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Aphid transmissibility of different European beet polerovirus isolates

Anna Kozłowska-Makulska · Monique Beuve · Jerzy Syller · Marek S. Szyndel · Olivier Lemaire · Salah Bouzoubaa · Etienne Herrbach

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Abstract Different field isolates of the 'beet poleroviruses' *Beet mild yellowing virus* (BMYV) and *Beet chlorosis virus* (BChV) (genus *Polerovirus*, family *Luteoviridae*) collected in France and Poland were evaluated for transmissibility from and to sugar beet plants by different aphid species. In general, both BMYV and BChV were efficiently transmitted by *Myzus persicae* and by a French clone of *Macrosiphum euphorbiae*. In contrast, transmissibility of the two poleroviruses by an English clone of *M. euphorbiae* was evidently weaker, although the aphid samples contained the virus as demonstrated by RT-PCR. None of the BMYV or BChV isolates was transmitted by *Aphis fabae* or *Myzus ascalonicus*. In attempting to correlate biological properties with molecular variations, the RT proteins were sequenced. Some amino acid point variations, presumably affecting aphid transmissibility, were identified.

Keywords BMYV · BChV · Transmission efficiency · Vector specificity · Detection in vector

A. Kozłowska-Makulska · M. S. Szyndel
Department of Plant Pathology,
Warsaw University of Life Sciences,
02-766 Warsaw, Poland

A. Kozłowska-Makulska · M. Beuve · O. Lemaire · E. Herrbach (✉)
INRA,
UMR 1131 SVQV, BP 20507,
68021 Colmar, France
e-mail: etienne.herrbach@colmar.inra.fr

A. Kozłowska-Makulska · M. Beuve · O. Lemaire · E. Herrbach
Université de Strasbourg,
UMR A1131,
67000 Strasbourg, France

J. Syller
Plant Breeding and Acclimatization Institute,
Centre Młochów,
05-831 Młochów, Poland

A. Kozłowska-Makulska · S. Bouzoubaa
Institut de Biologie Moléculaire des Plantes,
67084 Strasbourg, France

The so-called 'beet poleroviruses' *Beet mild yellowing virus* (BMYV) and *Beet chlorosis virus* (BChV), belonging to the genus *Polerovirus* in the family *Luteoviridae* (D'Arcy and Domier 2005), have both been reported to infect sugar beet in Europe (Hauser et al. 2000; Stevens et al. 2005; Kozłowska-Makulska et al. 2007). Poleroviruses are transmitted from plant to plant by aphids in a persistent, circulative and non-propagative manner (Gildow 1999; Brault et al. 2007) and the principal vector of BMYV is the green peach aphid *Myzus persicae* (Herrbach 1999). Two other polyphagous aphids, *Macrosiphum euphorbiae* and *Aphis fabae*, are considered to be poor vectors of BMYV (Heathcote 1988; Thielemann and Nagi 1979). As to BChV, the virus is transmitted by *M. persicae* but probably not by *M. euphorbiae* (Hauser et al. 2002;

Stevens et al. 2005), and reports on its other vectors, if any, are so far lacking. The purpose of this study was to evaluate the transmissibility of different field isolates of BMYV and BChV by several aphid species or clonal populations.

BMYV and BChV isolates sampled in Poland and in France were maintained on sugar beet plants (*Beta vulgaris* cv. Trestel). Virus-free clonal populations (hereafter referred to as 'clones') of *M. persicae, M. euphorbiae, A. fabae* and *Myzus ascalonicus* were maintained for several years at INRA Colmar in separate cages on virus-free sugar beet, eggplant, sugar beet and rape, respectively (temperature 20±2°C, photoperiod 16 h). The cultures were initiated from field populations, sampled from various crops in France more than 15 years ago, except for *A. fabae* sampled in 2001 on French sugar beet. In addition, an English clonal population of *M. euphorbiae* initially reared on eggplant was kindly provided by Dr Mark Stevens (Broom's Barn Research Centre, UK).

To acquire the virus, aphids were allowed a 24-h acquisition access period (AAP) on BMYV- or BChV-infected sugar beet plants, as described by Hauser et al. (2002). After the AAP, wingless aphids were transferred with a fine-tipped paint brush onto sugar beet seedlings for a 72 h inoculation access period (IAP). We used six individuals per plant for the efficient vector *M. persicae*, as normally used in our laboratory, 12 for *M. ascalonicus* and *M. euphorbiae,* both presumed to be less efficient vectors, and 20 individuals for *A. fabae*, known as a poor vector (Thielemann and Nagi 1979). Infection of plants was tested by ELISA 3 to 4 weeks post-inoculation.

Nearly all BMYV and BChV isolates under study were transmitted with high efficiency by *M. persicae* and by the French clone of *M. euphorbiae* (Table 1). In contrast, *A. fabae* and *M. ascalonicus* were found unable to transmit both BMYV and BChV isolates. Other isolates gave the same result (not shown).

Transmission experiments using the French and English clones of *M. euphorbiae* showed that the BChV isolates were transmitted with a higher efficiency by the French clone (Table 2). The English clone failed to transmit three out of four BChV isolates, whereas the isolate BChV-M26 was transmitted by both aphid clones less efficiently than BMYV, though the difference was not significant (Table 2). Transmission experiments with other BMYV and BChV isolates showed the same tendency (data not shown). Interestingly, all the aphid samples taken from the inoculated plants after completing a 3-day IAP and assayed with RT-PCR were found to harbour viral RNA (AKM and MB, data not shown), suggesting that the lack of infection of plants did not result from the failure of virus passage into the hemocoel.

Table 1 Transmissibility of different *Beet mild yellowing virus* and *Beet chlorosis virus* isolates by four aphid species (French cultures)

Virus isolates		Aphid species			
Name	Origin	*Myzus persicae*	*Macrosiphum euphorbiae*	*Aphis fabae*	*Myzus ascalonicus*
BMYV-2ITB	France 1980	**12/12**[a]	**15/16**	0/8	0/8
BMYV-N27	France 2005	**12/12**	**16/16**	0/8	0/8
BMYV-19K	Poland 2005	**12/12**	**16/16**	0/8	0/8
BMYV total transmissibility		**36/36 (100)**[b]	**47/48 (97.9)**	0/24 (0)	0/24 (0)
BChV-M26	France 2005	**12/12**	**15/16**	0/8	0/8
BChV-18K	Poland 2005	**12/12**	**15/16**	0/8	0/8
BChV-2a	UK 1997	**12/12**	**10/16**	0/8	0/8
BChV total transmissibility		**36/36 (100)**	**40/48 (83.3)**	0/24 (0)	0/24 (0)
European beet poleroviruses total transmissibility		**72/72 (100)**	**87/96 (90.6)**	0/48 (0)	0/48 (0)

[a] number of infected/inoculated plants; positive transmissions in bold

[b] percentage of infected plants in brackets

Table 2 Variation in the efficiency of transmission of different *Beet chlorosis virus* isolates by French and English clones of *Macrosiphum euphorbiae*

Virus isolate	Experiment 1		Experiment 2	
	French clone	English clone	French clone	English clone
BChV-2a	**8/8**[a]	0/8 ***[b]	**8/8**	0/8 ***
BChV-M26	**6/8**	**3/8** NS	**3/8**	**1/8** NS
BChV-M27[c]	**8/8**	0/8 ***	**7/8**	0/8 ***
BChV-18K	**8/8**	0/8 ***	**8/8**	0/8 ***
BMYV-N27[d]	**8/8**	**7/8** NS	not tested	**8/8**
BMYV-19K[d]	**8/8**	**7/8** NS	**8/8**	**8/8** NS

[a] number of infected/inoculated plants; positive transmissions in bold

[b] difference between the two clones is significant at $P<0.001$ (***), or not significant (NS), using Chi-square test

[c] origin France 2005

[d] BMYV isolates were included as references

To determine whether a nucleotide sequence motif was correlated with vector specificity, both the CP and P5 regions of two BChV isolates (-18K and -M26) and of two BMYV isolates (-19K and -N27) were sequenced and compared to the published sequences of BChV-2a and BMYV-2ITB (Hauser et al. 2002; Guilley et al. 1995). GenBank accession numbers for ORF 5 are as follows X83110 (BMYV-2ITB), AF352024 (BChV-2a), EU148508 (BMYV-19K), EU148509 (BMYV-N27), EU022509 (BChV-18K), EU022510 (BChV-M26). The comparisons of CP-RT sequences showed that the isolate BChV-M26 differed in 16 amino acids (1.3% divergence) from the non-transmitted isolates BChV-2a and -18K. Furthermore, seven amino acid point variations of BChV-M26, as compared to other BChV isolates (-2a and -18K), were identical to those in the sequence of BMYV isolates -2ITB, -19K and -N27. In two cases, basic amino acids were replaced by neutral ones (R37T and A53R), in one case acidic by neutral (N26S) and in another case basic by acidic (H197Q) (Fig. 1). In-depth research with further BChV isolates is needed to assess the outcomes of these variations.

To summarise, our findings on BMYV transmissibility by *M. euphorbiae* differ from those obtained by Schliephake et al. (2000), who reported much lower efficiency of BMYV transmission by *M. euphorbiae* than by *M. persicae*. However, this divergence may result either from different experimental conditions applied in the two studies, or from the use of different virus isolates, or as the effect of the variable efficiencies of virus transmission by French and German populations of *M. euphorbiae*. The existence of differences has been evidenced by our results that show a differential ability of the English clone of *M. euphorbiae*, as compared to the French one, to transmit BChV (Table 2). It is possible that such a marked difference between English and French aphid clones results from an independent co-evolution of aphid and virus populations under different environmental conditions and selection pressures, and reveals

26[a]	37	52	53	138	197	220	
N(a)[b]	**R**(b)	A(n)	**A**(n)	S(n)	**H**(b)	A(n)	BChV-2a & BChV-18K
S(n)	**T**(n)	T(n)	**R**(b)	A(n)	**Q**(a)	T(n)	BChV-M26; BMYV-2ITB, -19K & -N27

Fig. 1 Position of point variations in amino acid sequence for the BChV-M26 isolate, as compared to other BChV and BMYV isolates. [a] Amino acid numbering begins with the first residue of the CP of these isolates. [b] Charges of amino acids: (a) acidic, (b) basic and (n) neutral. Amino acids with change in acid/base property are in bold type

a genetic heterogeneity of both partners, as discussed for other *Luteoviridae* members, e.g. species of the Barley yellow dwarf complex (Gray 1999). Moreover, the failure of the English *M. euphorbiae* clone to transmit some BChV isolates is probably not due to a poor virus uptake from BChV-2a-infected plants, since the virus content of plants used as sources of acquisition for BChV-2a and -M26 was similar (OD values: 1.85 and 1.20, respectively). Moreover, the aphids still harboured the virus after a 3-day IAP as shown from our RT-PCR tests; the lack of transmission in certain viral isolate–aphid clone combinations could therefore be explained by a differential selectivity of the epithelium of the gut or, more likely, of the accessory salivary gland (Gildow 1999; Brault et al. 2007). To determine whether the particles of non-transmitted isolates are unable to cross either the gut wall or the salivary gland epithelium or both, further studies to quantify and compare the amounts of aphid-transmissible and non-transmissible virus particles are necessary with the use of real time RT-PCR on sampled haemolymph. Preliminary results of real-time RT-PCR on virus-fed *M. euphorbiae* indicate that whole-body aphid individuals of the English clone tested for the presence of viral RNA contained about 100 times less virus particles when fed on BChV-M26 as compared to BChV-2a (AKM and MB, data not shown).

The lack of transmission of BMYV and BChV by *A. fabae* was also found with two other French clones of this species despite using 100 aphid individuals per beet plant (MB, unpublished data). However, Thielemann and Nagi (1979) and Schliephake et al. (2000) reported that *A. fabae* was able to transmit BMYV, though at low rates. Again, this discrepancy can be explained by the use of different virus isolates and/or aphid populations; moreover, *A. fabae* is a biotype-rich species with no well delineated borders (Coeur d'Acier et al. 2007). Taken together, *A. fabae* and *M. ascalonicus* do not seem to play an important role in the spread of BMYV and BChV in sugar beet crops, despite the fact that *A. fabae* is the most abundant aphid species in sugar beet crops, and that *M. ascalonicus* can harbour ELISA-detectable beet polerovirus particles during its spring flight (Herrbach et al. 1992). Hence, the epidemiological studies on poleroviral diseases in beets should focus on the occurrence of *M. persicae* and *M. euphorbiae*, whereas *A. fabae* and *M. ascalonicus* may be considered as species of negligible significance.

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