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Occurrence of two different types of RNA-5-containing beet necrotic yellow vein virus in the UK*

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Summary. Two types of RNA-5-containing beet necrotic yellow vein virus (BNYVV) have been detected in the UK at different sites in Norfolk. On the basis of nucleotide (nt) sequence comparisons, one virus source (UK-MH) was clearly identified as P type BNYVV, a virus type that had previously only been detected in two widely separated parts of the world, France and Kazakhstan. The other virus source (UK-FF) has a complex genome composition. The analysed portions of its RNAs 2 and 4 are closely related to the corresponding portions in the RNAs of the East Asian A type isolate S, whereas those of its RNAs 1 and 3 resemble P type RNA 1 from Kazakhstan and European A type RNA 3, respectively. Interestingly, the P25 encoded on its RNA 3 has an unique TYHG tetrad in the highly variable amino acid positions 67–70. RNA 5 of the UK-FF BNYVV source shares properties with P type RNA 5, but also with East Asian types of RNA 5. The possible origin and epidemiology of BNYVV types is discussed.

Introduction

Beet necrotic yellow vein virus (BNYVV) causes a damaging disease of sugar beet (*Beta vulgaris*) known as rhizomania [29]. The disease was first observed in Italy in the early fifties [6] and now has a world-wide distribution [1]. Molecular

*The GenBank accession numbers for the 28 new sequences described in this paper are listed in the figures in which they are labelled by an asterisk to differentiate them from those of previously submitted sequences.

analyses of the four RNA species always present in natural BNYVV infections have revealed the existence of three major genotypes of BNYVV in Europe; named A, B and P types. These types were originally recognized by means of RFLP and SSCP analyses [14, 19]. The RFLP patterns of the B type correspond to the sequences published by Bouzoubaa et al. [3–5] for the French isolate F2, whereas those of the A type are predicted from the sequences of their isolate G1 for which only deletion mutants of RNAs 3 and 4 have been analysed. More detailed information on the sequence differences between European A, B and P types has been given by [16]; accession numbers for reference sequences are listed in the VIIIth report of the International Committee on Taxonomy of Viruses [17]. In Europe, A and B types were found to be highly conserved in geographically widely separated regions and recently determined sequences are practically identical to those determined two decades ago [3–5, 16, 22, 25, 28]. BNYVV sources from East Asia (Japan, China) resemble the European A and B types, but are not identical to them [16, 21–23]. In Japan and China, variants of a fifth RNA species have frequently been found to be associated with A- or B-type-like BNYVV [20, 23]. A distinct variant of RNA 5 has been found to be associated with P type BNYVV, a virus type which so far has been found only in a small area around the French town of Pithiviers and in Kazakhstan [15, 16]. There is evidence that RNA-5-containing BNYVV sources are more pathogenic than those lacking the fifth RNA [31].

In the UK, rhizomania disease was first observed in 1987 [12]. Economic crop losses in infected regions have been reported to be as high as 80% [11]. Up until 2001, all UK rhizomania outbreaks could be attributed to the presence of A or B type BNYVV which did not contain a fifth RNA species. RNA-5-containing BNYVV was detected for the first time in 2001 in Norfolk [9]. In the present study we have analysed genome properties of RNA-5-containing BNYVV sources from two different sites in the Norfolk area. One of these BNYVV sources (UK-MH) represents the P type, whereas the other (UK-FF) is clearly distinct and takes an intermediate position between European and East Asian BNYVV types.

Material and methods

Virus sources

Two sites with RNA 5-containing BNYVV located 18 km apart were identified in Norfolk, UK, during a routine aerial survey. Soil was collected from each site. The presence of RNA-5-containing BNYVV was confirmed using real-time RT-PCR [9, 24]. Infected sugar beet plants were obtained by bait testing using cv. Roberta as described by Henry et al. [10]. Briefly, 4-day-old sugar beet seedlings were planted into 200-ml pots containing each soil. Four seedlings were placed in each pot. The plants were grown in a glasshouse at 21 °C and watered daily. After four weeks, the plants were removed from the pots, and soil was removed from the roots by gentle washing.

Nucleic acid extraction

Root tissue (200 mg) was placed inside 2-ml screw cap microcentrifuge tubes with 3 × 3 mm tungsten carbide beads (Qiagen) and 1 ml of lysis buffer (Kingfisher ML, Thermo Labsystems, Vantaa, Finland). Tubes were then mounted onto a grinding mill (Qiagen) and shaken at an

amplitude of 30 beats per second for 3 minutes. The tubes were centrifuged at 6000 *g* at room temperature for 3 minutes to remove debris.

RNA was extracted from the cleared lysate using a silica-based RNA extraction kit in conjunction with a Kingfisher ML magnetic particle processor (Thermo Labsystems, Vantaa, Finland). A total nucleic acid programme was used. Briefly, 1000 µl of cleared lysate was mixed with 50 µl of MagneSil™ magnetic silica particles (Promega) in well 1 of the Kingfisher ML 5 tube strips. The other wells were loaded as follows: well 2 – 1000 µl of wash buffer 1, well 3/4 – 1000 µl of wash buffer 2 and well 5 – 100 µl molecular biology grade water (BDH, Lutterworth, Leicestershire, UK). RNA was collected in tube 5, transferred to a sterile 1.5-ml tube and stored at −20 °C prior to use.

PCR amplification

One-step RT-PCR was carried out in 50 µl reaction volumes using 2× ReddyMix™ (ABgene) containing 1 µl (50 units) of Reverse-iT™ RTase Blend (ABgene), 10 pmol of forward and reverse primers and 2 µl of RNA template. To obtain the almost entire sequence of RNA 5, two primer pairs were designed for the amplification of overlapping regions covering the 5′ and the 3′part, respectively, of this RNA. Additional primers allowed the amplification of the coding regions for the C-terminal portion of P220 on RNA 1, for P13/P15/ΔP14 on RNA 2,

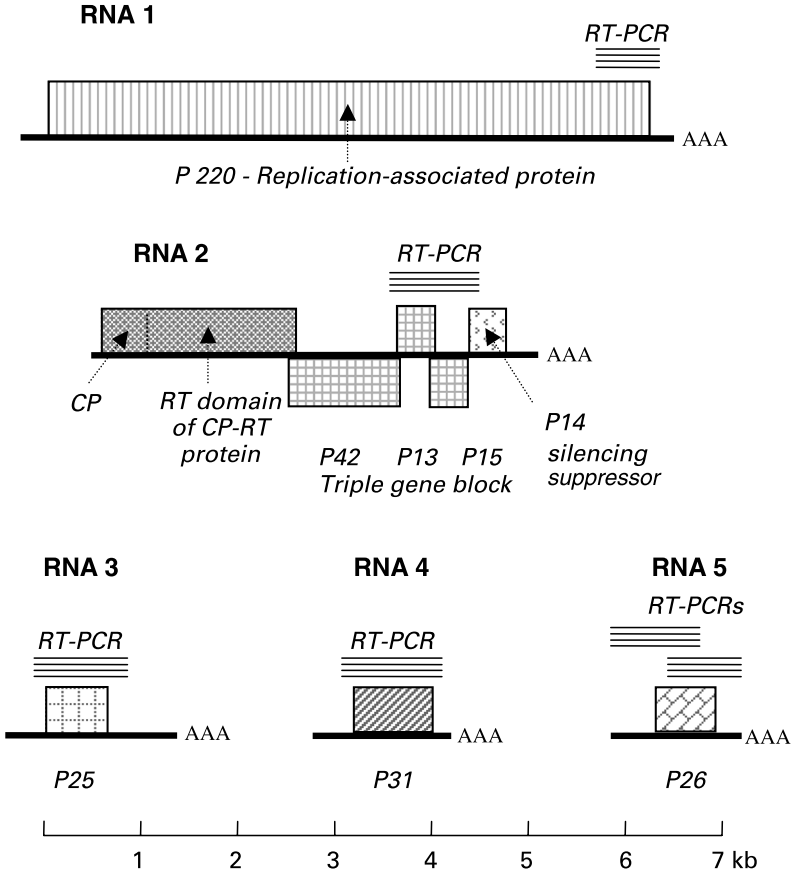


Fig. 1. Genome organization of BNYVV and overview of the regions amplified in this study by means of RT-PCR

Table 1. Nucleotide sequences of primers used for the amplification of various parts of the BNYVV genome

Primer	Nucleotide sequence (5′ to 3′)	Annealing on RNA position (Accession no. in parentheses)
RNA1 F	GAAGGGCGATGATGGTTT*	5940–5958 (NC_003514)
RNA1 R	ACCACAACAACCTTCATCAA*	6571–6590 (NC_003514)
RNA2 F	TGGGTCCTAACATTGCTGC*	3187–3295 (D84411)
RNA2 R	CAACACTTTCAGATCCCTCAG*	4086–4106 (D84411)
RNA3 F	TCGGAATATACAAGGTTTAAAAG	300–322 (D84412)
RNA3 R	GTCCCAACCAGATCAACAA*	1187–1205 (D84412)
RNA4 F	TTGGGTTTGTCACTGGGAT*	302–320 (D84413)
RNA4 R	CACATAAACCTTACCATAGCAA*	1313–1334 (D84413)
RNA5 511 F	AAATTCAAAGTACTTTCACATTGTA*	1–25 (U78293)
RNA5 523 F	TTTCGTGGACCTGGTAATTA*	900–919 (U78293)
RNA5 524 R	AGCACCGACCTCAACATTA	590–608 (U78293)
RNA5 525 R	GTCAATACACTGACAGAGAACCC*	1326–1348 (U78293)

*Indicates that the sequences of the RNA 1–4 primers were 100% identical in various European and Asian A types, European B and P types from Europe and Kazakhstan or that the sequences of the RNA 5 primers were 100% identical in European and East Asian RNAs 5, respectively. Primer RNA 3 F contains one mismatch with the sequence of isolate C-NM (underlined once). Primer RNA 5 524 R contains one mismatch with the sequences of most East Asian isolates (underlined once) and two mismatches with the sequence of isolate C-Baotou (underlined twice)

for P25 on RNA 3 and for P31 on RNA 4 (Fig. 1; Table 1). Multiple sequence alignments were performed on sequences from A types from Europe and Japan, B types from Europe and P types from Europe and Kazakhstan. Primers were designed to regions of the genome found to be either completely identical or nearly so (Table 1).

BNYVV RT-PCR products were purified using a QIAquick® PCR purification Kit (Qiagen) and sequenced by Sequiserve (Vaterstetten, Germany). Sequences were analysed by means of the LINEUP/PILEUP programs of the UWGCG software version 8 [7], and trees based on the neighbour-joining method [27] were generated by the program DNAMAN (Lynnon Bio/Soft). For visualizing nucleotide differences in sequence alignments, the program Boxshade (www.ch.embnet.org/software/BOX_form.html) was used.

Results

RNA5

Nearly complete nucleotide (nt) sequences, excluding terminal primer regions, were determined for RNA 5 of the UK-MH and UK-FF sources of BNYVV. BNYVV UK-MH came from the same location as the isolate previously studied by Harju et al. [9], and the partial 530-nt sequence (AJ439620) analysed by these authors proved to be 100% identical to the corresponding part of the 1300-nt sequence determined in this study. The 1293-nt sequence obtained for BNYVV UK-FF RNA 5 proved to be substantially different from the nucleotide sequence

for UK-MH. A cluster tree based on neighbour-joining analyses [27] using full-length or almost full-length sequences of BNYVV RNA 5 from various parts of the world indicated that the UK-MH RNA 5 is very closely related to the P-type-associated RNAs 5 from the Pithiviers area in France and from Kazakhstan (Fig. 2a) [16]. The percentages of sequence identities between these closely related RNA 5 species amount to more than 99% (Table 2). The UK-FF RNA 5 and the East Asian RNAs 5 from Japan and China are clearly distinct from the P-type-associated RNAs 5. UK-FF RNA 5 seems to have an intermediate position between the P type and the East Asian RNAs 5 (Fig. 2a).

Sequence alignment allowed a more detailed analysis of these relationships (Fig. 2b). Previous investigations [16] have revealed a number of positions in which the nts in P type RNAs 5 differ from those in East Asian RNAs 5. The UK-MH RNA 5 contains the P-type-specific nts in all these positions. The UK-FF RNA 5 also contains P-type-specific nts in many of these positions, labelled as '**P**' in the bottom rows of Fig. 2b. However, there are also a number of positions in which the nts of the UK-FF RNA 5 are identical to those in the East Asian sequences and not to those in the P type sequences; they are labelled by an '**A**' in the bottom rows of Fig. 2b. RNA 5 of the Chinese Baotou isolate has previously been shown to occupy an intermediate position between P type and typical East Asian RNAs 5 [16]. Interestingly, the UK-FF RNA 5 has many of those nts in

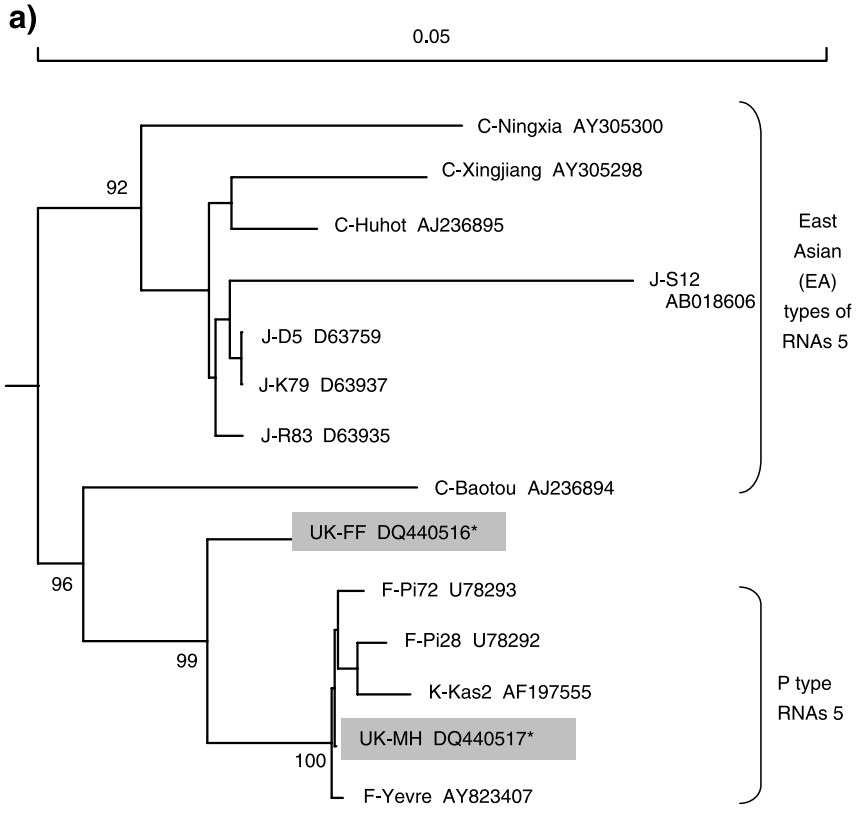


Fig. 2 (*continued*)

common with the Baotou RNA 5 which differentiate this RNA 5 from the other East Asian RNAs 5 and suggest a closer relationship between the Baotou and the P type RNAs 5. These positions are labelled by a 'b' in the bottom rows of Fig. 2b.

P type BNYVV RNA 5 differs from most of the East Asian BNYVV RNAs 5 not only by nts in specific positions, but also by a number of insertions or deletions in the 5′ untranslated regions (5′ UTR) and the P26-coding region (Fig. 2b). The UK-FF and C-Baotou RNAs 5 again exhibit a number of intermediate features. They both have unique insertions in the AU-rich region following nt 350 in the 5′ UTR (note that the numbering in Fig. 2b follows that of C-Huhot RNA 5) and – like the P type RNAs 5 – they both lack a 6-nt insertion (usually ACUAUA) c. 75 nts further downstream in the 5′ UTR following nt 428. This insertion is found in all East Asian RNAs 5 except for the Baotou RNA 5. In the coding region, they both lack an AUG insertion following nt 685, which is found in the P type

b)

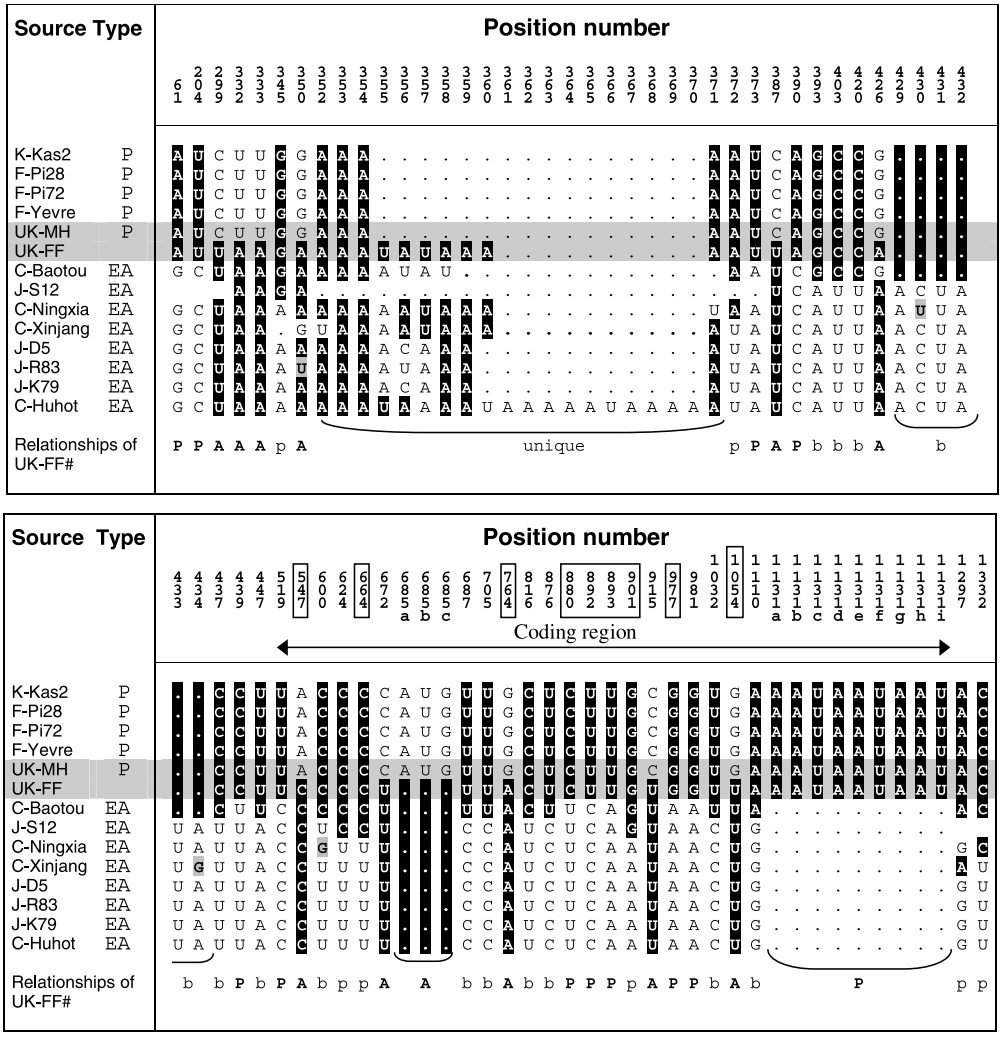


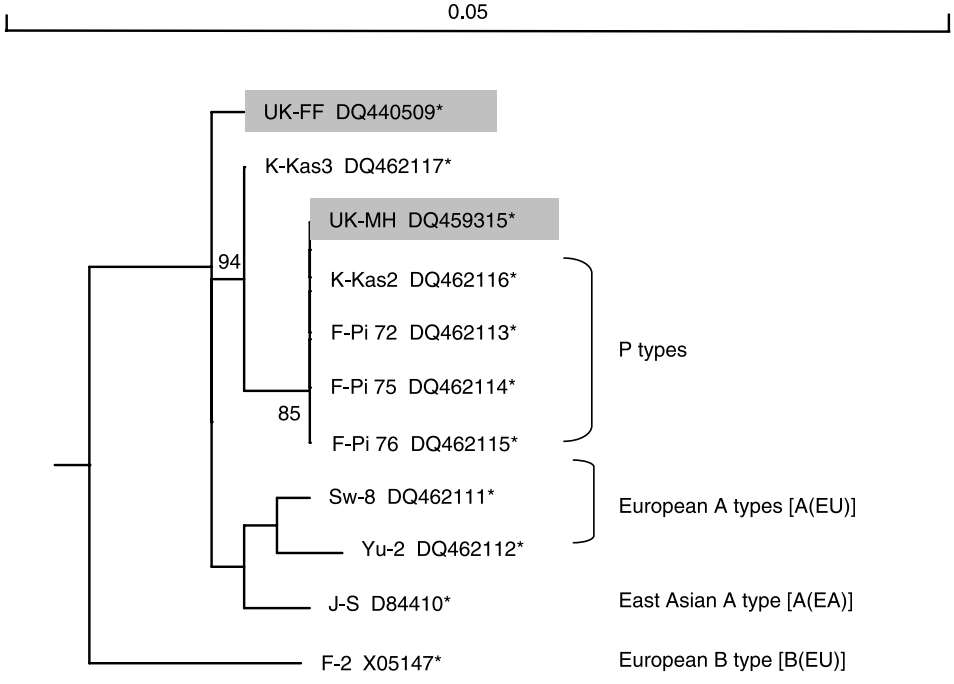
Table 2. Closest relationships of the RNAs of BNYVV UK-MH and UK-FF (percentages of sequence identities in the analysed regions in parentheses)

	UK-MH	UK-FF
RNA 1 (3′ portion of replicase gene and additional downstream nts)	European P type (100%)	P type Kazakhstan (K-Kas3) (99.6%)
RNA 2 (coding sequences for P13, P15 and part of P14)	European P type (99.5%)	East Asian A type (Japan) (99.9%)
RNA 3 (coding sequence for P25)	European P type (99.8%). The encoded P25 has a SYHG tetrad in pos. 67–70	European A types (99.5%). The encoded P25 has an unique TYHG tetrad in pos. 67–70
RNA 4 (coding sequence for P31)	European P type (99.9%)	East Asian A types (Japan; China) (99.2%; 99.4%)
RNA 5 (almost complete sequences)	European P type (99.8%)	Unique sequence with features of European P type as well as East Asian A type RNA 5

RNAs 5, but not in the typical East Asian RNAs 5. However, the UK-FF RNA 5 differs from the C-Baotou RNA 5 by having a 9-nt insertion close to the 3′end of the coding region, which is typical for the P types and is not found in the East Asian RNAs 5 including that of C-Baotou. Aside from the described similarities between the C-Baotou and the UK-FF RNAs 5, there are also many nt differences

Fig. 2. Sequence comparisons of full-length or almost full-length sequences of BNYVV RNA 5 from various parts of the world. **a** Tree based on neighbour-joining analyses [27]. The letters preceding the hyphen in the virus sources indicate the country of origin, i.e. *C* China, *F* France, *J* Japan, *K* Kazakhstan, *UK* United Kingdom. The letters or numbers following the hyphens designate the particular isolate; they are followed by the gene bank accession numbers. Newly determined sequences are labelled by an asterisk. The numbers on the branches give the percent bootstrap scores in 1000 trials; only values >85 are shown. The length of the branches can be estimated by means of the scale bar shown on the top of the figure. **b** Sequence alignments for the BNYVV RNA 5 sources shown in Fig. 2a. Only the nts in those positions are shown which allow a differentiation of various BNYVV sources. Dots signify missing nts, empty spaces portions of a sequence which were not analysed. The numbering of nts corresponds to that of C-Huhot RNA 5 (AJ236895). The additional nts present in some sources following positions 685 and 1131 are numbered by 685a, 685b etc. Positions in the coding regions in which mutations are leading to amino acid changes are boxed. The nts or deletions in the sequence of UK-FF RNA 5 are highlighted by white letters or dots on a black background. Nts or deletions in the other sequences that are identical to those in UK-FF are also highlighted by white letters or dots on a black background. P stands for P type, EA for East Asian; J-D5, J-R83, K79 and J-S12 represent the RNA 5 groups I-A, I-B, I-C and II described in Ref. [23]. # Relationships of UK-FF: In Fig. 2**b P** – signifies that the nt or insertion/deletion at this specific site corresponds to that in P type RNAs 5, **A** – that it corresponds to those in East Asian RNAs 5, b – that it corresponds to those in RNAs 5 of the P types and of C-Baotou, p – that it corresponds to those in RNA 5 of the P-types, of C-Baotou and of some additional East Asian sources

a)



b)

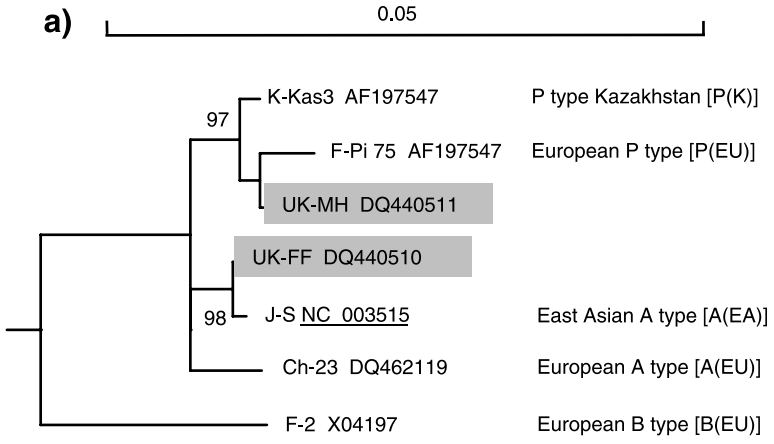
Source	Type	Position number in RNA 1 sequence
		6060 6105 6111 6240 6267 6273 6306 6312 6393 6394 6426 6459 6462 6465 6494 6513 6535 6541
UK-FF		U A A U U U U U G U G U U C A U G G
K-Kas3	P(K)	U A A U U C U U G U G U U C A U G G
UK-MH		C A G U U C U U G U G U U C A U G G
K-Kas2	P(K)	C A G U U C U U G U G U U C A U G G
F-Pi72	P(EU)	C A G U U C U U G U G U U C A U G G
F-Pi75	P(EU)	C A G U U C U U G U G U U C A U G G
F-Pi76	P(EU)	C A G U U C U U G U G U U C A U G G
Sw-S8	A(EU)	U A A U C U U G C G U U C A U G G
Yu-2	A(EU)	U A A U C U U G C G U U C A U G G
J-S	A(EA)	U A A U C U C U G U G C U C A U G G
F-2	B(EU)	C G A C C C C A U C C U U G C U A

Fig. 3. Comparison of the BNYVV sources UK-MH and UK-FF with BNYYV sources from different parts of the world on the basis of portions of RNA 1 corresponding to nts 5985–6552 of BNYVV RNA1 of the French B type isolate F-2. **a** A tree based on neighbour-joining analyses [27], **b** a sequence alignment showing only those nt positions which either differentiate between UK-MH and UK-FF or which differentiate between UK-MH and UK-FF on the one side and other BNYVV sources on the other side. The nts in the sequence of UK-FF and those that are identical to them in other BNYVV types are highlighted by white letters on a black background. For further information see legend to Fig. 2. Abbreviations not introduced in the legend to Fig. 2 are: *Sw* Sweden, *Yu* Yugoslavia, *EU* Europe

between them which reveal that the relationship of the UK-FF RNA 5 to the P type RNAs 5 is closer than that of the C-Baotou RNA 5 (Fig. 2a and b).

RNAs 1, 2, 3 and 4

In order to enable an assignment of the two UK RNA-5-containing BNYVV sources to known or possibly new BNYVV types, the following portions of their

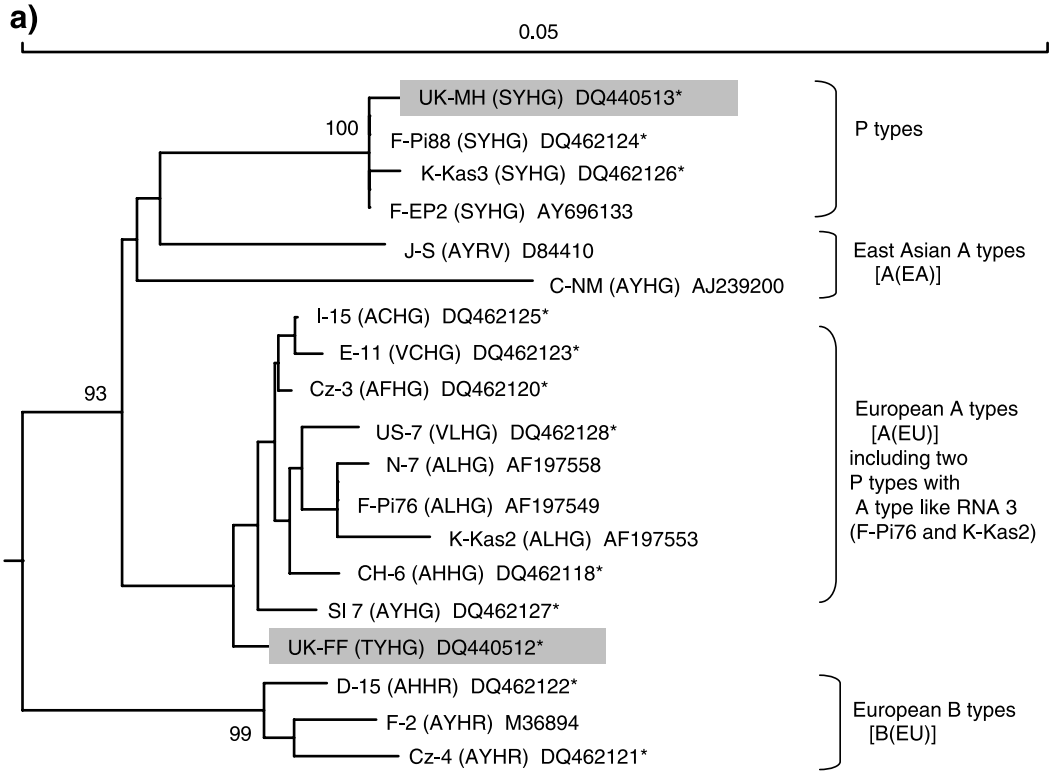


b)

BNVVY source	Type	Position number in coding sequence for P13	P15	P14
		1 1 2 6 7 9 2 1 1	1 2 3 3 9 5 0 6 8 3 7 4	3 9
K-Kas3	P(K)	U U G	G G A U	A
F-Pi75	P(EU)	U C G	G G A C	A
UK-MH		U C G	G G A C	A
UK-FF		C U A	A A C U	G
J-S	A(EA)	C U A	A A C U	G
Ch-23	A(EU)	U U A	A A A U	A
F-2	B(EU)	U U A	A A A U	A

Fig. 4. Comparison of the BNYVV sources UK-MH and UK-FF with BNYYV sources from different parts of the world on the basis of their coding sequences for P13, P15 and the 5′terminal part of P14 on RNA 2. These sequences correspond to nts 3287–4088 of BNYVV RNA2 of the French B type isolate F-2. **a** A tree based on neighbour-joining analyses [27], **b** a sequence alignment in which only those positions are shown in which the nts differ in UK-MH and UK-FF. The many positions in which differences occur between other virus types, but not between UK-MH and UK-FF, are not shown; there are e.g. more than 30 nt differences between Ch23 [A(EU)] and F2 [B(EU)]. The nts in the sequence of UK-FF and those that are identical to them in other BNYVV types are highlighted by white letters on a black background. Positions in which mutations are leading to amino acid changes are boxed. For further information see legend to Fig. 2. Abbreviations not introduced in the legend to Fig. 1 are: *Ch* Switzerland, *EU* Europe

a)



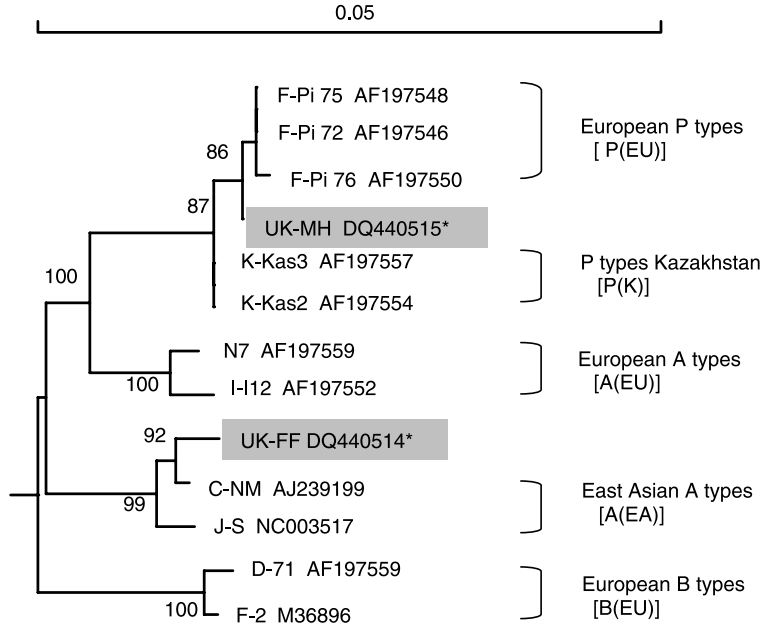
b)

Source	Type / amino acid tetrad in pos. 67–70 of P25		Position number in P25-coding sequence: 83 90 93 111 145 199 303 385 394 426 467 453 587 592
UK-MH		SYHG	C U G A U U C A A U C U U G
F-Pi88	P(EU)	SYHG	C U A A U U C A A U C U U G
K-Kas3	P(K)	SYHG	C U A A U U C A A U C U U G
F-EP2	P(EU)	SYHG	C U A A U U C A A U C U U G
J-S	A(EA)	AYRV	U C A G A G U A A U U U U G
C-NM	A(EA)	AYHG	G C A G A G U A A U U U U G
US-7	A(EU)	VLHG	U C A G A G U C U C U C C A
N-7	A(EU)	ALHG	U C A G A G U C U C U C C A
Ch-6	A(EU)	AHHG	U C A G A G U C U C U C C A
I-15	A(EU)	ACHG	U C A G A G U C U C U C C A
E-11	A(EU)	VCHG	U C A G A G U C U C U C C A
Cz-3	A(EU)	AFHG	U C A G A G U C U C U C C A
Sl-7	A(EU)	AYHG	C C A G A G U C U C U C C A
F-Pi76	A(EU)	ALHG	U C A G A G U C U C U C C A
K-Kas2	A(EU)	ALHG	U C A G A G U C U C U C C A
UK-FF		TYHG	U C A G A A U C U C U C C A
D-15	B(EU)	AHHR	U C A A U G U G A U U U C A
Cz-4	B(EU	AYHR	U C A A U G U G A U U U U G
F-2	B(EU)	AYHR	U C A A U G U G A U U U C A

Fig. 5. Comparison of the BNYVV sources UK-MH and UK-FF with BNYYV sources from different parts of the world on the basis of their P25-coding sequences on RNA 3. **a** A tree based on neighbour-joining analyses [27], **b** a sequence alignment in which only those nt positions are listed that differ in UK-MH and UK-FF. For further information see legend to Fig. 2. Abbreviations not introduced in the legend to Fig. 2 are: *I* Italy, *N* The Netherlands, *US* USA, *E* Spain, *Cz* Czech Republic, *Sl* Slovakia, *D* Germany

RNAs 1, 2, 3 and 4 were sequenced (Fig. 1): on RNA 1, the 521 3′ terminal nts of the coding region for the replication-associated P220 and 90 additional nts downstream of this coding region; on RNA 2, the second and third triple gene block genes, which encode the movement-associated proteins P13 and P15, including the downstream 5-terminal part of the coding sequence for P14, a putative suppressor

a)



b)

Source	Type	Position number in the p31-coding sequences
		120 123 191 254 310 313 390 394 499 457 423 477 440 400 510 563 555 554 667 695 714 747 734 744 819
F-Pi75	P(EU)	U U U C C A C G U C G G A A G C C A U U C U C A C
F-Pi72	P(EU)	U U U C C A C G U C G G A A G C C A U U C U C A C
F-Pi76	P(EU)	U U U C C A C G U C G G A A G C C A U U C U C A C
UK-MH		U U U C C A C G U C G G A A G C C A U U C U C A C
K-Kas3	P(K)	U U U C C A U G U C G G A A G C C A A U C U C A C
K-Kas2	P(K)	U U U C C A U G U C G G A A G C C A A U C U C A C
N-7	A(EU)	U U U U U G U G U C G A A A A C C A A U U C C A C
I-12	A(EU)	U U U U U G U G U C G A A A A C C A A U U C C A C
J-S	A(EA)	A A U U U G U A C A G A G G U A U G A U U C U A G
UK-FF		C A C U U G U A C A U A G G U A U G A C U C U G G
C-NM	A(EA)	C A U U U G U A C A G A G G U A U G A U U C U G G
D-71	B(EU)	U A U U U G U A U A G A A A U A C A A U U C C G C
F-2	B(EU)	U A U U U G U A U A G A A A U A C A A U U C C G C

Fig. 6. Comparison of the BNYVV sources UK-MH and UK-FF with BNYYV sources from different parts of the world on the basis of their P31-coding sequences on RNA 4. **a** A tree based on neighbour-joining analyses [27], **b** a sequence alignment in which only those nt positions are listed that differ in UK-MH and UK-FF. For further information see legend to Fig. 2. Abbreviations not introduced in the legend to Fig. 2 are: *I* Italy, *N* The Netherlands, *D* Germany

of virus-induced gene silencing [8]; on RNA 3, the coding region for P25, which is responsible for typical rhizomania symptoms [13, 32] and has an amino acid tetrad in positions 67–70 that is highly variable in different geographic locations [18, 21, 25, 28, 33]; on RNA 4, the coding region for P31 that plays a role in the transmission of the virus by *Polymyxa betae* [30].

Sequence alignments and cluster trees obtained with the above-described portions of RNAs 1–4 revealed that BNYVV UK-MH represents the P type of BNYVV. Previously described P-type-specific nt exchanges [16] were readily detected in its RNAs 1, 2 and 4 (Figs. 3, 4 and 6). No specific nt exchanges had previously been defined by Koenig and Lennefors [16] for P type RNA 3, because some P type sources from the Pithiviers as well as the Kazakhstan areas had contained an A-type-like RNA 3 (Fig. 5: F-Pi76 and K-Kas2), whereas others, like F-Pi88 and K-Kas3, contained an apparently P-type-specific RNA 3. The latter has recently been described by Schirmer et al. [28] as 'p25 IIb'-encoding RNA 3 for many BNYVV sources from the Pithiviers area. A very similar RNA 3 has also been found in UK-MH. The p25 encoded on these apparently P-type-specific RNAs 3 contains a SYHG tetrad in positions 67–70.

The analysed regions of RNAs 1–4 of BNYVV UK-FF differed from those of UK-MH and revealed relationships to different types of BNYVV. Its RNA 2 and 4 were closely related to the corresponding RNA regions of the Japanese virus source J-S [26] (Figs. 4 and 6). The percentages of identity between the UK-FF and the J-S nt sequences in these RNAs amount to more than 99% (Table 2). In addition, RNA 4 of UK-FF showed a close relationship to RNA 4 of the Chinese isolate C-NM (Fig. 6). Unfortunately, no data are available for the respective portions of RNAs 1 and 2 of this Chinese virus source. RNA 1 of BNYVV UK-FF has its closest relationship to RNA 1 of the BNYVV source K-Kas3 from Kazakhstan (Fig. 3), whereas its RNA 3 resembles European A type RNAs 3 (Fig. 5). Interestingly, the deduced amino acid sequence of the P25 encoded on the UK-FF RNA 3 contains a TYHG amino acid tetrad in position 67–70, which so far has not been observed in any other BNYVV source.

Inspection of the sequencing electropherograms of several PCR products gave no indications of mixed infections by BNYVV UK-MH and UK-FF.

Discussion

The results of this study, summarized in Table 2, indicate there are two types of RNA-5-containing BNYVV in the UK. UK-MH clearly represents the P type, which had previously only been found in two geographically widely separated regions, i.e. in the Pithiviers area in France and in Kazakhstan [15, 16, 28]. BNYVV UK-FF has a rather complex genome composition involving close relationships to East Asian A type RNAs 2 and 4, Kazakhstan P type RNA 1 and to European A type RNA 3 (Table 2, Figs. 3–6). RNA 5 of the UK-FF source has many unique properties that either relate it to the P type or relate it to East Asian RNA 5 types. BNYVV UK-FF has particular similarity to the Chinese virus source from Baotou, which was previously shown as the East Asian RNA 5 most closely related to P type RNA 5 [16]. The features of the UK-FF and the C-Baotou RNAs 5 that either

link them to or separate them from the P type and the typical East Asian RNA 5 types, respectively, are scattered throughout their sequences (see black columns in Fig. 2b). This suggests that the UK-FF and the C-Baotou RNAs 5 are probably not the result of recombination events, but rather the result of diverging phylogenetic developments.

The detection of both a known and a new type of BNYVV in the UK raises the question of from where these viruses originated. Viruliferous *P. betae* is readily transmitted by soil, for instance on agricultural equipment, planting stock or footwear of passers-by. Thus, UK-MH and UK-FF may have been introduced from any part of the world. P type BNYVV may well have been introduced to the UK-MH site from Pithiviers in France, where it has been established for many years. To date, BNYVV with properties resembling those of UK-FF has not been described from any other country. Introduction from far away regions, where the disease is already established, may not be the only reason for new rhizomania outbreaks. When rhizomania started to spread rapidly in Germany and France in the nineteen seventies, it was originally believed that it had been introduced from Italy, where the disease was first observed in the early fifties [6]. Subsequent molecular studies have indicated that this originally assumed transfer is unlikely. BNYVV in Italy has been shown to be of the A type, whereas B type BNYVV predominates in France and Germany [14, 19, 25, 28]. Also, P type BNYVV, which occurs in the Pithiviers area of France, has never been found in Italy. The nucleotide sequences of European BNYVV types have proved to be very stable during the past decades [16, 25, 28], which stands against the argument that the virus may have changed during its originally assumed transfer from southern to central Europe. It seems more likely that various types of BNYVV may have pre-existed in native hosts in different geographical regions long before sugarbeet growing began. Virus transmission to sugar beet from such native hosts may be a rare event, perhaps due to host preferences of the transmitting agent, presumably *Polymyxa betae*. However, once such a rare transmission event has taken place, the spread of the virus into neighbouring sugar beet growing areas would be very rapid. Climatic conditions in northern Europe may be less favourable for such transmission events from native plants than those found in southern European countries. It has been shown, for instance, that *P. betae* shows the highest infection rates at temperatures around 25 °C [2], which are reached over much longer periods of time in southern than in northern European countries. This may be one reason for the later appearance of rhizomania in the northern European countries. Various BNYVV types may have originated from different hosts, which allowed slightly different phylogenetic developments of ancestral BNYVV populations. Studies on the occurrence of BNYVV in the root systems of cultivated and wild native plants may provide further information on the origin of the various BNYVV types.

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