A comparison, using dsRNA analysis, between beet soil-borne virus and some other tubular viruses isolated from sugar beet

P. J. Hutchinson, C. M. Henry and R. H. A. Coutts

1 Department of Biology, Imperial College of Science, Technology and Medicine, Prince Consort Road, London SW7 2BB and 2 MAFF, Central Science Laboratory, Hatching Green, Harpenden, Hertfordshire AL5 2BD, U.K.

Double-stranded RNA preparations from Chenopodium quinoa leaves inoculated with two English isolates of beet soil-borne virus (BSBV), BSBV-N and BSBV-452N, a French isolate of beet necrotic yellow vein virus (BNYVV), a Swedish isolate of a tubular beet virus (86-109) or a Belgian isolate of a similar virus (1530) were compared following separation on non-denaturing polyacrylamide gels. The dsRNAs of BNYVV differed in mobility from those isolated from tissue infected with the other four tubular beet viruses, which possessed three major dsRNA species. The degree of sequence identity between BNYVV, BSBV-N, 86-109 and 1530 was investigated by RNA–RNA blot hybridization using 32P-5' end-labelled probes. Reciprocal hybridization experiments revealed similarity between the BSBV-N, 86-109 and 1530 isolates, but none between these isolates and BNYVV.

Beet soil-borne virus (BSBV) is a poorly characterized rod-shaped virus of sugar beet roots originally described by Ivanović & Macfarlane (1982). It has virus particles of 19 nm in diameter which, although of different lengths, have predominant lengths of 65 nm, 150 nm and 300 nm (Ivanović et al., 1983). The virus is transmitted by the plasmodiophorid fungus Polymyxa betae (Keskin) and infects spinach and sugar beet (Henry et al., 1986). BSBV is more widespread in England than originally thought, and also occurs in The Netherlands (Henry et al., 1986). Several similar poorly characterized viruses have been isolated from sugar beet in Sweden and West Germany (Lesemann et al., 1989), Belgium (Verhoyen et al., 1987) and California, U.S.A. (Duffus, 1988). These viruses and BSBV are morphologically similar to beet necrotic yellow vein virus (BNYVV); however, monoclonal antibodies to BNYVV do not cross-react with the original BSBV isolate from Norfolk, U.K. (BSBV-N; Torrance et al., 1988). Additionally, Lesemann et al. (1989) noted a lack of a serological relationship between BNYVV and isolates of BSBV, and reported preliminary evidence that cDNA clones of BNYVV RNAs do not hybridize with BSBV RNA.

Tests using polyclonal antisera against the German isolates Wierthe and Ahlum, and a Swedish isolate 86-109 in immunosorbent electron microscopy and ELISA have suggested the existence of at least two BSBV serotypes. One serotype contains the German isolates Wierthe and Dornheim, and the second group comprises the German isolate Ahlum, BSBV-N, the Swedish isolate 86-109 (Lesemann et al., 1989) and the Belgian isolate 1530 (Hutchinson et al., 1990). These serotypes are distinct from BNYVV and from the soil-borne sugar beet viruses isolated in California (Lesemann et al., 1989; Lindsten, 1989). However, in the absence of reciprocal serological tests (owing to the lack of availability of antisera to all of the isolates examined), such a subdivision is tentative.

We report here the analysis of dsRNA isolated from plants infected with BSBV-N, BSBV-452N, BSBV-86-109, BSBV-1530 or a French isolate of BNYVV (Torrance et al., 1988) to determine any genomic relationships between the viruses.

Single lesion BSBV isolates and BNYVV were propagated in Chenopodium quinoa leaves as described previously (Henry et al., 1986). The total amount of dsRNA isolated from 100 g infected tissue, as described by Valverde et al. (1986), was fractionated by PAGE on 5% acrylamide gels (Ratti & Buck, 1972), together with Aspergillus foetidus virus dsRNAs of known M, (Buck & Ratti, 1977).

Ribonuclease sensitivity tests confirmed the double-stranded nature of all dsRNAs investigated (results not shown). The dsRNA species (M, 1.8 × 10^6) present in the healthy C. quinoa preparation (Fig. 1, lane 3) was routinely found but its origin is not known. No other dsRNA species were detected in extracts of uninfected C. quinoa leaves (Fig. 1, lane 3).
Three major dsRNAs with $M_r$ values of $4.3 \times 10^6$, $2.0 \times 10^6$ and $1.7 \times 10^6$ (Fig. 1, lane 4) were regularly found in extracts of plants infected with BSBV-N after repeated passage in *C. quinoa*. These dsRNAs were designated 1, 2 and 3 in order of increasing mobility on gels. The dsRNA profile of *C. quinoa* tissue infected with BSBV-N freshly isolated from sugar beet roots was similar, apart from the presence of an additional smaller species (dsRNA 4) with an $M_r$ of $0.7 \times 10^6$. After three or more successive passages of freshly isolated BSBV-N, by mechanical inoculation into *C. quinoa*, the amounts of dsRNA 4 present in tissue extracts were undetectable following ethidium bromide staining or probing (results not shown). During subsequent transfers from *C. quinoa* to *C. quinoa*, the dsRNA pattern for BSBV-N was retained. These alterations in the dsRNA composition of BSBV-N were associated with the appearance of local necrotic lesions rather than the original symptoms, diffuse chlorotic/necrotic blots which tend to spread along leaf veins (Henry et al., 1986).

The four dsRNAs extracted from BNYVV-infected leaves had $M_r$ values of $4.6 \times 10^6$, $3.4 \times 10^6$, $1.2 \times 10^6$ and $1.0 \times 10^6$ (Fig. 1, lane 5), the sizes expected for dsRNA forms of the four genomic RNAs (Bouzoubaa et al., 1989). Variable RNA patterns have been observed in one proposed member of the furovirus group, BNYVV (Koenig et al., 1986; Kuszala et al., 1986), and the type member of the group, soil-borne wheat mosaic virus (SBWMV; Shirako & Brakke, 1984a, b). Koenig et al. (1986) have reported the tendency for small RNAs of BNYVV to be lost from local lesion isolates during progressive transfers from *C. quinoa* to *C. quinoa*. Deletion mutations of smaller RNAs have been correlated with changes in symptom expression of both BNYVV (Koenig & Burgermeister, 1989) and SBWMV (Shirako & Brakke, 1984a). Koenig & Burgermeister (1989) have also suggested that full-length BNYVV RNAs 3 and 4 may be necessary for vector transmission.

The isolates BSBV-452N, BSBV-1530 and BSBV-86-109 were passaged repeatedly in *C. quinoa*, and in each case three dsRNAs were detected in extracts from infected plants. The $M_r$ values of the dsRNAs were estimated to be: BSBV-452N, $4.2 \times 10^6$, $2.4 \times 10^6$ and $2.2 \times 10^6$; BSBV-86-109, $4.2 \times 10^6$, $2.3 \times 10^6$ and $2.0 \times 10^6$; BSBV-1530, $4.2 \times 10^6$, $2.4 \times 10^6$ and $2.2 \times 10^6$ (Fig. 1, lanes 1, 6 and 7 respectively). Following PAGE separation of dsRNA, ethidium bromide staining and photography, the nucleic acids were transferred by electroblotting onto Zeta-Probe membranes (Bio-Rad) according to the procedure of Bodkin & Knudson (1985). The blots were pre-hybridized and then hybridized with radiolabelled dsRNA probes and washed, assuming a G+C content of 67~ (similar to phytoareoviruses), using the methods described by Bodkin & Knudson (1986). Probes were produced by end-labelling total BSBV-specific dsRNA with $^{32}$P]ATP and T4 polynucleotide kinase using a procedure similar to that described by Sambrook et al. (1989), except that the kinase reaction buffer contained...
83 mM-Tris–HCl pH 7-6, 8.3 mM-DTT, 1.6 mM-MgCl₂ and 1.6 mM spermidine. The radiolabelled RNA probes were used directly following ammonium acetate precipitation and washing to remove unincorporated label (Okayama & Berg, 1982).

Hybridization experiments using ³²P-5' end-labelled probes constructed with a mixture of dsRNAs 1, 2 and 3 of, respectively, the BSBV-N, 86-109 and 1530 isolates revealed significant cross-hybridization between dsRNAs of these three BSBV isolates, but none between the dsRNAs of these isolates and those of BNYVV (Fig. 2). In Fig. 2, labelled denatured dsRNA of BSBV-1530 was used as a probe, but results with the dsRNAs of the other BSBV isolates were essentially the same (results not shown).

Some differential hybridization between the dsRNAs of the different isolates was observed in a number of probing experiments, particularly with reference to the efficiency of detecting dsRNA 1. Since, in the total dsRNA preparations utilized, the smaller dsRNA species 2 and 3 are presumably end-labelled more efficiently than the larger dsRNA 1, weaker signal intensity of dsRNA 1, as compared to that of the other two dsRNAs, would be expected on the blots.

The results presented here suggest that BNYVV is unrelated to any of the other four sugar beet viruses investigated. However, there may be some distant similarity between BNYVV and these isolates which may not have been detected under the stringent washing conditions used. Only further molecular characterization of BSBV will resolve this aspect. BSBV-N is related to the three sugar beet virus isolates 86-109, 1530 and 452N, and the genome of BSBV-N contains at least three genomic components, RNAs 1, 2 and 3.

We are grateful to Dr K. Lindsten and Dr M. Verhoyen for supplying, respectively, the Swedish 86-109 isolate and the Belgian 1530 isolate. This work was funded by the Science and Engineering Research Council, and the Sugar Beet Research and Education Committee. All work with BNYVV was conducted under licence number PHF 32A/67 (19) at MAFF, Central Science Laboratory, Harpenden, Hertfordshire.

References


**Short communication**


(Received 12 December 1991; Accepted 28 January 1992)