Gel Electrophoresis of Virus-Associated Polypeptides for Detecting Viruses in Bulbous Irises

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ABSTRACT

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Gel electrophoresis of virus-associated polypeptides enabled the detection and differentiation of three potyviruses, including iris mild mosaic virus (IMMV), iris severe mosaic virus (ISMV), and bean yellow mosaic virus (BYMV), and a carlavirus, narcissus latent virus (NLV), in leaf extracts from bulbous irises. In addition to the potyvirus coat protein, specific bands identical to those obtained from purified cylindrical inclusions were revealed. For NLV, a specific band was detected in addition to that of the virus coat protein. The method was employed to test about 100 plants and yielded reproducible results.

Additional key words: bulbous iris viruses, inclusion body proteins.

Four viruses have so far been identified in bulbous irises grown in Israel: iris mild mosaic virus (IMMV), iris severe mosaic virus (ISMV), bean yellow mosaic virus (BYMV) (1,9), and narcissus latent virus (NLV) (*unpublished*). All plants of commercial cultivars carry IMMV. Therefore, the other viruses occur always in combination with IMMV, and individual plants may carry three or four of these viruses. Infection by IMMV generally results in mild mosaic symptoms, while ISMV and BYMV cause severe yellow mosaic symptoms that are not specific to the infecting viruses. Infection by NLV did not produce symptoms under our conditions. Symptoms often appear only the following year after inoculation.

For propagation of iris plants free of ISMV, BYMV, and NLV, and especially for the future buildup of stocks also free of IMMV, it is essential to have a reliable diagnostic method that distinguishes between IMMV and ISMV, in particular. Both viruses (belonging to the potyvirus group) have flexuous particles, and are similar in length, ~750 nm (2,3). Inoculation of test plants also does not allow a clear distinction. In our experiments (*unpublished*), inoculation of plants with leaf extracts from plants infected only by IMMV, (without ISMV), to *Chenopodium quinoa* Willd., always resulted in chlorotic local lesions. According to Brunt and Phillips (3), ISMV but not IMMV produces chlorotic local lesions on *C. quinoa*. Comparing antisera against ISMV prepared in different laboratories also gave contradictory results.

As these viruses induce inclusion proteins in the plant, we examine the possibility of identifying them by electrophoretic separation of these proteins from leaf extracts. The method reported here enabled reliable identification of these viruses, after partial purification and electrophoretic separation of virus-associated proteins.

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MATERIALS AND METHODS

Plants. Infected iris plants were grown from bulbs in small plots at Bet Dagan. BYMV and NLV were identified by immuno-electron microscopy (IEM), while ISMV and IMMV were determined mainly by symptoms. Healthy plants were grown from seed and kept in a screened greenhouse.

Gladiolus plants (cultivar Eurovision, commercial stock), infected with BYMV only, as determined by ELISA, and healthy plants (from tissue culture progeny) were grown in greenhouses at Bet Dagan.

Preparation of protein extract. Five grams of infected leaves were homogenized in 40 ml of a solution containing 20 ml of 0.1 M phosphate buffer, pH 8.2, 0.1% 2-mercaptoethanol, and 20 ml of chloroform. The mixture was centrifuged at 2,000 *g* for 10 min. Polyethylene glycol (4% w/v; M.W. 6000) (PEG) and 0.2 M NaCl

were added to the aqueous phase and centrifuged after 1 hr at 12,000 *g* for 15 min. The pellet was resuspended in the phosphate buffer and brought to one-tenth of the initial volume. After additional precipitation with PEG and NaCl and low-speed centrifugation, the resulting pellet was resuspended in 0.2–0.5 ml of phosphate buffer and centrifuged at 2,000 *g* for 10 min. The supernatant was used for further analysis by gel electrophoresis. All procedures were conducted in the cold.

Extracts from healthy control plants were prepared similarly, using leaves from seed-grown irises.

Electrophoresis in polyacrylamide gel. To each volume of partially purified extract was added one-half volume of 0.06 M tris-HCl buffer, pH 6.8, containing 3% sodium lauryl sulfate (SDS), 5% 2-mercaptoethanol, 10% glycerol, and a few grains of bromophenol blue (disruption buffer). The mixture was boiled for 2 min and kept frozen at −20 C until use. Samples of 50–150 μl each were applied to a 1.5-mm-thick and 140-mm-long 5–15% polyacrylamide gradient gel with 10 wells of 10 mm each, containing 0.1% SDS, and separated by electrophoresis for 16 hr at 100 V in 0.025 M tris, 0.19 M glycine, 0.1% SDS buffer, pH 8.8, in a Hoefer SE600 refrigerated instrument with temperature maintained at 0 C (8,12). Gels were stained for 2 hr with 0.2% Coomassie brilliant blue (PAGE blue 83, BDH) in 50% methanol and 45% acetic acid, and rinsed in a solution containing 25% methanol and 7% acetic acid.

The following marker proteins (Pharmacia, numbers are daltons) were used: lysozyme (14,300), trypsin inhibitor (21,000), carbonic anhydrase (31,000), ovalbumin (44,000), albumin (68,000), and phosphorylase b (94,000). Potato virus Y coat protein was used as an additional marker in a separate lane.

Serology. The decoration method of IEM was performed with BYMV and NLV antisera obtained from Renate Koenig (Braunschweig, W. Germany), diluted 1:10, and the preparations were negatively stained with 2% aqueous uranyl acetate (10).

Purification of inclusion bodies. Cytoplasmic inclusion bodies from IMMV- and ISMV-infected iris plants and from BYMV-infected gladioli were prepared according to Hiebert and McDonald (6).

RESULTS AND DISCUSSION

Electrophoretic separations of polypeptides from IMMV-, ISMV-, BYMV-, and NLV-infected plants were compared with extracts from healthy plants, PYV coat protein, and molecular weight marker proteins (Fig. 1). The lower light bands represent viral coat protein. IMMV, ISMV, NLV, and PVY coat proteins were in the molecular range of 33,000 ± 500 daltons, while BYMV coat protein had an estimated size of 34,000 ± 500 daltons.

All infected plants had a slow-moving band with an estimated molecular weight of 69,000 daltons, similar to earlier published information (6). This band was not detectable in extracts from healthy plants. In extracts from IMMV-infected plants, only this band was apparent in addition to the coat protein band. Extracts from ISMV-, BYMV-, and NLV-infected plants had, in addition to the 69,000-dalton band, bands at 71,000, 73,000, and 44,000 daltons, respectively. Molecular weight was determined from at least three independent experiments. None of these bands was found in extracts from healthy plants. As all iris plants carry IMMV in addition to one of the other viruses, it seems that the 69,000-dalton band is associated with IMMV infection. Electrophoresis of protein extracts from *C. quinoa* inoculated with IMMV revealed the same 69,000-dalton band, in addition to viral coat protein. Electrophoresis of extracts from healthy *C. quinoa* did not show any of these bands.

IMMV, ISMV, and BYMV are potyviruses, with which the infection is accompanied by cytoplasmic cylindrical inclusions in the host cell (5). These structures are mostly composed of protein (13) that is virus-coded (4,7,11). To see if the slow-moving bands in our electrophoretograms were due to inclusion body protein, purified cytoplasmic inclusion bodies from IMMV, ISMV, and BYMV were subjected to gel electrophoresis. Cytoplasmic inclusion bodies were purified according to Hiebert and McDonald (6) with omission of the sucrose gradient step. Gel electrophoresis of these preparations, after disruption, revealed one band at 69,000 or two bands, one at 69,000 and the other at 71,000 daltons, for IMMV or ISMV preparations, respectively, with very little contamination of plant proteins and no viral coat protein. When extracts from BYMV-infected gladioli were analyzed (gladioli were

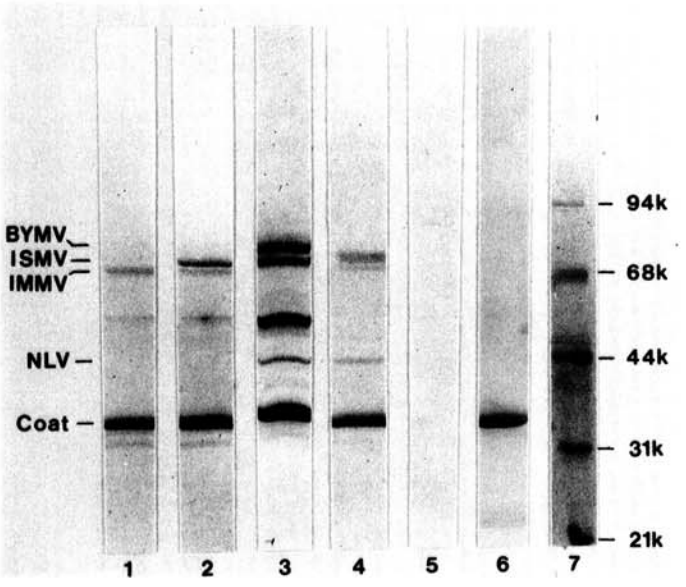

Fig. 1. Coomassie brilliant blue-stained polypeptides from iris extracts separated by electrophoresis on 5–15% gradient polyacrylamide gel containing 0.1% SDS: lane 1, coat and high-molecular-weight polypeptide from IMMV; lane 2, IMMV and ISMV; lane 3, IMMV, ISMV, BYMV, and NLV; lane 4, IMMV, ISMV, and NLV; lane 5, healthy plant; lane 6, coat protein of purified PVY; and lane 7, markers.

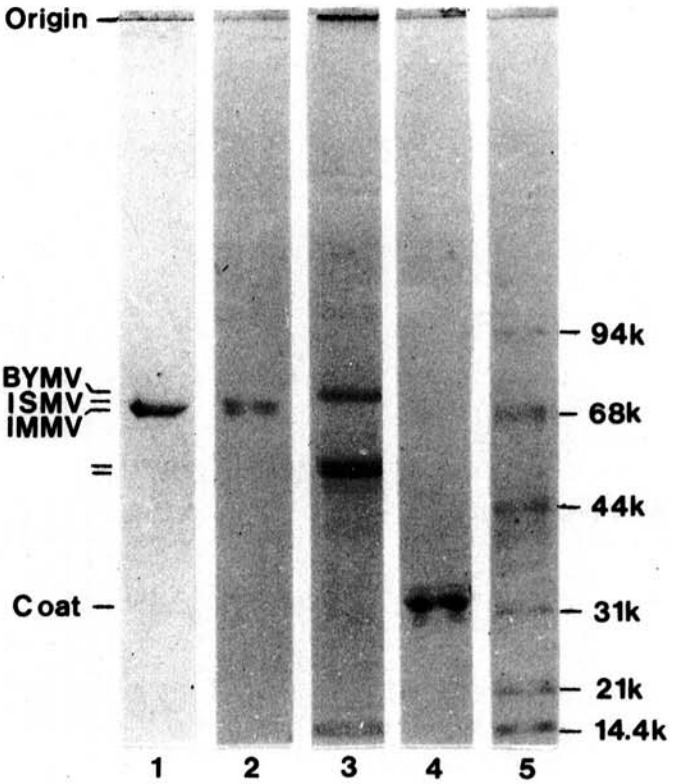

Fig. 2. Separation by SDS polyacrylamide gel electrophoresis of polypeptides from fractionated cylindrical inclusions: lane 1, extract from iris plant infected with IMMV; lane 2, IMMV and ISMV; lane 3, extract from gladiolus infected with BYMV; lane 4, IMMV coat protein; and lane 5, markers. The two unlabeled bars mark unidentified polypeptide bands.

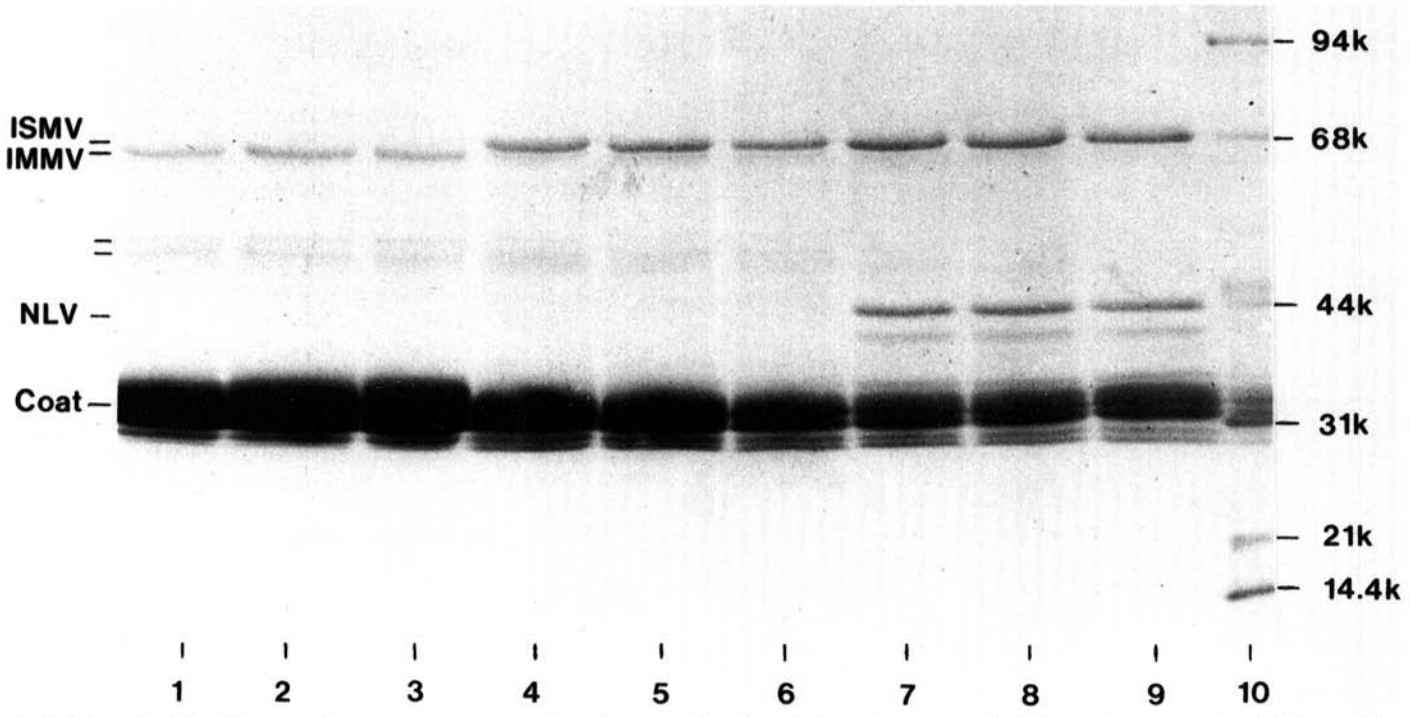

Fig. 3. Polyacrylamide gel showing the electrophoretic separation of polypeptides of a typical experiment: samples 1–3 were from an iris plant lacking typical symptoms, and samples 4–9 were from plants exhibiting ISMV symptoms. The presence of NLV in samples 7–9 was confirmed by serological reaction with NLV antibodies. The reproducibility of polypeptide separation is shown in this photograph, where the presence of NLV-specific protein does not interfere with the IMMV and ISMV separation. The two unlabeled bars mark unidentified polypeptide bands.

used because these plants were infected with BYMV only, while the iris plants that were infected with BYMV contained also IMMV and ISMV) a band of 73,000 daltons was revealed (Fig. 2). It seems, therefore, that the slow-moving bands are those of the potyvirus-associated cytoplasmic cylindrical inclusion proteins.

Additional polypeptide bands at 50–55,000 daltons did appear in the electrophoretograms for BYMV-infected gladioli (Fig. 2), but not in healthy plants. The possibility that these bands are the helper factor proteins was examined (14). Partially purified supernatant was centrifuged at 100,000 *g* for 1.5 hr. These polypeptides sedimented with the cytoplasmic inclusion bodies and did not remain in the supernatant as the helper factor protein. The origin of these polypeptides could be either from crystalline nuclear inclusions or cytoplasmic crystalline inclusions both of which BYMV is known to form (5). This possibility was not examined, since differentiation between the viruses in question could be accomplished by the separation of the cytoplasmic inclusion polypeptides.

Within 2 yr, extracts from 109 iris plants were tested by gel electrophoresis. Seventy plants indexed positively for IMMV only, while 14 were infected with both IMMV and ISMV. Eighteen plants reacted positively for NLV in addition to other viruses; and seven were positive for IMMV, ISMV, and BYMV. Repeated sampling of individual plants always gave similar protein bands.

It was often noted that with the advance of the growing period, the intensity of the inclusion protein band decreased relative to the virus coat protein band. Thus, it may be of advantage to select samples for gel electrophoresis when plants are young (Fig. 3).

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