Alfalfa Mosaic Virus Isolated from *Buddleia davidii* Compared with Other Strains

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ABSTRACT

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Alfalfa mosaic virus (AlMV) was isolated from *Buddleia davidii* in the south of France. Symptoms induced on different hosts by this isolate are reported; they differ from the symptoms described for AlMV isolated from *B. davidii* in East Germany in 1970. The isolate described in this report (AlMV-B) shows symptoms, RNA structure, and serological properties analogous to those of strain 425 from red clover. Strains S and B differ in their relative amounts of RNA 1 and 3.

We isolated alfalfa mosaic virus (AlMV) from *Buddleia davidii* (Franch.) showing symptoms of mosaic and leaf narrowing in the south of France. This strain has been used for comparative studies of translation efficiency in relation to the structure of the RNAs (*unpublished*). Structural characteristics of the RNA leader sequences have already been reported (10). It is therefore necessary to provide information on the symptoms induced by this virus and on its biophysical characteristics.

AlMV occurs naturally in many wild and cultivated species. Numerous strains have been described that have been differentiated on the basis of symptomatology and host range (4). Strains of AlMV with widely differing pathogenicity and geographical origin were shown to be closely related serologically (1,2,12), whereas amino acid composition of the coat protein allowed three groups of AlMV to be distinguished (5).

In this paper, we compare the symptoms induced by AlMV from *B. davidii* with those reported by Schmelzer (11) for AlMV isolated from *B. davidii* in East Germany. We also describe some physical properties of AlMV-B, which is compared with strain 425 isolated from red clover (3) and with strain S originating from lucerne (2).

MATERIALS AND METHODS

Leaves from *Chenopodium quinoa* (Willd.) inoculated with sap from *B. davidii* showing mosaic and distortion were used as a source for inoculating various plant species. AlMV isolates 425, Caldy, YSMV, and 15/64 obtained from E. M. J. Jaspars and AlMV-S from Strasbourg were maintained on *Nicotiana tabacum* (L.).

The virus was extracted from fresh leaves of *N. tabacum* harvested 8–10 days after inoculation. The extraction procedure was as described previously (9). The purified virus was recovered in 5 mM NaH_2PO_4 buffer containing 1 mM ethylenediaminetetraacetic acid (EDTA), pH 7.0. The nucleoprotein composition of the purified virus preparations was determined by observation of the Schlieren diagrams in a Spinco Model E ultracentrifuge. The components were identified by their sedimentation coefficients. A purified virus preparation was subjected to density-gradient centrifugation in a MSE-SW rotor (6 × 38 ml) for 5 hr at 105,000 *g*. Gradients consisted of five layers of sucrose from 50 to 10% in Sörensen's phosphate buffer 0.01 M, pH 7.2, diffused overnight.

AlMV-B, AlMV-425, and AlMV-S preparations purified by sucrose-gradient centrifugation were injected intramuscularly into rabbits at 7-day intervals. Each injection consisted of 1 ml of virus at 0.4–1.0 mg/ml; the first injection was mixed with Freund's incomplete adjuvant. Serological reactions were studied by the Ouchterlony double-diffusion technique (7).

AlMV RNAs were extracted from purified virus using phenol sodium dodecyl sulfate (SDS) and fractionated on gels of 2.4% polyacrylamide + 0.5% agarose as described previously (8). The relative amount of each RNA was obtained by high-resolution densitometric scanning at 560 nm of the O-toluidine-stained gel using a Shimadzu CS-390 dual-wavelength scanner.

Wheat germ translation was carried out for 2.5 hr at 25 C with extracts prepared as described by Marcu and Dudock (6). The translation reaction mixture contained in 10 μl was 2.7 mM of magnesium acetate, 14 mM of Hepes, pH 7.6, 50 mM of KCl, 0.025 mM of each amino acid, 2.5 mM of adenosine 5′-triphosphate (ATP), 0.375 mM of guanosine 5′-triphosphate (GTP), 5 mM of phosphoenolpyruvate, 0.5 mM of dithiothreitol, 0.65 mM of spermidine, 1.25 μCi of ^{35}S methionine, and 0.6 μg of unfractionated AlMV RNA. The products were analyzed on 10% polyacrylamide slab gels and revealed by autoradiography.

RESULTS AND DISCUSSION

AlMV-B could be transmitted to the following species in several families. In general, symptoms were similar to those induced by AlMV-425, except the time and intensity of symptom development differed in some species as noted below:

Amaranthaceae: *Gomphrena globosa* (L.)—necrotic local lesions, no systemic symptoms.

Aizoaceae: *Tetragonia expansa* (Murr.)—necrotic ring spots, no systemic

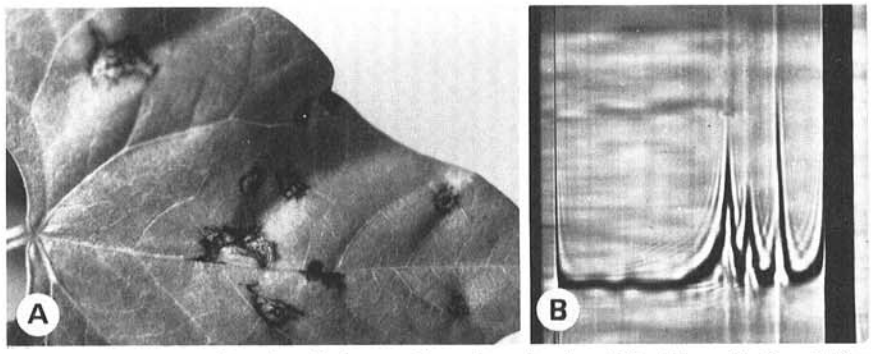

Fig. 1. (A) Appearance of large local lesions on *Phaseolus vulgaris* cv. Pinto 5 days after inoculation with crude sap of *Chenopodium quinoa* infected with alfalfa mosaic virus (AlMV) strain B. **(B)** Schlieren pattern of unfractionated AlMV-B. Photograph taken 10 min after the rotor reached a speed of 44,770 rpm; sedimentation from left to right. Peaks correspond to top b, middle, and bottom components from left to right.

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symptoms.

Chenopodiaceae: *C. amaranticolor* (Coste & Reyn.), *C. quinoa, C. foliosum* (Moench & Aschers)—chlorotic local lesions becoming necrotic with systemic flecking on the first two species. *C. murale* (L.)—chlorotic and necrotic ring spots. Symptoms produced by AlMV-B generally appeared earlier and were more pronounced than those produced by AlMV-425 and AlMV-S.

Cucurbitaceae: *Cucumis sativus* (L.)—chlorotic local lesions on cotyledonary leaves 4 days after inoculation, then vein yellowing and yellow blotching on upper leaves. *Cucurbita moschata* (Poir.)—veinclearing and mosaic.

Labiatae: *Ocimum basilicum* (L.)—no local lesions, systemic yellow mosaic.

Leguminoseae: *Vicia faba* (L.), *Vigna sinensis* (Endl.)—necrotic local lesions large and reddish 4 days after inoculation, extending to necrosis of whole inoculated leaves without systemic symptoms. *Phaseolus vulgaris* (L.)—(Fig. 1A) necrotic local lesions 4 days after inoculation, large and brownish on Pinto, and smaller, black, and extending to vein necrosis on Prince. Under some conditions, necrotic lesions coalesced and leaves were distorted and completely desiccated. No systemic infection. AlMV-425 and AlMV-S caused less pronounced symptoms than AlMV-B. On *P. vulgaris*, for example, local lesions were smaller and did not coalesce or cause leaf distortion.

Solanaceae: *Datura* spp.—chlorotic spots becoming necrotic. *N. tabacum* (cultivars Havana, Samsun, White Burley, and Xanthi n.c.)—necrotic local lesions or ring spots on inoculated leaves, line pattern on middle leaves, veinclearing and mottling on upper leaves followed in general by absence of symptoms in later leaves. No enations. *N. rustica* (L.), *N. sylvestris* (Speg. & Comes)—necrotic spots. *N. glutinosa* (L.)—chlorotic ring spots on inoculated leaves, yellow mosaic on upper leaves. *Petunia hybrida* (Hort.) cv. Rose du Ciel—typical necrotic ring spots, veinclearing, and leaf curling.

The yield of purified virus was about 100 mg/kg of fresh leaves. The Schlieren pattern shows the proportion of the components (Fig. 1B). Sucrose density-gradient fractionation yielded three peaks. Electron microscopy revealed that each peak contained predominantly one class of particles, corresponding to bottom, middle, and top b components. The sizes and the shapes were those of classical AlMV components, ranging from 18 to 60 nm measured on 2% uranyl acetate-stained virus preparations.

Antisera with titers of 1/128 for AlMV-B, 1/128 for AlMV-425, and 1/64 for AlMV-S were obtained. AlMV strains B, 425, S, Caldy, YSMV, and 15/64 were compared in crude sap from *C. quinoa* and *N. tabacum* in agar gel double-diffusion tests; no spur formation was detected in any of the possible combinations, suggesting close serological relationships among all the strains tested.

Purified AlMV-B contained four RNA species (RNA 1, 2, 3, and 4), which comigrated with the four major RNA species of AlMV strains 425 and S. There was no minor RNA species corresponding to RNA 3' of AlMV-S (Fig. 2A). Analysis of the 5'-end structure of the four RNAs indicated an extensive homology with AlMV-425 RNAs in the leader sequence except for the first 21 nucleotides of RNA 3. These sequence data have been reported separately (10).

The properties of AlMV-B described in this paper are typical for strains of AlMV. The symptoms induced by AlMV-B were more severe than those described by Schmelzer (11) on *Chenopodium murale, Cucumis sativus, N. tabacum*, and *P. hybrida*.

The relative proportion of the nucleoprotein components is characterized by the predominance of the bottom and top b components; the top a component was a minor one in this isolate (Fig. 1B). The distribution of the four RNA species is in agreement with the relative amounts of the nucleoprotein components.

The stained gels and the corresponding densitometric scans shown in Figure 2A illustrate the RNA species distribution in total RNA preparations from strains S and B. The two strains differ greatly in the ratio of RNA 1 and 3 or 3+3'; a ratio of 0.2 was calculated for strain S and 0.8 for strain B.

The translation of AlMV RNAs in a wheat germ system yields translation products with very similar mobilities for strains S and B (Fig. 2B) except for a minor protein (P'3), a P3-related protein that is still under investigation.

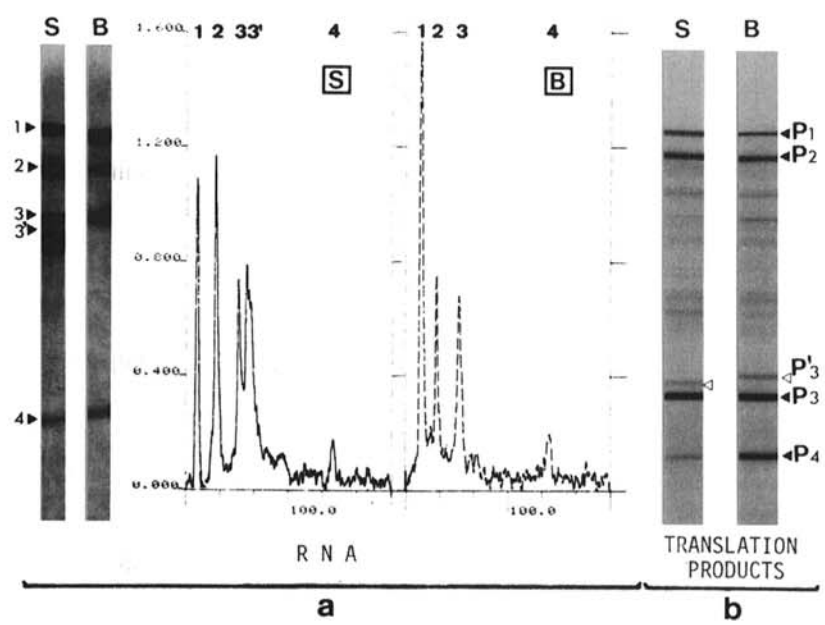

Fig. 2. (A) Polyacrylamide-agarose gel electrophoretic analysis and corresponding densitometric scans of unfractionated alfalfa mosaic virus (AlMV) strain S RNA (line and panel S) and AlMV-B RNA (line and panel B). The composite 0.5% agarose-2.4% acrylamide slab gel was run for 3 hr at 120V. The RNAs stained with O-toluidine are indicated by numbers 1–4. **(B)** Translation products obtained from total AlMV RNAs from strains S (line S) and B (line B) in wheat germ extracts. The major translation products corresponding to RNAs 1, 2, 3, and 4 are indicated by P1, P2, P3, and P4, respectively.

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