Relationships Among Australian and North American Isolates of the Bean Yellow Mosaic Potyvirus Subgroup

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ABSTRACT

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Relationships among 17 isolates from the bean yellow mosaic potyvirus subgroup were investigated by molecular hybridization, using randomly primed complementary DNA to each of the isolate-RNA's, and by direct enzyme-linked immunosorbent assay, using antisera to five of the isolates. The same general pattern of relationships among isolates was found by both methods. Six clover yellow vein virus (CYVV) isolates from Australia appeared to be related to a North American isolate, CYVV-Pratt, though they were more closely related to each other. Relationships among seven bean yellow mosaic virus (BYMV) isolates from Australia were more diverse, with some isolates appearing closely related to a North American isolate, BYMV-Scott. None of the Australian isolates, including pea mosaic virus I, were closely related to the North American pea mosaic virus (BYMV)-204-1. The Australian sweet pea mosaic virus was not closely related to BYMV, CYVV, or pea mosaic virus.

A bean yellow mosaic virus-subgroup (BYMV-subgroup) of the potyvirus group was proposed by Randles et al (30) on the basis of amino acid composition of coat proteins. The subgroup comprised bean yellow mosaic virus (BYMV), pea mosaic virus (PMV), and sweet pea mosaic virus (SPMV). These same viruses were also closely related serologically (24,25). Clover yellow vein virus (CYVV) is closely related to BYMV and PMV by serology (19,21) and therefore could be included in the BYMV-subgroup.

These four viruses have many properties in common. Their modal particle lengths of about 750 nm and their nonpersistent mode of aphid transmission place them in the potyvirus group (18). Cytoplasmic inclusion bodies (pinwheel inclusions with laminated aggregates but without scrolls) induced in cells by these viruses place them in Edwardson's potyvirus inclusion body subdivision II (12,25). Nuclear crystalline inclusions are found in some hosts infected with BYMV, CYVV, and PMV (5); the BYMV-subgroup viruses are the only viruses in inclusion body subdivision II with nuclear inclusions (12).

The experimental host ranges of these viruses are also very similar (5), though CYVV usually causes more severe symptoms on *Nicotiana clevelandii* Gray and systemic symptoms develop more quickly on *Chenopodium quinoa* Willd. than do those caused by BYMV or PMV. It is common to find more than one of these viruses in the same region though some strains seem to occur only in limited geographical areas where a particular strain may be the predominant BYMV-subgroup virus of those areas and crops (4,26,30). It is important to be able to differentiate between viruses in this subgroup because resistance to BYMV, PMV, and CYVV is controlled by different genes in *Phaseolus vulgaris* L. (29; in this reference PMV is termed BYMV-PV-2 and CYVV is termed BYMV-S). A similar situation occurs in *Pisum sativum* L. where recent evidence indicates different but closely linked genes for resistance to BYMV and CYVV (Provvidenti, *personal communication*).

BYMV-subgroup viruses appear closely related by some serological procedures but are readily distinguishable by others. With gel diffusion serology of sonicated virus particles, BYMV, PMV, and SPMV appear closely related (with serological differentiation indices [SDI] between 1 and 2), but the S and Q strains of BYMV (with SDI between 1 and 3) formed spurs that are indicative of a more distant relationship (24,25). The BYMV-subgroup, excepting SPMV, was considered by Jones and Diachun (19) to be a single virus, BYMV, with three distinct serotypes. Their serotypes were delineated with sodium dodecyl sulfate (SDS)-gel diffusion after intragel absorption: CYVV strains were in serotype I, BYMV strains in serotype II, and PMV strains in serotype III. Enzyme-linked immunosorbent assay (ELISA) readily separates PMV and CYVV isolates if sera to both viruses are used in the double antibody sandwich ELISA (23), but BYMV isolates yield results that are difficult to interpret when only PMV and CYVV sera are used (3).

Molecular hybridization of BYMV-subgroup virus RNA with randomly primed complementary DNA (cDNA) shows promise for identification of viruses in this subgroup. Abu-Samah and Randles (1) found considerable sequence homology among three strains of BYMV but no homology between these strains and PMV. Reddick and Barnett (31) found very little sequence homology among BYMV, CYVV, and PMV but close homology among isolates of CYVV and among isolates of PMV.

In the present work, we used molecular hybridization to investigate relationships among members of the BYMV-subgroup from North America and Australia and to identify several Australian BYMV-subgroup isolates that failed to show RNA sequence homology with cDNA prepared from BYMV-G, -Q, or -S (2). The use of molecular hybridization and ELISA for virus identification has also been evaluated.

MATERIALS AND METHODS

Virus isolates. The virus isolates used, the hosts from which they were isolated, and locations of origin are shown in Table 1. Isolates were sap inoculated by grinding infected tissue in 0.1 M sodium phosphate buffer, pH 8.0, then rubbing the sap on Carborundum-dusted leaves. Various isolates were maintained in different host plants: CYVV in *N. clevelandii*, PMV in broad bean, SPMV in pea, and BYMV in either broad bean or bean.

Virus purification. Virus isolates, propagated in various host plants (Table 2), were purified by Method 2 of Reddick and Barnett (31), usually with two cycles of cesium sulfate equilibrium

centrifugation. After the final concentration by high-speed centrifugation the virus pellet was resuspended in sterile double distilled water, and a portion was taken to measure virus concentration ($E = 2.4\ [mg/ml]^{-1}\ cm^{-1}$ at 260 nm after correction for light scattering by boiling for 2 min in 1% SDS [7] or not corrected when virus yields were low). SDS was added to 1%, and the virus preparation frozen.

RNA extraction. Virus preparations were thawed and one-third volume of a freshly made and sterilized, concentrated disruption buffer was added (final concentration 0.25 M Tris-Cl, 0.1 M NH_4HCO_3, 1 mM EDTA, 1% SDS). The virus in the disruption buffer was layered immediately onto a sucrose step gradient previously equilibrated 4–8 hr at room temperature (2.7, 2.9, 3.0, 3.4 ml of 7.5, 15, 22.5, and 30 g of sucrose per 100 ml of solution in 0.5 M Tris-Cl buffer, pH 9.0, respectively) and centrifuged at 24,000 rpm for 18 hr at 14 C in a Beckman SW 41 rotor. The RNA band was collected, ethanol precipitated (2 vol redistilled ethanol, 0.1 M sodium acetate), phenol extracted, precipitated twice with ethanol, and taken up in sterile double distilled water for storage at −20 C. Concentration of RNA was estimated spectrophotometrically, $E = 25\ (mg/ml)^{-1}\ cm^{-1}$ at 260 nm.

Synthesis of cDNA from virus RNA. RNA (1–5 μg, but 5 μg for most preparations) plus 70 μCi (3 nmoles) ^{3}H-dCTP (Amersham, Arlington Heights, IL) was lyophilized after adding an equal volume of water. The cDNA was prepared by the procedures of Abu-Samah and Randles (1) using the random primer method (33) with avian myeloblastosis virus reverse transcriptase. The cDNA made from BYMV-Q RNA was that prepared by Abu-Samah and Randles (1). The RNAs of BYMV-Scott, CYVV-Pratt, and PMV-204-1 were extracted from virus in South Carolina, lyophilized, and cDNA made in Australia.

Hybridization of cDNA with virus RNA. Hybridizations were performed in 0.01 M Tris-Cl, pH 7.0, 1 mM EDTA, 0.05% SDS, and either 0.18 M or 0.54 M NaCl (14). RNA at 0.2 μg/ml and cDNA (about 2,600 counts per minute) in a final volume of 40 μl were hybridized in a 0.5-ml conical plastic microcentrifuge tube overlaid with paraffin oil. After heating at 100 C for 3 min, hybridization occurred during incubation at 65 C for 120 hr or longer (R_0t exceeding 0.27 mol·s·l^{-1}). The S1 nuclease reaction was performed according to procedures of Reddick and Barnett (31) with 20 units of S1 nuclease per milliliter in 30 mM sodium acetate, pH 4.6, 1 mM zinc sulfate, 5% glycerol, 0.05 M or 0.54 M NaCl for 1 hr at 45 C. The amount of hybrid product resistant to S1 nuclease was calculated by dividing the counts per minute of the S1 treated part of the sample by the counts per minute of the untreated portion. Hybridization percentages were corrected for background counts per minute and S1 nuclease resistance of the cDNA. More than a single run was required to test 17 RNAs with 17 cDNAs so hybridization percentages were adjusted for run-to-run variation by least squares analysis (not all RNAs or cDNAs were present in every run). Each test was replicated twice and sometimes three or four times.

A similarity matrix was derived from these adjusted hybridization percentages by scaling (using the formula of Gonda and Symons [14]) reactions of the RNAs within each cDNA, setting the homologous RNA reaction to 100. The resulting matrix was tested for symmetry (by comparing the increase in residual sum of squares under the symmetry restrictions with the unrestricted sum of squares) and found to be nonsymmetric. The clustering method of King (20), applied without the assumption that similarity matrices are symmetric, was used to produce a hierarchical clustering of the 17 isolates together with the corresponding dendrogram.

Serology. Antisera to PMV-204-1 and CYVV-Pratt were those used by McLaughlin et al (22). Antiserum to BYMV S was that used by Randles et al (30). Antisera to BYMV-G and CYVV-PQ were made by subcutaneous and intramuscular injection of rabbits with virus emulsified in Freund's adjuvant. Several injections were made and bleedings made several months after the initial injection were used for ELISA. Serum titers in microprecipitin tests ranged from 1:128 to 1:512.

Gel diffusion tests were in Gooding's SDS medium (15) with plant tissue ground in 0.02 M Tris buffer, pH 7, or 0.01 M sodium phosphate buffer, pH 7.4. Serum preparation and ELISA procedures were performed as described by McLaughlin et al (22). Infected plant tissue was ground in 0.02 M sodium phosphate, pH 7.0, 0.03% NaN_3, 0.02 M DIECA, 0.05% Tween 20. Three assays were made, each of which included most but not all of the serum/antigen combinations. Different antigens were in various plants (broad bean, snap bean, garden pea, *N. clevelandii*, *C. quinoa* or *C. amaranticolor*). The appropriate optical densities of healthy plant extracts were subtracted from optical densities of virus infected plant extracts and values from different assays were averaged.

RESULTS

Host reactions. The only virus that infected Greenfeast pea was SPMV (Table 2). CYVV isolates caused more pronounced

TABLE 1. Bean yellow mosaic virus-subgroup isolates, their original host and place of origin

Isolate[a]	Original host	Collection site[b]	Source & Reference[c]
SPMV-Aust.	*Lathyrus odoratus* L.	S.A., Aus.	R. I. B. Francki[1] (24)
CYVV-Pratt	*Trifolium repens* L.	Can.	M. J. Pratt[7] (28)
-PQ	*Pisum sativum* L.	Shepparton, Vic., Aus.	P. R. Smith[2] (2)
-P3	*P. sativum*	Shepparton, Vic., Aus.	P. R. Smith[2] (2)
-Q1	Unknown	Gatton, Qld., Aus.	G. M. Behncken[3] (2)
-RL1	*Lupinus* sp.	Vic., Aus.	P. R. Smith[2]
-L1	*Lupinus* sp.	Rutherglen, Vic., Aus.	P. R. Smith[2] (2)
-NSW	*Lupinus luteus*	Lismore, N.S.W., Aus.	G. M. Behncken[3] (2)
PMV-204-1[d]	*T. incarnatum* L.	KY, USA	S. Diachun[8] (19)
-I	*P. sativum*	Vic., Aus.	P. R. Smith[2] (34)
BYMV-Scott	USDA type isolate	USA	R. O. Hampton[9] (19)
-Q	*Canna* sp.	Brisbane, Qld., Aus.	D. S. Teakle[4] (24)
-G	*Gladiolus communis*	Adelaide, S. A., Aus.	T. C. Lee[5] (24)
-G-81-1	*Gladiolus* sp.	Vic., Aus.	P. R. Smith[2]
-RL7	*Lupinus* sp.	Rutherglen, Vic., Aus.	P. R. Smith[2] (2)
-WA8	*T. subterraneum*	Narrikup, W. A., Aus.	G. D. McLean[6]
-WA22	*Lupinus* sp.	Bindoon, W. A., Aus.	G. D. McLean[6]
-S	*Vicia faba*	Naracoorte, S. A., Aus.	J. W. Randles[1] (30)

[a] SPMV = sweet pea mosaic virus, CYVV = clover yellow vein virus, PMV = pea mosaic virus, BYMV = bean yellow mosaic virus.
[b] States of Australia: S.A. = South Australia; Vic. = Victoria; N.S.W. = New South Wales; Qld. = Queensland; W.A. = Western Australia.
[c] [1]Waite Agricultural Research Institute, The University of Adelaide; [2]Plant Research Institute, Department of Agriculture, Victoria; [3]Department of Primary Industries, Indooroopilly, Queensland; [4]University of Queensland, St. Lucia, Queensland; [5]Botanic Garden, Adelaide; [6]Department of Agriculture, Perth, Western Australia; [7]Agriculture Canada, Vancouver; [8]University of Kentucky, Lexington; [9]Oregon State University, Corvallis.
[d] PMV-204-1 is the designation for the isolate originally described as BYMV-204-1 by Diachun.

symptoms on inoculated leaves and usually more pronounced effects on systemically infected portions of Hawkesbury Wonder bean than did BYMV isolates. CYVV isolates also caused more pronounced symptoms on *N. clevelandii* than those caused by PMV or BYMV isolates. Most BYMV isolates caused wilting of Unicrop lupine, which was not observed with CYVV or PMV infections. All CYVV isolates caused systemic symptoms in *C. quinoa*, but only two BYMV isolates caused systemic symptoms in this plant. In general, the symptoms were considered typical of BYMV-subgroup isolates.

RNA extraction and cDNA production. All virus strains were purified by the one procedure in sufficient quantities to yield RNA for cDNA production and subsequent hybridization with the various cDNAs. Purifications of the isolates were from different propagation hosts (Table 2). Several isolates were purified from bean, broad bean, and *N. clevelandii*. Yields of virus ranged from 0.008 to 5 mg per 100 g of bean tissue, 0.002 to 2.8 mg per 100 g of broad bean tissue, and 0.1 to 7.6 mg per 100 g of *N. clevelandii* tissue. RNA extraction in alkaline gradients gave between 29 and 86% recovery. Size distribution of cDNA was determined from the distribution of ^{3}H-cDNA in polyacrylamide gels after electrophoresis (16). Some cDNAs had major peaks of radioactivity between the 25S and 4S size markers, whereas other cDNAs had major peaks smaller than the 4S marker.

TABLE 2. Host reactions caused by bean yellow mosaic virus-subgroup isolates

Isolate[a]	*Phaseolus vulgaris* 'Hawkesbury Wonder'	*Pisum sativum* 'Greenfeast'	*Lupinus angustifolius* 'Unicrop'	*Vicia faba major* 'Aquadulce'	*Nicotiana clevelandii*	*Chenopodium amaranticolor*	*C. quinoa*
SPMV		+*			-;-		
	-;-[b]	-;VC,Mo	-;Ep,W,C	-;-	.	RNLL;-	NLL;ChSp
CYVV							
-Pratt	Ep;Mo				ChLL;Mo +*	+	+
	-;VC	-;-	-;Mo,Ma,N	-;-	-;Mo	RNLL;-	Ch/NLL;ChSp,N
-PQ	-;Mo,C *			NLL;ChSp,C	+*	RNLL;-	-;ChSp
	NLL;Mo,S	-;-	NLL;Mo,N,C	-;VC,N	-;Mo	ChLL;-	ChLL;-
-P3	*			NLL,NRS;VC	+		
	Ep,ChLL;S,VC,N	-;-	NLL;Mo,N	-;-	-;Mo	RNLL;-	ChLL;ChSp
-Q1	*			NLL;Mo,N	+*		
	Ep,ChLL;Mo,S	-;-	-;Mo,N,C	-;-	-;Mo	RNLL;ChSp	NLL;ChSp,VN
-RLI	Ch,N;Mo,S *			NLL;N,C +	+*		N/ChLL;-
	ChLL;Mo,S	-;-	-;Mo,N,W	-;-	ChLL;Mo	RNLL;ChSp	-;-
-LI	ChLL,VN;Mo,S *			NLL;N,C	+		
	-;Mo	-;-	NLL;Mo,N,C	-;-	-;Mo	RChLL;-	ChLL;ChSp
-NSW	ChLL;Mo,Ma *			-;Mo +	+*		
	Ep,VC;S,Mo,VC	-;-	-;FMo	-;-	ChLL;Mo	RNLL;-	Ch/NLL;ChSp
PMV							
-204-1				-;Mo +*		RNLL;-	
	-;-	-;-	-;Ep,FMo	-;-	-;FMo	NLL;-	ChLL;-
-I				+			+
	.	.	.	-;Mo	.	.	ChLL;-
BYMV							
-Scott	*			+		NLL;ChSp	ChLL;ChSp
	ChLL;Mo	-;-	W,C;W,Ma,Mo,C	-;VC,Mo	-;FMo	ChLL;-	ChLL;-
-Q	+*						
	ChLL;Mo	-;-	W;Ep,Mo	-;-[c]	-;-	ChLL;-	ChLL;-
-G				+*			
	Ch/NLL;Mo,VN	-;-	W,N,C;W,VN,C	Ch;VC,Mo,S	-;-	Ch/RNLL;-	ChLL;-
-G81-1	Ch,VN;			+*	ChLL;Mo		
	-;-	-;-	N;Ep,Mo	-;VC,Mo	-;FMo	ChLL;-	ChLL;-
-RL7	EpChLL;Mo *			+		ChLL;VB	ChLL;-
	-;Mo	-;-	C;W,Mo	-;VC,Mo	-;FMo	-;-	-;-
-WA8	ChLL;Mo +*			-;Mo	-;ChSp	ChLL;ChSp	ChLL;-/ChSp
	-;Mo,S	-;-	W,Ch;Ch,N,Ma	-;-	-;-	ChLL;-	ChLL;-
-WA22	+*			-;C		ChLL;VB,ChSp	
	ChLL;Mo	-;-	W,Ch,C;Ep,W,Ch	-;-	-;FMo	ChLL;-	ChLL;-
-S	ChLL;-			-;Mo,Ma +*			
	-;FMo	-;-	-;Mo,W,C	-;-	-;-	ChLL;-	ChLL;-

[a] Virus abbreviations and isolates as for Table 1.

[b] Symptom designations: the semicolon separates local from systemic reactions; -, no symptom; Mo, mosaic, VC, vein clearing or chlorosis, W, wilt; Ep, epinasty; N, necrosis, C, collapse of tissue and death, ., not done; ChLL, chlorotic local lesions; NLL, necrotic local lesions; ChSp, chlorotic spots; R, red rimmed; Ch, chlorotic or chlorosis; F, faint, VN, veinal necrosis, S, stunt; VB, vein banding; Ma, leaf or tip malformation, *, propagation host for purification, +, host for serology, /, symptoms varied as shown on either side of slash.

[c] Previously this strain infected Aquadulce (2) but since has been maintained in bean.

TABLE 3. Sequence homology among bean yellow mosaic virus subgroup isolates

RNA used in hybridization reaction	RNA used for cDNA preparation: SPMV-Aust.	CYVV-Pratt	-PQ	-P3	-Q1	-RLI	-LI	-NSW	PMV-204-1	BYMV-Scott	-Q	-G	-G81	-RL7	-WA8	-WA22	-S
I - Adjusted Mean Hybridization Percentages[y]																	
SPMV-Aust.	73.5[a] *	0.1[a]	1.6[a]	2.1[a]	1.7[a]	2.9[a]	1.0[a]	- 0.1[a]	2.5[a]	2.3[a]	3.9[a]	1.3[a]	2.2[a]	2.8[a]	3.6[a]	9.2[a] *	-3.0[a]
CYVV-Pratt	2.9[a]	50.8[c] *	13.8[c] *	9.1[c] *	13.9[c] *	11.8[c] *	8.6[c] *	4.6[c] *	-3.4[a]	-2.5[a]	-4.4[a]	-4.2[a]	-3.2[a]	-1.2[a]	2.9[a]	4.0[a]	-5.0[a]
-PQ	2.9[a]	7.1[c] *	68.5[c] *	58.7[c] *	44.1[c] *	52.3[c] *	35.5[c] *	58.0[c] *	4.9[a]	4.9[a]	6.4[a]	4.6[a]	4.1[a]	5.9[a]	4.6[a]	3.2[a]	-2.5[a]
-P3	2.5[a]	10.4[c] *	67.7[c] *	64.6[c] *	57.4[c] *	59.8[c] *	36.8[c] *	58.4[c] *	-3.8[a]	-4.2[a]	-3.1[a]	-5.2[a]	-3.2[a]	-4.7[a]	3.3[a]	4.2[a]	-3.9[a]
-Q1	2.4[a]	9.9[c] *	45.4[c] *	45.7[c] *	70.9[c] *	61.8[c] *	37.0[c] *	47.2[c] *	0.1[a]	-3.8[a]	-3.1[a]	-4.1[a]	-4.8[a]	-6.2[a]	3.0[a]	5.3[a]	-4.1[a]
-RLI	2.9[a]	6.5[c] *	59.6[c] *	48.8[c] *	58.3[c] *	67.6[c] *	46.9[c] *	52.9[c] *	-3.1[a]	-2.7[a]	-1.1[a]	-1.8[a]	-1.0[a]	0.4[a]	2.4[a]	1.4[a]	-5.0[a]
-LI	2.8[a]	8.3[c] *	59.0[c] *	53.3[c] *	56.3[c] *	54.9[b] *	46.2[c] *	49.3[c] *	-2.5[a]	-2.4[a]	-3.6[a]	-5.3[a]	-5.0[a]	-4.9[a]	1.1[a]	4.7[a]	-7.5[a] *
-NSW	2.5[a]	9.0[c] *	57.6[c] *	60.1[c] *	54.7[c] *	59.1[c] *	34.3[c] *	61.8[c] *	-3.3[a]	-6.0[a]	-3.2[a]	-2.9[a]	-3.1[a]	-3.9[a]	2.0[a]	7.8[a]	-6.5[a]
PMV-204-1	2.6[a]	-5.4[a]	-4.2[a]	-6.2[a]	-11.0[a] *	-3.3[a]	-4.7[a]	-23.6[a] *	53.3[a] *	0.9[a]	2.9[a]	4.1[a]	7.6[a] *	0.0[a]	5.2[a]	7.9[a] *	-5.0[a]
BYMV-Scott	2.7[a]	-7.2[a] *	-7.9[a] *	-8.0[a] *	-14.7[a] *	-5.2[a]	1.6[a]	-24.0[a] *	0.8[a]	43.6[c] *	25.4[c] *	49.0[c] *	49.1[c] *	21.5[c] *	14.1[a] *	22.7[a] *	7.5[a] *
-Q	1.9[a]	1.2[a]	1.1[a]	2.3[a]	1.4[a]	0.6[a]	2.9[a]	2.9[a]	6.9[a] *	19.5[a] *	52.7[a] *	51.8[a] *	48.4[a] *	34.5[a] *	26.5[a] *	25.9[a] *	-1.0[a]
-G	2.9[a]	-1.6[a]	-6.2[a]	-2.4[a]	-0.9[a]	-1.2[a]	1.8[a]	-1.4[a]	2.7[a]	25.3[c] *	41.2[c] *	70.6[c] *	56.2[c] *	31.0[c] *	27.9[a] *	23.7[a] *	2.3[a]
-G81	3.4[a]	-1.9[a]	-4.8[a]	-2.9[a]	-2.4[a]	-0.7[a]	1.4[a]	0.2[a]	1.3[a]	25.0[c] *	26.4[c] *	57.2[c] *	68.3[c] *	30.6[c] *	19.9[a] *	24.0[a] *	7.2[a] *
-RL7	2.4[a]	-2.1[a]	-6.2[a]	-2.9[a]	-2.4[a]	-0.8[a]	0.1[a]	-0.7[a]	0.3[a]	7.1[c] *	24.2[c] *	27.8[c] *	32.8[c] *	58.9[c] *	32.6[a] *	41.9[a] *	-0.8[a]
-WA8	4.1[a]	0.6[a]	2.4[a]	1.8[a]	3.4[a]	2.6[a]	0.9[a]	0.7[a]	4.6[a]	12.5[a] *	40.1[a] *	34.5[a] *	26.1[a] *	48.1[a] *	59.2[a] *	58.5[a] *	1.5[a]
-WA22	2.6[a]	-0.3[a]	2.7[a]	1.5[a]	2.3[a]	4.6[a]	1.2[a]	0.3[a]	5.1[a]	8.4[a] *	28.8[a] *	35.3[a] *	27.1[a] *	53.8[a] *	39.8[a] *	51.7[a] *	-0.6[a]
-S	5.2[a]	1.7[a]	1.9[a]	2.9[a]	1.4[a]	3.1[a]	3.4[a]	0.1[a]	4.5[a]	15.6[a] *	8.2[a] *	23.4[a] *	26.4[a] *	17.4[a] *	16.6[a] *	12.3[a] *	30.7[a] *
Healthy	1.1[a]	0.2[a]	1.8[a]	2.3[a]	3.6[a]	1.9[a]	0.1[a]	0.4[a]	2.1[a]	1.2[a]	2.5[a]	0.9[a]	4.3[a]	1.7[a]	1.8[a]	3.7[a]	2.0[a]
II - Similarity Matrix[z]																	
SPMV-Aust.	100	0	2	3	2	4	2	0	5	5	7	2	3	5	6	18	0
CYVV-Pratt	4	100	20	14	20	17	19	8	0	0	0	0	0	0	5	8	0
-PQ	4	14	100	91	62	77	77	94	9	11	12	7	6	10	8	6	0
-P3	3	20	99	100	81	89	80	94	0	0	0	0	0	0	6	8	0
-Q1	3	20	66	71	100	91	80	76	0	0	0	0	0	0	5	10	0
-RLI	4	13	87	76	82	100	101	86	0	0	0	0	0	1	4	3	0
-LI	4	16	86	83	79	81	100	80	0	0	0	0	0	0	2	9	0
-NSW	3	18	84	93	77	89	74	100	0	0	0	0	0	0	3	15	0
PMV-204-1	4	0	0	0	0	0	0	0	100	2	6	6	11	0	9	15	0
BYMV-Scott	4	0	0	0	0	0	3	0	2	100	48	69	72	37	24	44	25
-Q	3	2	2	4	2	1	6	5	13	45	100	73	71	59	45	50	0
-G	4	0	0	0	0	0	4	0	5	58	78	100	82	53	47	46	8
-G81	5	0	0	0	0	0	3	0	2	57	50	81	100	52	34	46	23
-RL7	3	0	0	0	0	0	0	0	1	16	46	39	48	100	55	81	0
-WA8	6	1	3	3	5	4	2	1	9	29	76	49	38	82	100	113	5
-WA22	3	0	4	2	3	7	3	0	10	19	55	50	40	91	67	100	0
-S	7	3	3	4	2	5	7	0	8	36	15	33	39	30	28	24	100

[y]Hybridization was in 0.18 M NaCl and the S1 nuclease reaction was in 0.05 M NaCl. Adjusted mean hybridization percentages are given with superscripts identifying their standard errors (a: ± 3.49, b: ± 2.85, c: ± 2.47) and the presence of * indicating that a difference from zero hybridization was detected at $P < 0.05$.

[z]Adjusted mean hybridization percentages were converted to a similarity matrix for purposes of dendrogram construction (Fig. 1). All values in each cDNA column were scaled to yield a value of 100 for the homologous entry in that column; negative estimates were replaced by zeros during this conversion. For dendrogram construction values greater than 100 were set at 100.

Incorporation of ^{3}H-dCTP into cDNA ranged from 15 to 34%. Log_{10} $R_0t_{½}$ values for homologous hybridizations were between −2.2 and −2.6 except for the CYVV-PQ reaction, which was −1.5.

Relationship of isolates by molecular hybridization. Hybridization percentages of 17 cDNAs with 18 RNAs under stringent conditions (low salt, 65 C) are given in Table 3, part I. Most of the hybridization percentages greater than zero occurred in two groups; one group consisted of seven isolates of CYVV, and the other group consisted of eight isolates of BYMV. None of the cDNA reactions with healthy plant RNA were different from zero.

The dendrogram constructed from Table 3, part II branched into four clusters below 6% hybridization (Fig. 1), which is in the range of values not different from zero hybridization. Two of these clusters contained a single isolate each (PMV-204-1 and SPMV-Aust). The other two clusters are products of subsequent branching, one contained the seven CYVV isolates and the other contained the eight BYMV isolates.

Results of the hybridization of selected isolates under high stringency (low salt) and low stringency (high salt) conditions are shown in Table 4. Even in low stringency conditions, where partial homologies should give more hybridization, homologies were low among BYMV, CYVV, PMV, and SPMV; though PMV did show detectable homology with BYMV. CYVV-Pratt and BYMV-S were distantly related to other isolates in their respective clusters (Fig. 1), but both of these isolates showed more homology with other isolates of their clusters in low rather than high stringency conditions (Table 4).

Hybridization assays with RNA from PMV-I were done separately from the other hybridization reactions. cDNA to PMV-I, under the conditions in Table 3, gave 71% hybridization in homologous tests with RNA from PMV-I but did not hybridize with RNA from PMV-204-1, BYMV-S, BYMV-G, or CYVV-PQ (1.5%, 3.0%, 5.4%, and 2.4% hybridization, respectively). In tests done at the same time, cDNA to PMV-204-1 hybridized with homologous RNA (62%) but not with RNA from PMV-I or BYMV-S (1.2% and 3.2%, respectively).

Relationship of isolates by serology. In SDS-gel diffusion tests, antiserum to BYMV-S reacted with BYMV-S and BYMV-G (their precipitin bands were confluent) and weakly with BYMV-G81-1 but not with SPMV-Aust., BYMV-RL7, or six CYVV isolates. Antiserum to PMV-204-1 gave distinct precipitin bands with BYMV-S, -G81-1, and CYVV-LI, but weaker precipitin bands with SPMV-Aust., BYMV-WA8, -RL7, CYVV-NSW, -P3, -PQ, and -RLI. Spurs were formed at some of the junctions between distinct and weak precipitin bands (the distinct band continued over the weak band). Of particular note was the spur formed by the BYMV-G band over the BYMV-RL7 band. Antiserum to CYVV-Pratt gave distinct precipitin bands with CYVV-NSW, -P3, -PQ, -Q2, and -LI, weak bands with CYVV-RLI, BYMV-S, -G, -G81-1, and -RL7, but no bands were formed with SPMV-Aust. or BYMV-WA8. Spurs were often formed when the distinct precipitin bands continued over the weak bands at the junctions. The SDS-gel diffusion tests were not repeated with all isolates and the homologous isolates for all antisera were not used. None of the antisera reacted with healthy plant extracts.

Results of double antibody sandwich, direct ELISA with five antisera in three different tests are given in Table 5. Homologous reactions for four of the antisera were near an optical density of 1 but the homologous reaction of BYMV-S antiserum was very weak. Antiserum to CYVV-PQ reacted with most CYVV isolates and also with some BYMV isolates.

For each antiserum, a symmetric dissimilarity matrix was prepared from ELISA results by taking pairwise absolute differences between scaled isolate means (Table 5) and standardizing to a maximum dissimilarity of 100; dissimilarity of an isolate from itself is zero by this definition and similarity is (100 − dissimilarity). Cluster analysis and dendrogram construction, as described previously for the RNA-cDNA hybridization percentages, were repeated using the aggregate similarities derived from antisera to BYMV-G, BYMV-S, PMV-204-1, and CYVV-Pratt (equally weighted), and using similarities derived from the antiserum to CYVV-PQ alone (Figs. 2 and 3).

For purposes of comparison with the dendrogram in Figure 1, the arbitrary horizontal scales of these two dendrograms were chosen so that their first (#1) and last (#16) nodes coincided with those in Figure 1; the resulting scale is labeled 'serological distance index.' The dendrogram in Figure 2 was quite similar to that using RNA-cDNA hybridization percentages as the similarity measure, but that in Figure 3 differs in that BYMV-Scott was grouped with the Australian CYVV isolates and the other BYMV isolates were divided into two groups, one of which also included SPMV-Aust., and CYVV-Pratt was removed from the CYVV cluster.

DISCUSSION

Bean yellow mosaic virus subgroup members exhibit different interrelationships among themselves, their crop hosts, and their wild plant hosts. The biological properties that establish these interrelationships allow certain members to be distinguished from other isolates and may lead to certain members being predominant in a particular geographical area. For instance, in a region in South Australia and western Victoria, Australia BYMV-S-like isolates predominate in *Vicia faba* L. crops, whereas this strain of BYMV was not found in other parts of Australia, even in *V. faba* (2). In another example, in the southeastern United States, CYVV is the

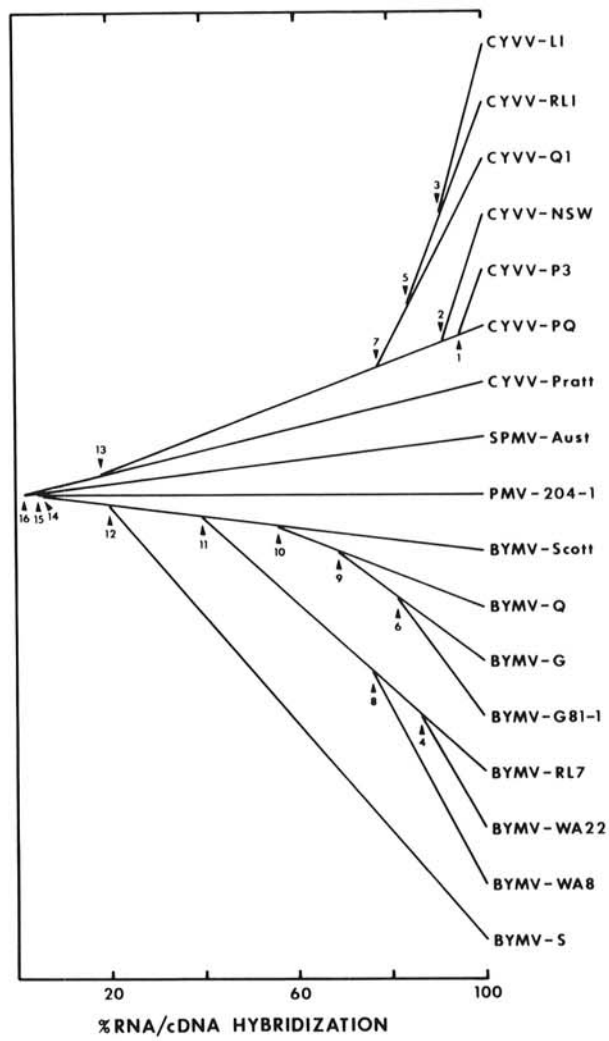

Fig. 1. Dendrogram of clusters among 17 bean yellow mosaic virus subgroup potyvirus isolates, using RNA-DNA hybridization as the measure of similarity. Arrows denote points of isolate divergence. The percent hybridizations at these points are as follows: 16) 1.6, 15) 4.5, 14) 5.2, 13) 16.6, 12) 19.3, 11) 39.1, 10) 55.2, 9) 68.0, 8) 76.0, 7) 77.7, 6) 81.5, 5) 83.0, 4) 86.0, 3) 90.5, 2) 91.2, and 1) 95.0. Horizontal distances between points are representative of relationships.

predominant BYMV-subgroup virus found in annual forage legumes where white clover is the major perennial forage legume, but PMV predominates in annuals where red or crimson clovers are widely grown (4). Also, cultivated gladiolus plants in the United States are infected predominantly with BYMV isolates rather than with PMV or CYVV isolates (26). These examples illustrate the necessity for proper identification, not only of the members of the bean yellow mosaic virus subgroup, but also of their strains to develop an understanding of epidemiological interactions within the bean yellow mosaic virus subgroup.

TABLE 4. Sequence homologies of selected bean yellow mosaic virus subgroup isolates in high and low salt hybridization conditions[a]

RNA used in hybridization reaction		RNA used for cDNA preparation						
		SPMV-Aust.	CYVV-Pratt	-PQ	PMV-204-1	BYMV-Scott	-G	-S
SPMV-Aust.	H	100	−0.4	−9.2	−0.2	−0.1	−0.7	0.3
	L	100	0.8	−6.7	−0.1	1.4	−1.2	−5.4
CYVV-Pratt	H	−6.2	100	54.2*	17.8	−0.9	6.2	3.0
	L	1.8	100	16.1*	5.8	−0.5	−1.5	−2.3
CYVV-PQ	H	−6.9	15.8	100*	−0.8	2.4	6.4	−0.6
	L	0.7	6.6	100*	2.9	−0.6	−2.4	−5.9
PMV-204-1	H	−4.3	−5.5	−4.7	100	7.8	30.1	13.6
	L	3.4	−0.8	−8.8	100	7.1	11.9	1.6
BYMV-Scott	H	−4.5	−2.2	−2.6	20.0	100	83.7	46.4
	L	0.2	3.3	−7.7	6.6	100	68.8	39.1
BYMV-G	H	−1.9	2.0	1.2	7.7	50.6	100	52.0
	L	2.2	1.4	−7.0	8.3	62.0	100	26.8
BYMV-S	H	−4.9	−0.6	−3.1	19.0	39.2	62.2	100
	L	0.1	1.5	−7.1	6.0	40.7	36.3	100

[a] Hybridization and S1 nuclease conditions were as in Materials and Methods except that NaCl concentrations were 0.18 M and 0.05 M, respectively, for low salt (L) and 0.54 M and 0.54 M, respectively, for high salt (H). All values in each column were scaled to yield a value of 100 for the homologous entry in that column. All values are means of two replicates (except those with *, which had four) and the scaled mean hybridization percentages had standard errors of ± 8.39 (for *: ± 5.94).

TABLE 5. Reaction of bean yellow mosaic virus subgroup isolates with five sera in enzyme linked immunosorbent assays[a]

Virus	Antisera									
	BYMB-G		BYMV-S		PMV-204-1		CYVV-Pratt		CYVV-PQ	
	Mean	Scaled	Mean	Scaled	Mean	Scaled	Mean	Scaled	Mean	Scaled
SPMV-Aust.	0.00^{f}	0.00	0.00^{e}	0.00	0.01^{d}	1.33	0.03^{e}	2.24	-0.02^{f}	0.00
CYVV-Pratt	-0.02^{d}	0.00	-0.02^{c}	0.00	0.16^{b}	16.81	1.49^{c}	100	0.20^{d}	10.15
-PQ	-0.02^{d}	0.00	-0.01c	0.00	0.04^{b}	4.42	0.98^{c}	65.64	1.97^{d}	100
-P3	-0.03^{f}	0.00	-0.04f	0.00	0.05^{f}	5.31	1.34^{f}	89.93	1.94^{f}	98.48
-Q1	-0.04^{f}	0.00	-0.01^{e}	0.00	0.06^{d}	5.84	0.75^{d}	50.11	1.64^{f}	83.00
-RLI	-0.02^{f}	0.00	-0.01e	0.00	0.06^{d}	6.37	1.11^{e}	74.27	1.93^{f}	97.97
-LI	-0.04^{f}	0.00	-0.02e	0.00	0.07^{d}	7.17	0.99^{e}	66.44	1.32^{f}	67.00
-NSW	-0.04^{f}	0.00	0.00^{e}	0.00	0.20^{d}	20.71	0.71^{e}	47.43	1.91^{f}	96.95
PMV-204-1	0.23^{d}	21.56	0.05^{c}	18.37	0.94^{b}	100	0.21^{c}	13.96	0.24^{d}	11.93
-I	0.22^{b}	20.54	0.01^{b}	3.97	0.26^{b}	27.79	0.12^{b}	8.39	0.05^{b}	2.71
BYMV-Scott	0.42^{d}	39.81	0.10^{c}	32.65	0.28^{b}	29.91	0.02^{c}	1.34	1.74^{d}	88.58
-Q	0.92^{f}	86.73	0.10^{f}	32.31	0.08^{f}	7.96	0.04^{f}	2.35	0.80^{f}	40.61
-G	1.06^{d}	100	0.12^{c}	40.14	0.08^{b}	8.14	0.14^{c}	9.40	0.78^{d}	39.34
-G-81	0.03^{d}	2.84	0.19^{c}	64.63	0.14^{b}	14.51	0.22^{c}	15.03	0.04^{d}	2.16
-RL7	0.02^{d}	2.37	0.16^{c}	55.78	0.14^{b}	15.40	0.17^{c}	11.54	0.04^{d}	2.16
-WA8	0.46^{d}	43.60	0.34^{c}	116.33	0.19^{b}	20.53	0.12^{c}	7.78	0.80^{d}	40.86
-WA22	0.15^{d}	14.22	0.21^{c}	70.75	0.18^{b}	19.65	0.23^{c}	15.57	0.02^{d}	1.27
-S	0.19^{d}	18.01	0.29^{c}	100	0.31^{b}	32.74	0.16^{c}	11.01	0.50^{d}	25.51

[a] Means in each column were scaled to yield a value of 100 for the homologous entry in that column. Standard errors of the means are b: ± 0.081, c: ± 0.089, d: ± 0.100, e: ± 0.115, f: ± 0.141.

Serology has been the main criterion for identification and separation of potyviruses (18). However, serological relationships among potyviruses with different biological properties make the distinction of potyviruses by serology alone difficult (5,36). These serological relationships may be due to reactions of group antigens common to widely different potyviruses (11,32). Thus, another technique is needed to supplement serology for potyvirus differentiation.

Potyvirus RNA genomes contain nucleotide sequences that code for at least six proteins, with the capsid protein being the smallest (11). Molecular hybridization analysis allows comparisons of the sequence homologies of the total viral genomes, not just of that portion responsible for the capsid protein, provided that cDNA is produced against randomly primed RNA and an S1 nuclease assay is used to analyze the RNA-cDNA hybrid (17).

Cucumber mosaic virus (CMV) strains and tomato aspermy virus (TAV) show various sequence homologies by this technique (14). With cDNA complementary to each of the four RNAs of CMV-Q, the four RNAs of CMV-P could not be distinguished from the corresponding RNAs of CMV-Q; the RNAs of CMV-M only gave partial sequence homologies (15–30%) with the RNAs of CMV-Q; and the RNAs of TAV showed even less homology (2–14%) with RNAs of CMV-Q.

Molecular hybridization analysis of tobamoviruses revealed little sequence homology among tobacco mosaic virus (= U1), para-tobacco mosaic virus (= U2), tomato mosaic virus (= dahlemense), cucumber mosaic virus 4 (= CV4), Sunn-hemp mosaic virus, and Frangipani mosaic virus but considerable sequence homologies among strains of tobacco mosaic virus, strains of para-tobacco mosaic virus, and strains of tomato mosaic virus (27,35). These relationships agree closely with relationships established by the amino acid compositions of capsid proteins and serology (13,37).

Molecular hybridization and serology also lead to similar conclusions regarding relationships among nepoviruses (10).

By molecular hybridization analysis and serology, six Australian isolates were identified as CYVV. Although their relationship with CYVV-Pratt, the Canadian isolate, was definite, the six isolates from Australia were more closely related to each other than to CYVV-Pratt. These six isolates include five isolates previously shown not to be related to BYMV (2). Three Australian isolates (-Q, -G, and -S) previously characterized as BYMV, were confirmed as BYMV isolates by their relationship to BYMV-Scott. Four other Australian isolates were identified as BYMV by molecular hybridization analysis and serology; in a previous study, one of these isolates, -RL7, did not show sequence homology with

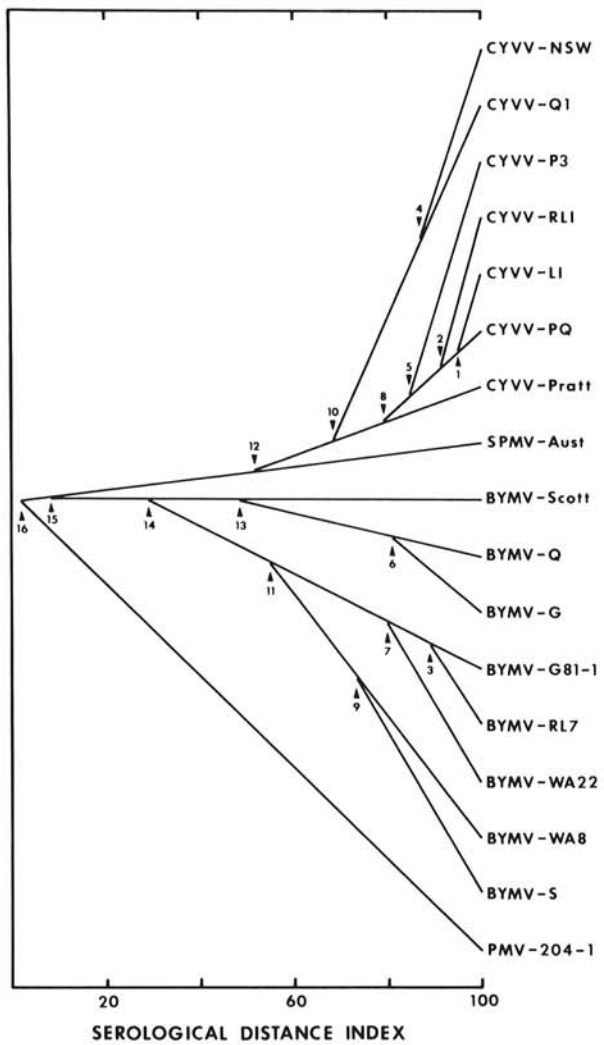

Fig. 2. Dendrogram of clusters among 17 bean yellow mosaic virus subgroup potyvirus isolates, using serological relationships by quantitative ELISA with antisera to BYMV-G, BYMV-S, PMV-204-1, and CYVV-Pratt. Arrows denote points of isolate divergence. The serological distance indices at these points are: 16) 1.6, 15) 7.8, 14) 28.1, 13) 47.4, 12) 50.8, 11) 54.9, 10) 68.1, 9) 72.8, 8) 78.7, 7) 79.8, 6) 80.3, 5) 84.7, 4) 86.7, 3) 89.0, 2) 91.4, and 1) 95.0. Values for PMV-I were omitted for uniformity with Figure 1.

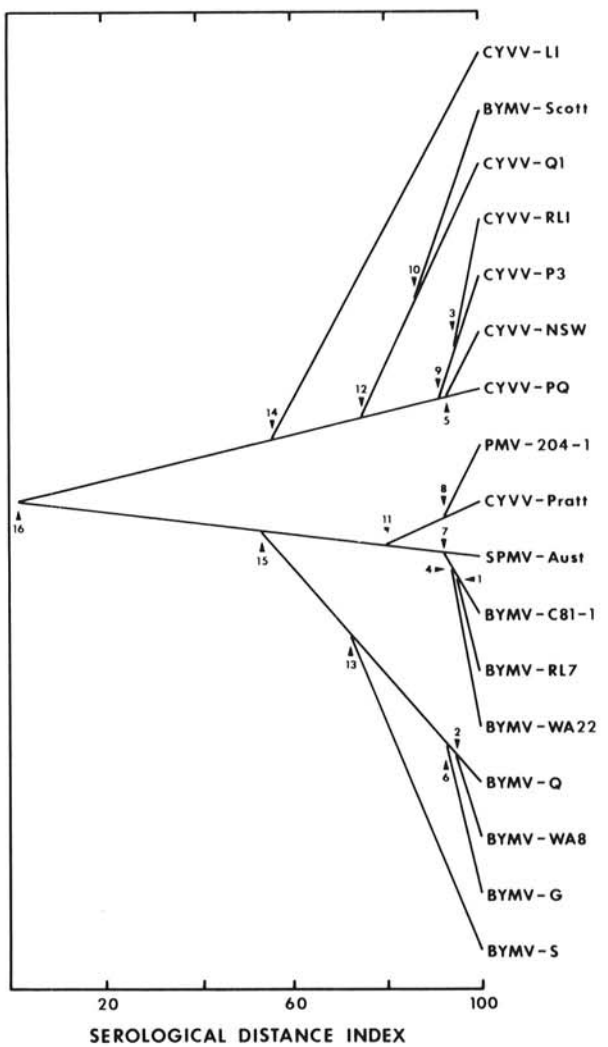

Fig. 3. Dendrogram of clusters among 17 bean yellow mosaic virus subgroup potyvirus isolates, using serological relationships by quantitative ELISA with antiserum to CYVV-PQ. Arrows denote points of isolate divergence. The serological distance indices at these points are: 16) 1.6, 15) 53.4, 14) 55.8, 13) 72.5, 12) 74.9, 11) 79.2, 10) 86.4, 9) 91.3, 8) 92.2, 7) 92.3, 6) 92.8, 5) 93.0, 4) 93.6, 3) 94.2, 2) 94.6, and 1) 95.0. Values for PMV-I were omitted for uniformity with Figure 1.

BYMV-G, -Q, or -S, but the source of RNA was a crude virus preparation (2).

No Australian isolate used in this study exhibited a close relationship with PMV(BYMV)-204-1. This isolate originated from red clover collected in Kentucky (8,9). Other isolates collected from forage legumes in the southeastern United States are similar to this isolate by direct ELISA tests (23; BYMV in Table 5 of that reference refers to serum produced against PMV-204-1) and an isolate from Canada (PMV [BYMV]-Pratt) is closely related to PMV-204-1 by molecular hybridization analysis and serology (4,31). Since PMV-204-1 was serologically grouped (19) with Bos' E-198 isolate of the pea yellow mosaic strain of BYMV (6) and because of the low sequence homology between BYMV-Scott and PMV-204-1, it seemed appropriate to designate -204-1-like isolates as pea mosaic virus (31). However, because PMV-I, a well-characterized pea mosaic virus isolate from Australia (24,25,30,34), was not closely related to PMV-204-1 by molecular hybridization or by direct ELISA, further comparisons are needed to determine the relationships among viruses that cause pea mosaic symptoms.

Reactions in SDS gels roughly paralleled results from direct ELISA, but their interpretation was more difficult because the weak bands and spurs did not occur in all replications. Double antibody sandwich, direct ELISA was used to study serological relations among isolates quantitatively because direct ELISA distinguished among bean yellow mosaic-subgroup viruses better than indirect ELISA (21). Our direct ELISA results illustrated some difficulties with potyvirus identification by serology. A dendrogram (not shown) constructed from results with all five sera (in Table 5) was very similar to that in Figure 2 (after omitting results from CYVV-PQ serum). Serological relations indicated by the CYVV-PQ serum (Fig. 3) were quite different, however, and so it is less useful for virus identification but could be useful for strain identification; Australian and North American strains of CYVV can be distinguished, for example. We infer that serological identification of viruses in the BYMV-subgroup, and possibly of potyviruses in general, requires use of antisera of known performance.

Based on both molecular hybridization and direct ELISA, we conclude that BYMV-subgroup isolates can be subdivided into three viruses that have several strains (BYMV-Scott, PMV-204-1, and CYVV-Pratt typify these viruses here) and one virus for which only one isolate is known (SPMV-Aust.). Groups of isolates or strains delineated by these two methods seem to have similar properties related to pathogenesis (see first paragraph of discussion) although biological properties such as host range are inadequate for identification.

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