Etiology

Comparison of Potato Yellow Dwarf Virus Serotypes

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ABSTRACT

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Three isolates of potato yellow dwarf virus (PYDV) were compared. The California (SYDV-CA) and New York (SYDV-NY) isolates of PYDV were representative of the SYDV serotype, while the New Jersey isolate (CYDV-NJ) represented the CYDV serotype. Serotype differences were readily observable by immunodiffusion and double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) tests, but were less apparent by indirect ELISA. However, SDS polyacrylamide gel electrophoresis of virion proteins showed distinct molecular weight differences for at least two virion proteins between the two PYDV serotypes. Proteins G and M_2 of CYDV-NJ had molecular weights of 92,500 and 29,000, respectively, compared to 85,000 and 24,000 for the corresponding proteins of the SYDV-CA and SYDV-NY serotypes. Despite their physical differences, the G proteins of the two serotypes cross reacted in indirect ELISA. Virion protein molecular weights, as well as serological properties, can be used to separate PYDV serotypes.

Additional key word: Rhabdoviridae.

Members of the Rhabdoviridae family have unique virion morphology, specific physicochemical properties, and a negative strand, single-stranded RNA genome (3,14,18). Five protein species, generally designated L, G, N, NS, and M, also are found on rhabdovirus virions. Members within the Rhabdoviridae are often not related serologically (eg, vesicular stomatitis virus and sowthistle yellow vein virus [15]). However, members within a specific group are serologically related and share group-specific antigens (3,18). Type-specific antigens separate the members of a group by being present only on individual serotypes or isolates within the group.

Potato yellow dwarf virus (PYDV) was one the first plant viruses demonstrated to be a member of the Rhabdoviridae (2). Two PYDV isolates representing two specific serotypes have been previously examined (2,6,9,10). Recently an isolate of PYDV was discovered in California (SYDV-CA) (5). Preliminary studies

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indicated that SYDV-CA was more closely related to SYDV-NY (the New York isolate of PYDV) than to CYDV-NJ (the New Jersey isolate of PYDV). Further studies, including SDS-polyacrylamide gel electrophoresis of virion proteins for the three PYDV isolates, suggested that individual virion protein molecular weights as well as serological relationships might be used to separate the two PYDV serotypes.

In this report, serological relationships and properties of individual virion proteins for the three PYDV isolates are presented.

MATERIALS AND METHODS

The SYDV-NY and CYDV-NJ isolates of PYDV and antiserum to these isolates were kindly provided by Hei-ti Hsu. All virus isolates were maintained in greenhouse-grown *Nicotiana debneyi* Domin by mechanical transfer (carborundum abrasive and 0.03 M phosphate [pH 7.0] inoculation buffer).

Virion purification. Tissues were harvested approximately 1 mo after inoculation when leaves showed curling and vein yellowing, which are characteristic of systemic infection. All three virus isolates were purified, as described earlier (5), by using a modification of the method used for sonchus yellow net virus (8). Tissues were homogenized in 0.1 M Tris-HCl, 0.01 M $MgCl_2$, 0.04 M Na_2SO_3, pH 8.4 (solution A), and clarified by squeezing the slurry through cheesecloth and centrifuging it at 8,000 rpm for 10 min in a JV-20 rotor. Discontinuous sucrose gradient centrifugation was done by layering samples on 6 ml of 60% and 5 ml of 30% sucrose (w/v) in solution B (0.1 M Tris-HCl, 0.01 M $MgCl_2$, 0.04 M Na_2SO_3, pH 7.5) and centrifuging in a Beckman type 30 rotor at 27,000 rpm for 1 hr. The 30–60% interface was removed and filtered through a Celite® (manufactured for Fisher Scientific by Johns-Manville, New York, NY 10016) pad. The filtrate was centrifuged at 27,000 rpm for 30 min in the type 30 rotor. Pellets were resuspended in solution B and subjected to rate-zonal sucrose density gradient centrifugation in gradients made of 5, 10, 10, and 10 ml of 5, 10, 20, and 30% (w/v) sucrose in solution B for 20 min at 26,000 rpm in an SW 28 rotor. The virion-containing zone was removed and centrifuged at 39,000 rpm for 45 min with a type 40 rotor. Virions were resuspended in solution B and used for immediate analysis or stored at −20 C.

Antiserum production. Antiserum to SYDV-CA was produced in rabbits by three weekly intramuscular injections of purified virions. One milliliter of purified virions (~0.5 mg/ml) was mixed thoroughly with 1 ml of Freund's incomplete adjuvant and injected into the hind leg of the rabbit. Bleedings began 1 wk after the third injection. Serum was collected after the blood had been stored

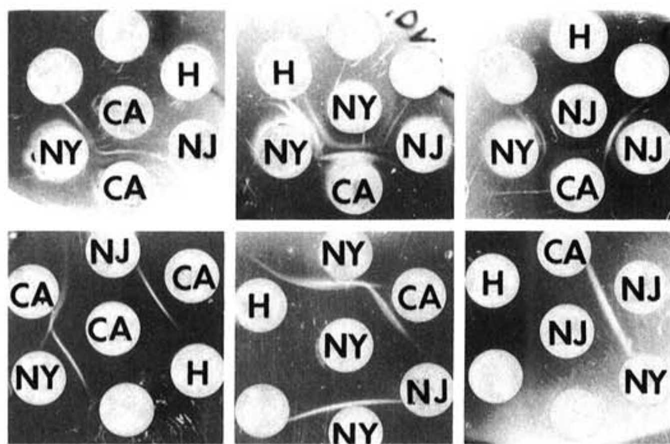

Fig. 1. Immunodiffusion reactions of antigens from potato yellow dwarf virus infected and healthy *Nicotiana debneyi*. Top row contains antigens in plant sap and bottom row contains soluble antigens. CA is SYDV-CA, NY is the SYDV-NY isolate of PYDV, and NJ is the CYDV-NJ isolate. H is antigens from healthy sap. Antigens are in outer wells and antisera are in the center wells.

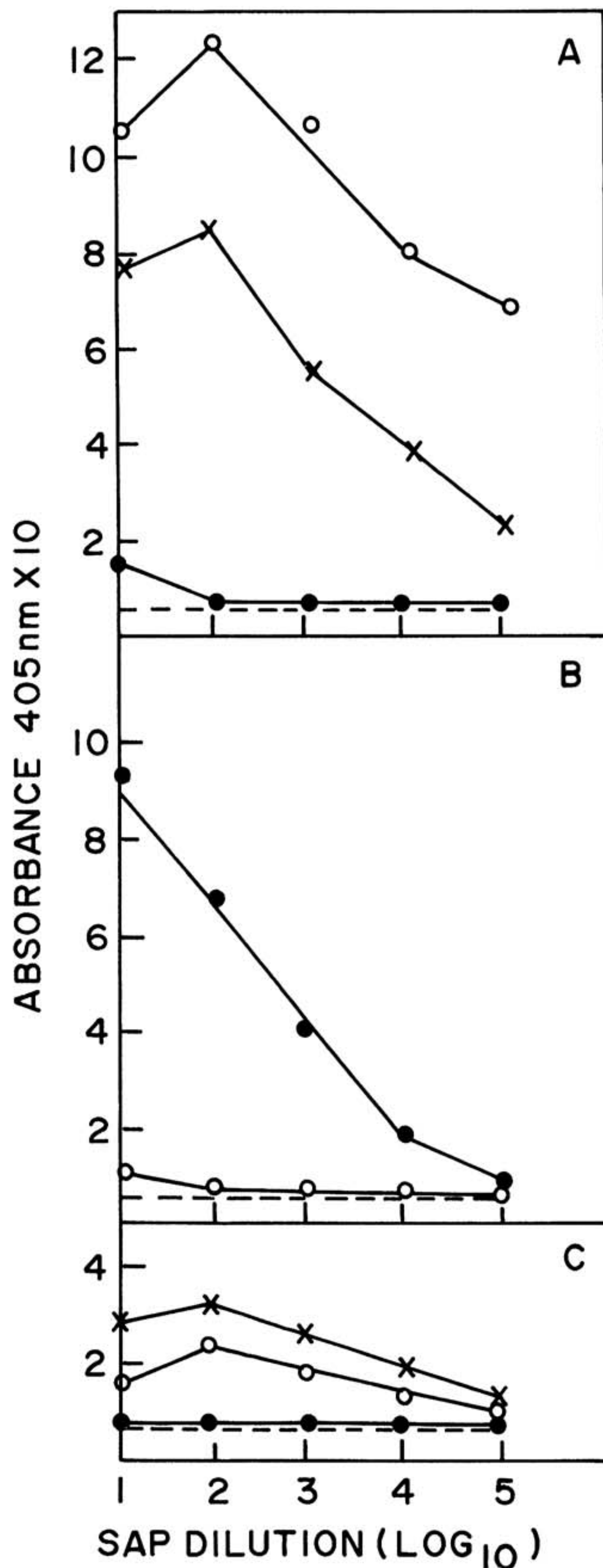

Fig. 2. Double antibody sandwich ELISA $A_{405\ nm}$ values for samples reacted with alkaline phosphatase conjugated gamma globulins to: **A**, SYDV-NY; **B**, CYDV-NJ; and **C**, SYDV-CA. o——o represents SYDV-NY sap dilutions, ×——× represents SYDV-CA sap dilutions, •——• represents CYDV-NJ sap dilutions, and —— represents sap from healthy *Nicotiana debneyi*.

overnight at 3 C, and stored frozen at −20 C.

Immunodiffusion tests. Immunodiffusion tests were done using crude sap and soluble antigens from infected plants. Crude sap antigens were prepared by grinding leaves with a mortar and pestle and squeezing the sap through cheesecloth. Soluble antigens were prepared by using a modification of the procedure described by Knudson and MacLeod (10). Two grams of tissue were ground in solution A, squeezed through cheesecloth, and centrifuged for 75 min at 16,000 rpm in an SS-34 rotor. One volume of saturated ammonium sulfate was added to the supernatant. This solution was kept at 0 C for 1 hr and centrifuged at 5,000 rpm for 10 min. The pellet was resuspended in solution A and used for immunodiffusion tests.

Immunodiffusion tests were done in 0.6% Ionagar in distilled water containing 0.02% sodium azide. Test antigens were placed in the outside wells and antiserum was placed in the center well. Plates were observed daily and kept at room temperature for up to 1 wk.

Enzyme-linked immunosorbent assay. Double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) was done essentially as described by Clark and Adams (4), and indirect ELISA was done according to Voller et al (17) and Koenig (11). Immunoglobulins purified from antiserum to each of the PYDV isolates and goat antirabbit IgG (Miles Laboratories, Inc., Elkhart, IN 46514) were conjugated separately with alkaline phosphatase (Sigma Chemical Co., St. Louis, MO 63178). DAS-ELISA was done by coating polystyrene plates (COOKE microtiter) with purified IgG at 1 μg/ml in coating buffer (0.05 M sodium carbonate, pH 9.6), for 3–6 hr at 37 C, followed by washing with PBS-Tween (0.02 M phosphate, 0.15 M NaCl, 0.05% Tween-20, pH 7.4). Test antigens were prepared in PBS-Tween containing 2% polyvinylpyrrolidone-40 (PBST-PVP) and incubated in plates overnight at 6 C. *P*-nitrophenyl phosphate substrate, at 0.6 mg/ml in diethanolamine buffer, was added to plates. The reaction was stopped after 0.5–2.0 hr and results were assessed at $A_{405 nm}$ by using a Beckman double-beam spectrophotometer. Indirect ELISA was done by coating plates with test antigens in coating buffer overnight at 6 C. Plates were washed and purified antiviral IgG (1 μg/ml) in PBS-Tween was added to the plates for 3 hr at 20 C. Plates were washed, and conjugated goat antirabbit IgG was added at 1/2,000 dilution in PBST-PVP for 2 hr at 20 C. Plates were washed and *p*-nitrophenyl phosphate (0.6 mg/ml) in diethanolamine buffer was added. Results were assessed after 1–3 hr.

Polyacrylamide gel electrophoresis of virion proteins. SDS polyacrylamide gel electrophoresis (SDS-PAGE) of virion proteins was done on 1.5-mm-thick slab gels by using the system of Laemmli (12). The resolving gel was prepared in 0.375 M Tris-HCl, pH 8.9, and the stacking gel was prepared in 0.125 M Tris-HCl, pH 6.7. The electrophoresis buffer was 0.025 M Tris, 0.38 M glycine, 0.1% SDS, pH 8.2. The sample buffer was 0.0625 M Tris-HCl, 1% SDS, 15% glycerol, 0.5% 2-mercaptoethanol, and 0.001% bromophenol blue, pH 6.7. One volume of protein was mixed with one volume of sample buffer, boiled for 1 min, and loaded onto the gels. Electrophoresis was for 4–5 hr at 100 V. Gels were stained overnight with 0.1% Coomassie brilliant blue R250 in 50% methanol, 7% acetic acid, and 43% water, and destained in the same solution without the Coomassie brilliant blue.

Purified, separated proteins of all three isolates were prepared for serological experiments by the method of Talbot and Yphantis (16). Purified virions of SYDV-CA, CYDV-NJ, and SYDV-NY (in sample buffer) were added one part to 0.02 parts of 10% dansyl chloride in acetone, thoroughly mixed, and placed in boiling water for 1 min. The sample was then electrophoresed as before. Separated proteins were visualized by exposure to ultraviolet light at 295 nm. Fluorescing bands were excised from the gel and proteins were eluted by crushing the gel in 10 ml water with a mortar and pestle and freezing the slurry overnight. The solution was allowed to thaw and centrifuged at 8,000 rpm for 10 min to remove contaminating acrylamide. Five volumes of methanol containing 3% sodium acetate was added to the supernatant and samples were incubated for at least 2 hr at −20 C and then centrifuged at 8,500 rpm for 15 min. Proteins were resuspended in distilled water and examined by SDS-PAGE or ELISA.

RESULTS

Virion purification. SYDV-CA, SYDV-NY, and CYDV-NJ were all purified by using the same procedure. Yields of SYDV-CA and SYDV-NY were comparable (~20 μg/g) but yield of purified virions of CYDV-NJ was considerably less (~6 μg/g).

Immunodiffusion. The three PYDV isolates were easily distinguished by immunodiffusion tests (Fig. 1). None of the antisera used reacted with healthy sap. SYDV-NY and SYDV-CA were closely related and reacted strongly with their homologous and heterologous antisera. CYDV-NJ reacted slightly with antisera to SYDV-NY, but very strongly with its homologous serum. The major antigen reacting in immunodiffusion plates was the soluble antigen and spur formation occurred for the soluble antigen of SYDV-CA and SYDV-NY with the homologous combination spurring over the heterologous antigen in every test. No reaction occurred when soluble antigens of CYDV-NJ were tested with either SYDV-NY or SYDV-CA antiserum and, similarly, no reactions occurred when soluble antigens of SYDV-CA or SYDV-NY were tested with antiserum to CYDV-NJ.

ELISA. DAS-ELISA reactions using fresh sap and soluble antigens from infected plants were similar to reactions obtained with immunodiffusion tests. No reactions occurred between CYDV-NJ and either SYDV-NY or SYDV-CA when tested in all combinations of IgG and sap antigens (Fig. 2). SYDV-NY and SYDV-CA did cross react, but were distinguished by the intensity of reactions.

Indirect ELISA experiments in which plant sap or purified virions was used, differentiated between PYDV serotypes, but slight cross reactions occurred between members of the different serotypes, especially when purified virions were used as the test antigen. When saps from infected plants were used as test antigens, homologous reactions were stronger than heterologous reactions. $A_{405 nm}$ values from one of four representative experiments of SYDV-NY, SYDV-CA, and CYDV-NJ saps diluted 1/1,000 with PBST-PVP were 1.06, 0.94, and 0.42, respectively, when tested with SYDV IgG, and 0.4, 0.38, and 0.94 when tested with CYDV-NJ IgG. Values for healthy sap controls were 0.36 and 0.21 using SYDV and CYDV-NJ IgG, respectively. When purified virions (20 μg/ml) were tested, $A_{405 nm}$ values for SYDV-NY, SYDV-CA, and CYDV-NJ were 1.6, 1.46, and 1.22, respectively, when tested using SYDV IgG, and 0.76, 0.68, and 1.15 using CYDV-NJ IgG. Purified tobacco mosaic virus at 10 μg/ml was used as a control and $A_{405 nm}$ values were 0.03 and 0.2 for SYDV-NY and CYDV-NJ IgG, respectively.

Indirect ELISA tests using individual virion proteins failed to demonstrate serotype differences, but showed that isolated proteins of both serotypes were serologically related. Results from one of four representative experiments are given in Table 1. Purified turnip yellow mosaic virus coat protein was used as a control and gave $A_{405 nm}$ values of 0.06. Individual proteins failed to react with homologous or heterologous serotype IgG when tested by DAS-ELISA.

Polyacrylamide gel electrophoresis. When virion proteins were examined by SDS-PAGE, SYDV-CA, and SYDV-NY were indistinguishable. However, CYDV-NJ was easily distinguished from the SYDV-CA and SYDV-NY isolates using 12, 15, or 7.5–15% gradient gels (Fig. 3). The molecular weights for individual proteins of each isolate were estimated in 12% gels by plotting the mobilities of standard proteins vs the $\log_{10}$ of their respective molecular weights. PYDV proteins were then fit to the standard regression curve by measuring their mobilities in the same gel. Although all the CYDV virion proteins have higher estimated molecular weights than SYDV serotype virion proteins, the CYDV-NJ G and M_2 proteins had obviously higher molecular weights than their SYDV serotype counterparts in all experiments (Table 2).

DISCUSSION

These data demonstrate that SYDV-CA is very closely related to SYDV-NY and is a member of the SYDV serotype. SYDV-CA is much more distantly related to CYDV-NJ. In addition to

TABLE 1. Indirect ELISA $A_{405\,nm}$ values for isolated proteins of three potato yellow dwarf virus isolates

	Protein species[a]											
	G			N			M_1			M_2		
Immunoglobulin	CA	NY	NJ	CA	NY	NJ	CA	NY	NJ	CA	NY	NJ
NJ	0.38[b]	0.82	0.21	0.17	0.165	0.195	0.12	0.05	0.075	0.19	0.15	NT[c]
NY	0.55	1.92	0.79	0.42	0.35	0.225	0.2	0.105	0.09	0.36	0.21	NT

[a]Individual proteins were isolated from SDS-gels as described in Materials and Methods. Proteins of the same species for the three PYDV isolates were adjusted to approximately equal $A_{280\,nm}$ concentrations and used for the indirect ELISA.
[b]CA represents the protein species from the SYDV-CA isolate, NY is the SYDV-NY isolate, and NJ is the CYDV-NJ isolate of PYDV.
[c]NT indicates not tested.

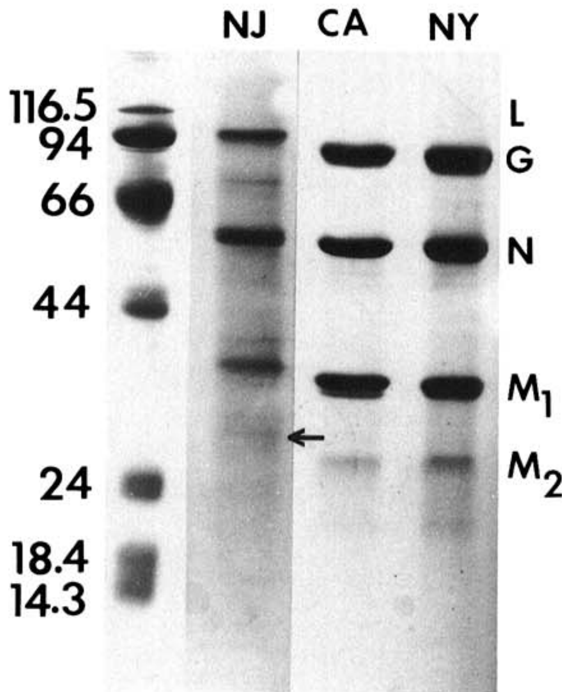

Fig. 3. SDS polyacrylamide slab gel (12% acrylamide) showing proteins from the SYDV-NY (NY), SYDV-CA (CA), and CYDV-NJ (NJ) isolates of potato yellow dwarf virus. Migration was from top to bottom. Electrophoresis was done for 5 hr at 100V. Virion proteins are labeled L, G, N, M_1, and M_2. Arrow shows location of CYDV-NJ M_2 protein. L proteins are not visible in this gel. Standard marker proteins (from the same gel) from top to bottom are: β-galactosidase, phosphorylase B, bovine serum albumin, ovalbumin, trypsinogen, β-lactoglobulin, and lysozyme. Numbers shown are molecular weights (daltons) times 10^{-3}.

serological differences between PYDV serotypes, PYDV serotypes were distinguishable by SDS-PAGE of virion proteins. The G protein was easily distinguishable for each serotype. The G protein also has been shown to be one of the major antigenic determinants for most rhabdoviruses (3,9,18). Knudson and MacLeod (10) demonstrated that the SYDV-NY G protein reacted with antiserum to CYDV-NJ. The data from indirect ELISA experiments presented here show that the G proteins of all three isolates reacted with antisera to SYDV-NY and CYDV-NJ, although the homologous serotype combinations reacted more strongly than heterologous combinations. Koenig (11) has shown, however, that indirect ELISA is useful for detecting slight serological relationships that are not detected by other serological techniques, but indirect ELISA reactions cannot be used quantitatively to estimate degrees of serological relatedness.

Despite their serological relatedness, differences in G proteins between serotypes were detected by SDS-PAGE. The observed higher molecular weight for the CYDV-NJ G protein is a physical property that can be used to differentiate the CYDV-NJ serotype from the SYDV serotype. The higher molecular weight for the CYDV-NJ G protein is unlikely due merely to the anomalous migration that is sometimes observed for glycoproteins in SDS-gels (13), because recently Adam and Hsu (1) also observed differences in G protein molecular weights for SYDV-NY and CYDV-NJ. They also found differences in G proteins by limited peptide mapping experiments.

TABLE 2. Molecular weights of potato yellow dwarf virus proteins determined by SDS-polyacrylamide gel electrophoresis

	Virion protein				
Virus isolate	L	G	N	M_1	M_2
SYDV-CA	>100,000[a]	85,000	59,000	34,000	24,000
SYDV-NY	>100,000	85,000	59,000	34,000	24,000
CYDV-NJ	>100,000	92,500	61,000	36,000	29,000

[a]Average molecular weights (daltons) determined from nine experiments.

It was also observed that the M_2 protein of CYDV-NJ had a higher molecular weight than the M_2 proteins of SYDV-CA and SYDV-NY. Such a difference in molecular weight for the M_2 protein had not been noticed previously (12); however, Adam and Hsu (1) did observe a 1,000-dalton difference and found substantial differences in CYDV-NJ and SYDV-NY M_2 proteins when analyzed by proteolytic peptide mapping. We obtained weak positive reactions for SYDV-NY and SYDV-CA M_2 proteins tested against antiserum to CYDV-NJ by indirect ELISA; however, Knudson and MacLeod (10) found SYDV M_2 proteins did not react with CYDV-NJ antiserum by immunodiffusion tests. Because the molecular weight differences were observed in all experiments and because Knudson and MacLeod (10) found that the M_2 proteins of SYDV-NY and CYDV-NJ are not serologically related by immunodiffusion, it is likely that the M_2 proteins of different serotypes are not as closely related as are the N and M_1 proteins.

It has been shown by numerous workers that the CYDV-NJ and SYDV (-CA and -NY) serotypes of PYDV have many properties that allow them to be distinguished (1,2,6,9,10). We have also shown that some of the virion proteins differ in molecular weight between PYDV serotypes. Our data also raise questions about the location and relationships of PYDV virion proteins for the two serotypes. The G protein is known to be the most exterior protein and a major antigenic determinant for rhabdoviruses. The M proteins of many rhabdoviruses are believed to be associated with the matrix immediately interior to the G protein (3,7,18,19) and, for SYNV, M_2 appeared to the external to M_1 and partially exposed to the surface (7). If G and M_2 are more external proteins of PYDV virions, then our data showing the molecular weight differences of these two proteins between the two serotypes may help to explain the distinct serological differences between the two serotypes.

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