Uniform RNA Patterns of Beet Necrotic Yellow Vein Virus in Sugarbeet Roots, but Not in Leaves from Several Plant Species

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SUMMARY

Northern blot hybridization experiments with cDNAs to the four RNA species of a Yugoslavian isolate of beet necrotic yellow vein virus (BNYVV) revealed identical virus RNA patterns in root extracts from field-grown sugarbeets and sugarbeet seedlings grown in soil from rhizomania-affected fields in various regions in Germany and abroad. In contrast, in leaf extracts from mechanically infected Chenopodium quinoa, Tetragonia expansa and sugarbeet and from a naturally infected sugarbeet we observed great variations in the number, size and relative concentration of the small BNYVV RNAs, which suggests that they may undergo deletion mutations when the virus is propagated in leaf tissues.

Beet necrotic yellow vein virus (BNYVV), the causal agent of sugarbeet rhizomania, is a tubular virus with particles of different sizes (e.g. Tamada, 1975) and several, typically four, different RNA species (Putz, 1977). Under natural conditions it is transmitted from infected to uninfected sugarbeet roots by the fungus Polymyxa betae. Infections usually remain confined to the roots; leaf infections are only rarely observed. Local lesions only are formed when the virus is transmitted mechanically to leaves of sugarbeets, Chenopodium quinoa or Tetragonia expansa (e.g. Tamada, 1975; Kuszala & Putz, 1977).

Richards et al. (1985) found that in both a French and a German isolate of BNYVV, RNA 1 consisted of 7100 nucleotides and RNA 2 of 4800 nucleotides; the sizes of RNAs 3 and 4, however, differed between the two isolates which they had propagated in C. quinoa. RNA 3 contained 1800 and 1400 and RNA 4 1500 and 1150 nucleotides in the two isolates, respectively. Bouzoubaa et al. (1985) determined the nucleotide sequences of approximately full-length cloned cDNAs to RNA 3 and 4 of these isolates. They concluded that the German isolate had a deletion of 354 nucleotides in RNA 3 and of 324 nucleotides in RNA 4 compared to the French isolate. Otherwise the nucleotide sequences of RNAs 3 and 4 appeared closely similar in the two isolates.

We have recently used cloned cDNAs to the four RNA species of a Yugoslavian isolate of BNYVV to study the RNA patterns of 13 isolates of BNYVV by Northern blot analysis (Burgermeister et al., 1986). These isolates were obtained from sugarbeets received from various countries in Europe. They were separated by local lesion transfer in C. quinoa and were maintained in this host. The sizes of RNA 1 and 2, respectively, were identical in all 13 isolates, but the smaller RNAs that hybridized with cDNAs to RNA 3 and 4 of the Yugoslavian isolate showed a great variation not only in size, but also in number and relative concentration. In one of the isolates we could not detect any of the small RNAs, but other isolates contained up to four small RNA species of different sizes. Even local lesion isolates obtained from the same sugarbeet showed pronounced differences in the patterns of the small RNAs. During successive transfers from C. quinoa to C. quinoa, using sap either from single lesions or entire leaves for
Northern blot hybridization analysis of BNYVV RNAs in infected plant tissues using a mixture of four $^{32}$P-labelled plasmids with cDNAs specific for each of the four RNAs of the Yu 2 isolate of BNYVV. Phenol extracts from the following plant materials were applied to the lanes: (a) leaf from field-grown sugarbeet showing symptoms of BNYVV infection; (b, f) roots from field-grown sugarbeets in northern (Braunschweig) and southern (Regensburg) Germany, respectively; (c, d, e, g) roots from sugarbeet seedlings grown in soils which were collected from rhizomania-affected fields in the Danube valley near Plattling, the upper River Main valley, the Danube valley near Regensburg and upper Rhine valley near Gross Gerau, respectively; (h, i, j, l) leaves from C. quinoa infected mechanically with various local lesion isolates of BNYVV; (k) sugarbeet leaf infected mechanically with the same isolate as in (j). Identical patterns as in (b) to (g) were obtained with extracts from side and tap roots, respectively, from sugarbeets grown in eight different fields in northern Germany near Braunschweig and with root extracts from seedlings grown in a soil sample from Yugoslavia.

Inoculations, the RNA pattern typical for each isolate was usually retained; occasionally, however, we observed qualitative changes in the intensity of individual RNA bands or even quantitative changes in the number of bands (Burgermeister et al., 1986). On the whole, there seems to be a tendency for small RNAs to be lost from local lesion isolates during progressive transfers from C. quinoa to C. quinoa. In this way, we have now obtained a second isolate which is devoid of small RNAs and three of our other isolates have lost one of several small RNA species during the past year (C. Kothe, W. Burgermeister & R. Koenig, unpublished observations).

With a few field-grown sugarbeets which had all originated from the same region, we obtained preliminary evidence that the RNA patterns of BNYVV are more stable in sugarbeet roots which are infected through P. betae (Burgermeister et al., 1986). This has now been confirmed with root extracts obtained from more field-grown sugarbeets and from sugarbeets which were grown in the greenhouse in soil samples collected from rhizomania-affected fields in different regions of Germany and abroad.

The cloning of cDNAs to the four RNA species of the Yugoslavian BNYVV isolate Yu 2, extraction of total RNAs from plant tissues, the electrophoretic separation of RNAs in formamide-containing agarose gels, the Northern blot transfer of the separated RNAs to nitrocellulose membranes and the hybridization of the RNAs with mixtures of cDNAs specific for the individual RNAs of the Yu 2 isolate have been described in detail by Burgermeister et al. (1986).

Field-grown sugarbeets were obtained from several fields near Braunschweig in northern Germany and near Regensburg in southern Germany shortly before harvest time in September and October. Soil samples were collected from rhizomania-affected fields in the upper Rhine.
valley, Franconia, the Danube valley near Regensburg and near Plattling and in Yugoslavia. Clay pots were filled with soil which had been stored in plastic bags for 1 to 3 years and each pot was placed on another pot turned upside down to avoid cross contamination of the soils with zoospores from other pots. Sugarbeet seed was sown into these soils and the roots of the developing seedlings were harvested about 10 weeks later.

Fig. 1 (b to g) shows that the BNYVV RNA patterns for all root extracts from field-grown sugarbeets as well as from sugarbeet seedlings grown in the greenhouse in soil samples from various parts of Germany were identical. Further experiments with naturally infected sugarbeets from eight different fields revealed identical BNYVV RNA patterns in extracts from the small side roots and the tap root. However, an extract from a naturally infected sugarbeet leaf lacked one of the small RNAs found in sugarbeet roots (Fig. 1a). RNAs 3 and 4 of BNYVV from sugarbeet roots were calculated to have 2100 and 1700 nucleotides, respectively, when the RNAs of tobacco mosaic virus with 6395 nucleotides (Goelet et al., 1982) and brome mosaic virus with 3234, 2865, 2114 and 876 nucleotides (Ahlquist et al., 1984), respectively, were used as size markers.

For comparison, Fig. 1 also shows the RNA patterns of four local lesion isolates of BNYVV in C. quinoa in which we either failed to detect small RNAs (Fig. 1i) or detected one (Fig. 1j), two (Fig. 1j) or three (Fig. 1h) small RNA species. Several of these RNA species differed in size from those found in root extracts from sugarbeets. None of these or nine other Chenopodium isolates we have studied (Burgermeister et al., 1986) showed the typical BNYVV RNA pattern seen with extracts from sugarbeet roots.

From the sugarbeet root whose RNA pattern is shown in Fig. 1 (f), we obtained two local lesion isolates. One was the isolate that failed to show small RNAs in leaf extracts when cDNAs to the Yu 2 isolate of BNYVV were used as probes (Fig. 1i) and also in extracts from purified virus preparations after staining of gels with ethidium bromide (R. Koenig & W. Burgermeister, unpublished). The other isolate, named Regensburg 2, contained one small RNA (Burgermeister et al., 1986).

After mechanical transfer of Chenopodium isolates to leaves of sugarbeets or T. expansa the RNA patterns of the Chenopodium isolates were usually retained as shown in Fig. 1 (k, j) for a Chenopodium isolate which was transmitted mechanically to sugarbeet leaves.

Several hypotheses have been advanced to explain the role of the small BNYVV RNAs which show no sequence homologies with RNA 1 or 2 of the virus (Richards et al., 1985; Burgermeister et al., 1986). The possibility that they may be satellite RNAs has been considered by Richards et al. (1985), Bouzoubaa et al. (1985) and Burgermeister et al. (1986). The latter authors have also discussed the possibility that the small RNAs may be necessary for the transmission of the virus by P. betae or its multiplication and spread in sugar beet roots, but not in leaves. Our present results lend support to the hypothesis that under natural conditions of soil transmission and root infection the small RNAs are essential parts of the viral genome, but that they may become partially or completely redundant under unnatural conditions of mechanical transfer to leaves in which the infection remains localized or in rarely occurring cases when the virus is translocated from sugarbeet roots to leaves. Under such conditions the small RNAs may persist as satellites, may undergo deletions or may eventually be lost.

Our observations on the variable RNA patterns of BNYVV bear certain similarities to those made by Shirako & Brakke (1984b) with soil-borne wheat mosaic virus (SBWMV) which like BNYVV has been considered as a member of the proposed furovirus group (Shirako & Brakke, 1984a). The genome of SBWMV is somewhat less complex than that of BNYVV. SBWMV has an RNA I which is found in all preparations. SBWMV RNA II with a length of 0.5L is found in leaves of field-grown wheat plants harvested early in spring. In the leaves of field-grown plants harvested later in spring or in field-infected plants which were grown in the greenhouse during the winter the 0.5L RNA was partially or completely replaced by a shorter 0.35L RNA. A lack of transmissibility of virus with 0.35L RNA II by the vector P. graminis has been discussed by Shirako & Brakke (1984b) as one possible explanation for the observation that recently infected wheat plants always contain 0.5L RNA II.

Our results with BNYVV indicate that a virus mechanically transmitted to an experimental
host may not be identical in all properties to the virus in the natural host. The fact that Chenopodium isolates are usually deletion mutants may explain our failures to infect sugarbeet roots with such isolates.

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REFERENCES


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