



Variation in the transmission of barley yellow dwarf virus-PAV by different *Sitobion avenae* clones in China



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ABSTRACT

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Fourteen *Sitobion avenae* Fabricius (Hemiptera: Aphididae) clonal lines (clones) originating from China were tested for their ability to transmit BYDV-PAV (one isolate from Belgium and another from China) using wheat plants. By sequence analysis, the coat protein gene of BYDV-PAV-BE was distinguishable from BYDV-PAV-CN. All of the clones could transmit BYDV-PAV, and the transmission varied from 24.42% to 66.67% with BYDV-PAV-BE and from 23.55% to 56.18% with BYDV-PAV-CN. These data suggest that *S. avenae* has no specialty in BYDV-PAV isolate. Significant differences in the transmission frequencies between the clones with BYDV-PAV-BE and BYDV-PAV-CN were observed. The transmission efficiencies of aphid clones from the middle-lower reaches of Yangtze River (AH, HD, HDE, HZ, JZ, JY and SJ) and Yunnan province (YH) were similar. Nevertheless, differences in the virus transmission efficiencies of the clones from northern (ST and STA) and northwestern (QX, SB and XS) regions were assessed. The transmission efficiency of *S. avenae* from northern and northwestern China, where BYDV impact is more important, was higher than that from the middle-lower reaches of the Yangtze River and Yunnan province. This work emphasizes the importance of considering aphid vector clonal diversity in addition to virus strain variability when assessing BYDV transmission efficiency.

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1. Introduction

Barley yellow dwarf is one of the most damaging cereal diseases worldwide. It is caused predominantly by different viruses currently classified into two genera, *Luteovirus* and *Polerovirus* of the plant virus family *Luteoviridae* (Mayo and D'Arcy, 1999). BYDVs display a high degree of vector specificity among different aphid species. Each BYDV strain is transmitted efficiently by only a limited number of aphid species. Nevertheless, one aphid species can efficiently transmit more than one virus strain (Rochow, 1959). Four BYDV (GPV, PAV, GAV, and RMV) isolates exist in China according to Rochow's system (Zhou et al., 1987; Wang et al., 2001; Liu et al., 2007). BYDV-GAV is similar to BYDV-MAV, which is transmitted nonspecifically by the *Sitobion avenae* and *Schizaphis*

graminum aphids (Wang et al., 2001), whereas the BYDV-GPV strain is more closely related to BYDV-RPV, which is transmitted by both *S. graminum* and *Rhopalosiphum padi* (Wang et al., 1998). *R. padi* and *S. avenae* efficiently transmit PAV, whereas RMV is best transmitted by *R. maidis* (Wang and Zhou, 2003; Zhou et al., 1984, 1987; Wang and Zhou, 2003). Lastly, the BYDV-GPV strain has only been observed in China (Zhou et al., 1987).

BYDVs are transmitted by aphids in a persistent or circulative manner. Acquisition and infection feeding periods of 48 hours or more are required to maximize the transmission rate. Once acquired, the virus is retained for numerous days, often for the entire life of the vector. The circulative viral route through the aphid body has been partially characterized. Aphids acquire the viruses from infected phloem cells while feeding using their piercing-sucking stylets. The virions travel up the stylet food canal and into the aphid's gut lumen. Subsequently, the virions traverse the lining of the hindgut, are released into the body cavity (hemocoel), and begin to circulate in the hemolymph. The virions suspended in the hemolymph that contact the paired accessory salivary glands (ASG) are actively endocytosed into the ASG cells, transported into

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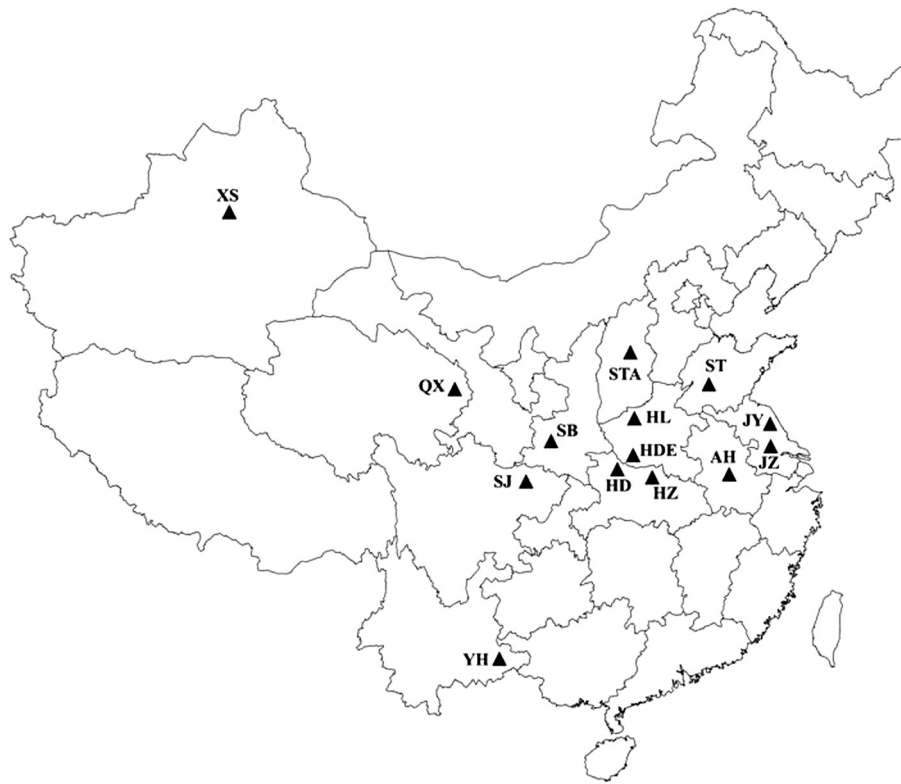


Fig. 1. Locations (Chinese province & city) of *Sitobion avenae* clones collection. AH, Anhui Hefei; HD, Hubei Danjiangkou; HDE, Henan Dengzhou; HL, Henan Luoyang; HZ, Hubei Zaoyang; JY, Jiangsu Yancheng; JZ, Jiangsu Zhenjiang; QX, Qinghai Xining; SB, Shaanxi Baoji; SJ, Sichuan Jiangyou; ST, Shandong Taian; STA, Shanxi Taiyuan; XS, Xinjiang Shihezi; YH, Yunnan Honghe.

the salivary duct, from which they can infect potential host plants (Gildow, 1985, 1993; Gildow and Gray, 1993; Yang et al., 2008). Virus-aphid specificity likely results from the recognition between virions of a specific isolate and the viral receptors in the accessory salivary glands of a particular aphid species (Gildow and Rochow, 1980; Gildow and Gray, 1993; Peiffer et al., 1997; Bencharki et al., 2000).

Although the viral transmission efficiency is well known to differ between aphid species (even if the molecular mechanisms are still unknown), the mechanisms that differentiate the ability of distinct aphid clones to transmit various BYDV variants remain unknown. At present, studies on intra-specific variation of BYDV transmission that were performed mainly with *S. graminum* and *R. padi* always used one virus strain, which did not always correspond to origin of the tested aphid clones (Gildow and Rochow, 1983; Guo and Moreau, 1996; Guo et al., 1997a, 1997b; Bencharki et al., 2000; Smyrnioudis et al., 2001; Papura et al., 2002; Dedryver et al., 2005; Du et al., 2007).

Given that *S. avenae* is one of the most common and destructive wheat pests and is a primary vector for BYDV-PAV, we assessed the viral transmission efficiency of a diversity of aphid clones and virus strains using a wheat model in this study. A large collection of *S. avenae* aphid clones throughout China were attained and tested for BYDV-PAV transmission in two different geographic isolates, one from China and one from Europe (Belgium).

2. Materials and methods

2.1. Collection and rearing of *S. avenae*

Fourteen *S. avenae* clones were collected from winter wheat (*Triticum aestivum* L.) fields in the wheat-growing areas of China, including the Huang-Huai winter (autumn sowing) wheat area,

Yangtze River winter (autumn sowing) wheat area, Xinjiang winter-spring wheat area, Qinghai-Tibet spring-winter wheat area and the Southwest winter (autumn sowing) wheat area (Fig. 1) in 2009. All clones were maintained separately on seedlings from a susceptible aphid wheat cultivar (cv. Toison d'or). All of the aphids were reared under conditions that minimized the contamination risk between clones, i.e., the aphid isolates were transferred to pots of wheat seedlings at the second stage, and each pot was separated with a transparent plastic cylinder cage covered with gauze (12 cm height and 24 cm in diameter). The aphids and plants were maintained in a greenhouse compartment at 22 ± 1 °C, 60–70% relative humidity and a 16 hour light photoperiod.

2.2. Virus strains

The BYDV strains were obtained from Belgium, Louvain-la-Neuve (BYDV-PAV-BE) in 2009 and China, Yangling - Shanxi province (BYDV-PAV-CN) in 2011. They were maintained separately on wheat seedlings cv. Toison d'or infested with *S. avenae* in a greenhouse compartment at 20 ± 1 °C and a 16 h light photoperiod.

2.3. RT-PCR

RT-PCR was used to identify the BYDV strain using the primer pair P5 (5'-CCAGTGGTTGTGGTC-3') and P3 (5'-GGAGTCTACCTATTT-3') (Du et al., 2007). Total RNA was extracted from the plant material using the RNeasy plant mini-kit (Qiagen, Germany) following the manufacturer's instructions. cDNA synthesis with RT-PCR and PCR were performed as described by Robertson et al. (1991) and Du et al. (2007), respectively. The amplified products were purified and sequenced, and the sequences were aligned using Clustal W. The aligned RNA sequences were imported into

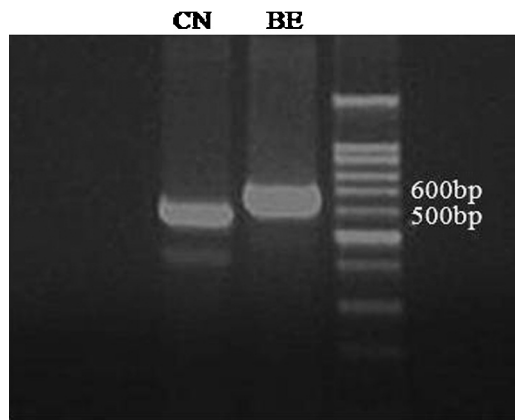


Fig. 2. RT-PCR products of the coat protein gene from the BYDV-PAV-BE isolate using the primer pair P5 and P3. CN: BYDV-PAV-China isolate (503 bp); BE: BYDV-PAV-Belgium isolate (534 bp).

MEGA4.0 (Tamura et al., 2007) for sequence comparison and variation analysis.

2.4. Viral transmission efficiency assays

The plants were assessed for the presence of the virus with DAS-ELISA (DSMZ, Braunschweig). Plants with similar optical densities (OD) were used as virus inoculums in the transmission experiments. Two-day-old *S. avenae* nymphs were fed an artificial diet (infected tissue ground in a 15% sucrose-containing solution) through paraffin membrane for a 48 hour acquisition access period. Aphids were removed from the membrane, and three were transferred onto each test plant. Thirty plants were used for each condition. After a 72 h infection access period, the aphids were killed. The wheat plants were stored in a greenhouse for 15 days before observation. Transmission by each *S. avenae* clone was repeated in three separate experiments.

The presence of the BYDV-PAV virus in the leaves of infected plants was assessed using DAS-ELISA following the manufacturer's instructions (Dr S. Winter, DSMZ, Braunschweig, Germany). The samples were considered positive when the OD values were greater than three times the mean of the results from uninfected control leaves.

2.5. Data analyses

An analysis of variance (ANOVAs) was performed using the GLM procedure in the SAS system (SAS Institute Inc. 2001). A one-way analysis of variance with treated aphid clones was conducted for all variables, and the means were separated by Tukey's studentized range test (HSD) at $P=0.05$.

3. Results

3.1. Molecular diagnosis of BYDV strain

Two unique 534 bp and 503 bp RT-PCR product were amplified for the BYDV strain from Belgium and China (Fig. 2) and were separately sequenced. In comparisons with other known *Luteoviridae* members sequences, the BYDV strain sequence from Belgium was similar to the BYDV-PAV-Sweden isolate (Accession number: AJ563413) sequence, and the BYDV strain sequence from China was similar to the BYDV-PAV-CN isolate (Accession number: EU332318.1) sequence. This study employed the BYDV-PAV strain. Genetic distance analysis based on the coat protein gene sequences indicated that the BYDV-PAV-BE isolate was distinguishable from

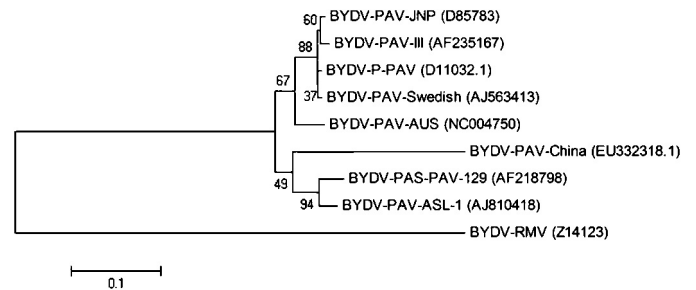


Fig. 3. Genetic distance analysis for BYDV-PAV in this study and other PAV isolates based on coat protein gene sequences. Accession numbers of previously sequenced BYDV coat protein gene sequences are in parentheses. BYDV-RMV (Z14123) was used as outgroup. Their genetic distances were analyzed with MEGA 4.0 using the neighbor-joining method with a bootstrap value of 1000.

the BYDV-PAV-CN isolate (Fig. 3), but the nucleotides of the two BYDV-PAV gene were 78% similar (Fig. 4).

3.2. Transmission efficiency of different aphid clones

Fourteen geographically separate *S. avenae* clones were submitted to transmission experiments. All aphid clones transmitted the BYDV-PAV-BE isolate and BYDV-PAV-CN isolate (Table 1). The average transmission rate of BYDV-PAV-BE isolate was 42.06%. A one-way variance analysis of transmission rates revealed a significant effect for the *S. avenae* clone ($df=13$, $MS=42.326$, $F=10.36$, $P<0.001$). The STA clone transmitted at $66.67 \pm 3.84\%$, whereas the HDE clone transmitted at only $24.42 \pm 2.21\%$. The most efficient *S. avenae* clone transmitted BYDV-PAV approximately three times more efficiently than the least efficient aphid strain. The average transmission rate for the BYDV-PAV-CN isolate was 35.08%, which was lower than for the BYDV-PAV-BE isolate. A significant effect of the clone on transmission rate was observed ($df=13$, $MS=8.219$, $F=23.5$, $P<0.001$). The STA clone transmitted at $56.18 \pm 5.22\%$, whereas the HDE clone transmitted at only $23.55 \pm 1.36\%$. The most efficient *S. avenae* clone transmitted the BYDV-PAV approximately 2.4 times more than the least efficient clone. The transmission efficiency of the clones from the middle-lower reaches of the Yangtze River (AH, HD, HDE, HZ, JZ, JY and SJ) and the Yunnan province (YH) were not significantly different, and neither were the transmission efficiencies of the clones from the northern (ST and STA) and northwestern (QX, SB and XS) regions. Lastly, the transmission efficiency of *S. avenae* from the northern and northwestern regions was higher than from the middle-lower reaches of the Yangtze River and the Yunnan province.

4. Discussion

BYDVs have been previously demonstrated to display high degrees of vector specificity among different aphid species, where each virus is transmitted efficiently by only one or a few aphid species. In China, Du et al. (2007) reported that BYDV-PAV was efficiently transmitted by *R. padi*-La and *S. avenae*-La but poorly by *S. graminum*-La, *S. graminum*-La and *S. avenae*-La. *M. dirhodum* exhibited transmission rates of 75% with BYDV-GAV, and BYDV-GPV was transmitted by *S. graminum*-La and *R. padi*-La at approximately 80% and 50%, respectively. The Chinese BYDV-PAV and BYDV-GAV isolates are members of *Luteovirus* and distinguishable from their relatives isolated in other countries. BYDV-GPV is a distinct virus in China. It harbors a polerovirus-like coat protein gene and is closest to CYDV-RPV and CYDV-RPS but lacks a serological relationship all U.S. isolates (Du et al., 2007).

In this study, the *S. avenae* clones were from China, and the BYDV-PAVs were from China and Belgium, Europe. The genetic

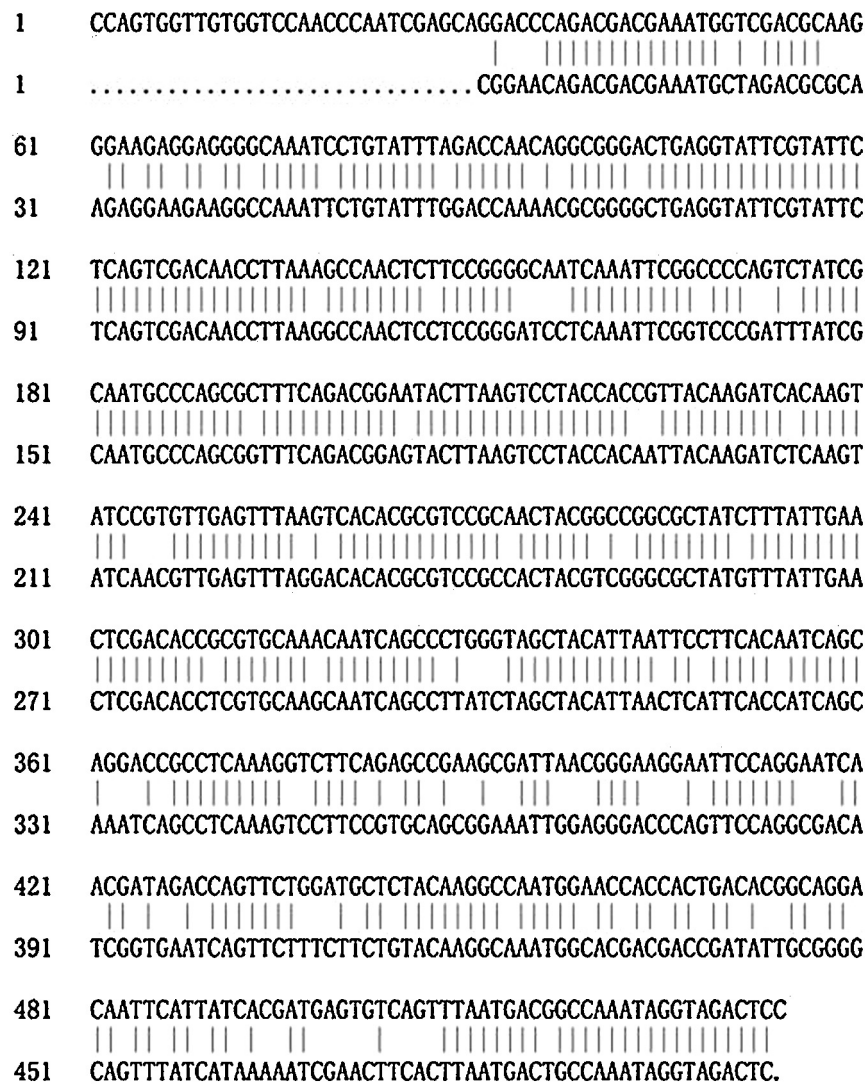


Fig. 4. Alignment of BYDV-PAV-BE and BYDV-PAV-YL-CN. Upper line: BYDV-PAV-BE, lower line: BYDV-PAV-YL-CN. Identity = 77.93% (392/503), Gap = 5.81% (31/534).

distance analysis based on the coat protein gene sequences indicated that the BYDV-PAV-BE isolate was distinguishable from the BYDV-PAV-CN isolate, but the nucleotide sequences of the two BYDV-PAV genes were 78% similar. The Belgian virus strain was used to assess the transmissibility of non-vector-associated aphid clones. Our results demonstrated that all Chinese clones were able to transmit BYDV-PAV-BE at different efficiency rates. The transmission rates varied from 24 to 67% with the BYDV-PAV-BE isolate and from 23 to 56% with the BYDV-PAV-CN isolate. These results are partly consistent with previous BYDV-PAV transmission studies using different *S. avenae* clones. Guo and Moreau (1996) and Guo et al. (1997a, 1997b) observed a transmission range of 0 to 76% among 21 clones collected from 4 French regions. Papura et al. (2002) tested 39 F1 progeny and observed a 0% to 88% of viral transmission rates. Notably, the BYDV-PAVs did not possess any non-vector strains in this study. Indeed, all strains transmitted both BYDV-PAV isolates at least at a 20% transmission rate, whereas an extremely large area for aphid strain collection was investigated. This finding provides some context to the results from Bencharki et al. (2000), who reported that the transmission efficiency of *S. avenae* was generally dependent upon the PAV isolate. Considering a pool of aphid clones, the final transmission rate was extremely similar even for different BYDV-PAV strains.

Little is known about why such diversity in transmission ability exists. van den Heuvel et al. (1994, 1997) have demonstrated that symbionin, a GroEL homologous protein synthesized by endosymbiotic bacteria and secreted into the aphid hemolymph, is essential for efficient *Luteovirus* transmission. Additionally, they revealed that symbionin from different aphid species could bind to *Luteoviridae* in vitro with different affinities. Bencharki et al. (2000) suggested that the observed intraspecific variability in transmission efficiency may be related, at least in part, to differences in the movement ability of the BYDV virus throughout the epithelial cell barriers at the hindgut and/or the accessory salivary glands in the different clones or to the stability of the virus in the hemolymph. Symbionin of the inefficient aphid sub-clones may be released at low concentrations or exhibit a low binding affinity for the virus. Although endosymbionts may participate in stabilizing virus particles, Burrows et al. (2007) did not believe that they determine vector competence because they observed that the hindgut and accessory salivary gland barriers to transmission were genetically controlled and separated in F2 *S. graminum* hybrid. Further functional experiments using omics tools are merited to elucidate the molecular mechanisms of the virus-aphid interactions and to characterize the participating proteins.

Taken together, the results suggest that both the *S. avenae* clone and the BYDV-PAV strains must be considered when assessing

Table 1Comparison of transmission efficiency of BYDV-PAV between *Sitobion avenae* clones.

Locality	Code	Transmission rates of BYDV-PAV-BE (%)				Transmission rates of BYDV-PAV-CN (%)			
		Repeat 1	Repeat 2	Repeat 3	Mean ± SE ^b	Repeat 1	Repeat 2	Repeat 3	Mean ± SE ^c
Huang-Huai winter (autumn sowing) wheat area and Yangtze River winter (autumn sowing) wheat area									
Anhui Hefei	AH	40.00 (10 ^d)	33.33 (10)	40.00 (10)	37.78 ± 2.22 CDE	34.19 (10)	32.96 (10)	30.83 (10)	32.66 ± 1.70 C
Henan Dengzhou	HDE	26.67 (8)	26.67 (8)	20.00 (6)	24.42 ± 2.21 E	22.89 (10)	25.11 (10)	22.65 (9)	23.55 ± 1.36D
Henan Luoyang	HL	40.00 (10)	46.67 (10)	40.00 (10)	42.23 ± 2.23 BCDE	31.61 (10)	29.32 (10)	37.76 (10)	32.90 ± 4.36 C
Hubei Danjiangkou	HD	33.33 (10)	26.67 (8)	40.00 (10)	33.32 ± 3.85 DE	27.59 (10)	29.36 (10)	30.79 (10)	29.54 ± 1.60 CD
Hubei Zaoyang	HZ	40.00 (10)	33.33 (10)	26.67 (8)	33.33 ± 3.85 DE	30.16 (10)	31.08 (10)	34.23 (10)	31.82 ± 2.13 CD
Jiangsu Yancheng	JY	40.00 (10)	40.00 (10)	33.33 (10)	37.78 ± 2.22 CDE	37.30 (10)	36.82 (10)	38.03 (10)	37.38 ± 0.61 C
Jiangsu Zhenjiang	JZ	26.67 (8)	26.67 (10)	40.00 (10)	31.12 ± 4.44 DE	29.61 (10)	31.10 (10)	33.35 (10)	31.35 ± 1.88 CD
Shaanxi Baoji	SB	66.67 (10)	53.33 (10)	60.00 (10)	60.00 ± 3.87 AB	30.61 (8)	28.31 (10)	36.91 (10)	31.94 ± 4.45 CD ^a
Shandong Taian	ST	46.67 (10)	53.33 (10)	46.67 (10)	48.89 ± 2.21 ABCD	46.46 (10)	49.65 (10)	42.72 (10)	49.61 ± 3.13 B
Shanxi Taiyuan	STA	73.33 (10)	66.67 (10)	60.00 (10)	66.67 ± 3.84 A	50.36 (10)	60.45 (10)	57.74 (10)	56.18 ± 5.22 A
Xinjiang winter-spring wheat area									
Xinjiang Shihezi	XS	53.33 (10)	46.67 (10)	33.33 (10)	44.43 ± 5.87 BCD	29.39 (10)	30.83 (10)	36.44 (10)	32.22 ± 3.72 C
Qinghai-Tibet spring-winter wheat area and Southwest winter (autumn sowing) wheat area									
Sichuan Jiangyou	SJ	46.67 (10)	40.00 (10)	40.00 (10)	42.23 ± 2.23 BCDE	36.77 (10)	36.81 (10)	36.64 (10)	36.74 ± 0.09 C
Qinghai Xining	QX	66.67 (10)	53.33 (10)	46.67 (10)	55.56 ± 5.89 ABC	38.13 (10)	34.12 (10)	37.90 (10)	36.72 ± 2.25 C ^a
Yunnan Honghe	YH	26.67 (10)	40.00 (10)	26.67 (8)	31.12 ± 4.44 DE	30.65 (10)	28.83 (10)	27.73 (10)	29.07 ± 1.47CD

Horizontal, Two-sample *t*-test.^a Significantly different transmission efficiency between the two BYDV-PAV isolate are indicated ($n=3$, $P<0.05$). Vertical: One-way ANOVA, Tukey's strudentized range test (HSD).^b Means within columns followed by the same letter are not significantly different ($df=13$, $MS=42.326$, $F=10.36$, $P<0.0001$).^c Means within columns followed by the same letter are not significantly different ($df=13$, $MS=8.219$, $F=23.5$, $P<0.0001$).^d No. of viruliferous aphids.

aphid-virus interactions. Both the aphid species and the viral clones should be considered for viral transmission assessment. Moreover, the heterogeneity of aphid clones from a common given species, such as *S. avenae*, is extremely high and thus must be significantly sampled for thorough representation. Indeed, if most of the clones displayed extremely similar virus transmission efficiencies between the BYDV-PAV-BE and BYDV-PAV-CN strains, where half of the clones displayed a 10% or less changes in viral transmission efficiency, and more than a quarter of the clones (4 of 14 clones) exhibited a 20 to 30% virus transmission variation among the BYDV-PAV strains. Moreover, two clones (from Shaanxi Baoji and Qinghai Xining) displayed highly significant changes (from 40 to 90% rate increases) when switching from a BYDV-PAV to other strain. These results were systematically observed with BYDV-PAV-BE, which is a strain that does not occur co-geographically with other tested aphid clones. This finding is important given that aphids are dispersed regionally and internationally. Because a two to three fold increase in viral transmission is due to a new efficient aphid clone in a particular area, extensive damage and yield losses could occur. In this study, the transmission efficiency of *S. avenae* from northern and northwestern China was higher than that from the middle-lower reaches of the Yangtze River and Yunnan province. Coincidentally, BYDVs predominantly caused wheat yellow dwarf diseases throughout the northern and northwestern provinces of China (Jin et al., 2004). Du et al. (2007) also demonstrated that the clones that display the highest transmission efficiency were from a highly prevalent BYDV area. These results suggest that assessing variation for viral transmission by identifying the aphid species will be immensely interesting for epidemiological studies.

Analysis of aphid transmission efficiencies is particularly significance for researching its migration path. Using microsatellite markers, Wang (2007) inferred that: (i) *S. avenae* are highly migratory throughout the middle-lower reaches of the Yangtze River wheat growing area of China; (ii) *S. avenae* are highly migratory throughout the north and northwest wheat growing area of China; (iii) the microsatellite marker analyses did not support the conclusion that *S. avenae* overwinters in the south and migrates north in the spring. The above conclusion coincides with the result of our study: (i) the transmission efficiencies of the clones from the

middle-lower reaches of the Yangtze River (AH, HD, HDE, HZ, JZ, JY and SJ) did not differ; (ii) the transmission efficiencies of the clones from the northern (ST and STA) and northwestern (QX, SB and XS) areas of China did not differ; (iii) and the transmission efficiency of *S. avenae* from the northern and northwestern regions was higher than from the middle-lower reaches of the Yangtze River.

In conclusion, further analysis of the virus-aphid interactions are merited to elucidate the mechanisms of virus acquisition, transport and availability in the aphid vectors by integrating high and low transmitting efficiency clones and comparing them. Further research may provide further context for monitoring the occurrence of important transporters and/or receptors in efficient aphid vectors and for identifying potential inhibitor/competitors of virus-binding proteins to control virus dispersion. A better understanding of viral transmission efficiency in aphids may change epidemiological models that are applied to plant viruses in regional areas and may improve control strategies for aphid-virus associations.

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