EVALUATION OF APHID TRANSMISSION ABILITIES AND VECTOR TRANSMISSION PHENOTYPES OF BARLEY YELLOW DWARF VIRUSES IN CHINA

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SUMMARY

Barley yellow dwarf viruses (BYDVs) are economically important viruses that infect cereal crops worldwide, including wheat grown in China. These viruses include several members of the *Luteoviridae* and are transmitted only by aphids. In this study, BYDV isolates and their aphid vectors were collected from different regions of China and assayed. BYDV isolates from China were generally transmitted by more than one aphid species except for BYDV-RMV and a few BYDV-PAV. Seven vector transmission phenotypes (VTPs) were identified involving five serotypes; BYDV-GAV, BYDV-GPV and BYDV-PAV were predominant in China. Aphid clones differing in virus transmission abilities were occasionally observed for three laboratory-maintained species although they generally showed similar virus transmission profiles. Degenerate primers similar to Lu1 and Lu4 were designed to amplify coat protein gene fragments from all in the virus collection. Serotypes could be distinguished after cleavage of the resulting PCR products with *Hinf*I. Sequence variations among Chinese BYDVs were also deduced from differences among their restriction patterns. To clarify this, intact coat protein genes of a few isolates were amplified with serotype-specific primers, cloned and sequenced. Similarity and genetic distance analyses confirmed that the three major Chinese BYDVs possess coat protein genes distinct from those of BYDV from other countries.

Key words: Luteoviridae, aphid, interaction, serotype, RFLP, barley yellow dwarf disease.

INTRODUCTION

Barley yellow dwarf viruses (BYDVs) are naturally transmitted by at least 25 aphid species in a highly specific, circulative, non-propagative manner, and have been found infecting agronomically important small grains (Gray and Gildow, 2003). BYDVs are single-stranded positive sense RNA viruses that are currently classified in family *Luteoviridae,* which contains three genera (*Luteovirus*, *Polerovirus*, and *Enamovirus*) (D'Arcy and Domier, 2005). BYDVs comprise BYDV-PAV, BYDV-MAV and BYDV-PAS in genus *Luteovirus*; *Cereal yellow dwarf virus*-RPV (CYDV-RPV, formerly BYDV-RPV) and CYDV-RPS in genus *Polerovirus* as well as BYDV-SGV, BYDV-GPV and BYDV-RMV that have not yet been grouped into a genus. Aphid-virus relationships for BYDVs have been well documented in the past 50 years. Rochow (1969) grouped New York BYDVs into strains based on their aphid transmission efficiencies and specificities. Subsequently, variations in virus-aphid interactions were reported by others (Guo *et al.* 1997; Moon *et al.* 2000). One example is BYDV-GAV, which is serologically related to BYDV-MAV transmitted only by *Sitobion* (formerly *Macrosiphum*) *avenae*, but is transmissible by *Schizaphis graminum* as well as *S. avenae* (Zhou *et al.* 1987; Wang *et al.* 2001).

In China, wheat is the major crop that is infected by BYDV-GPV, BYDV-PAV and BYDV-GAV, and less frequently by BYDV-RMV (Zhou *et al.* 1984; 1986; 1987). BYDV-GPV from China, does not cross-react with antibodies to BYDVs from New York. BYDV-PAV and BYDV-RMV in China are similar in biology and serology to those from New York. The coat protein-encoding gene of BYDV-PAV-CN shared only 71~74% amino acid identity with other isolates of BYDV-PAV (Jia *et al.* 2003). BYDV-GAV sequences were similar to, but distinguishable from, BYDV-MAV (Wang *et al.*, 2001; Jin *et al.* 2004). Furthermore, the coat protein gene of BYDV-GPV was unlike those of any other BYDV (Cheng *et al.* 1996). In this study, field samples of BYDVs and their vector aphid species were collected throughout China, compared to laboratory strains and aphid vectors initially by virus transmission experiments. Viruses were further characterized by using immunological techniques, by comparing restriction patterns of RT-PCR products and by sequencing the coat protein-encoding genes.

MATERIALS AND METHODS

Aphids, viruses, host plants and antibodies. Labora-

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Table 1. BYDV transmission efficiencies of aphid genotypes.

Aphid[a]	BYDV-PAV	BYDV-GAV	BYDV-GPV	Aviruliferous
Rhopalosiphum padi clones:				
Jiangdu, Jiangsu	14/15[b]	9/17	17/22	0/15
Yangzhou, Jiangsu	10/18	3/16	5/15	0/15
Zhengzhou, Henan	10/10	4/12	5/13	0/12
Jinan, shandong	6/12	4/13	6/18	0/6
Huaiyin, Jiangsu	11/11	0/4	3/4	0/8
Jingjiang, Jiangsu	6/9	0/10	2/12	0/12
Weigang, Jiangsu	11/14	0/13	2/14	0/10
Juye, Shandong	6/9	0/8	4/10	0/9
Luohe, Henan	15/15	0/9	1/18	0/8
Xinyang, Henan	10/16	0/16	2/13	0/13
Yichuan, Shaanxi	15/28	0/26	13/27	0/21
Weinan, Shaanxi	3/6	0/4	3/5	0/3
Linfen, Shanxi	15/15	0/12	11/12	0/12
Guangyuan, Sichuan	23/26	0/20	5/19	0/20
Hangzhou, Zhejiang	23/26	0/13	3/18	0/16
Zhangye, Gansu	14/15	0/10	9/12	0/9
Fengzhen, Inner Mongolia	3/15	0/4	0/4	0/8
Shanxi	8/10	0/4	0/4	0/6
R. padi-La	89/102	0/60	29/64	0/93
Schizaphis graminum clones:				
Kangbao, Hebei	7/19	16/19	19/19	0/15
Zhangye, Gansu	3/12	9/12	12/12	0/9
Fengzhen, Inner Mongolia	2/3	2/3	2/3	0/3
Weinan, Shaaxi	2/10	4/8	5/8	0/9
Yichuan, Shaaxi	0/13	8/17	9/13	0/12
Jinan, Shandong	0/10	5/12	6/7	0/6
S. graminum-La	7/75	33/43	30/39	0/78
Sitobion avenae clones:				
Guangyuan, Sichuan	3/11	11/17	8/14	0/15
Jinan, Shandong	1/9	6/12	4/7	0/6
Linfen, Shanxi	2/6	6/7	3/7	0/7
Longxian, Shaanxi	9/19	19/21	0/26	0/18
Zhidan, Shaanxi	6/20	11/16	0/19	0/19
Ganquan, Shaanxi	8/17	18/23	0/26	0/15
Weinan, Shaanxi	3/8	9/9	0/10	0/6
Haerbin, Heilongjiang	5/9	9/11	0/13	0/9
Gangu, Gansu	2/9	2/4	0/8	0/6
Hainan	3/5	5/5	0/5	0/3
Changping Beijing	10/15	11/13	0/13	0/9
S. avenae -La	69/101	44/50	0/47	0/79

[a]Location from which aphid clone was isolated or laboratory aphid; [b]Diseased/total inoculated seedlings. Data from multiple tests are combined and "aviruliferous" means inoculation with aviruliferous aphids.

tory aphid clones of *Rhopalosiphum padi* (*R. padi*-La), *Sitobion avenae* (*S. avenae*-La) and *Schizaphis graminum* (*S. graminum*-La) have been reared continuously for nearly 20 years as BYDV vectors. *Rhopalosiphum maidis* and *Metapolophium dirhodum* were recently isolated from Beijing and Gansu, China, respectively. Field aphid clones, listed in Table 1, were collected from different regions of China during 1996-1997. Laboratory BYDV-GPV, BYDV-GAV, BYDV-PAV and field virus isolates collected from different regions of China during 1996-1997 are listed in Table 2, and the two field GAV-like isolates, 30W and 38W, were obtained from Beijing in 2003. The two Italian virus isolates were provided by Dr. Piero Caciagli (Istituto di Virologia Vegetale, CNR, Italy). Oat (*Avena sativa* cv. Coast Black) was used as a host for BYDV infection and wheat (*Triticum aestivum* cv Fengkang 8) was used for rearing aphids. Antibodies to BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV and CYDV-RPV were provided by Dr. R.M. Lister (Purdue University, Lafayette, USA).

Virus transmission experiments and serotyping. Aviruliferous aphid populations were generated each from a newborn nymph and confirmed as being aviruliferous by a negative virus transmission test (Rochow 1969). Aphids were reared on wheat seedlings in an incubator (2000 lux, 12 h light per day at 20°C). Plant leaf tissues infected with each virus isolate were kept at -20°C, and isolate regeneration was done from leaf tissue extracts by allowing aphids to feed through a membrane and then on a host plant as described by Rochow (1960). In virus transmission experiments, after an acquisition feeding in the dark for 48 h at 15°C, oat plants at the 2-leaf stage were each inoculated for 72 h using 5 viruliferous aphids. Aphids were subsequently killed with pesticide and the inoculated oats were kept at 18°C in 20000 lux for about 15 days before observation.

Double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was done using each of the five antibodies for virus serotyping (Aapola and Rochow 1971). Optical densities at 405 nm (OD_{405}) were measured with an ELISA reader (Bio-Rad, Hercules, CA, USA). A positive reaction was recorded when the OD_{405} of a sample was twice that given by samples from a healthy control plant (Zhou *et al*. 1984).

Molecular analysis. General molecular methods were as described by Sambrook *et al.* (1989). Because previously designed *Luteoviridae* degenerate primer pairs (Robertson *et al*. 1991; Naidu *et al*. 1997) might not allow the amplification of the coat protein genes of all BYDV isolates, Chinese BYDV sequences (Cheng *et al.*, 1996; Wang *et al*., 2001) were aligned with those of 16 other viruses in family *Luteoviridae* (Vincent *et al.*, 1990; 1991; Ueng *et al*., 1992) and the consensus regions of the 18 sequences were obtained by using the software MACAW (Lipman *et al*., 1989). Accordingly, two short 15-mer primers were designed in the coat protein-coding region. The upstream primer P5 (5'-CCAGTGGTTGTGGTC-3') covers sequence from +67 to +114 (base A of the start codon as +1) and the downstream primer P3 (5'-GGAGTCTACCTATTT-3') overlaps the amber codon UAG (corresponding to underlined bases). Viral RNA extraction and cDNA synthesis plus RT-PCR were done as described by Cheng and Zhou (1986) and Robertson *et al*. (1991) respectively. The PCR program was: 94°C for 45 sec, 38°C for 1 min, 72°C for 1 min; 35 cycles followed by 10 min extension. PCR products were cleaved with *Hinf*I, *Apa*I or *Taq*I, and separated by electrophoresis in 10% neutral polyacrylamide gel (PAGE). To sequence the coat protein genes, virus-specific primers (Wang *et al*. 1998; Cheng *et al*. 1996; Jin *et al*. 2004) were used in RT-PCR and the amplified fragments were cloned into pGEM-T and sequenced by the dideoxy-chain termination method using an A.L.F. express automatic sequencer (Amersham Pharmacia Biotec, Uppsala, Sweden). Sequences were aligned by using Clustal X (Jeanmougin *et al*., 1998). Genetic distance was calculated by the neighbor-joining method and an evolutionary tree was then built by using Treecon 1.3b (van de Peer and De Wachter 1997) with a bootstrap of 1000 resamplings.

RESULTS

Chinese BYDVs are generally transmitted by multiple aphid species. Most Chinese BYDVs can be transmitted by more than one aphid species (Zhou *et al*. 1984; 1986; 1987), which suggested biological dissimilarity of either the viruses or their vectors from those previously described. To exclude differences caused by experimental conditions, we first tested the impact of acquisition access period (AAP) and inoculation access period (IAP) on vector transmission phenotype (VTP). Twenty-five AAP/IAP combinations were tested for the transmission of BYDV-GAV by virus-free *S. avenae*-La. Longer AAP and IAP resulted in higher transmission rates and more stable VTPs. Minimal AAP and IAP for a successful transmission were 24 h and 12 h, respectively. A transmission rate of 87.5% was obtained for an AAP/IAP of 48/48 h and 100% for 48/72h. This indicated the 48/72h of AAP/IAP used here was reliable and previously observed virus-vector relationships are real. Secondly, three laboratory isolates, BYDV-GPV, BYDV-GAV and BYDV-PAV were transmitted by aviruliferous *S. graminum*-La, *S. avenae*-La, *R. padi*-La and *M. dirhodum* (Table 2). None of the viruses could be transmitted by *R. maidis*. BYDV-PAV was efficiently transmitted by *R. padi*-La and *S. avenae*-La (with a frequency of 70 to 80%) but poorly by *S. graminum*-La (5-7%). *S. graminum*-La, *S. avenue*-La and *M. dirhodum*

showed transmission rates of 75% with BYDV-GAV. BYDV-GPV was transmitted by *S. graminum*-La and *R. padi*-La, with a frequency of about 80% and 50%, respectively. *M. dirhodum* were able to transmit BYDV-GAV but not the other two viruses. Thus, the major Chinese BYDVs are transmitted by multiple aphid vectors in a specific manner.

Differences of geographical aphid clones in BYDV transmission. Virus-free field isolates of aphids belonging to three species were then tested for their abilities to transmit the laboratory strains of BYDV-GPV, BYDV-PAV and BYDV-GAV (Table 1). Twelve *R. padi* clones resembled *R. padi*-La in their efficient transmission of BYDV-PAV and BYDV-GPV, but inefficient transmission of BYDV-GAV. In contrast, *R. padi* clones from Jiangdu, Yangzhou, Zhengzhou and Jinan were able to transmit all three tested viruses, and BYDV-GAV and BYDV-GPV at similar frequencies. *R. padi* clones from Fengzhen and Shanxi only transmitted BYDV-PAV, but only a few seedlings were inoculated. However, their BYDV-GPV transmission abilities were lower than other *R. padi* isolates. For *S. avenae*, 9 of 11 clones displayed typical virus transmission profiles like that of *S. avenae*-La. *S. avenae* clones from Guangyuan, Jinan and Linfen were able to vector all the three virus isolates. Four *S. graminum* clones, like *S. graminum*-La, could transmit all the three laboratory BYDV strains, with fairly low frequencies of BYDV-PAV transmission. However, the other two, isolated from Yichuan and Jinan, did not transmit BYDV-PAV.

These data confirmed the multiple-vector feature of Chinese BYDVs. Clearly, the VTPs of BYDVs are usually very stable for the majority of the aphid genotypes, but clonal variations of aphids with distinct BYDV transmission abilities were occasionally observed.

Table 2. Vector transmission phenotypes (VTPs) and serotypes of BYDV isolates.

Virus[a]	Host	Transmitted by [c]					VTP	Serotype
		Sg-La	Sa-La	Rp-La	Md	Rm		
Italy	wheat	0/16[b]	0/27	24/36	0/20	0/11	P	RPV
Guiyang	wheat	0/31	0/34	34/52	0/26	0/27	P	PAV
Guiyang	wheat	0/34	0/32	24/42	0/28	0/19	P	PAV
Linfen	wheat	0/16	0/16	18/18	0/14	0/14	P	PAV
Italy	wheat	0/24	12/45	24/33	12/41	0/12	PAD	PAV
BYDV-PAV	wheat	7/75	69/101	89/102	0/45	0/21	PAG	PAV
Beijing	grass	0/21	0/27	0/18	0/27	5/32	M	RMV
Zhengzhou	wheat	7/24	17/26	0/22	17/26	0/20	GAD	MAV
Linfen	wheat	8/22	15/27	0/17	9/15	0/17	GAD	MAV
Linfen	wheat	9/12	8/12	0/12	12/17	0/12	GAD	MAV
BYDV-GAV	wheat	33/43	44/50	0/60	14/20	0/18	GAD	MAV
Beijing	wheat	16/17	0/18	7/20	0/21	0/22	GP	GPV
BYDV-GPV	wheat	30/39	0/47	29/64	0/18	0/18	GP	GPV
Beijing	wheat	14/14	0/14	8/14	0/19	0/12	GP	GPV
Beijing	grass	17/27	0/27	4/26	0/18	0/15	GP	GPV
Zhengzhou	wheat	18/21	0/24	11/24	0/18	0/16	GP	GPV
Zhengzhou	wheat	30/32	0/17	7/18	0/22	0/12	GP	GPV
Beijing	grass	15/15	0/15	11/15	0/18	0/15	GP	GPV
Beijing	wheat	18/20	0/22	6/16	0/23	0/15	GP	GPV
Beijing	grass	15/17	0/15	12/15	0/18	0/15	GP	GPV
Beijing	wheat	19/23	1/19	10/18	0/20	0/17	GPA	GPV
Beijing	wheat	12/17	1/15	4/15	0/21	0/15	GPA	GPV
Aviruliferous control		0/78	0/79	0/93	0/54	0/54		

[a]Location from which virus was sampled or laboratory strain; [b]Diseased/total inoculated seedlings. Here shows the combined data from multiple tests. [c]Sg-La, Sa-La, Rp-LA, Md and Rm indicate laboratory aphid clones of *S. graminum*, *S. avenae*, *R. padi*, *M. dirhodum* and *R. maidis* respectively. VTP: the letters signify the species that transmitted the isolate (P = *R. padi*; A = *S. avenae*; G = *S. graminum*; D = *M. dirhodum*; M = *R. maidis*), the order shows the relative efficiencies of transmission, from greatest on the left to lowest on the right.

The same serotype may display diverse aphid transmission patterns. Field BYDV samples from diseased wheat seedlings and grasses were assayed for their VTPs and serotypes. In VTP assay, field virus isolates were initially isolated by transmitting viruses with aviruliferous aphids for a few generations until a stable VTP was observed. Their VTPs were then tested by virus transmission experiments using virus-free vectors of *S. graminum*-La, *S. avenae*-La, *R. padi*-La, *M. dirhodum* and *R. maidis*. As shown in Table 2, 7 VTPs were found based on virus transmission patterns and designated as P, PAD, PAG, GAD, GP, GPA and M, based on the acronyms of vector species name. VTP usually referred to a stable and high virus transmission frequency (around 50-80%) in most cases, but an unstable and low rate (5-10%) was observed for PAG transmitted by *S. graminum*-La and GPA by *S. avenae*-La. The GAD and PAD (Italian isolate), but not other phenotypes, could be transmitted by *M. dirhodum*. *R. maidis*, however, could only transmit the sole M isolate obtained from grass in Beijing. Two Italian isolates had a VTP of P and PAD that is unique among the tested viruses. For the 20 Chinese isolates including the three laboratory strains, 6 VTP types were observed. Among them, 9 GP, 4 GAD and 3 P-type isolates were found. Two isolates showed a VTP of GPA, but only 1 M-type isolated from grass in Haidian, Beijing, was seen. In this study, we did not find an isolate that resembled BYDV-PAV.

The five polyclonal antibodies raised originally against New York BYDV-PAV, BYDV-MAV, BYDV-SGV, BYDV-RMV, and CYDV-RPV could react with Chinese BYDVs. A typical result with laboratory BYDVs is shown in Table 3. Specific positive reactions were seen for antibodies against BYDV-PAV, BYDV-MAV, BYDV-RMV and CYDV-RPV. However, BYDV-SGV antibody did not recognize any of the five tested virus strains. Obviously, BYDV-RMV and CYDV-RPV (from Italy) are serologically related to their New York relatives. Chinese BYDV-PAV and BYDV-GAV are serologically close to New York BYDV-PAV and BYDV-MAV, respectively. However, BYDV-GPV did not show any cross-reaction with any of the five antibodies, confirming that it is a unique BYDV virus in China.

Serotypes of the 22 virus isolates were subsequently determined by DAS-ELISA (Table 2). The two Italian isolates with the VTP type of P and PAD showed a serotype of RPV and PAV as expected. Of 20 Chinese isolates tested, 1 RMV, 4 MAV, 4 PAV and 11 GPV serotype-isolates were identified. Apparently, GPV, MAV and PAV are the major serotypes and GPV is the most abundant in China. BYDV-RMV isolated from grass resembled New York BYDV-RMV in VTP. Serotypes RMV, MAV and RPV had VTPs of M, GAD and P, respectively. GPV serotypes were of two VTPs, GP and GPA. PAV serotypes were of three VTPs, PAD, PAG and P. The serotypes of BYDVs did not always correspond to a VTP, suggesting that aphid transmission of BYDVs is not always linked to antigenic features.

Molecular diagnosis of BYDVs. Multiple-vector and diverse VTPs strongly suggested genetic differences between Chinese BYDVs and their New York relatives. To clarify this, coat protein gene fragments were amplified with the designed primer pairs. A unique 530-bp RT-PCR product could be amplified for all the 22 isolates listed in Table 2 (Fig. 1 left). The amplified fragments were then subjected to digestion with *Hinf*I. This way, five serotypes could be discriminated after 10% neutral PAGE (Fig. 1 left). BYDV-GAV and BYDV-RMV showed restriction patterns similar to their New York counterparts. Four bands were observed for CYDV-RPV (approximately 370, 130, 110 and 50 bp), a pattern similar but not exactly the same as that predicted from RPV-NY sequence. BYDV-GPV displayed two visible bands at positions of 400 and 140 bp. Polymorphism of the restriction patterns was found among isolates of PAV serotype but not of BYDV-GAV isolates (Fig. 1

Table 3. Viral serotypes determined by quantitative DAS-ELISA.

Virus	[a]OD_{405} against antiserum of				
	PAV	MAV	SGV	RPV	RMV
BYDV-PAV	0.91±0.069	0.20±0.022	0.15±0.005	0.16±0.010	0.15±0.013
BYDV-GAV	0.17±0.009	0.52±0.027	0.18±0.022	0.18±0.019	0.13±0.014
BYDV-GPV	0.18±0.013	0.21±0.082	0.17±0.017	0.20±0.029	0.13±0.010
CYDV-RPV	0.19±0.012	0.18±0.008	0.17±0.003	0.39±0.017	0.14±0.004
BYDV-RMV	0.21±0.031	0.22±0.018	0.15±0.006	0.16±0.007	0.45±0.023
Oat extract	0.15±0.015	0.20±0.013	0.17±0.009	0.18±0.006	0.13±0.006
Blank	0.000	0.000	0.000	0.000	0.000

[a]Mean ± SD is normalized to 1 g starting leaf tissue from three independent experiments; positive reactions are indicated by underlining.

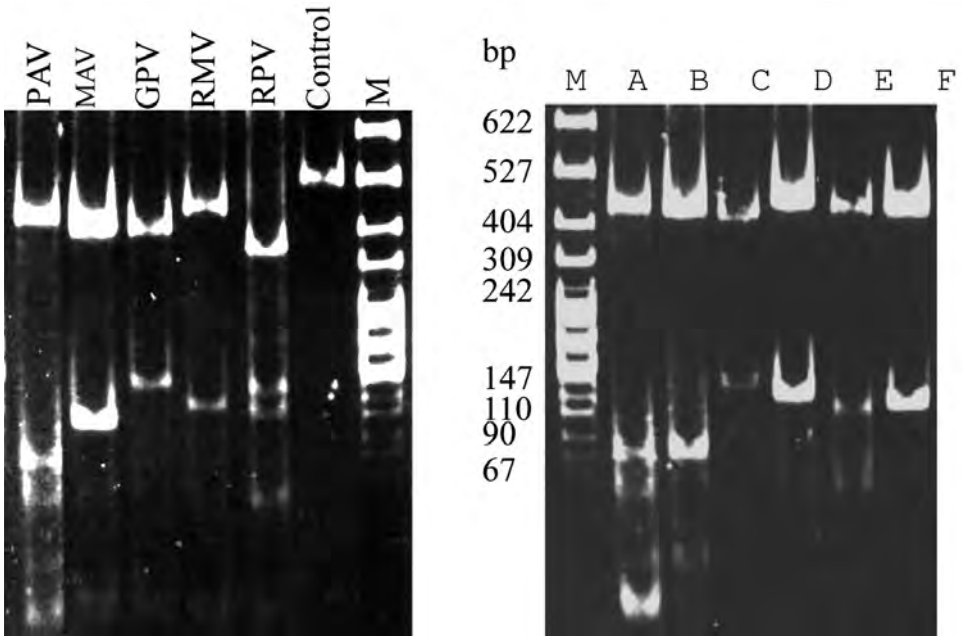

Fig. 1. *Hinf*I digestion patterns of the coat protein gene RT-PCR fragments. All the 22 virus isolates were assayed and results from the five serotypes (Laboratory strain and CYDV-RPV from Italy) are shown in the left panel. Control is undigested RT-PCR product of the laboratory PAV isolate. M is DNA size marker of pBR322/MsPI. The right panel represents results for 4 PAV and 2 MAV isolates. PAV isolate Guiyang-1 (A) and Guiyang-2 (B) are from Guiyang. The other two PAV isolates are from Italy (C) and the laboratory strain (D), respectively. The two MAV serotype isolates are from Linfen (E) and Zhengzhou (F), respectively.

right). The laboratory strain with the VTP type of PAG, but not other VTP types of PAV serotype, displayed a digestion pattern similar to that predicted from the sequence of New York BYDV-PAV.

To clarify this variation, sequences corresponding to coat protein genes were amplified using primers specific for the laboratory BYDV-PAV strain and two field isolates (30W and 38W) of BYDV-GAVs, cloned into pGEMT and sequenced. In comparisons with other known sequences of *Luteoviridae* members, BYDV-PAV sequence showed 98% identity at the amino acid level to previously sequenced Chinese BYDV-PAV sequence, and both were less similar to other PAV-serotype strains (isolates). Similarly, 30W and 38W (accession number: DQ305494 and DQ305495) are quite similar (95% nucleotide identity) although 38W contained a 3'terminal deletions and base variations. Both sequences were similar to those of previously sequenced laboratory BYDV-GAV (Wang *et al*., 2001), but less similar to those of BYDV-MAV and BYDV-PAV. Genetic distance analysis based on the coat protein gene sequences (Fig. 2) indicated that Chinese BYDV-PAV and BYDV-GAV isolates are members of genus *Luteovirus* and distinguishable from their relatives isolated in other countries. BYDV-GPV has a polerovirus-like coat protein gene and is closest to CYDV-RPV and CYDV-RPS in the genetic tree. Combining the biological and immunological data, we conclude that BYDV-GPV is a distinct virus in China, but that it should not be put it into genus *Polerovirus* until its whole genome is sequenced.

DISCUSSION

Aphid transmission of luteoviruses is often said to be vector-specific, i.e. most luteoviruses are transmitted specifically by one or, at most, a few aphid species, but the transmission specificity is rarely absolute and, in most cases, only refers to that aphid species which most efficiently transmits the luteoviruses (Gray, 1999). Several studies have reported differences in transmission characteristics among clones of a vector species where an inefficient virus-vector relationship has been established. For example, Saksena *et al.* (1964) tested four biotypes of *R. maidis* for their ability to transmit CYDV-RPV, and found transmission efficiencies of 28-87%. Significant variability in BYDV-MAV transmission efficiency was identified in *R. padi* clones collected in the Midwestern USA (Rochow and Eastop, 1966). In the most cases, studies on the intraspecific variability of BYDV transmission of efficient vector-virus combination have reported no differences among aphid clones (Rochow, 1958; Smith and Richards, 1963; Gildow *et al*., 1987).

In the present work, we collected BYDV isolates and their aphid vectors from different regions of China and assayed the vector transmission phenotypes (VTPs). The results obtained indicate that Chinese BYDVs constitute a complex and diverse virus group, generally transmissible by more than one aphid species. Aphid genotypes occasionally exhibited different BYDV transmission rates in spite of the generally stable VTP, suggesting genetic differences among certain aphid species.

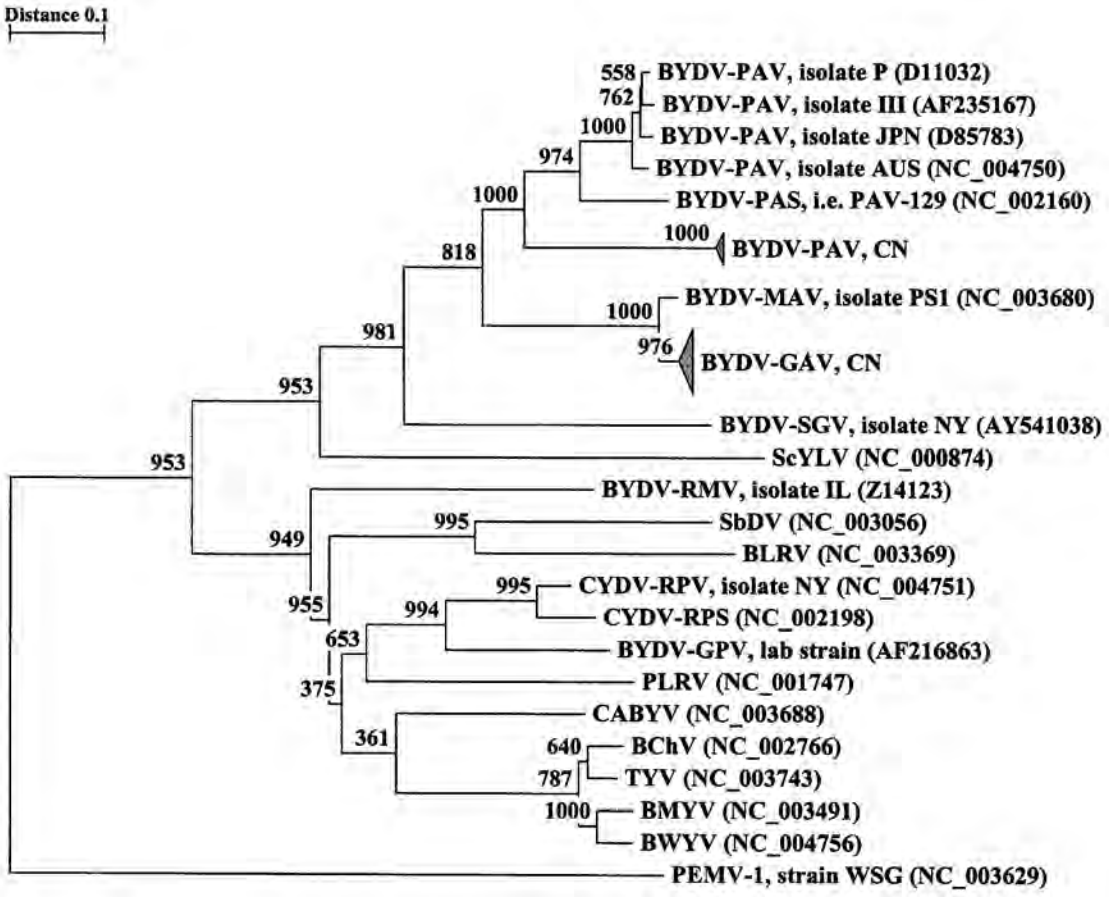

Fig. 2. Genetic distance analysis for Chinese BYDVs and other viruses in family *Luteoviridae* based on coat protein gene sequences. The BYDV-PAV sequence and sequences of the field isolates of BYDV-GAV, 30W and 38W have accession numbers of AY855920, DQ305494 and DQ305495, respectively. Both the two Chinese PAV isolates and the three GAV isolates that were clustered together are combined and shown as indicated by triangles. Accession number of previously sequenced Chinese BYDV-PAV and BYDV-GAV coat protein gene sequence are AF192967 and AY220739, respectively. Accession numbers of other sequences used in this analysis are shown in parentheses. Sequences were aligned with Clustal X and their genetic distances were analyzed with Treecon 1.3 by neighbor-joining method with a bootstrap of 1000 as described in methods.

As found in this study, VTP variants have been reported (Gray and Gildow 2003). Other recent examples are two isolates, PAV-DK1 (Moon *et al*., 2000) and PAV-RG isolate (Guo *et al*., 1997), differing in transmission by *R. padi*. An *S. graminum* clone from South Carolina does not transmit any tested BYDV serotypes (Gray *et al*., 1998). Probably, BYDVs that are transmitted by multiple aphid species have a higher chance of being transmitted, which would accelerate their spread and increase the risk of disease breakouts. This needs further investigation and evaluation. Considering potential aphid transmission variations, disease diagnosis and prediction should rely mainly on ELISA and molecular techniques rather than biological methods.

BYDV-aphid relationships are complex, involving trilateral interactions. The luteovirus proteins involved in aphid transmission have been identified, and suggested interactions of viral coat protein and readthrough protein with specific aphid receptors might determine VTPs (Brault *et al*., 1995; Chay *et al*., 1996; Peiffer *et al*., 1997; Liu & Miller 2002). And potential virus receptors have been identified in *S. avenae* and *S. graminum* (Li *et al*., 2001; Wang and Zhou, 2003). Further study might elucidate detailed virus-aphid interaction mechanisms, which would increase our knowledge base and possibly help us to design new approaches to control yellow dwarf and other similar diseases.

ACKNOWLEDGEMENTS

We thank Dr. R. M. Lister (Purdue University, Lafayette, USA) for providing virus antibodies, and Dr. P. Caciagli (Istituto di Virologia Vegetale, CNR, Torino, Italy) for providing virus samples. Financial supports were provided by the National Key Basic Research of China (973 contract 2006CB100203) and Nature Science Foundation of China (NSFC 30470081).

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Received August 31, 2006
Accepted December 19, 2006

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