Eur J Plant Pathol (2007) 118:1–10
DOI 10.1007/s10658-006-9095-3

RESEARCH PAPER

A new furovirus infecting barley in France closely related to the Japanese soil-borne wheat mosaic virus

Djabbar Hariri · Michel Meyer

Received: 1 September 2006 / Accepted: 18 December 2006 / Published online: 10 March 2007

Abstract In April 2001, stunted barley plants bearing mosaic symptoms were observed in a field in France (Marne Department, 51). Rod-shaped and flexuous particles were visualized by electron microscopy and positive serological reactions were detected by ELISA with *Barley yellow mosaic virus* (BaYMV) and *Soil-borne cereal mosaic virus* (SBCMV) polyclonal antisera. The tubular virus which was soil transmissible to barley cv. Esterel was separated from BaYMV by serial mechanical inoculations to barley cv. Esterel. This furo-like virus, in contrast to a French isolate of SBCMV, could be transmitted to *Hordeum vulgare*, *Avena sativa*, *Beta vulgaris* and *Datura stramonium*. RT-PCR was used to amplify the 3′-terminal 1500 nucleotides of RNA1 and the almost complete sequence of RNA2. Nucleotide and amino acid sequence analyses revealed that the French virus infecting barley is closely related to a Japanese isolate of *Soil-borne wheat mosaic virus* (SBWMV-JT) which was originally isolated from barley. This French isolate was named SBWMV-Mar. The 3′ UTRs of both RNAs can be folded into tRNA-like structures which are preceded by a predicted upstream pseudoknot domain with seven and four pseudoknots for RNA1 and RNA2, respectively. The four pseudoknots strongly conserved in RNAs 1 and 2 of SBWMV-Mar show strong similarities to those described earlier in SBWMV RNA2 and were also found in the 3′ UTR of *Oat golden stripe virus* RNAs 1 and 2 and Chinese wheat mosaic virus RNA2. Sequence analyses revealed that the RNAs 2 of SBWMV-Mar and -JT are likely to be the product of a recombination event between the 3′ UTRs of the RNAs 2 of SBWMV and SBCMV. This is the first report of the occurrence of an isolate closely related to SBWMV-JT outside of Japan.

Keywords *Soil-borne cereal mosaic virus* · Serology · Nucleotide sequence

D. Hariri (✉) · M. Meyer
Institut National de la Recherche Agronomique (INRA), Route de Saint Cyr, 78026 Versailles Cedex, France
e-mail: hariri@versailles.inra.fr

Introduction

Several furoviruses naturally infecting cereal species have been described in the world: *Soil-borne wheat mosaic virus* (SBWMV) in wheat, rye and barley (Brakke, 1971; Koenig & Huth, 2003; Shirako & Ehara, 1986), *Oat golden stripe virus* (OGSV) in oats (Plumb, Catherall, Chamberlain, & MacFarlane, 1977), *Soil-borne cereal mosaic virus* (SBCMV) in wheat and rye (Koenig, Pleij, & Huth, 1999; Lapierre, Courtillot, Kusiak, & Hariri, 1985), and Chinese wheat mosaic virus

(CWMV) in wheat (Diao et al., 1999). These viruses are naturally transmitted by *Polymyxa graminis*, a eukaryotic obligate biotrophic plasmodiophorid parasite of plant roots (Rao & Brakke, 1969). The viral genome is divided into two RNA species that are individually encapsidated (Shirako & Wilson, 1993). The particles of furoviruses are tubular and rigid, 18–20 nm diam and 300 nm or 160 nm in length.

SBWMV, the type-member of the genus *Furovirus*, was first recognized in Illinois and Indiana in 1919 (McKinney, 1937). It has been identified as the cause of a major disease of winter wheat in parts of the USA, in Japan (Saito, Takanashi, Iwata, & Okamoto, 1964), China (Cai, Peng, & Mang, 1983) Egypt, Brazil (Reis, Pereira Dos Santos, & Pereira, 1985) and in European countries (Chen et al., 1997; Koenig and Huth, 2003). The disease usually results in a 10–30% yield loss, but may cause up to 80% yield loss in seriously infested fields (Brakke, 1971; Chen, 1993; Hunger et al., 1989). The 300 nm particles contain the 7.2 kb RNA1, which codes for two N-terminally overlapping 150-kDa and 209-kDa replicase proteins in the 5′-terminal region and a 37-kDa putative cell to cell movement protein (MP) in the 3′-terminal region (Shirako & Wilson, 1993; An et al., 2003). The 160 nm particles contain the 3.6 kb RNA2, which codes for the 19-kDa capsid protein (CP) in the 5′-terminal region, and a 19-kDa cysteine-rich protein in the 3′-terminal region. The UGA termination codon of the CP is occasionally read through, at 10–20% efficiency, to produce an 84-kDa readthrough protein (RT) thought to be required for furovirus transmission by *P. graminis* (Torrance & Koenig, 2005).

Recently, a new viral disease on barley was described in France and a preliminary characterization of this virus provisionally called soil-borne barley mosaic virus was reported (Hariri, 2004; Hariri, Meyer, & Rivard, 2005). This paper reports the partial sequence analyses for the genome of this virus renamed SBWMV-Mar and investigates its host range and its taxonomic relationship with other furoviruses.

Materials and methods

Virus isolates, maintenance, and host study

The French strain of SBCMV was obtained from infected wheat plants collected in a field in France (Indre Department, 36) in 2001. The Marne isolate of SBWMV was derived from infected barley plants collected in a field in Marne (Department, 51) in 2001. SBCMV and SBWMV-Mar were maintained by mechanical inoculation on wheat (*Triticum aestivum*) cv. Soissons and on barley (*Hordeum vulgare*) cv. Esterel, respectively. To compare the biological properties of these two viruses, five monocotyledonous and twelve dicotyledonous species were inoculated mechanically with these viruses (Table 1). After inoculation, the plants were kept in growth chambers controlled at 15°C. The presence or absence of the viruses in these plants was assessed by enzyme-linked immunosorbent assay (ELISA) using polyclonal antiserum to SBCMV obtained in our laboratory at

Table 1 Various species inoculated mechanically with SBWMV-Mar and SBCMV

Plant species	SBCMV	SBWMV-Mar
Arachis hypogaea	0/5	0/5[a]
Avena sativa (cv. Peniarth)	0/5	1/5-1/5[b]
Beta vulgaris	0/5	2/5
Brassica napus	0/5	0/5
Datura stramonium	0/5	2/5
Hordeum vulgare (cv. Esterel)	0/5	5/5
Lactuca sativa	–	0/5
Lycopersicon esculentum	0/5	0/5
Nicotiana benthamiana	5/5	3/5
Nicotiana tabacum (cv. Xanthi)	2/5	0/5
Pisum sativum	0/5	0/5
Secale cereale	0/5	0/5
Spinacia oleracea	3/5-3/5	1/5-3/5
Tetragonia tetragonioides	–	0/5
Triticum aestivum (cv. Soissons)	5/5	1/5-0/5
Vicia faba	0/5	0/5
Zea mays	0/5	0/5

–: not tested; [a] Number of infected plants/ number of plants tested; [b] Two repetitions

Samples are considered to be positive when the OD value is equal or more than three times that of the healthy control

Versailles. These tests were performed 20–30 days post-inoculation.

ELISA

Double antibody sandwich ELISA was performed with the SBCMV or BaYMV antisera (kindly supplied by M J Adams). For each species studied, systemically infected leaves of five plants were ground in a citrate buffer (0.1 M, pH 7.4, containing 0.5 M urea) and centrifuged at 10,000 *g* for 3 min. The supernatants were collected and 100 µl added to each well of plates, which previously had been coated with SBCMV or BaYMV IgG (1 µg ml^{-1}). The plates were incubated at 4°C overnight, washed, filled with alkaline phosphatase conjugated IgG of SBCMV or BaYMV and incubated for 3 h at 37°C. After a further washing, *p*-nitrophenyl phosphate substrate was added and absorbance measured at 405 nm. Samples were considered to be positive when the OD value was equal or more than three times that of the healthy control.

Electron microscopy

Leaves of diseased barley plants were ground in phosphate buffer 0.01 M pH 7.2. Drops of this extract were placed on a grid for 15 min and stained using 2% potassium phosphotungstate. Preparations were examined using a Philips EM 420 transmission electron microscope.

Soil transmission

Soil from the infected field of Marne in which both viruses were detected was collected and seeds of barley cv. Esterel were sown in 1:1 contaminated soil/sterile sand mixture (v/v). The plants were grown in a temperature controlled greenhouse at 15°C. After 2 months, the presence of each virus was checked by ELISA.

Nucleic acid extraction, PCR amplification and sequence analysis

Total RNA was extracted from infected leaves of barley (cv. Esterel) 3–4 weeks after mechanical inoculation (Schenk et al., 1995). First-strand cDNAs were reverse transcribed using oligonucleotide primer sb11 corresponding to the 3′-terminal 16 nucleotides of RNAs 1 and 2 of viruses belonging to the genus *Furovirus*. Different primer pairs were used to amplify by polymerase chain reaction (PCR) different portions of RNA1 and RNA2 of SBWMV-Mar (Table 2) (Koenig et al., 1999; Shirako, Suzuki & French, 2000). After PCR amplification, the resulting fragments were gel purified, inserted into pGEMT-easy (Promega), and sequenced from

Table 2 The oligonucleotide primers used in RNA1- and RNA2 RT-PCR amplification

Primer	Sequence	Positions[a]
RNA1-1F	GAGACTTTGAATGGTAATCGAG	5629–5651
RNA1-1R	CTCGTCGTTGATCGGTATGC	6129–6110
RNA2-1F	ATAAGGTAACTGCGGAGAGC	268–287
RNA2-1R	ATCTGGGCTCTCAACTTTCC	895–876
RNA2-2F	TGGAATCGTCAGAAAAAGG	1376–1394
RNA2-2R	TCGTTCCTTTTTCTGATC	1957–1938
RNA2-3F	AGATCAGAAAAAGAGGAACG	1937–1956
RNA2-3R	CTACCAATTCCACTTCATCC	2551–2532
RNA2-4F	TGCTGGTGTTGTGAGTTACG	2486–2505
RNA2-4R	GGAGCAGTGTTGGCCTTAGC	3025–3006
RNA2-5F	CAGGTTGGAAAAGAGGGAGG	2948–2967
RNA2-5R	GTAATAGCAGCCGCGACACC	3519–3500
sb11	TGGGCCGGATAACCCT	3574–3559
sb20[b]	AGTGGGAAGGTACGAGTTGA	820–839
sb20[b]	CCACGCTTTCCCATTCATCAAATTG	1443–1419

[a] Relative to SBWMV-JT sequences AB033689 (RNA1) and AB033690 (RNA2)

[b] Relative to the De-O source RNA2 of SBRMV AF146283 (Koenig et al., 1999)

both ends. Nucleotide and amino acid comparisons were performed using the GCG programmes CLUSTALW and GAP and were also refined manually. Phylogenetic trees were constructed by a distance method (NEIGHBOR) using the original data set and 100 bootstrap data sets generated by the programme SEQBOOT. The consensus tree was generated by the programme CONSENSE. Trees were displayed as phylograms in the programme TREEVIEW. The secondary structures of the 3′ upstream pseudoknot domains of the viral RNAs were predicted and refined manually using the mfold programme (http://www.bioinfo.rpi.edu/applications/mfold/old/rna). The GenBank accession numbers for the RNA1 and RNA2 sequences of SBWMV-Mar are AJ749658 and AJ749657, respectively.

Results

Symptom expression and virus detection

In April 2001, in a French field, severe mosaic and stunting symptoms reminiscent of an infection by soil-transmitted viruses were observed on plants of the barley cv. Esterel. Electron microscopy observations showed the presence of rod-shaped and flexuous particles. ELISA tests performed with the polyclonal antisera against BaYMV and SBCMV confirmed that these plants were infected by BaYMV in mixture with a furovirus. Mechanical inoculation of barley plants cv. Esterel allowed the separation of the furovirus and BaYMV. Plants only infected by the furovirus (SBWMV-Mar) were used to study host range and for cDNA cloning.

Transmission of SBWMV-Mar by soil in controlled conditions

Plants of cv. Esterel were grown in infected soil in controlled conditions. Out of twenty plants analysed by ELISA, seven were infected by SBWMV-Mar, three by BaYMV and ten by both viruses. All the plants infected with BaYMV or SBWMV-Mar alone or with both viruses showed identical symptoms.

Host range of SBWMV-Mar and SBCMV

The reactions of different monocotyledonous and dicotyledonous species to SBWMV-Mar and a French strain of SBCMV after mechanical inoculation are summarized in Table 1. Symptoms were not clearly observed and ELISA tests were performed to detect both viruses. Out of the five cereal species used in this study, two (*Secale cereale* and *Zea mays*) were not infected, one (*T. aestivum*) was a common host and two (*H. vulgare* cv. Esterel and *Avena sativa*) were only susceptible to SBWMV-Mar. Among the dicotyledonous species tested, two (*Nicotiana benthamiana* and *Spinacia oleracea)* were infected by both viruses. Only SBWMV-Mar was detected in new emerging leaves of *Beta vulgaris* and *Datura stramonium*.

Sequence analyses

In order to better characterise SBWMV-Mar, reverse transcription-PCR (RT-PCR) was carried out using the primer pair sb40/sb20, which matches regions for the RT and CP genes in RNA2 of both SBWMV and SBCMV (Koenig and Huth, 2000). A PCR product of the expected size (624 nt) was obtained and sequence analyses revealed that this region of SBWMV-Mar shared a high degree of identity with the Japanese strain of SBWMV (SBWMV-JT) (92.2% and 96.1% nucleotide and amino acid identity, respectively). Amplifications by RT-PCR of various regions of both RNAs of SBWMV-Mar were then performed. In total, the 3′-terminal 1500 nt of RNA1 and the almost complete sequence of RNA2 were determined. Analysis of these sequence data showed that the two RNA species were organised in a similar manner as SBWMV. The partial sequence of SBWMV-Mar RNA1 contained one ORF corresponding to a predicted MP whereas the almost complete sequence of SBWMV-Mar RNA2 carried three ORFs including the CP, the RT protein and the cysteine-rich protein.

Pairwise comparisons of these four ORFs were made with those of other sequenced furoviruses [six isolates of SBWMV, seven isolates of SBCMV, CWMV, OGSV and *Sorghum chlorotic spot virus* (SrCSV)]. The highest amino acid

identities were always found with the corresponding ORFs of SBWMV-JT and ranged from 98.3% for the CP to 84.4% for the MP. The comparison of the cysteine-rich proteins showed that those of the SBCMV isolates were nine amino acids shorter at their C-terminal end than those of other furoviruses, except for SrCSV. Nucleotide sequence alignments indicated that for all the SBCMV isolates this difference can be attributed to the presence of a conserved extra nucleotide, which leads to a translation frameshift and to the translation arrest of the cysteine-rich protein gene just after this nucleotide (Fig. 1A). If this supplementary nucleotide is removed, the next stop codon found in the cysteine-rich protein genes of the SBCMV isolates corresponded to that of other furoviruses and the predicted peptide (DSSPRKCGAI) showed only four amino acid differences with respect to the last ten amino acids (DSSPRKSKPL) of the cysteine-rich proteins of SBWMV-Mar and -JT.

The close relationship between SBWMV-Mar and -JT was shown by phylogenetic analyses of these four coding regions (Fig. 2). Different tree topologies were obtained, but SBWMV-Mar and -JT always grouped together. For the three RNA2-encoded proteins, this cluster was more closely related to the cluster containing the different isolates of SBCMV with the average identities of 92.3% in the CP, 75.4% in the RT protein, and 89.6% in the cysteine-rich protein. It is interesting to note that the RT protein of the G isolate of SBCMV whose sequence was only 78% identical to those of the other SBCMV sources (Koenig et al., 1999) grouped with those of SBWMV-Mar and -JT, the percentage of identity between these proteins amounting to about 85%. In the case of the MP, a distant relationship was found between the cluster including SBWMV-Mar and -JT and all the other furoviruses.

Analysis of the non-coding regions of SBWMV-Mar showed that the 3′-untranslated region (UTR) of RNA2 was shorter than the UTR of RNA1 (375 nt and 467 nt, respectively excluding the 3′-terminal 16 nt of primer sb11). RNA1 and RNA2 shared 84.7% identity in the terminal 255 nt. Higher sequence identity was found with the 3′ non-coding regions of other furoviruses. Thus, in the 3′ UTR of RNA2, SBWMV-Mar was more similar to SBWMV-JT (97.6% identity) than to the American and German isolates of SBWMV (86.9% identity) and high sequence identity was also found with CWMV (79.9% in the terminal 360 nt) and OGSV (72.1% in the terminal 230 nt). A lower sequence identity (58.6%) was found with the 3′ UTR of SBCMV RNA2. The 3′ UTR of SBWMV-Mar RNA1 was shorter than that of SBWMV-JT RNA1 but these non-coding regions again showed considerable sequence identity (95.8%). Alignment of these sequences showed that this difference in size was due to either a putative deletion of 76 nt located in the 5′-terminal third of the 3′ UTR of SBWMV-Mar RNA1 or an insertion of extra-nucleotides in the 3′ UTR of SBWMV-JT RNA1 (Fig. 1B). High sequence identity was also found with OGSV (80.9% in the terminal 260 nt) whereas the 3′ UTRs of the RNA1s of SBWMV-Mar and SBCMV shared only 68.9% sequence identity between one another.

Previous studies have shown that the predicted secondary structure of the 3′ UTRs of RNAs 1 and 2 of some furoviruses may consist of a 3′-terminal tRNA-like structure containing four stem loops and an upstream pseudoknot domain (UPD) (Koenig et al., 1999). In the case of SBWMV-Mar and -JT, upstream of their tRNA-like structure, the UPDs of RNAs 1 and 2 were predicted to form seven and four pseudoknots, respectively (Fig. 1C). The four terminal pseudoknots of the UPDs of RNAs 1 and 2 were found to be conserved for SBWMV-Mar and -JT and corresponded to those (named PKb, PKi, PKk and PKl) described in the UPD of RNA2 of the Nebraska isolate of SBWMV(SBWMV-Neb) (Koenig et al., 1999). These four conserved pseudoknots were also found to be present in the UPDs of CWMV RNA2 and OGSV RNAs 1 and 2. The nucleotide covariations seen in the putative conserved pseudoknots of the different viral RNAs strongly support these predictions of structure.

For RNA2, the alignment of the 3′ UTRs for all the SBWMV and SBCMV strains showed that the region located between the highly conserved domain of 49 nucleotides found in the 3′ UTRs of SBWMV and SBCMV (Koenig et al., 1999) and

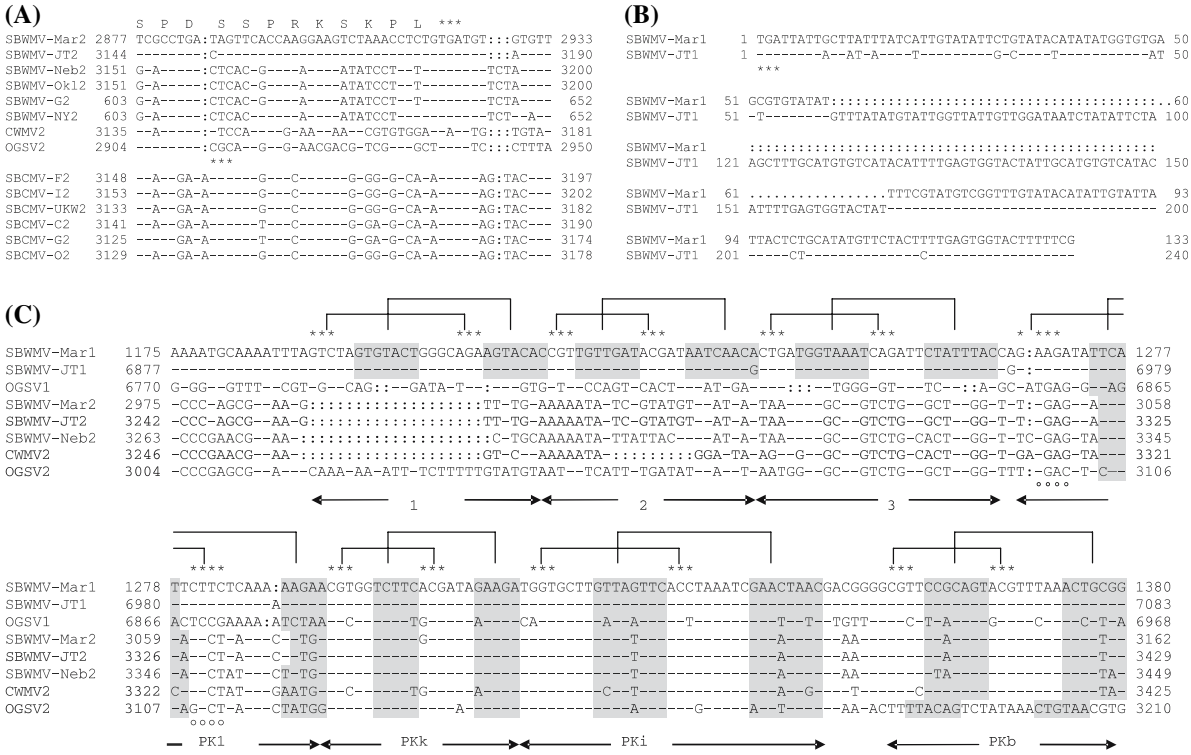

Fig. 1 (**A**) Nucleotide alignment of the region surrounding the termination codon of the cysteine-rich protein gene for SBWMV-Mar and other furoviruses. Virus names and accession numbers for the RNA2 sequences are as follows. SBWMV-JT (Japanese strain, Tochigi isolate), AB033689; SBWMV-Neb (USA strain, Nebraska isolate), L07938; SBWMV-Okl (USA strain, Oklahoma isolate), X81639; SBWMV-NY (USA strain, New-York isolate), AF361642; SBWMV-G (German strain), AF519800; CWMV, AJ012005; OGSV, AJ132579; SBCMV-F (French strain, Poitiers isolate), AJ132577; SBCMV-I (Italian strain, Ozzano isolate), AJ252152; SBCMV-UKW (UK strain, Wiltshire isolate), AJ298069; SBCMV-C (German strain, Eikeloh isolate), AF146282; SBCMV-G (German strain, Braunschweig isolate), AF146281; SBCMW-O (German strain, Osnabrück isolate), AF146283. The deduced amino acid sequence of the C-terminal region of the cysteine-rich protein of SBWMV-Mar is shown above the nucleotide sequence alignment. Dashes indicate identical nucleotides. Gaps to allow optimal alignment are indicated by a colon and the stop codon is indicated by asterisks. (**B**) Alignment of the 5′-terminal nucleotide sequences of RNAs 1 of SBWMV-Mar and -JT. Only those nucleotides which differ from the sequence of SBWMV-Mar are shown. The termination codon is indicated by asterisks. (**C**) Alignment upstream of the tRNA-like structure of the upstream pseudoknot domains of the RNAs 1 and 2 of SBWMV-Mar, -JT and OGSV and of the RNAs 2 of SBWMV-Neb and CWMV. Only those nucleotides which differ from the sequence of RNA1 of SBWMV-Mar are shown. The elements involved in seven possible pseudoknots are indicated below the sequence. Nucleotide sequences possibly forming stem structures are indicated on a grey background and connected by lines. The complementary regions putatively involved in pseudoknot formation are indicated by asterisks and connected by lines except for the 5′ proximal pseudoknot of RNAs 2 for which the putative complementary regions are indicated by dots. The four 3′ proximal pseudoknots are named according to the nomenclature used by Koenig et al., (1999). Gaps to allow optimal alignments are indicated by a colon

the tRNA-like structure was highly different (Fig. 3). For the SBWMV-Mar, -JT and the SBWMV isolates, this region contained four pseudoknots (see above) whereas seven pseudoknots (PKc, PKd, PKe, PKf, PKg, PKh and PKb) were present for the SBCMV isolates (Koenig et al., 1999). In addition to the conserved PKb, PKk of the SBWMV-Mar, -JT and SBWMV isolates and PKg of the SBCMV isolates are likely to correspond to a conserved pseudoknot and the region located between PKk/PKg and PKb consisted of one (PKi) and four (PKf, PKe, PKd, PKc) pseudoknots in the SBWMV and SBCMV isolates, respectively. We predict that the 3′ UTRs

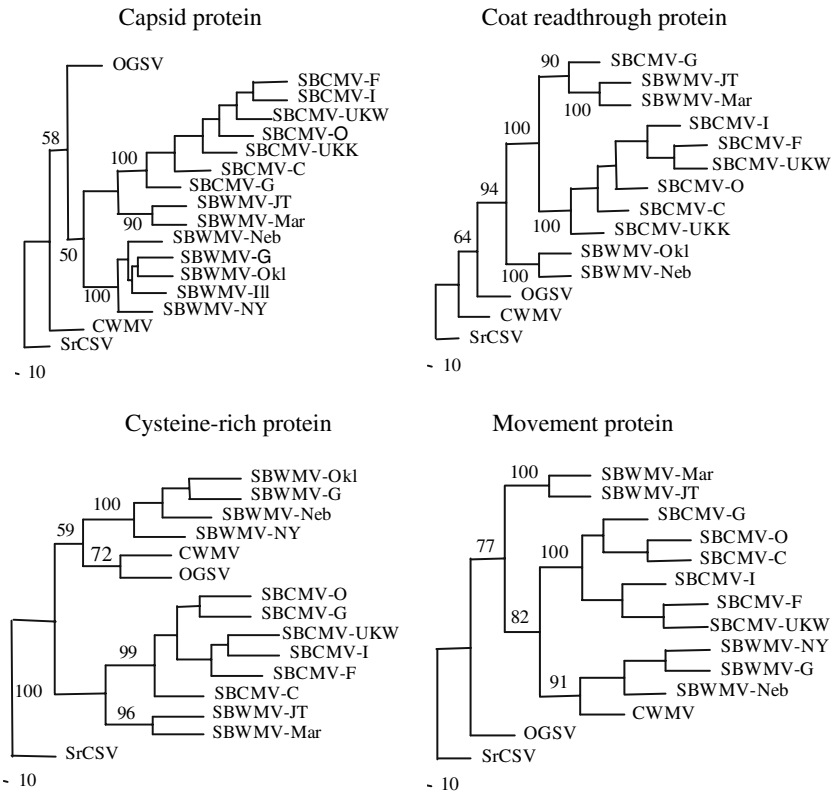

Fig. 2 Phylograms of aligned amino acid sequences of four proteins of SBWMV-Mar and other furoviruses (trees rooted with SrCSV). The bootstrap values for 100 replicates are shown above each branch. The bars represent distances scaled as substitutions per amino acid residue. Virus names and accession numbers not mentioned in Fig. 1A are as follows. SBWMV-Neb, L07937; SBWMV-NY, AF361641; SBWMV-Ill (USA strain, Illinois isolate), AB002812; SBWMV-G, AF519799; SBWMV-JT, AB033689; CWMV, AJ012005; SrCSV, AB033691 and AB033692; OGSV, AJ132578; SBCMV-F, AJ132576; SBCMV-I, AJ252151; SBCMV-UKW, AJ298068; SBCMW-O, AF146280; SBCMV-UKK (UK strain, Kent isolate), AG298070; SBCMV-G, AF146278; SBCMV-C, AF146279

of the SBWMV-Mar and -JT have been generated by a recombination between the highly conserved domains of SBCMV and SBWMV resulting in the replacement of the UPD and the tRNA-like structure of SBCMV by those of SBWMV.

Discussion

In this paper, we report biological properties and partial sequence of SBWMV-Mar naturally infecting barley in Europe. Our molecular data show unambiguously that this virus is closely related to SBWMV-JT considered to be a strain of SBWMV because it has been transmitted to wheat by mechanical inoculation. Interestingly, this virus was also originally isolated from naturally infected barley in 1982 (Shirako & Ehara, 1986).

The appearance of spontaneous deletion mutants obtained after manual inoculation has been reported for Japanese and American isolates of SBWMV (Shirako & Ehara, 1986; Tsuchizaki, Hibino, & Saito, 1972). These deletions were found in the CP-RT domain in the case of two isolates from the USA (Chen, MacFarlane, & Wilson, 1994, 1995; Yamamiya, Miyanishi, & Shirako, 2005). Sequencing of SBWMV-Mar RNA2 indicated that the complete RT protein was conserved although this isolate was maintained by mechanical inoculation to barley plants. We have no simple hypothesis to explain why

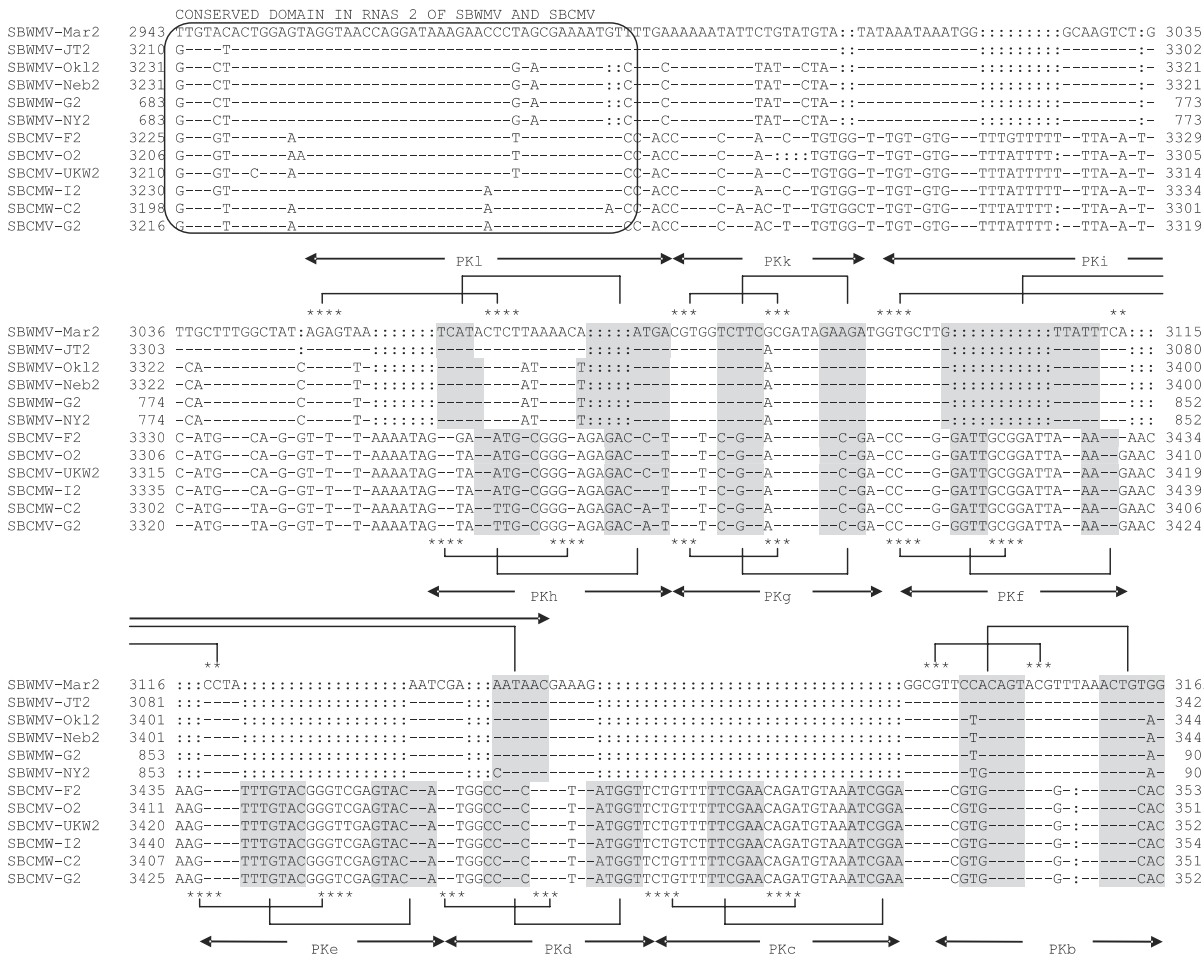

Fig. 3 Nucleotide sequence alignment (using the Clustal W programme) from the conserved domain to the tRNA-like structure of the 3′ UTRs of the RNAs 2 of SBWMV-Mar, -JT, -Okl, -Neb, -G and -NY and SBCMV-F, -UKW, -I, -O, -C and -G. Nucleotide sequences possibly forming stem structures are boxed and connected by lines above and below the boxes for the SBWMV and SBCMV isolates, respectively. The complementary regions putatively involved in pseudoknot formation are indicated by asterisks. The pseudoknots are named according to the nomenclature used by Koenig et al., (1999). Gaps to allow optimal alignments are indicated by a colon

internal deletions were not detected in this protein gene.

Sequence comparisons showed that the 3′ non-coding regions of RNAs 2 of all the furoviruses (namely SBWMV, OGSV, CWMV) which have a 3′ UTR of RNA2 shorter than the 3′ UTR of RNA1 were similar not only in their tRNA-like structure but also in their UPD which may be folded in similar predicted secondary structure. Our analysis showed also that the RNAs 2 of SBWMV-Mar and -JT were likely to have been generated by a recombination between the 3′ UTRs of the RNAs 2 of SBWMV and SBCMV.

At present, the *Furovirus* classification is still a matter of debate for the wheat-infecting viruses. Some authors consider that CWMV and SBCMV may be considered as strains of SBWMV whereas others consider that it would be more appropriate to consider the wheat-infecting viruses from the USA (SBWMV), from Europe (SBCMV) and from China (CWMV) as distinct virus species (Shirako et al., 2000; Koenig, Bergstrom, Gray, & Loss, 2002). Among SBWMV isolates, it has been suggested that SBWMV-JT may represent a distinct species due to its genetically distant relationship with SBWMV-Neb, the type member of the

genus *Furovirus* (Shirako et al., 2000; Torrance & Koenig, 2005). However, the fact that recombinant viruses can be formed between SBWMV-Neb and -JT on the one side and between SBWMV and SBCMV on the other (Miyanishi, Roh, Yamamiya, Ohsato, & Shikaro, 2002; Torrance & Koenig, 2005) suggests that these wheat-infecting virus sources from the USA, Japan and Europe belong to one single virus species.

Our analysis confirms that the taxonomic position of SBWMV-Mar and -JT is not easy to delineate. The phylogenetic relationships found with the RT proteins encoded by RNA2 of furoviruses clearly showed that SBWMV-Mar and -JT were the most closely related to SBCMV. However, this relatedness was not conserved for the two other proteins encoded by RNA2. A similar situation was found for the proteins encoded by RNA1. Thus, a closer relationship between the RNA polymerase of SBWMV-JT and OGSV has previously been noticed (Shirako et al., 2000) and the phylogenetic trees obtained with the sequences of the MPs supported the separation of SBWMV-Mar and -JT from other furoviruses. Based on nucleotide sequence comparison, we also showed that the 3′ UTRs of the RNAs 1 and RNAs 2 of SBWMV-Mar and -JT have a close similarity to that of OGSV and SBWMV, respectively. In addition to these unique molecular characteristics, SBWMV-Mar and -JT have also a clearly distinct host range compared to that of other wheat and oat-infecting furoviruses. Thus, we found that oat which is a natural host of OGSV can also be infected mechanically by SBWMV-Mar. However, the host range of OGSV is quite different from that of SBWMV-Mar and -JT. Barley is a natural host of SBWMV-Mar and -JT and wheat can be infected mechanically by these two isolates whereas OGSV does not infect either barley or wheat by mechanical inoculation (Plumb et al., 1977). It would be interesting to determine if SBWMV-JT is also able to infect oat. Recently, transcripts of SBWMV-JT were found to be infectious on *Chenopodium quinoa* whereas the European isolates of SBCMV cannot infect this dicotyledoneous species (Koenig et al., 2002; Yamamiya & Shirako, 2000). We report also that two dicotyledoneous species (*Beta vulgaris*, *Datura stramonium*) can specifically be infected by SBWMV-Mar. On the other hand, rye plants are infected by soil-borne rye mosaic virus (a strain of SBCMV) (Koenig et al., 1999) whereas SBWMV-Mar was not infectious on this cereal species.

Taken together, all these data indicate that SBWMV-Mar and -JT share common genome properties with at least three other furoviruses and that their genomic RNAs may have been generated by past inter-viral recombinations between the same components of SBWMV, SBCMV and OGSV. That the RT domain of SBCMV-G is more closely related to SBWMV-Mar and -JT than to two strains of SBCMV might also be explained by an ancient reassortment between the RNAs 2 of SBWMV and SBCMV. The fact that the Japanese and French isolates of SBWMV had high sequence identity in several genes and showed a close relationship in phylogenetic analyses strongly suggests that they share a common origin. Such a situation is reminiscent of what was observed between the French (Sil) and the Japanese (Ka1) strains of *barley mild mosaic bymovirus* which is also transmitted by *P. graminis* (Hariri, Meyer, & Prud'homme, 2003). Exchange of plant material infested with adhering infected soil or seed transmission are possible sources of contamination. Further investigations on the occurrence of SBWMV-JT in various parts of France and Europe will provide further interesting evidence concerning the origin of this SBWMV variant in Europe.

Acknowledgement We thank Dr. M Guilleroux for critically reading this manuscript, Dr. B Gélie for her kind help with electron microscopy, E. Mendes-Pereira for sequencing assistance and H. Willigsecker for plant management.

References

An, H., Melcher, U., Doss, P., Payton, M., Guenzy, A. C., & Verchot-Lubicz, V. (2003). Evidence that the 37 kDa protein of soil-borne wheat mosaic virus is a virus movement protein. *Journal of General Virology*, *84*, 3153–3163.

Brakke, M. K. (1971). Soil-borne wheat mosaic virus. *CMI/AAB Description of Plant Viruses*, *77*, 1–4.

Cai, W., Peng, X., & Mang, K. (1983). Identification of soil-borne wheat mosaic virus causing wheat mosaic in Shandong province, China. *Acta Phytopathologica Sinica*, *13*, 7.

Chen, J. (1993). Occurrence of fungus-transmitted wheat viruses in China. *Annals of Applied Biology*, *123*, 55–61.

Chen, J., MacFarlane, S. A., & Wilson, T. M. (1994). Detection and sequence analysis of a spontaneous deletion mutant of soil-borne wheat mosaic virus RNA2 associated with increased symptom severity. *Virology*, *202*, 921–929.

Chen, J., MacFarlane, S. A., & Wilson, T. M. (1995). An analysis of spontaneous deletion sites in soil borne wheat mosaic virus RNA2. *Virology*, *209*, 213–217.

Chen, J., Torrance, L., Cowan, G. H., MacFarlane, S. A., Stubbs, G., & Wilson, T. M. (1997). Monoclonal antibodies detect a single amino acid difference between the coat proteins of soilborne wheat mosaic virus isolates: implications for virus structure. *Phytopathology*, *87*, 295–301.

Diao, A., Chen, J., Gitton, F., Antoniw, J. F., Mullins, J., Hall, A. M., & Adams, M. J. (1999). Sequences of European wheat mosaic virus and oat golden stripe virus and genome analysis of the genus *Furovirus*. *Virology*, *261*, 331–339.

Hariri, D. (2004). Soil-borne barley mosaic. In: H. Lapierre, P-A. Signoret (Eds.), *Viruses and virus diseases of Poaceae* (pp. 477–478). Gramineae: INRA editions.

Hariri, D., Meyer, M., & Prud'homme, H. (2003). Characterization of a new barley mild mosaic virus pathotype in France. *European Journal of Plant Pathology*, *109*, 921–928.

Hariri, D., Meyer, M., & Rivard, M. (2005). The new French barley-infecting furovirus is closely related to the Japanese strain of soilborne wheat mosaic virus. In: C. M. Rush(Ed.), *Proceedings of the Sixth Symposium of the International Working group on plant Viruses with Fungal Vectors* (pp. 38–41). American Society of Sugar Beet Technologists, Denver: USA.

Hunger, R. M., Armitage, C. R., & Sherwood, J. L. (1989). Effects of wheat soil-borne mosaic virus on hard red winter wheat. *Plant Disease*, *73*, 949–952.

Koenig, R., Bergstrom, G. C., Gray, S. M., & Loss, S. (2002). A New York isolate of soil-borne wheat mosaic virus differs considerably from the Nebraska type strain in the nucleotide sequences of various coding regions but not in the deduced amino acid sequences. *Archives of Virology*, *147*, 617–625.

Koenig, R., & Huth, W. (2000). Soil-borne rye mosaic and European wheat mosaic virus: Two names for a furovirus with variable genome properties which is widely distributed in several cereal crops in Europe. *Archives of Virology*, *145*, 689–697.

Koenig, R., & Huth, W. (2003). Natural infection of wheat by the type strain of soil-borne wheat mosaic virus in a field in Southern Germany. *European Journal of Plant Pathology*, *109*, 191–193.

Koenig, R., Pleij, C. W. A., & Huth, W. (1999). Molecular characterization of a new furovirus mainly infecting rye. *Archives of Virology*, *144*, 2125–2140.

Lapierre, H., Courtillot, M., Kusiak, C., & Hariri, D. (1985). Résistance au champ des blés en semis d'automne au virus de la mosaïque du blé (*wheat soil-borne mosaic virus*). *Agronomie*, *5*(7), 565–572.

McKinney, H. H. (1937). Mosaic disease of wheat and related cereals. *US Department of Agriculture Circular*, *442*, 23.

Miyanishi, M., Roh, S. H., Yamamiya, A., Ohsato, S., & Shirako, Y. (2002). Reassortment between genetically distinct Japanese and US strains of soil-borne wheat mosaic virus: RNA1 from a Japanese strain and RNA2 from a US strain make a pseudorecombinant virus. *Archives of Virology*, *147*, 1141–1153.

Plumb, R., Catherall, P. L, Chamberlain, J. A., & MacFarlane, I. (1977). A new virus in oats in England and Wales. *Annals of Phytopathology*, *9*, 365–370.

Rao, A. S., & Brakke, M. K. (1969). Relation of soil-borne wheat mosaic and its fungal vector Polymyxa graminis. *Phytopathology*, *59*, 581–587.

Reis, E., Pereira Dos Santos, H., & Pereira, L. R. (1985). Rotagao de cultures: Efeito sobre mosaico e doencas radiculares do trigo em 1983. *Fitopatologia Brasileira*, *10*, 637–642.

Saito, Y., Takanashi, K., Iwata, Y., & Okamoto, H. (1964). Studies on soil-borne virus disease of wheat and barley. I. Several properties of viruses. *Bulletin of the National Institute of Agricultural Sciences (Japan) Series C*, *17*, 1–19.

Schenk, P. M., Antoniw, J. F., deBatista, M., Jacobi, V., Adams, J., & Steinbiss, H. H. (1995). Movement of barley mild mosaic and barley yellow mosaic viruses in leaves and roots of barley. *Annals of Applied Biology*, *120*, 291–305.

Shirako, Y., & Ehara, Y. (1986). Comparison of the in vitro translation products of wild-type and a deletion mutant of soil-borne wheat mosaic virus. *Journal of General Virology*, *67*, 1237–1245.

Shirako, Y., & Wilson, M. A. (1993) Complete nucleotide sequence and organization of the bipartite RNA genome of soil-borne wheat mosaic virus. *Virology*, *195*, 16–32.

Shirako, Y., Suzuki, N., & French, R. C. (2000) Similarity and divergence among viruses in the genus *Furovirus*. *Virology*, *270*, 201–207.

Torrance, L. & Koenig, R. (2005) Genus Furovirus. In: C. M., Fauquet, M. A., Mayo, J., Maniloff, U., Desselberger, & L. A. Ball (Eds.) *Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses* (pp. 1027–1032). Elsevier Academic Press.

Tsuchizaki, T., Hibino, H., & Saito, Y. (1972). Comparisons of soil-borne wheat mosaic virus isolates from Japan and the United States. *Phytopathology*, *63*, 634–639.

Yamamiya, A., & Shirako, Y. (2000). Construction of full-length cDNA clones of soil-borne wheat mosaic virus RNA1 and RNA2, from which infectious RNAs are transcribed in vitro: Virion formation and systemic infection without expression of the N-terminal and C-terminal extensions to the capsid protein. *Virology*, *277*, 66–75.

Yamamiya, A., Miyanishi, M., & Shirako, Y. (2005). Stable deletions arising in the readthrough region of soil-borne wheat mosaic virus RNA2 define the 5′ limit of the functional promoter for the p19 subgenomic RNA. *Archives of Virology*, *150*, 1871–1884.