Electrophoretic Variability Among Dianthoviruses

H. R. Pappu and C. Hiruki

Graduate research assistant and professor, Department of Plant Science, University of Alberta, Edmonton, Alberta T6G 2P5, Canada.
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ABSTRACT

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Seven distinct members of the dianthovirus group were purified and their mobilities analyzed by virion electrophoresis. Each virus or strain possessed a single electrophoretic form as determined by differential staining of encapsidated RNA and coat protein by ethidium bromide and Coomassie Brilliant Blue, respectively. Two strains of sweet clover necrotic mosaic virus (SCNMV-38 and SCNMV-59) were electrophoretically distinct. Carnation ringspot virus (CRSV) strain A comigrated with SCNMV-38, whereas CRSV-N had the slowest mobility of all the seven strains tested. Among the three red clover necrotic mosaic virus (RCNMV) strains used in this study, RCNMV-TpM 34 and RCNMV-TpM 48 had similar mobilities, whereas RCNMV-Aus was faster. The isoelectric points of all the strains were in the pH range 4.75–5.1 except that of CRSV-N, which was between 6.0–6.2. The single coat protein of all the dianthoviruses tested was estimated to have a molecular mass of about 39,000 daltons.

Additional keywords: agarose gel electrophoresis, legume viruses, strain relationships.

The dianthovirus group consists of carnation ringspot virus (CRSV), the type member, red clover necrotic mosaic virus (RCNMV), and sweet clover necrotic mosaic virus (SCNMV), and they share the following general properties: two species of single-stranded RNA molecules encapsidated in isometric particles measuring about 30–35 nm in diameter, with a single coat protein of about 39,000 daltons (8,16). Several strains of each virus have been characterized based on biological and serological properties. Serological relationships among the members and the strains revealed varying degrees of relationships as assessed by monoclonal (9,11) and polyclonal antibodies (7,11,18–20,23,26). In a previous paper, we reported the electrophoretic variability between virions of two strains of SCNMV (23). We extended this observation to several other strains of RCNMV and CRSV, and in this communication we describe the distinct differences in virion electrophoretic mobilities among dianthoviruses as determined by agarose gel electrophoresis. Preliminary results have appeared in an abstract form elsewhere (22).

MATERIALS AND METHODS

Viruses. The following virus strains were used in this study: carnation ringspot virus (CRSV), strains A and N (31); sweet clover necrotic mosaic virus (SCNMV), strains 38 and 59 (23); and red clover necrotic mosaic virus (RCNMV), strains TpM 34, TpM 48 (18), and Aus (7). Each virus was multiplied separately on *Phaseolus vulgaris* 'Red Kidney' and purified according to a previously published procedure (7). Final virus preparations were suspended in 50% glycerol and stored at −20 C.

Coat protein analysis. Purified virus preparations were dissociated at 100 C in the presence of sodium dodecyl sulfate (SDS) and 2-mercaptoethanol and analyzed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) by using the discontinuous buffer system of Laemmli (14). Bands were located by staining with Coomassie Brilliant Blue R250 and destaining overnight in a destaining solution (methanol:acetic acid:water, 10:10:80).

Isoelectric focusing. Isoelectric focusing of purified virus preparations was done as previously described (23). Each virus preparation (2–5 μg) was subjected to focusing in 1% agarose gel (Isogel

agarose, FMC Corporation) containing 10% glycerol and 2% Bio-Lyte ampholytes with pH ranges of 3–10 or 5–7 (Bio-Rad Laboratories, Richmond, CA) using Bio-Rad's Mini IEF cell (model 111). Focusing and detection of proteins were done according to the protocol supplied by the manufacturer. Focusing was done under constant voltage conditions, with voltage being gradually increased, initially at 100 V for 15 min, 200 V for 15 min followed by 450 V for 1 hr. After focusing was complete, the gel was fixed for 15 min in a fixative solution (30% methanol, 5% trichloroacetic acid, and 3.5% sulfosalicylic acid) followed by a brief rinse in 95% ethanol. The gel was stained for 30 min with 0.2% Coomassie Brilliant Blue R250 made in 28% ethanol and 14% acetic acid followed by destaining in 28% ethanol and 14% acetic acid. The isoelectric point of each virus, calculated from an average of three separate experiments, was determined by a regression analysis of the pH gradient of the gel as previously described (24).

Agarose gel electrophoresis of virions. Nondenaturing agarose gel electrophoresis of purified virus preparations was done as previously described (23). Agarose (Seakem LE, FMC Corporation) gels (0.8%) were cast in electrophoresis buffer (10 mM Na_2HPO_4, NaH_2PO_4 buffer, pH 7.0). Samples were prepared by mixing 5 μg of virus and 1 μl of tracking dye (0.05% Bromophenol blue and 40% sucrose in electrophoresis buffer) and were electrophoresed at a constant voltage of 3 V/cm at 4 C with buffer recirculation (100 ml/min) to avoid the development of a pH gradient during electrophoresis. After electrophoresis, the gel was stained with ethidium bromide (0.5 μg/ml) in the presence of 1 mM disodium ethylenediaminetetraacetic acid to locate the encapsidated RNA. After a brief rinse with distilled water, the same gel was restained with Coomassie Brilliant Blue R250 and destained overnight in destaining solution to locate the coat protein. Gels were photographed using Polaroid instant film (Type 57) using a red filter (for ethidium bromide-stained gels) or an orange filter (for Coomassie Blue-stained gels).

RESULTS

Coat protein profiles. A single polypeptide species of about 39,000 daltons was detected for each virus (Fig. 1). No significant mobility differences could be seen among the viral proteins.

Isoelectric focusing. The isoelectric points of SCNMV-38, SCNMV-59, CRSV-A, RCNMV-TpM 34, RCNMV-TpM 48, and RCNMV-Aus were in the pH range of 4.75–5.1 (average of three experiments), whereas that of CRSV-N was between 6.0 and 6.2 as determined by the regression analyses of the pH gradients of 3 and 10 or 5 and 7 following focusing (Fig. 2A and B). Each virus preparation showed one major polypeptide band and occasionally one minor band (Fig. 2B, lane 2). The differences in the focusing pattern were consistent among the strains and the most discernible differences were found between CRSV strains, A and N and, RCNMV strains, TpM 48 and Aus (Fig. 2A and B).

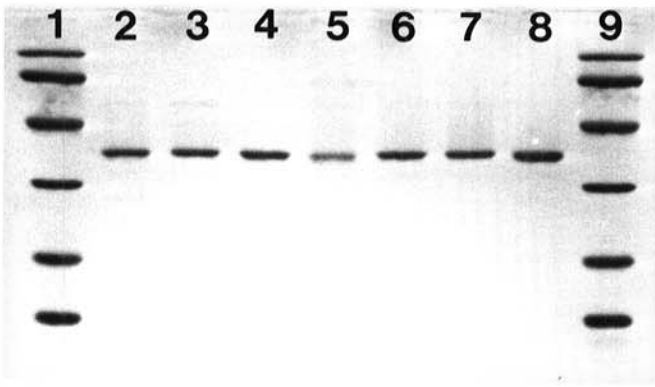

Fig. 1. SDS-PAGE (12.5%) of dissociated coat proteins of dianthoviruses. Lanes 1 and 9, Protein molecular weight standards: (from top to bottom) phosphorylase b (97.4 kDa), bovine serum albumin (66.2 kDa), ovalbumin (42.6 kDa), carbonic anhydrase (31 kDa), soybean trypsin inhibitor (21.5 kDa) and lysozyme (14.4 kDa). Lane 2, sweet clover necrotic mosaic virus (SCNMV)-38; Lane 3, SCNMV-59; Lane 4, carnation ringspot virus (CRSV)-A; Lane 5, CRSV-N; Lane 6, red clover necrotic mosaic virus (RCNMV)-TpM 34; Lane 7, RCNMV-TpM 48; Lane 8, RCNMV-Aus. Gel stained with Coomassie Brilliant Blue and destained overnight. Migration was from top (negative) to bottom (positive).

Agarose gel electrophoresis. When purified virus preparations were electrophoresed in agarose gels under nondenaturing conditions, a single electrophoretic form was detected. The migrating component is presumed to be a virion based on the observation that it was stained by ethidium bromide (viral RNA) and Coomassie Brilliant Blue (viral protein). Significant mobility differences were noticed among the strains of all three viruses (Fig. 3A and B). All virions migrated from cathode to anode indicating a net negative charge for the virions under the electrophoretic conditions used. SCNMV-38 and SCNMV-59 were electrophoretically distinct as reported earlier (23) and CRSV-A showed similar mobility to that of SCNMV-38. CRSV-N had the slowest mobility of the strains tested in this study. RCNMV-TpM 34 and RCNMV-TpM 48 migrated identically, whereas RCNMV-Aus had a greater mobility and was similar to those of SCNMV-38 and CRSV-A (Fig. 3A and B). The electrophoretic profile of each strain and the relative mobility differences among all the strains were consistent and highly reproducible when the experiment was run several times during a period of 1 yr.

To confirm whether the mobility pattern displayed by each strain is a characteristic feature, the electrophoretically distinct strains were mixed in various combinations and then subjected to nondenaturing agarose gel electrophoresis. After differential staining as described above, each individual virus preparation in a mixture retained its characteristic mobility pattern and migrated as a single but distinct electrophoretic form (Fig. 4A and B).

DISCUSSION

Results of the physical characterization of dianthoviruses by agarose gel electrophoresis and isoelectric focusing provide evidence for differences in electrophoretic mobilities among their various strains. Similar results have been obtained for other groups of plant viruses (2–4,15,27). The taxonomic grouping of the dianthoviruses is based mainly on their serological relationships, and the differences in electrophoretic mobility support this taxonomy to some extent. The two electrophoretically distinct strains of SCNMV were also serologically distinguishable by immunodiffusion tests (23). Similarly, two closely related strains of CRSV, CRSV-A, and CRSV-N, which showed striking mobility differences in this study, were serologically distinguishable (31).

The reason for conflicting data on RCNMV-TpM 34 and TpM 48 is unknown. Previous reports stated that RCNMV-TpM 34 and TpM 48 were serologically and electrophoretically distinct (5,6,18,19); however, we found that the migration patterns of these two strains were similar, and consistent and reproducible among different batches of virus preparations that were made over a period of 1 yr. Also, in our study, the isoelectric points of RCNMV-TpM 34 and TpM 48 were very similar. A possible explanation for this apparent discrepancy might be that one or both of the strains used in this study could be mutants of the RCNMV strains originally described (17), since the symptomatology produced by our strains on selected host plants was different from that of the original strains (25). This discrepancy must be resolved by further investigation by using the original strains since RCNMV-TpM 34 and TpM 48 are grouped into two different serotypes (serotype A and serotype B respectively). RCNMV-Aus, belonging to serotype D, has a different mobility pattern and a significantly different isoelectric point from those of RCNMV-TpM 34 and TpM 48.

There are several possible reasons for the origin of charge heterogeneity. The host passage effect may have occurred, leading

to an adaptive mutation in a particular virus strain (32). In the case of SCNMV, the type strain (SCNMV-38) was originally isolated from sweet clover (10), whereas its new serotype was isolated from alfalfa (23). Of the two CRSV strains, CRSV-A forms stable 12-particle aggregates at acidic pH, whereas CRSV-N is a nonaggregating strain. They are also serologically distinguishable as determined by reactions of partial identity in gel diffusion tests (31). The striking differences in virion mobilities and isoelectric points of CRSV-A and CRSV-N are probably caused by differences in the amino acid composition of their respective coat proteins (30). Mobility differences have been correlated with differences in amino acid composition for strains of hibiscus chlorotic ringspot virus (HCRV) (13), thus the observed differences among the three strains of RCNMV may be explained similarly. Because the three strains were originally reported from Czechoslovakia (RCNMV-TpM 34 and TpM 48) (17) and Australia (RCNMV-Aus) (7), this geographical isolation may have allowed for some genetic drift in their coat proteins.

Virion electrophoresis has been used to detect tobacco mosaic virus in crude plant extracts (1), to study its in vitro disassembly products (12), and to characterize variants of HCRV (13), and strains of panicum mosaic virus and their associated satellites (29). It has also been used extensively in characterizing bacteriophages (28). It is easy to perform, rapid and sensitive, since only small amounts, as low as 15–30 ng/ml, of virus preparations are required (21). Furthermore, it has potential application as an aid in distinguishing electrophoretically distinct strains of a virus in mixed infections that may occur under field conditions.

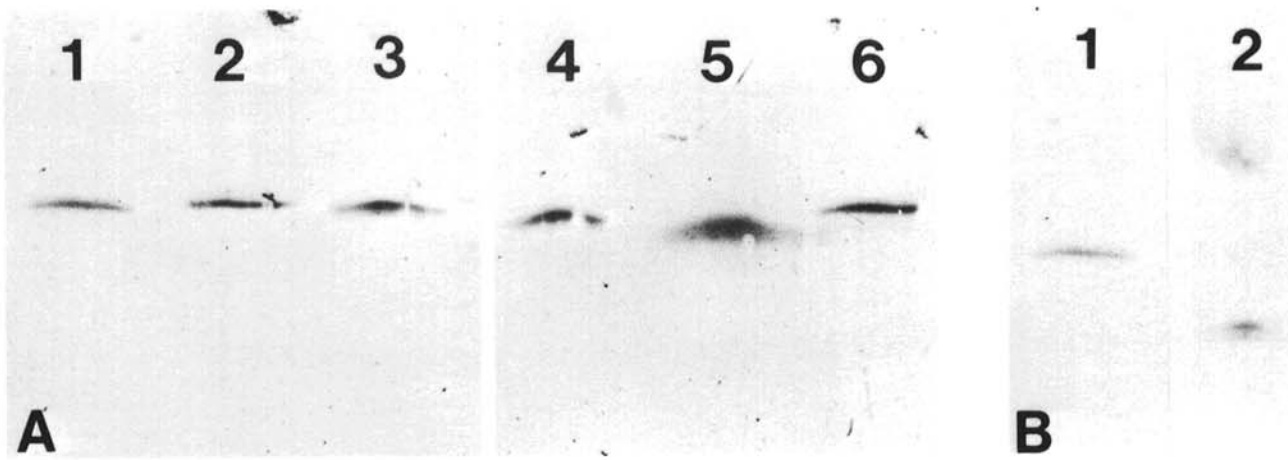

Fig. 2. Isoelectric focusing of purified preparations of dianthoviruses. **A,** Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-59; lane 3, carnation ringspot virus (CRSV)-A; lane 4, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 5, RCNMV-TpM 48; lane 6, RCNMV-Aus. The pH gradient was generated using Bio-Lyte 5-7 ampholytes. **B,** Lane 1, carnation ringspot virus (CRSV)-A; lane 2, CRSV-N. The pH gradient was generated using Bio-Lyte 3-10 ampholytes. Both gels were stained with Coomassie Brilliant Blue and destained overnight. Migration was from top (positive) to bottom (negative).

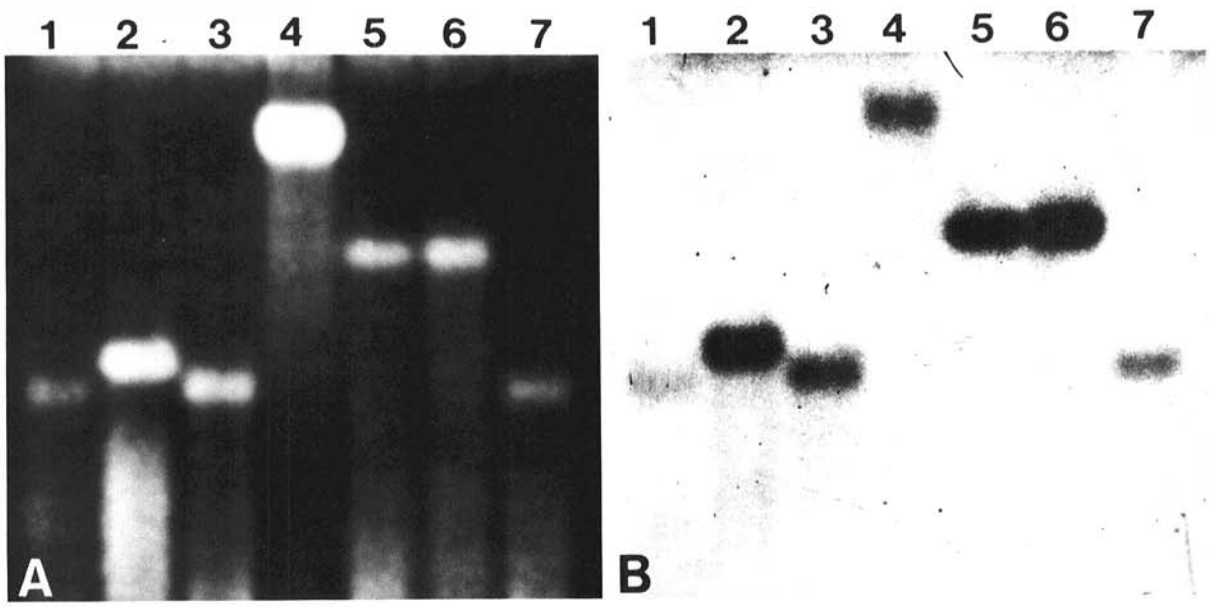

Fig. 3. Agarose gel (0.8%) electrophoresis of purified preparations of dianthoviruses. Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-59; lane 3, carnation ringspot virus (CRSV)-A; lane 4, CRSV-N; lane 5, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 6, RCNMV-TpM 48; lane 7, RCNMV-Aus. **A,** Gel stained with ethidium bromide (0.5μg/ml in water containing 1 mM disodium EDTA). **B,** The same gel restained with Coomassie Brilliant Blue and destained overnight. Migration was from top (negative) to bottom (positive).

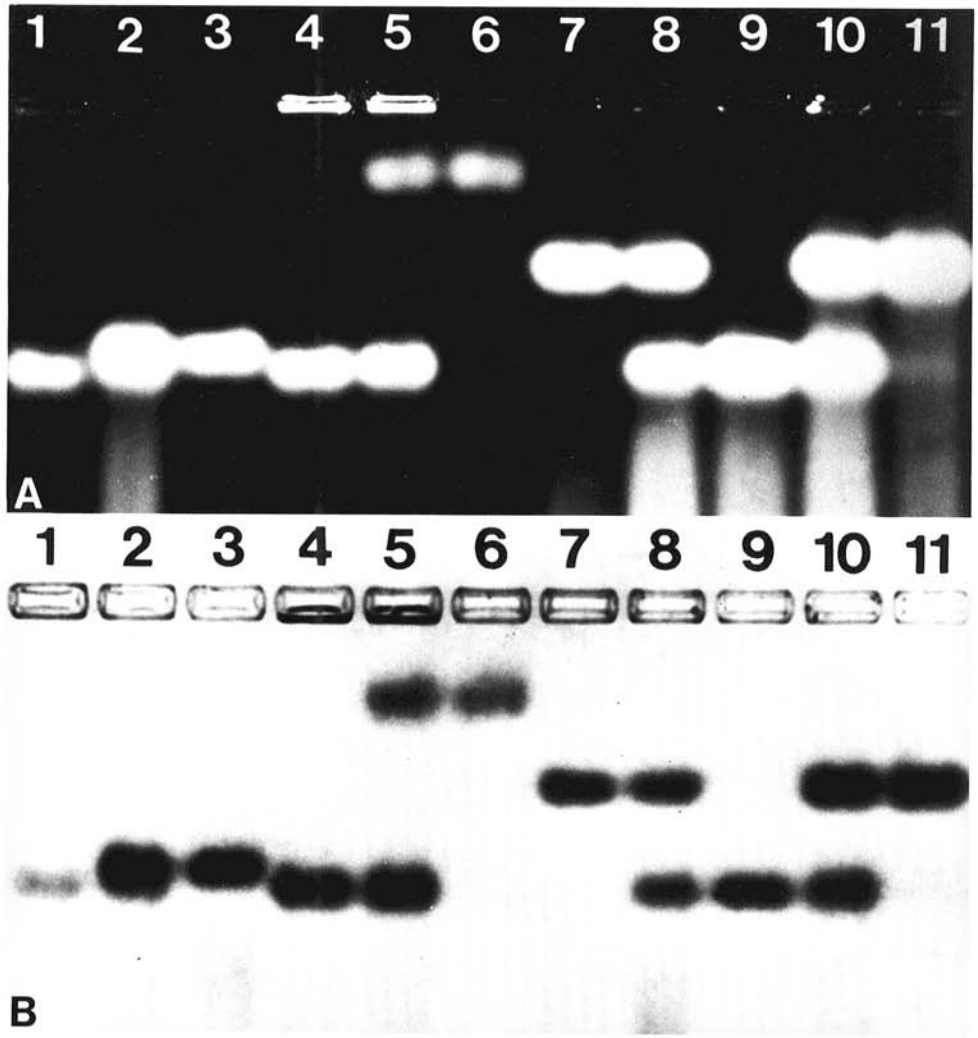

Fig. 4. Agarose gel (0.8%) electrophoresis of mixtures of purified dianthoviruses. Lane 1, sweet clover necrotic mosaic virus (SCNMV)-38; lane 2, SCNMV-38 + SCNMV-59; lane 3, SCNMV-59; lane 4, carnation ringspot virus (CRSV)-A; lane 5, CRSV-A + CRSV-N; lane 6, CRSV-N; lane 7, red clover necrotic mosaic virus (RCNMV)-TpM 34; lane 8, RCNMV-TpM 34 + RCNMV-Aus; lane 9, RCNMV-Aus; lane 10, RCNMV-TpM 48 + RCNMV-Aus; lane 11, RCNMV-TpM 48. **A,** Gel stained with ethidium bromide (0.5 μg/ml in water containing 1 mM disodium EDTA). **B,** The same gel restained with Coomassie Brilliant Blue and destained overnight. Migration was from top (negative) to bottom (positive).

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