Evaluation of Heat Therapy of Poinsettia Mosaic and Characterization of the Viral Components

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Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison. We are grateful for the support and cooperation of Paul Ecke Poinsettias. We thank Steven A. Vicen for preparing graphs.
Accepted for publication 16 June 1981.

ABSTRACT

Pfannenstiel, M. A., Mintz, K. P., and Fulton, R. W. 1982. Evaluation of heat therapy of poinsettia mosaic and characterization of the viral components. Phytopathology 72:252-254.

Poinsettia mosaic virus (PMV) was not transmitted to poinsettias by standard commercial propagating practices. Foliage or root contact over periods of several months also did not transmit PMV. Growing infected poinsettias at 32 C for 9 wk or more resulted in virus-free shoots, which were propagated by grafting to virus-free rootstocks. The protein subunit of PMV had a molecular weight of 21,700 daltons and there was a single species of RNA with a molecular weight of 2.0×10^6 daltons.

Additional key words: tymovirus, ribonucleic acid.

Poinsettia mosaic virus (PMV) was first described by Fulton and Fulton (5), who suggested that it be considered a member of the tymovirus group. It is commonly found in commercial poinsettia (*Euphorbia pulcherrima* Wild.) in the United States and has been reported in West Germany (7). Symptoms in poinsettia may be inapparent or consist of mild mottling. Although *Euphorbia* spp. are not highly susceptible to experimental mechanical inoculation (5), some poinsettia cultivars are completely infected, probably the result of vegetative propagation from symptomless plants. Poinsettias are commercially propagated by rooting 6–7 cm stem tips from stock plants. Because successive crops of stem tips are removed by hand, virus might be manually transferred among stock plants. It seemed important to determine if such transmission occurred.

This paper describes attempts to determine whether commercial propagation practices result in mechanical transmission of PMV from plant to plant. A method for producing virus-free plants by heat therapy is described. Also, we present additional data characterizing the virus.

MATERIALS AND METHODS

Poinsettias were propagated by applying rooting hormone to excised stem tips 6–8 cm long and rooting them in urea formaldehyde foam cubes embedded in damp sphagnum moss. Rooted poinsettias and seedlings of *E. cyathophora* were grown in a composted soil-sand-peat moss mixture in a greenhouse kept at about 24 C.

Plants were inoculated by rubbing corundum-dusted leaves with extracts of young infected *E. cyathophora* leaves prepared in 0.03 M sodium phosphate buffer, pH 8.

The ELISA we used to test for the presence of PMV in plant extracts was the double antibody sandwich method as described by Clark and Adams (3). Tests always included both positive and negative controls.

Protein of PMV was prepared by heating virions at 37 C for 2 hr in 1% sodium dodecyl sulphate (SDS), 1% 2-mercaptoethanol and 6M urea in neutral sodium phosphate buffer. The dissociated virus was then electrophoresed in 12.5% polyacrylamide running gel (pH 8.8) with a 3% stacking gel (pH 6.9) (4,15). Electrophoresis buffer contained 0.192 M glycine, 0.025 M Tris, 0.1% SDS, pH 8.3 (8). Electrophoresis was for 5 hr in cylindrical 0.6×9-cm gels at 8 mA per gel. After electrophoresis, gels were stained overnight with 0.25% Coomassie Brilliant Blue and destained in 50% methanol, 7.5% acetic acid. The molecular weight of the protein was determined by the least squares method with lysozyme, β-lactoglobulin, trypsinogen, pepsin, ovalbumin, and bovine albumin (Sigma Chemical Co., St. Louis, MO 63178) as internal standards.

For amino acid analysis, protein prepared from PMV bottom particles (PMV-B) by phenol extraction (14) was precipitated from the phenol phase with six volumes of ethanol. The precipitate was dissolved in 67% acetic acid and dialyzed for 48 hr against 0.1% 2-mercaptoethanol. The protein was hydrolyzed at 110 C for 20, 47, and 78 hr in 6 N HCl containing 0.2% phenol (10). Amino acids were determined in a Durrum D-500 amino acid analyzer. Loss of serine was corrected for by extrapolating to zero time.

Viral nucleic acid was extracted as described by Brakke and Van Pelt (2) and precipitated with ethanol. Electrophoresis of nucleic acid (1) was done in 2.5% polyacrylamide, 6 M urea cylindrical gels (0.6×9-cm) at 6 mA per gel for 5 hr. The electrophoresis buffer was 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA, pH 7.2. After electrophoresis, gels were stained with 0.1% Toluidine Blue O and destained with 1% acetic acid in 40% methanol. Nucleic acids similarly extracted from alfalfa mosaic and turnip yellow mosaic viruses served as molecular weight standards.

RESULTS

In an effort to duplicate commercial practice, stem tips were snapped by hand alternately from PMV-infected and healthy poinsettias. After 8 wk, healthy plants were tested for virus by enzyme-linked immunosorbent assay. The 8-wk period was judged sufficient because symptoms in poinsettias inoculated by leaf rubbing became detectable in 4 wk or less. None of 25 healthy poinsettias became infected by the handling involved in removing the tops.

It also seemed possible that PMV might be transmitted by foliage contact between infected and healthy plants. To test this, *E. cyathophora* seedlings were grown in rows of plastic containers, one plant per container. This species develops conspicuous mosaic in 10–12 days after infection. One row of plants was mechanically inoculated and the adjacent row remained uninoculated. As the plants grew, foliage of one row came into contact with that of the other. None of the uninoculated plants developed symptoms, indicating that no infection had occurred by leaf contact.

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0031-949X/82/02025203/$03.00/0

In another experiment, foliage of PMV-infected poinsettias was allowed to mingle with foliage of healthy poinsettias for 4–5 mo. No virus was detectable by ELISA in eight plants thus exposed.

To test the possibility of virus transmission by root contact, *E. cyathophora* seedlings were grown in pairs in 10.2-cm (4-inch) plastic containers. Foliage of each pair member was shielded from its neighbor by a cellophane sheet. One plant of each pair was inoculated with PMV. Plants were cut back several times on the assumption that new growth might induce movement of virus from the roots to apical meristems along with food materials. None of 24 uninoculated plants developed symptoms in the more than 2 mo the experiment was continued.

In another experiment, five *E. cyathophora* seeds were sown in each of six pots from which commercially propagated, PMV-infected poinsettias had been removed. None of the resulting seedlings became infected.

These trials indicated that virus transmission from plant to plant during normal propagation was unlikely. Selected stocks could probably be maintained virus-free without difficulty. This is consistent with previous observations (5) that mechanical transmission was never obtained to all the plants inoculated even with what were considered the most efficient techniques.

Production of PMV-free poinsettias. When the efficiency of ELISA for detecting PMV was demonstrated (5), attempts were made to select virus-free poinsettias of as many cultivars as possible. One or more virus-free plants were found of cultivars Dark Hegg, Jingle Bells, Rochford, and V-10. For certain other cultivars we could find no plants that tested virus-free. We then tried heat treatments in an attempt to produce virus-free plants.

Young PMV-infected poinsettias were kept at 36 ± 1 C, with 12-hr days for various periods in growth chambers controlled to within one degree. Stem tips 2–3 cm long were removed after 2–3 wk and grafted on the tops of poinsettias known to be virus-free. At 36 C, poinsettias deteriorated within a few weeks. Most scions from these plants failed to heal when grafted and they died. The one stem tip that did grow was still infected.

Treatment temperature was then lowered to 32 C. At 32 C, poinsettias grew rapidly, producing elongated, somewhat etiolated stem tips. After 4–6 wk at 32 C, two of eight excised stem tips were virus-free. After 9–10 wk at 32 C, seven of seven excised stem tips were virus-free. The 2–3 cm of stem of these plants that was grafted was growth that had occurred at 32 C. Repeated testing of these scions and plants propagated from them showed them consistently virus-free.

Stock plants that had been 10 wk at 32 C and had supplied virus-free scions were transferred to a normal greenhouse environment, in which they resumed normal growth. After several weeks, ELISA showed they were infected. Evidently virus multiplication and movement were inhibited at 32 C and tissue produced at this temperature remained virus-free.

Further characterization of PMV. We could find no report of heat therapy of a tymovirus. Therefore, additional characterization of PMV was done to verify its identification as a tymovirus.

The protein. The molecular weight of the protein subunit, estimated by concurrent electrophoresis with standard protein markers was 21,700 daltons. This was the average from several separate extractions and electrophoresis runs (Fig. 1).

The values for amino acid composition of PMV (Table 1) were compared with the published values for 10 tymoviruses (12) by the chi square test. PMV is most similar to the two isolates of eggplant mosaic virus, although it is not serologically related to it or 11 other tymoviruses (5).

The nucleic acid. A single species of nucleic acid was isolated from unfractionated PMV and from PMV-B separated by density gradient centrifugation. When subjected to electrophoresis in 2.5% polyacrylamide gels with nucleic acids from turnip yellow mosaic (TYMV) and alfalfa mosaic viruses, PMV nucleic acid migrated at the same rate as TYMV nucleic acid (Fig. 2). A molecular weight of 2.0×10^6 daltons for PMV nucleic acid was determined after several runs, which is consistent with values obtained for other tymoviruses (6).

After PMV nucleic acid or yeast transfer RNA were incubated with 10 μg/ml ribonuclease (Mann Research Laboratories) for 30 min at room temperature, no fluorescent bands staining with ethidium bromide were seen after electrophoresis in polyacrylamide gels. Incubation with 10 μg/ml deoxyribonuclease (DNAse I, Sigma) with 0.03 N added $MgCl_2$ (9) for 30 min at room

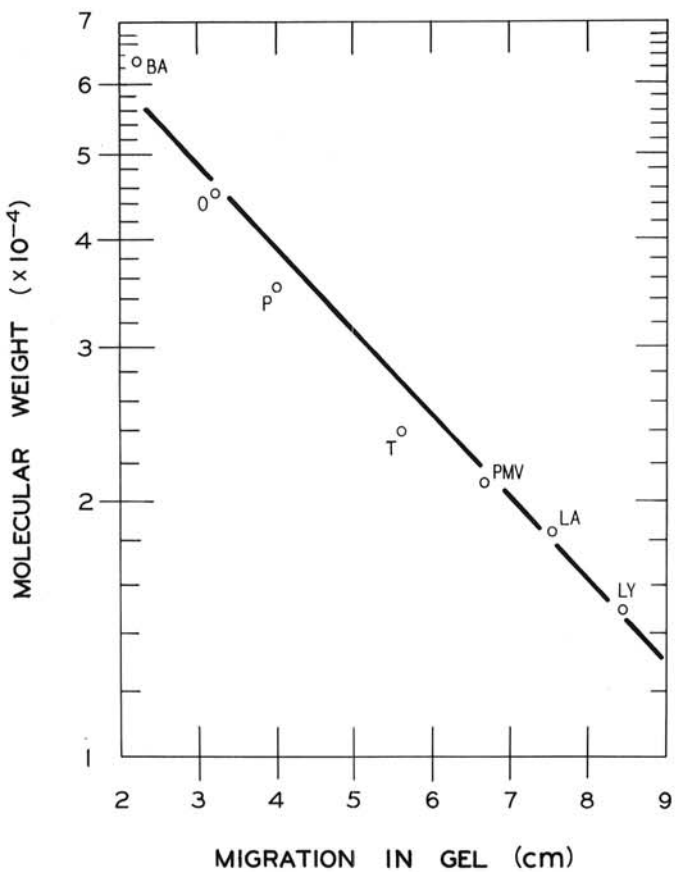

Fig. 1. Molecular weight vs electrophoretic migration in 12.5% polyacrylamide gels of bovine albumin (BA), ovalbumin (O), pepsin (P), trypsin (T), poinsettia mosaic virus capsid protein (PMV), β-lactoglobulin (LA), and lysozyme.

TABLE 1. Amino acid composition of poinsettia mosaic virus protein

Amino acid	Mol/100 mol of amino acid recovered after: 20 hr	47 hr	78 hr	Avg[a]
Asx	9.52	9.10	8.95	9.00
Thr	10.36	10.83	10.77	10.43
Ser	11.66	11.03	10.72	11.61
Glx	7.50	7.27	7.20	7.17
Pro	7.67	7.35	7.20	7.26
Gly	8.45	8.20	8.09	8.08
Ala	10.41	10.07	9.83	9.90
Cys	ND[b]	ND	ND	ND
Val	5.53	7.10	7.74	7.58
Met	0.97	0.55	0.52	0.65
Ile	2.83	3.27	3.64	3.57
Leu	10.97	11.03	11.08	10.81
Tyr	2.60	2.73	2.81	2.66
Phe	3.26	3.19	3.13	3.13
Lys	3.26	3.32	3.31	3.23
His	1.75	1.75	1.80	1.73
Arg	3.26	3.30	3.21	3.19
Trp	ND	ND	ND	ND

[a] Correction for loss of serine was obtained by extrapolation of 20, 47, and 78 hr values to zero time. Values for isoleucine and valine were not maximal until 78 hr of hydrolysis. The remaining values were averages from each hydrolysis period.

[b] ND, not done.

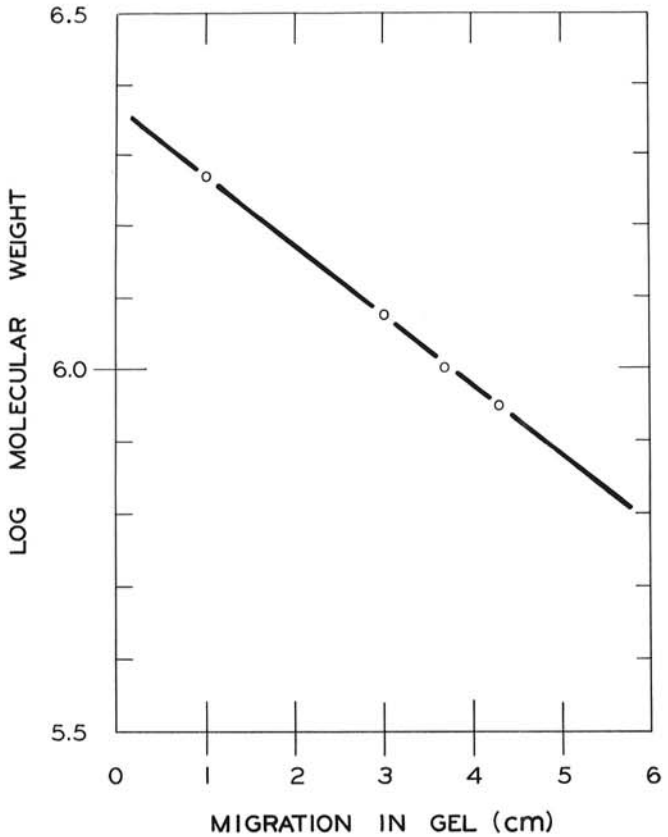

Fig. 2. Log molecular weight vs electrophoretic migration in 2.5% polyacrylamide, 6 M urea gels of turnip yellow mosaic virus, poinsettia mosaic virus (upper left point), and alfalfa mosaic virus RNA's (three lower points) with molecular weights of 1.3, 1.1, and 0.9×10^6 daltons.

temperature did not affect the occurrence of a fluorescing nucleic acid band upon electrophoresis. We conclude that PMV contains RNA.

No measurable residual RNA was isolated from PMV-T. Since top and bottom particles have the same shape, percent RNA was estimated by the method of Reichmann (13) with the equation: $(X = (S_f/S_e - 1)/(S_f/S_e + 1)$, in which S_f = sedimentation coefficient of PMV-B and S_e = sedimentation coefficient of PMV-T. With S_f = 112 and S_e = 50 (3) the calculated RNA content of PMV is approximately 38%.

When top and bottom components were separated, the A_{260}/A_{280} nm ratio for top was 1.10 and for bottom was 1.78 (an average of four preparations). With Paul's (11) equation: absorbance ratio A_{260}/A_{280} nm = 0.9320 + 0.0454 (% RNA) − 0.006 (% RNA)^2, the RNA contents of PMV-T and PMV-B were calculated as 4 and 34%, respectively. A discrete RNA species was not detected in PMV-T by gel electrophoresis, but fibrillar material was seen in electron micrographs of top particles (5). This was not apparent after PMV-T was incubated with ribonuclease. Presumably the fibrillar material was nucleic acid adhering to top particles and is reflected in the value of 4% RNA in PMV-T found with Paul's equation. The value of 34% for the percentage of RNA for PMV-B is close to the 35%, which is characteristic of the tymovirus group (6).

DISCUSSION

Difficulty in getting 100% transmission of PMV with the mechanical inoculation methods that are effective for many other viruses (5) suggested that maintaining stocks of poinsettia free from virus might be relatively easy. This was confirmed by experiments that failed to demonstrate infection resulting from normal propagation practices. This contrasts with the apparently high incidence of infection in many poinsettia cultivars. Possibly vectors of the virus not previously (5) tested are present and effective in some situations.

The production of virus-free stem tips of poinsettia at 32 C provides a method for obtaining uninfected plants of cultivars that appear to be completely infected with PMV. This temperature apparently restricts virus multiplication and movement so that when shoot tips elongate sufficiently at 32 C to provide a graftable stem, it is virus-free. Failure to obtain virus-free plants at 36 C may have been due to the reduced amount of new growth at this temperature. The rooting of stem tips was not attempted because they were distinctly etiolated. Even when grafted to healthy poinsettias at 24 C, excised shoot tips required 3–4 wk to begin normal growth.

The size of protein subunits and nucleic acid and their proportion confirm earlier suggestions that PMV be considered a tymovirus.

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