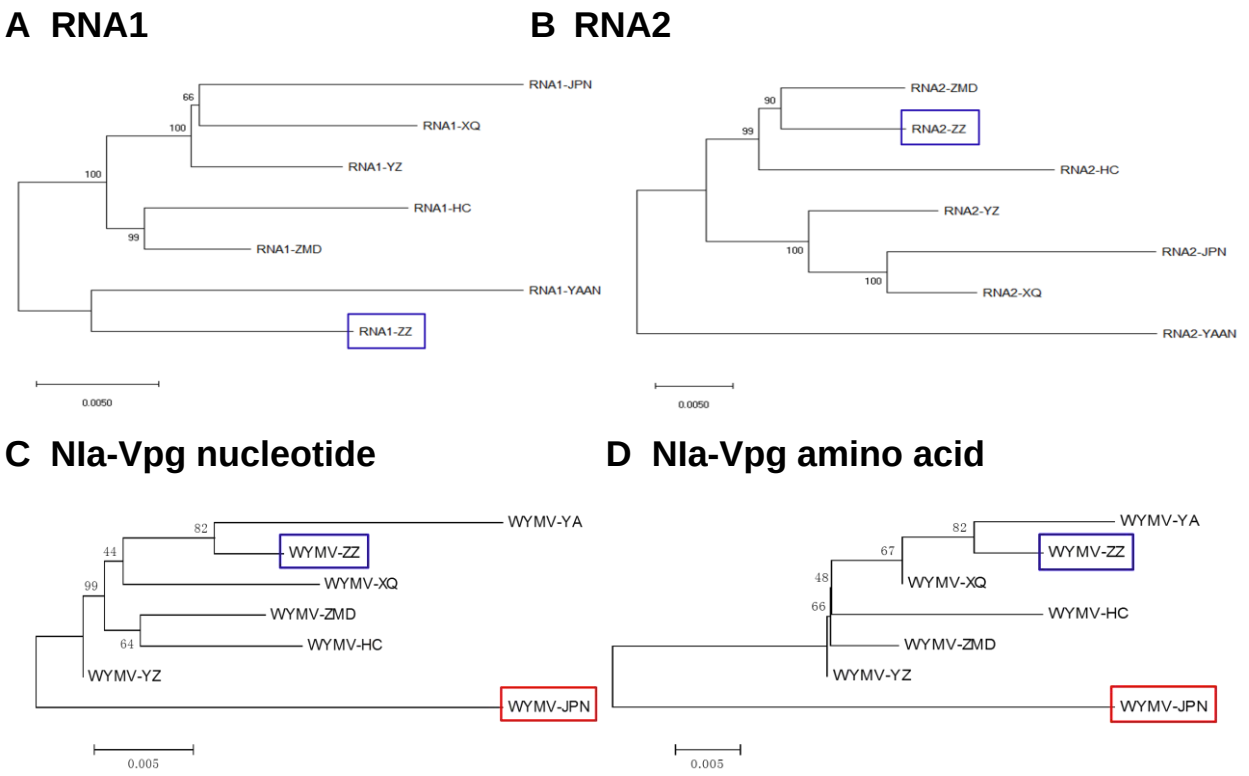

Figure 2. Phylogenetic trees generated by seven WYMV isolates. A, B: The phylogenetic trees for RNA1 and RNA2 nucleotide of seven WYMV isolates. Blue frame: WYMV-ZZ; C, D: Nucleotide and amino acid of VPg neighbor-joinging trees generated and the respective amino acid of seven WYMV isolates. Blue frame: VPg of WYMV-ZZ. Red frame: VPg of WYMV-JPN.

during the life cycle of virus. VPg of tobacco etch potyvirus is a host genotype-specific determinant for long-distance movement (Schaad et al., 1997; Daròs et al., 1999), and may also participate in inhibiting viral gene silencing (Germundsson et al., 2006). Sequences of VPg region were used for distinguishing two Japanese pathotypes (WYMVY-T and WYMV-M) (Ohto et al., 2005). Moreover, WYMV VPg accumulated in both the nucleus and cytoplasm of infected cells but exclusively localized in the nucleus when expressed alone in plants, and VPg interacted with WYMV coat protein (CP) and proteinase 1 (P1) proteins in vitro and in planta assays, WYMV-P1 may adjust to facilitate VPg activity through regulating VPg sub-cellular distribution (Rong, 2011). The structural and subcellular distribution of VPg protein was analysed that VPg protein contained a nuclear localization signal and a nuclear export signal, that VPg protein was detected in both cytoplasm and nucleus in virus infected leaves of wheat plant cells (Bian, 2013). In addition, the WYMV-Nib8 gene was trasformed into the transgenic wheat line N12-1, and this transgenic wheat can effectively control the wheat yellow mosaic virus disease (Fu et al., 2016).

Recently, the nucleotide sequences encoding CP and VPg of WYMV collected from five provinces of China was determined; the results showed the low level of genetic diversity and inferred that the WYMV in China was genetic stability or recent emergence (Sun et al., 2013). From this study, the seven WYMV isolates showed high sequence identity comparison with nucleotide and with amino acid and VPg may be the breakthrough point of WYMV ongoing molecular evolution.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

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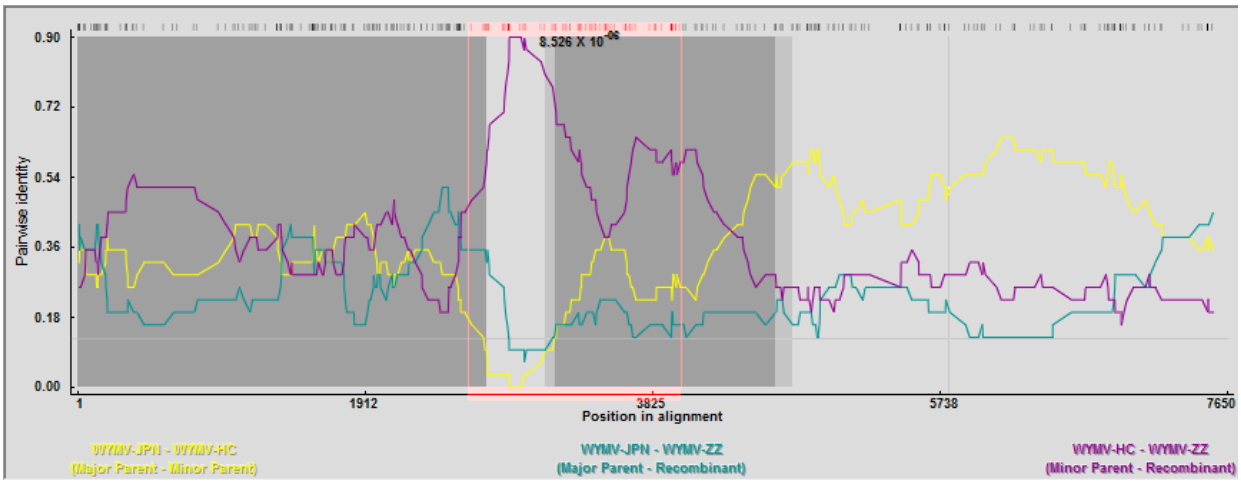
Abbreviations

WYMV, Wheat yellow mosaic virus; **WYMV-HC,** WYMV

A



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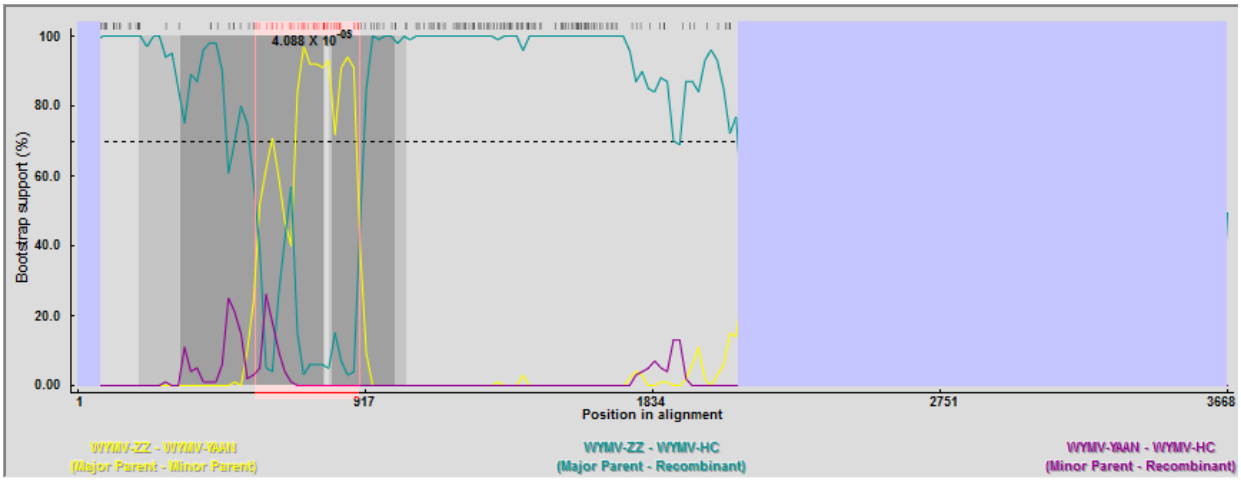

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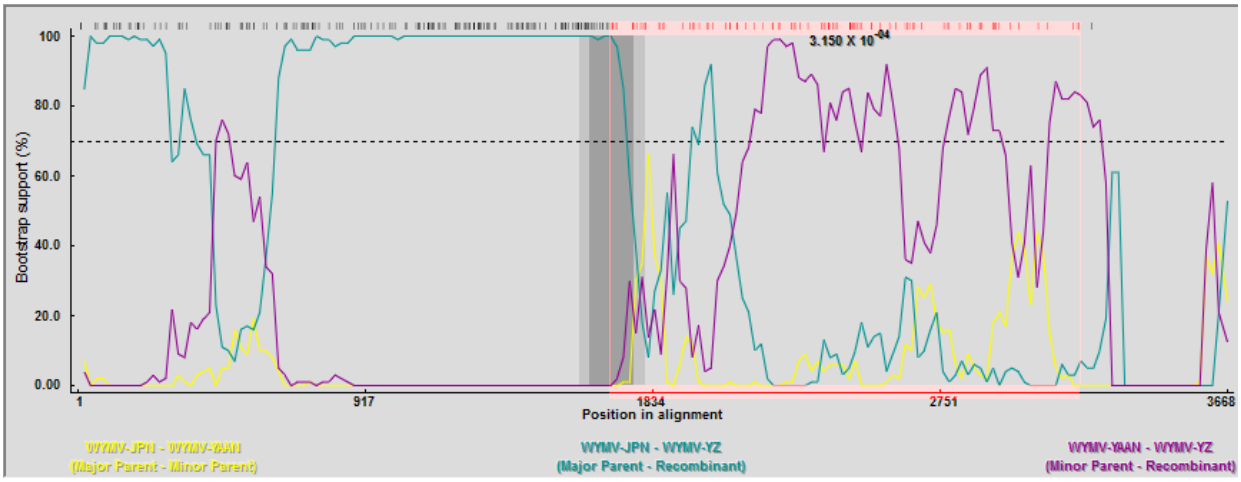

Figure 3. Recombination of WYMV analyzed using RDP4.97. A-D: BOOTSCAN plot for the recombinant of WYMV-ZZ within RNA1, of WYMV-HC within RNA2, and of WYMV-YZ within RNA2. The left and the right boundaries of the pink region indicate breakpoint positions. The yellow line is the major parent: minor parent plot, the green line is the recombinant plot; the dotted line indicates the bootstrap cut off value.

Table 4. Recombination events detected in WYMV isolates.

Event	RNA1	RNA2	RNA2
Recombinant	WYMV-ZZ	WYMV-HC	WYMV-YZ
Major parent	Unknown (WYMV-YZ)	WYMV-ZZ (98.4%)	WYMV-JPN (98.1%)
Minor parent	WYMV-HC (95%)	Unknown (WYMV-YAAN)	WYMV-YAAN (96.9%)
***P*-values determined using seven different programs**			
RDP	8.526×10^{-06}	$5.\times10^{-04}$	3.147×10^{-05}
GENECONV	1.031×10^{-06}	ND	ND
BootScan	3.214×10^{-08}	ND	3.150×10^{-04}
MaxChi	1.695×10^{-07}	2.601×10^{-04}	4.474×10^{-11}
Chimaera	9.839×10^{-06}	1.302×10^{-04}	2.923×10^{-09}
Siscan	2.500×10^{-08}	ND	4.204×10^{-05}
3Seq	1.901×10^{-03}	1.264×10^{-03}	2.041×10^{-08}
Beginning breakpoint (nt)	2598	564	1698
Ending breakpoint (nt)	4019	898	3196

ND: Recombination was not detected by this program.

isolate of Huangchuan, Henan province; **WYMV-YZ,** WYMV isolate of Yangzhou, Jiangsu province; **WYMV-YA,** WYMV isolate of Ya'an, Sichuan province; **WYMV-ZMD,** WYMV isolate of Zhumadian, Henan province; **WYMV-XQ,** WYMV isolate of Xiaqiao, Jiangsu province; **WYMV-JPN,** WYMV isolate of Japan; CP, coat protein; **VPg,** viral genome-linked protein.

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