Etiology

Comparative Potyvirus Host Range, Serology, and Coat Protein Peptide Profiles of White Lupin Mosaic Virus

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ABSTRACT

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A potyvirus isolated from white lupin (*Lupinus albus*) plants with severe mosaic symptoms was purified; compared with other selected potyviruses in terms of host range, serology, and coat protein peptide profiles; and found to be distinct. Accordingly, it has been assigned a new name, white lupin mosaic virus (WLMV). In reciprocal enzyme-linked immunosorbent assays with polyclonal antisera, WLMV behaved similarly to the Scott isolate of bean yellow mosaic virus (BYMV) and was distinguishable from the PMV-1 isolate of pea mosaic virus and the Pratt isolate of clover yellow vein virus (CYVV). In preliminary host range and pathogenicity tests, however, WLMV was distinct from BYMV, lacking the capacity to infect standard BYMV-susceptible cultivars of bean (*Phaseolus vulgaris*) and attacking BYMV-resistant cultivars of pea (*Pisum sativum*). When tested against a panel of 22 potyvirus-differentiating monoclonal antibodies, WLMV evidenced an absence of two epitopes common to all examined members of the BYMV subgroup of potyviruses and the presence of one epitope not previously known among BYMV subgroup members. In comparisons of trypsin digests of WLMV coat protein by high-performance liquid chromatography with those of other selected potyviruses, peptide profiles of WLMV most closely resembled a severe isolate (BYMV-S) of BYMV, but the terminal regions of its coat protein structure were distinctive.

Relationships among members of the potyvirus group have been notorious for their complexity (3). In many cases, shared antigenic determinants, and thus polyclonal serological relationships, have had very little bearing on other principal potyvirus characteristics such as host range. In recent years, however, a greater degree of order and understanding of potyviruses has resulted from high-performance liquid chromatography (HPLC) analyses of peptides derived from trypsin digestion of potyvirus coat proteins (10–12). Likewise, the simultaneous characterization of monoclonal antibodies and the potyviruses with which they react has led to an improved understanding of specific epitopes (9,15). In addition, complete and partial RNA nucleotide sequences are being published for a growing list of potyviruses, providing the ultimate basis for taxonomic relationships (18).

White lupin mosaic virus (WLMV) was isolated from experi-

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mental white lupin (*Lupinus albus* L. 'Ultra') plants being evaluated as an alternative crop species for the Pacific Northwest at the University of Idaho Experiment Station, Kimberly. Symptoms induced in white lupin by WLMV included severe mosaic and distortion of emerging leaves followed by stunting, localized systemic necrosis, and death of most infected plants. Such symptoms were reproducible under greenhouse conditions by three isolates of WLMV and by isolates of bean yellow mosaic virus (BYMV), pea mosaic virus (PMV), and clover yellow vein virus (CYVV). WLMV was unable to infect selected bean cultivars that were susceptible to BYMV and CYVV and that caused more severe disease (i.e., very rapid plant death) of inoculated pea, lentil, and chickpea cultivars than any known member of BYMV subgroup of potyviruses. Each isolate of WLMV produced identical results in preliminary tests, and subsequent characterization was completed with a single isolate.

Until recently, when new viruses such as WLMV were isolated, investigators often were undecided on true virus distinctions and reluctant to proliferate new virus terms. Typically, decisions on new terms were subjective and were dependent on numerous considerations (5). Recent distinctions among related viruses based on monoclonal antibodies (9) and peptide profiles of coat protein (10–12) are consistent with nucleotide sequences of viral coat protein genes (13) and also delineate known potyviruses from those not previously described. Such methods, consequently, provide more critical examination of intravirus, intervirus, intragroup, and intergroup variations than previously had been possible.

The objective of this study was to characterize WLMV by host range and pathogenicity, by serology using polyclonal and monoclonal antibodies, and by peptide profiles of coat protein. This paper describes the uniqueness of WLMV and provides the basis for its differentiation from other potyviruses.

MATERIALS AND METHODS

Potyvirus reference isolates and antisera. The Scott isolate of bean yellow mosaic virus (BYMV-Scott) was obtained from W. J. Zaumeyer in 1961, and it has since been used as a typical bean-infecting isolate of this virus. The VFS-11 isolate of PMV was seedborne in two commercial seedlots of *Vicia faba* L. (R. O. Hampton, *unpublished*). The Pratt isolate of clover yellow vein virus (CYVV-Pratt) was obtained as PV-123 from the American Type Culture Collection, Rockville, MD. The PMV-1, PMV-204-1, and PMV-Provv isolates of PMV and a severe isolate of BYMV (BYMV-S) were provided for this study by O. W. Barnett, along with antisera to CYVV-Pratt, BYMV-Scott, PMV-1, and PMV-204-1. The B-25 isolate of BYMV was provided by L. Bos. Coat protein of pepper mottle virus (PeMV) was provided by W. G. Dougherty. Sources of potyvirus isolates included in comparisons with monoclonal antibodies were as indicated by Jordan and Hammond (9). Preparations of WLMV and BYMV-Scott for tests with polyclonal and monoclonal antibodies and antiserum to WLMV were provided by R. Hampton. WLMV antiserum was prepared in rabbits by a series of injections (one intradermal, two intramuscular, with complete adjuvant) averaging 530 μg of whole virus per injection and extending over a 6-mo interval. IgG for this study was derived from blood taken 6 wk after the initial antigen injection (i.e., after two injections).

Potyvirus purification. For enzyme-linked immunosorbent assays (ELISAs) of purified potyviruses, the viruses were purified by a protocol developed from several published methods by Karen Keller (R. Hampton's laboratory). In essence, the method consists of extracting virus from prechilled infected plant tissue with cold buffer A (0.2 M borate buffer, pH 8.0, containing 0.5% mercaptoethanol, 1 mM ethylenediaminetetraacetate [EDTA], 1% Triton × 100, 1% Antifoam B, and 20% [v/v] chloroform [latter two components added just before extraction]). Tissue homogenate was kept cold and centrifuged 10 min at 3,000 *g*. The supernate was filtered through glass wool, precipitated for 45 min (stirring) with 6% polyethylene glycol (PEG, relative molecular weight [Mr] 8,000), and centrifuged 10 min at 16,000 *g*. The PEG pellet was suspended (45 min, 4 C, with stirring) in cold buffer B (10 mM borate buffer, pH 8.0, containing 0.05% mercaptoethanol, 1 mM EDTA, and 1% Triton × 100). The suspension was clarified by centrifugation for 10 min at 3,000 *g*. The supernate was again filtered through glass wool and centrifuged 90 min at 118,000 *g*, and the pellet was suspended in buffer B. The suspension was layered onto 10–40% sucrose density gradients (prepared in buffer B) and centrifuged 150 min at 104,000 *g*. Bands associated with virions were manually collected, diluted 1:1 with buffer C (10 mM borate buffer, pH 8.0, without additives), and centrifuged 90 min at 118,000 *g*. Pellets were suspended in buffer C, and virus concentration was estimated spectrophotometrically using an extinction coefficient of 2.4. The virus was lyophilized and stored at −30 C.

Monoclonal antibody panel. WLMV was compared with six other potyviruses (PTYs) by means of 22 monoclonal antibodies (MAbs), designated PTY MAbs. This panel of MAbs distinguishes epitopes that are 1) specific to BYMV strains, 2) characteristic of recognized BYMV isolates, 3) characteristic of members of the BYMV subgroup, 4) distinctive for unique potyviruses, or 5) shared by all recognized potyviruses (9). In addition, these viruses were tested against MAb TBV 7 (provided by H. T. Hsu), generated against tulip breaking virus (TBV) and shown to react with several distinct potyviruses (8; R. L. Jordan, *unpublished*).

Preliminary host range test. WLMV was tested for its capacity to infect 23 standardized legume virus hosts (6) in comparison with the VFS-11 isolate of PMV, the B-25 isolate of BYMV, and CYVV-Pratt (Table 1).

Indirect ELISA. WLMV was compared with selected members of the BYMV subgroup by reciprocal indirect ELISA (2), the optimal form of ELISA for probing multiple degrees of viral relatedness. Extracts from infected and control plants were diluted 100-fold (w/v) with antigen buffer (2), placed into duplicate wells per sample, and incubated 4 h at 37 C. Antisera were diluted 2,000-fold with antigen buffer and incubated in wells for 1 h at 37 C. Secondary antibody, goat antirabbit antibody conjugated to alkaline phosphatase, was added to wells at a dilution of 1:2,000 in antigen buffer and incubated in wells for 2 h at 37 C. After each respective incubation of the plant extracts, antisera, and antibody-alkaline phosphatase conjugate, plates were washed and then blocked for 1 h at 37 C with 0.1% dry milk in antigen buffer. Substrate, *p*-nitrophenyl phosphate, was used at 400 μg/ml. Plates were incubated for the first 6 h at 22 C and A_{405} values recorded. Plates were stored at 4 C and A_{405} values were recorded during each of the subsequent 5 days.

Antigen-coated plate ELISA. Virus samples were evaluated by indirect antigen-coated plate ELISA (9). Purified viruses were dissociated in 0.2% sodium dodecyl sulfate at 56 C for 15 min before diluting to 2 μg/ml in 0.5 M sodium carbonate-bicarbonate (coating) buffer and dispensing to duplicate wells of Nunc MaxiSorp polystyrene ELISA plates (A/S Nunc, Roskinde, Denmark). Plant samples were prepared as previously described (9), except that 0.2% sodium diethyldithiocarbamate (DIECA) was added to coating buffer containing 2% polyvinylpyrrolidone (PVP; Mr 40,000). The protocol with and without sodium DIECA was compared for sensitivity of antigen detection.

Isolation and digestion of viral coat protein. Coat proteins were isolated from freshly purified viral preparations, reduced, and carboxymethylated according to the methods of Gough and Shukla (4). Samples of 0.5–1.0 mg of coat protein were digested with trypsin (TPCK, Worthington Biochemicals, Freehold, NJ) in 250–500 μl of 0.05 M ammonium bicarbonate, pH 8.0, at 37 C for 18 h at an enzyme/substrate ratio of 1:50 (w/w). Each solution then was dried, and the residue was vortexed with 0.1% trifluoroacetic acid (TFA). After centrifugation at 10,000 *g* for 15 min, peptides in the soluble fraction were subjected to HPLC separation (14).

Analysis of peptides. Peptides from coat proteins were separated on a C_{18} reverse-phase column (model 218 TP, 5 μm, 4.6 mm × 25 cm; VYDAC, Hesperia, CA) connected to an LC4 chromatography system (Perkin Elmer, Norwalk, CT) (14). Peptides were eluted using a linear gradient of 0–33% acetonitrile in 0.1% TFA

over a period of 60 min at a flow rate of 1.0 ml/min and a column temperature of 45 C. Products separated by chromatography were monitored at 214 nm.

RESULTS

Host range comparisons. The host range of WLMV was most like that of PMV-VFS-11 but was distinguished from it by the latter's ability to induce mosaic symptoms in Black Turtle bean and chlorotic local lesions on *Chenopodium amaranticolor,* and by the inability of VFS-11 to produce symptoms in two pea cultivars (Table 1). WLMV was differentiated from BYMV B-25 primarily by its ability to infect and kill the two BYMV-resistant pea cultivars and its inability to infect the three cultivars of *Phaseolus* bean. WLMV was differentiated from CYVV-Pratt by its inability to establish infection in inoculated *C. amaranticolor* leaves, its ability to infect and kill Dark Skin Perfection pea, and its inability to infect *Trifolium repens.*

Polyclonal serological comparisons. Reciprocal indirect ELISAs of WLMV and four representative isolates of the BYMV subgroup suggested that antigenic determinants of WLMV were similar to those of BYMV-Scott (Table 2). WLMV antigen was consistently more serologically reactive than the other four antigens, when reacted with their homologous antisera. Like WLMV antigen, BYMV-Scott also reacted with antisera to all five viruses. Dip-prep electron microscopy of the five viruses in faba bean extracts indicated that virion concentrations were similar. Likewise, WLMV antiserum was more broadly reactive than the other four antisera, exhibited a range of reactivity, and was least reactive with CYVV-Pratt.

Monoclonal serological comparisons. The reaction profile of WLMV against the MAb panel is distinct from any potyvirus previously evaluated (Table 3) (9). Reactions of WLMV with 22 MAbs indicate that the virions possess two of three BYMV subgroup-specific epitopes, five of seven BYMV-specific epitopes, and one virus strain-specific epitope (defined by PTY 43) previously detected only on virions of BYMV-GDD. Moreover, WLMV does not possess two epitopes common to many potyviruses (defined by PTYs 5 and 9) that were possessed by all previously tested members of the BYMV subgroup. Further, an epitope of WLMV (defined by TBV 7) was not previously detected on any other tested members of the BYMV subgroup.

Extracts from plants infected by WLMV, BYMV-GDD, and BYMV-Scott were also compared against the MAb panel. Characteristic reaction profiles were consistent, whether the viruses were tested against the panel as plant extracts or as purified viruses.

Effect of DIECA on sensitivity of virus detection. Extracts from BYMV-GDD-infected *Nicotiana benthamiana* and from BYMV-Scott- and WLMV-infected white lupin plants were prepared in coating buffer containing 2% PVP, with and without 0.2% sodium DIECA. Virus-specific A_{405} values produced after 1 h of substrate incubation by samples containing DIECA were 1.7–3.4 times (average, 2.4 times) greater than those without DIECA (Table 4). A_{405} values for healthy plant extracts were unaffected by DIECA (data not shown).

Comparison of coat protein peptide profiles. The peptide profile of WLMV was compared with those of four members of the BYMV subgroup of potyviruses and an unrelated potyvirus, PeMV (Fig. 1). Coat protein profiles are clearly distinguishable, but with similarities among BYMV subgroup members, especially peptides retained in the column from 15 to 40 min. Peptides from PMV-204-1 (not shown) and BYMV-S exhibited the greatest similarity to WLMV among isolates tested, particularly among peptides with retention times between 35 and 60 min. At the same time, the WLMV peptide profile is clearly distinct from that of PMV-204-1 (not shown) or BYMV-S.

Although WLMV has two epitopes (defined by MAb PTYs

TABLE 1. Host ranges and host reactions of white lupin mosaic virus and three selected viruses of the bean yellow mosaic virus subgroup tested on 23 standardized plant differentials[a]

Hosts tested[c]	Host responses to inoculations[b]			
	WLMV	PMV	BYMV	CYVV
Antirrhinum majus	−/−[d]	−/−	−/−	1/−[e]
Chenopodium amaranticolor	−/−	LLc/−	NS/Mo,LC,N	LLn/(VB)
Glycine max I	?/−	?/s	?/VB,VC,Mo	−/−
G. max II	?/−	?/Mo	?/VN,LC,Mo,Stu	−/−
Medicago sativa	−/−	−/−	−/−	1/Mo,RSc
Nicotiana tabacum	−/−	−/−	1/−	RSc,NS/−
Phaseolus vulgaris I	−/−	−/−	LLc/Mo,Ma,Stu	LLc/ChlS
P. vulgaris II	−/−	−/Mo	VN/VN,Mo,Ma,Stu	LLc/Mo,Stu
P. vulgaris III	−/−	−/−	1/Chls,Mo,Stu	1/ChlS
Pisum sativum I	n/N+	−/−*	−/−*	LLc/Chl
P. sativum II	n/N+	−/−*	−/−*	−/−
Spinacia oleracea	−/−	−/−	1/−	−/−
Trifolium pratense	−/−	−/−	−/−s	1/s−/−
T. repens	−/−	−/−	−/−	1/Mo
Vicia faba	1/Mo,Stu+	−/Mo	1/VC,Mo	LLn/Mo,Stu,RSn
Vigna unguiculata	−/−	−/−	1/s	−/−

[a]Test plants selected in 1975 by members of the International Working Group on Legume Viruses (6). Plants were inoculated mechanically with the respective viruses, maintained in glasshouses at 20–25 C, and provided with supplemental fluorescent lighting, 16 h/day, ~150 langleys. Symptoms were recorded 7–30 days after inoculations.

[b]BYMV, bean yellow mosaic virus B-25 (L. Bos); CYVV, clover yellow vein virus PV-123 (M. Pratt); PMV, pea mosaic virus VFS-11; and WLMV, white lupin mosaic virus. BYMV B-25 is pathogenically equivalent to BYMV-Scott, and PMV-VFS-11 is equivalent to PMV-1.

[c]*G. max* I and II, cvs. Bragg and Davis, respectively. *P. vulgaris* I, II, and III, cvs. Bountiful, Black Turtle, and Pinto 111, respectively. *P. sativum* I and II, cvs. Perfected Wales and Dark Skin Perfection, respectively. Plant species not susceptible to any of the viruses evaluated included cucumber (Chicago Pickling), *Datura stramonium* L., *Gomphrena globosa* L., tomato (Marglobe), *N. glutinosa* L., petunia (King Henry), and phlox (Tall Mixed Colors).

[d]Plant responses to inoculations: −/− = no symptoms induced on either inoculated leaves or on subsequent foliage. Latent infection in WLMV-inoculated, symptomless plants was tested by indirect ELISA using immunoglobulin G from antiserum to WLMV. −/−* = Plants not tested for latent systemic infection. + = WLMV detected in plants with symptoms by indirect ELISA.

[e]Virus-induced symptoms: − = No symptoms; 1 = latent (symptomless) infection of inoculated leaves; ChlS = chlorotic spots; Chl = chlorosis; ( ) = lesions; LC = leaf curl (upward); LLc = chlorotic local lesions; LLn = necrotic local lesions; Ma = malformation (distortion, crinkle, savoying, strap leaf, fern leaf, etc.); Mo = mottle or mosaic; n = necrosis of inoculated leaves; N = necrosis, systemic, general; NS = necrotic spots (restricted systemic necrosis; zones, spots, flecks); RSc = chlorotic ring spot; RSn = necrotic ring spot; s = systemic latent infection; Stu = stunt; VB = vein banding; VC = vein clearing; VN = vein necrosis; ? = abnormal leaf appearance, nondescript.

TABLE 2. Comparison of enzyme-linked immunosorbent assay A_{405} values[a], produced by extracts from faba bean plants infected with virus isolates representative of the bean yellow mosaic virus subgroup of potyviruses, when tested against their homologous and heterologous polyclonal antisera

	Antisera				
Virus[b]	WLMV	BYMV-Scott	PMV-204-1	PMV-1	CYVV-Pratt
WLMV	>2.00	>2.00	>2.00	>2.00	1.79
BYMV-Scott	>2.00	>2.00	0.75	0.52	0.42
PMV-204-1	1.42	0.26	1.05	0.71	0.13
PMV-1	1.33	0.33	0.44	0.88	0.11
CYVV-Pratt	0.56	0.20	0.12	0.13	0.51
Faba bean	0.14	0.10	0.18	0.02	0.15
Buffer	0.03	0.00	0.00	0.03	0.00
T values[c]	0.279	0.100	0.243	0.074	0.171

[a]Average A_{405} values from duplicate wells after 5-day substrate incubation. Homologous reactions underlined. Two hundred microliters per well of the following reagents were added successively: coating antibody, 500 μg/ml in antigen buffer; infected/healthy faba bean extract, 100-fold dilution in antigen buffer; goat antirabbit antibody, 2,000-fold dilution in antigen buffer; and *p*-nitrophenyl phosphate, 400 μg/ml in substrate buffer. Plates were blocked between reagents with 0.1% dry milk in antigen buffer. A selected 5-day incubation was based on standardizations that enhanced antigen differentiation.

[b]WLMV = white lupin mosaic virus. BYMV-Scott = Scott isolate of bean yellow mosaic virus. PMV-204-1 = 204-1 isolate atypical of both pea mosaic virus and bean yellow mosaic virus. PMV-1 = pea mosaic virus (PV-89). CYVV-Pratt = clover yellow vein virus; North American type isolate.

[c]Antigen-detection thresholds (*T*) (i.e., herein, measures of serological relatedness) were calculated as: $T = X_h + 3 \times s$. Thus, for each antiserum, virus relationships were indicated by A_{405} values exceeding average healthy-plant control A_{405} values (X_h) by three times the standard deviations for X_h.

21 and 33) also shared by specific isolates of BYMV and PeMV, the peptide profile of PeMV coat protein bears no resemblance to WLMV or to other BYMV subgroup members (Fig. 1).

DISCUSSION

In this report, we describe results of biological, serological, and biochemical analyses that distinguish WLMV from other well-characterized, bean/pea-infecting members of the BYMV potyvirus subgroup. WLMV induces the same symptoms in white lupin as those induced by other BYMV subgroup members, but it is devastating to BYMV-resistant pea cultivars and is unable to infect BYMV-susceptible bean cultivars. It was similar to BYMV-Scott in indirect ELISAs with polyclonal antibodies, was somewhat similar to BYMV-GDD in indirect ELISAs with monoclonal antibodies (distinguished by MAb PTYs 5, 9, 17, 18, and 21–35), and was similar to, but distinguishable from, BYMV-S in peptide profiles of coat protein. Current comparisons among numerous other members of the BYMV subgroup now suggest that some isolates of PMV and BYMV have virtually indistinguishable HPLC peptide profiles, suggesting unconventional intergraded viral relationships. These comparisons also suggest the likely necessity of reliance on sequences of whole viral genomes for taxonomic accuracy and the respeciation of selected potyviruses.

As outlined by Jordan and Hammond (9), MAb PTYs 1–12 and PTY 19 each define specific epitopes common to members of the potyvirus group. PTYs 14, 17, and 18 define two epitopes common to 11–12 of 12 members of the BYMV subgroup. PTY 13 defines an epitope shared by all tested members of the BYMV subgroup and by tobacco vein mottling and tulip chlorotic blotch viruses. PTYs 21 and 33 define two epitopes shared by certain isolates of BYMV and by PeMV. PTYs 24, 30, 35, and 37 define four epitopes shared by most BYMV isolates, and PTY 43 defines

TABLE 3. Differentiation of purified potyviruses in antigen-coated plate enzyme-linked immunosorbent assay (ELISA)[a] by a monoclonal antibody panel

Monoclonal antibody[b]	ELISA A_{405} values for seven viruses[c]						
	WLMV	PMV-PV89	BYMV-GDD	BYMV-G	CYVV-Pratt	PeMV-Nc165	TEV-NAT
PTY 1	1.72[d]	>2.00	>2.00	>2.00	1.98	1.72	>2.00
PTY 2	1.34	1.24	1.51	1.49	1.57	0.02	1.49
PTY 3	0.75	1.44	1.00	1.37	1.05	0.86	0.04
PTY 4	1.05	1.41	1.01	1.36	1.02	0.33	0.02
PTY 5	0.07	0.88	1.20	1.11	0.85	0.02	0.79
PTY 8	0.15	0.48	0.70	1.69	0.67	0.01	0.66
PTY 9	0.02	0.45	0.80	0.61	0.37	0.45	0.67
PTY 10	0.02	0.01	0.01	0.12	0.09	>2.00	1.23
PTY 11	0.34	0.61	0.92	1.18	0.81	0.03	0.45
PTY 12	0.37	0.28	0.20	0.18	0.20	0.68	0.61
PTY 13	1.27	0.56	1.18	>2.00	0.56	0.01	0.02
PTY 14	1.50	0.61	1.52	>2.00	0.61	0.01	0.02
PTY 17	0.01	1.40	0.98	1.39	1.04	0.02	0.01
PTY 18	0.01	0.38	0.58	1.66	0.98	0.01	0.01
PTY 19	0.77	0.01	0.99	1.10	0.54	0.01	0.02
PTY 21	0.57	0.02	>2.00	>2.00	0.01	0.52	0.01
PTY 24	0.01	0.01	1.88	>2.00	0.01	0.01	0.01
PTY 30	0.12	0.01	1.49	1.70	0.01	0.01	0.02
PTY 33	0.23	0.02	>2.00	>2.00	0.01	0.21	0.01
PTY 35	0.01	0.01	1.45	1.72	0.01	0.01	0.01
PTY 37	0.26	0.02	0.88	0.68	0.02	0.01	0.02
PTY 43	1.04	0.01	1.42	0.01	0.01	0.01	0.01
Myeloma	0.01	0.01	0.01	0.02	0.04	0.02	0.02
PTY 1 ASC	1.87	1.59	>2.00	>2.00	>2.00	1.75	1.89
TBV 7 ASC	1.84	0.08	0.02	0.04	0.03	0.11	1.93
NS1 ASC	0.02	0.03	0.01	0.02	0.02	0.02	0.02

[a]Purified virus dissociated with 0.2% SDS at 56 C, 10 min before dilution in coating buffer to 2.0 μg/ml and direct coating of Nunc Maxisorp ELISA plate.

[b]Tissue culture supernatant liquid from hybridoma or NS1 myeloma diluted 1:10 and ascites fluids (ASC) diluted 1:5,000. NSC ASC antibody obtained from ascites fluid, from NS1 myeloma-injected control mouse.

[c]Sources and information reported by Jordan and Hammond (9) for the six viruses other than white lupin mosaic virus (WLMV).

[d]Average A_{405} values for duplicate wells after 30-min substrate incubation. Duplicate trials produced comparable data and identical virus differentiation.

TABLE 4. Effect of diethyldithiocarbamate (DIECA) in extraction buffer on detection of three potyviruses with four monoclonal antibodies by antigen-coated plate enzyme-linked immunosorbent assay

Monoclonal antibody[b]	Substrate incubation (h)	A_{405} values without and with DIECA[a]					
		WLMV[c]		BYMV-Scott		BYMV-GDD	
		−	+	−	+	−	+
PTY 1	1.0	0.69	1.22	0.80	0.88	0.65	1.39
	2.5	1.76	>2.00	1.93	>2.00	1.88	>2.00
PTY 13	1.0	0.54	1.25	0.15	0.30	0.13	0.38
	2.5	1.45	>2.00	0.39	0.78	0.40	1.18
PTY 14	1.0	0.76	1.30	0.35	0.41	0.21	0.68
	2.5	1.91	>2.00	0.98	1.12	0.71	1.90
PTY 43	1.0	0.52	1.06	0.01	0.01	0.29	0.99
	2.5	1.45	>2.00	0.01	0.01	1.02	>2.00

[a] Average A_{405} values from duplicate wells after substrate incubation.
[b] Culture supernatant from hybridoma diluted 1:10.
[c] Virus-infected plant samples extracted 1:10 (w/v) for 2 h at 23 C in coating buffer (CB) with 2% PVP, with (+) or without (−) 0.2% sodium DIECA, before preparing the final dilution of 1:100 in CB for direct coating of Nunc Maxisorp ELISA plates. White lupin mosaic virus (WLMV) and the Scott isolate of bean yellow mosaic virus (BYMV-Scott) were tested in tissues from infected white lupin (*Lupinus albus* L.) plants, and the GDD isolate of bean yellow mosaic virus (BYMV-GDD) was tested in tissues from infected *Nicotiana benthamiana* Domin plants.

an epitope previously detected only on a single isolate of BYMV, BYMV-GDD. MAb TBV 7 defines an epitope shared by 20 of 33 distinct potyviruses (R. Jordan, *unpublished*). The reaction profile of WLMV with the MAb PTY panel indicates a unique serological relationship of WLMV with other members of the BYMV subgroup.

Use of sodium DIECA was first recommended by Banttari and Franc in 1981 (1) and was considered advantageous by Hobbs et al (7). Although it has been optional in some ELISA systems, we had recently noticed that some tests simply failed to work at all without DIECA. Although its presence was not indispensable in our direct comparisons (Table 4), we (R. O. Hampton and R. L. Jordan) have incorporated it into most of our ELISA protocols. Although sodium DIECA functions as an antioxidant and may as such benefit virus purification protocols, the exact function and benefit in ELISA (particularly indirect ELISA) is not known to us.

While WLMV is recognizably distinct from other known potyviruses, we believe there will be many more examples of uniqueness as potyvirus isolates are compared. At the same time, we are confident that more virus-term amalgamations (10,12,19) are inevitable and equally useful to systematic plant virology.

Our experience with field populations of potyviruses, especially within food legume crops, suggests that an unrecognized magnitude of diversity exists among members of the BYMV subgroup of potyviruses. Accordingly, we anticipate that there are many WLMV-like potyvirus distinctions in principal agroecosystems waiting to be noted and defined.

A recent comparison of the complete genome sequences of potyviruses (13,18) has shown that strains share a high degree of sequence similarity irrespective of their exact gene products, while distinct potyviruses have a significantly lower degree of similarity of gene products. A like trend exists in comparing nucleic acid sequences of coding and noncoding regions for viral strains versus distinct potyviruses (13). This suggests that the relatedness of potyviruses and their strains deduced from studies of coat protein only (16–18) are valid. Prior studies have shown that distinct potyviruses in general possess 38–71% sequence homology, whereas the homology for strains is >90%. This results in a bimodal distribution of sequence homologies between distinct viruses on one hand and viral strains on the other (13). The delineation between distinct and similar potyviruses can now be achieved with greater confidence than ever before. Biological

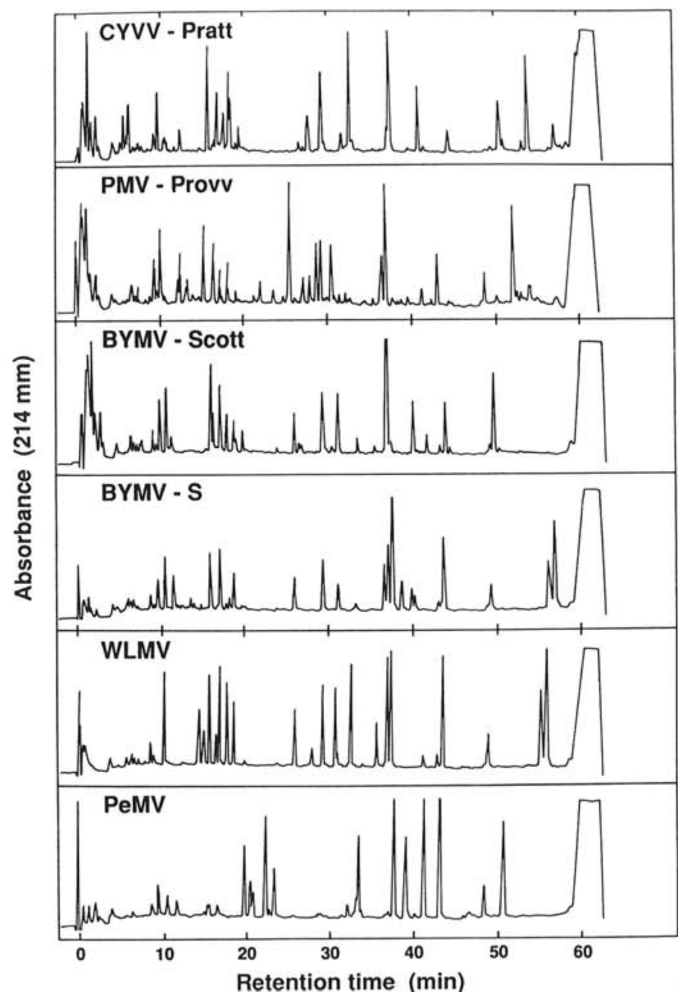

Fig. 1. High-performance liquid chromatography peptide profiles of tryptic digests of viral coat proteins illustrating differences between peptides of white lupin mosaic virus (WLMV) protein and those of selected isolates of clover yellow vein virus (CYVV), pea mosaic virus (PMV), bean yellow mosaic virus (BYMV), and pepper mottle virus (PeMV). Note the substantial similarity between the profiles of WLMV and BYMV-S, but clear distinctions between peptides eluting early in the chromatogram.

distinctions now can be further defined by poly- and monoclonal antibodies and coat protein peptide characterization, eventually including the sequences of the coat protein gene and the whole viral genome. We have accordingly presented the potyviral distinctiveness of WLMV by the best approaches currently available to us.

LITERATURE CITED

1. Banttari, E. E., and Franc, G. D. 1981. Single or combined antisera for detection of potato viruses S and X in plants or tubers using enzyme-linked immunosorbent assay. (Abstr.) Phytopathology 71:201.
2. Converse, R. H., and Martin, R. R. 1990. ELISA methods for plant viruses. Pages 179-196 in: Serological Methods for Detection and Identification of Viral and Bacterial Plant Pathogens. R. Hampton, E. Ball, and S. De Boer, eds. American Phytopathological Society, St. Paul, MN.
3. Edwardson, J. R. 1974. Some properties of the potato virus Y Group. Fla. Agric. Exper. Stn. Monogr. Ser. 4. Institute of Food and Agricultural Sciences, University of Florida, Gainesville.
4. Gough, K. H., and Shukla, D. D. 1981. Coat protein of potyviruses. 1. Comparisons of the four Australian strains of sugarcane mosaic virus. Virology 111:455-462.
5. Hamilton, R. I., Edwardson, J. R., Francki, R. I. B., Hsu, H. T., Hull, R., Koenig, R., and Milne, R. G. 1981. Guidelines for the identification and characterization of plant viruses. J. Gen. Virol.

54:223-241.
6. Hampton, R., Beczner, L., Hagedorn, D., Bos, L., Inouye, T., Barnett, O., Musil, M., and Meiners, J. 1978. Host reactions of mechanically transmissible legume viruses of the northern temperate zone. Phytopathology 68:989-997.
7. Hobbs, H. A., Reddy, D. V. R., Rajeshwari, R., and Reddy, A. S. 1987. Use of direct antigen coating and protein A coating ELISA procedures for detection of three peanut viruses. Plant Dis. 71:747-749.
8. Hsu, H. T., Franssen, J. M., van der Hulst, C. T. C., Derks, A. F. L. M., and Lawson, R. H. 1988. Factors affecting selection of epitope specificity of monoclonal antibodies to tulip breaking potyvirus. Phytopathology 78:1337-1340.
9. Jordan, R. L., and Hammond, J. 1991. Comparison and differentiation of potyvirus isolates and identification of strain-, virus-, subgroup-specific, and potyvirus group-common epitopes using monoclonal antibodies. J. Gen. Virol. 72:25-36.
10. McKern, N. M., Shukla, D. D., Barnett, O. W., Vetten, H. J., Dijkstra, J., Whittaker, L. A., and Ward, C. W. Coat protein properties suggest that adzuki bean mosaic virus, blackeye cowpea mosaic virus, peanut stripe virus, and three potyvirus isolates from soybean are all strains of the same potyvirus. Intervirology. In press.
11. McKern, N. M., Shukla, D. D., Toler, R. W., Jensen, S. G., Tosic, M., Ford, R. E., Leon, O., and Ward, C. W. 1991. Confirmation that the sugarcane mosaic virus subgroup consists of four distinct potyviruses by using peptide profiles of coat proteins. Phytopathology 81:1025-1029.
12. McKern, N. M., Whittaker, L. A., Strike, P. M., Ford, R. E., Jensen, S. G., and Shukla, D. D. 1990. Coat protein properties indicate that maize dwarf mosaic virus-KS1 is a strain of johnsongrass mosaic virus. Phytopathology 80:907-912.
13. Shukla, D. D., Frenkel, M. J., and Ward, C. W. 1991. Structure and function of the potyvirus genome with special reference to the coat protein coding region. Can. J. Plant Pathol. 13:178-191.
14. Shukla, D. D., McKern, N. M., Gough, K. H., Tracy, S. L., and Letho, S. G. 1988. Differentiation of potyviruses and their strains by high performance liquid chromatographic peptide profiling of coat proteins. J. Gen. Virol. 69:493-502.
15. Shukla, D. D., Tribbick, G., Mason, T. J., Hewish, D. R., Grayson, H. M., and Ward, C. W. 1989. Localization of virus-specific and group-specific epitopes of plant potyviruses by systematic immunochemical analysis of overlapping peptide fragments. Proc. Natl. Acad. Sci. USA 86:8192-8196.
16. Shukla, D. D., and Ward, C. W. 1988. Amino acid sequence homology of coat proteins as a basis for identification and classification of the potyvirus group. J. Gen. Virol. 69:2703-2710.
17. Shukla, D. D., and Ward, C. W. 1989. Identification and classification of potyviruses on the basis of coat protein sequence data and serology. Arch. Virol. 106:171-200.
18. Ward, C. W., and Shukla, D. D. 1991. Taxonomy of potyviruses: Current problems and some solutions. Intervirology 32:269-296.
19. Yu, M. H., Frenkel, M. J., McKern, N. M., Shukla, D. D., Strike, P. M. and Ward, C. W. 1989. Coat protein of potyviruses. 6. Amino acid sequences suggest watermelon mosaic virus 2 and soybean mosaic virus-N are strains of the same potyvirus. Arch. Virol. 105:55-64.