Luteovirus Relationships Assessed by cDNA Clones from Barley Yellow Dwarf Viruses

F. A. Fattouh, P. P. Ueng, E. E. Kawata, D. J. Barbara, B. A. Larkins, and R. M. Lister

Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907.
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ABSTRACT

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The results of reciprocal dot blot hybridization tests using genomically mapped cDNAs derived from cultures of P-PAV, MAV-PS1, and NY-RPV, three isolates of barley yellow dwarf virus (BYDV), were consistent with their grouping by serology and other characteristics. Thus, cDNAs from the nonimmunogenic regions of the genomes of P-PAV and MAV-PS1 reacted homologously and also heterologously. MAV-PS1 cDNAs from the nonimmunogenic region also weakly detected a related BYDV type, namely NY-SGV. However, representative cDNAs derived from the immunogenic regions of the MAV-PS1 or P-PAV genomes reacted significantly only to the homologous viruses. None of the MAV-PS1 or P-PAV-derived cDNAs tested detected the NY-RPV isolate although they represented most of the viral genome. Also, no cDNAs derived from any part of the NY-RPV genome detected either MAV-PS1 or P-PAV. In tests of infected leaf samples, cDNAs representative of the MAV-PS1, P-PAV, or NY-RPV genomes did not detect any other luteoviruses tested.

Barley yellow dwarf virus (BYDV) comprises a group of viruses regarded as typifying the luteovirus group (21), which includes other economically important viruses such as beet western yellows virus (BWYV) and potato leaf roll virus (PLRV) (20). Luteoviruses are isometric particles about 25 nm in diameter with a single capsid protein of relative molecular mass about 22 kDa and a positive sense RNA of relative molecular mass about 2×10^6. They are also characterized by being restricted to the phloem of their hosts and are obligately aphid transmitted in a circulative manner (20).

BYDV occurs widely in gramineous hosts as a spectrum of luteoviruses that differ in various ways and comprise at least five distinguishable types or variants. A fundamental criterion separating these types is vector relationship (17). On this basis, five isolates exemplifying the division have been described (17,18): MAV, specifically transmitted by *Macrosiphum (Sitobion) avenae*; RPV, specifically transmitted by *Rhopalosiphum padi*; PAV transmitted by both *S. avenae* and *R. padi*; RMV, specifically transmitted by *R. maidis*; and SGV, specifically transmitted by *Schizaphis graminum*. Based on serological interactions and other criteria, isolates can also be organized into two subgroups, with the representative isolates MAV, PAV, and SGV in "Subgroup 1" and RMV and RPV in "Subgroup 2" (1,6,17,19,20).

Genomic similarities among these viruses, as among the luteoviruses in general, are not yet clear, but information on genomic organization is currently emerging (2,16). In our laboratory, a library of cDNA clones was produced in bacteriophage λgt11 from the RNA of a subculture of an MAV isolate, by random priming with calf thymus DNA (2). Subcloning into the plasmid pUC18 and restriction enzyme mapping and hybridization tests identified overlapping inserts collectively representing at least 85% of the MAV genome (a total of 5.2 kb). Such mapping enables identification of the location of each cDNA within the genome of the virus, so that in hybridization tests of genomic homologies, all parts of the genome can be represented. Here we present further genomic mapping data for this MAV isolate, together with similar maps for PAV and RPV types of BYDV. We also describe the use of mapped cDNAs, representing different parts of these genomes, in hybridization tests to identify relationships between BYDV isolates and other luteoviruses.

MATERIALS AND METHODS

Viruses and cDNAs. Viruses used in this study are listed in Table 1. They included isolates of BYDV, BWYV, PLRV, strawberry mild yellow edge virus (SMYEV), and pea enation mosaic virus (PEMV). Those used for cDNA probe production were subcultures of the MAV and RPV isolates of Rochow (1) and of the Purdue isolate (P-PAV) of Hammond et al (8). For clarity of reference herein, all BYDV isolates obtained from the Cornell collection established by Dr. Rochow are prefixed "NY" (New York). All BYDV cultures were maintained in oats (*Avena sativa* L. 'Clintland-64') in constant environment chambers at 18 C and transferred as required with the appropriate vectors: *S. avenae* for MAV, and *R. padi* for NY-RPV and P-PAV. The cultures were tested regularly by enzyme-linked immunosorbent assay (ELISA) (12) with antisera capable of distinguishing the isolates to ensure that no cross-contamination had occurred. Recent data indicates that our (Purdue) subculture of NY-MAV (distinguished as "MAV-PS1" herein, see Table 1), as used for cDNA production, differs slightly from NY-MAV in its reactions with a panel of monoclonal antibodies (13). The cDNAs used were inserts subcloned in plasmid pUC18 (2). Their locations in the restriction endonuclease maps for each isolate are shown in Figures 1–3.

Production of cDNA clones. Virus purification, RNA extraction, and cDNA clone production were essentially as described by Barbara et al (2). RNA was extracted by dissociation of virus purified according to Hammond et al (8), in either 0.1 M Tris-HCl, pH 9.5, on ice for 2–5 min (4), or in 0.5% sodium dodecyl sulphate and 100 μg/ml proteinase K for 30 min at 37 C. The RNA was then deproteinized by extracting twice with phenol/chloroform and once with chloroform, and was concentrated by precipitation from 66% ethanol. Cloning into the plasmid pUC18 was as described by Vieira and Messing (24).

For MAV-PS1, initial cloning was into the bacteriophage λgt11. Synthesis and cloning of the cDNAs and clone selection were largely as described by Huynh et al (9). For cloning, first strand

cDNA was produced by random priming of the RNA with calf thymus DNA fragments (22), so that most of the genome of each isolate would be represented in its library of clones. After complementary strand synthesis with avian myeloblastosis virus reverse transcriptase (BRL Life Technologies, Inc., Gaithersburg, MD), a second strand of cDNA was produced by using a mixture of RNase H and DNA polymerase I as described by Gubler and Hoffman (7). The cDNA was then treated with S1 nuclease to create blunt ends and methylated to prevent internal restriction enzyme digestion. *Eco*R1 compatible linkers were then ligated to both termini and digested with *Eco*R1. For initial clonings into λgt11, the largest cDNAs were cloned into the unique *Eco*R1 site in this phage (26). This vector was chosen as an initial cloning vehicle because of its high cloning efficiency and its potential

TABLE 1. Sources and hosts of virus isolates used in determinations of relationships by dot blot hybridizations with selected mapped cDNA clones representing the genomes of isolates of barley yellow dwarf virus[a]

Virus isolate	Acronym	Host	Source collection (supplier)
Barley yellow dwarf virus			
-PAV (New York)	NY-PAV	Oat	Cornell Univ. (W. F. Rochow)
-MAV (New York)	NY-MAV	Oat	Cornell Univ. (W. F. Rochow)
-RPV (New York)	NY-RPV	Oat	Cornell Univ. (W. F. Rochow)
-RMV (New York)	NY-RMV	Oat	Cornell Univ. (S. Gray)
-SGV (New York)	NY-SGV	Oat	Cornell Univ. (S. Gray)
-PAV (Indiana)	P-PAV	Oat	Purdue Univ.
-MAV (Purdue subculture)	MAV-PS1[b]	Oat	Purdue Univ.
-RMV (Idaho)	I-RMV	Oat	Univ. of Idaho (S. Halbert)
-SGV (Idaho)	I-SGV	Oat	Univ. of Idaho (S. Halbert)
Potato leaf roll virus			
-(C and D)	...	*Physalis floridana* Rydb.	Univ. of Wisconsin (S. Slack)
-(NZ 1 and 2)	...	Potato	Lincoln College, New Zealand (G. Webby)
-(Martin)	...	*P. floridana*	Agriculture Canada (R. Martin)
Beet western yellows virus			
-(81-50 and 81-54)	...	*Capsella bursa-pastoris* (L.) Med.	Univ. of California (B. Falk)
-(NZ-2)	...	*Crambe abyssinica* Hoscht	Lincoln College, New Zealand (G. Webby)
-(Martin)	...	*P. floridana*	Agriculture Canada (R. Martin)
Strawberry mild yellow edge virus	...	Strawberry	Univ. of California (B. Falk)
Pea enation mosaic virus	...	Pea	Univ. of Wisconsin (S. Slack)

[a]Virus isolates NY-MAV, MAV-PS1, P-PAV, and NY-RPV were routinely subcultured in our laboratory by mass transfer of *Sitobion avenae* or *Rhopalosiphum padi* (see Methods). The corresponding purified virus preparations were made by chloroform clarification of sap extracts, followed by concentration with polyethylene glycol, and separation on rate-zonal sucrose density gradients (8). All other virus isolates and control samples were kindly supplied in the form of infected or noninfected plant leaf, respectively.
[b]MAV-PS1 is a subculture of NY-MAV, derived at Purdue University, that shows serological differences from NY-MAV (13).

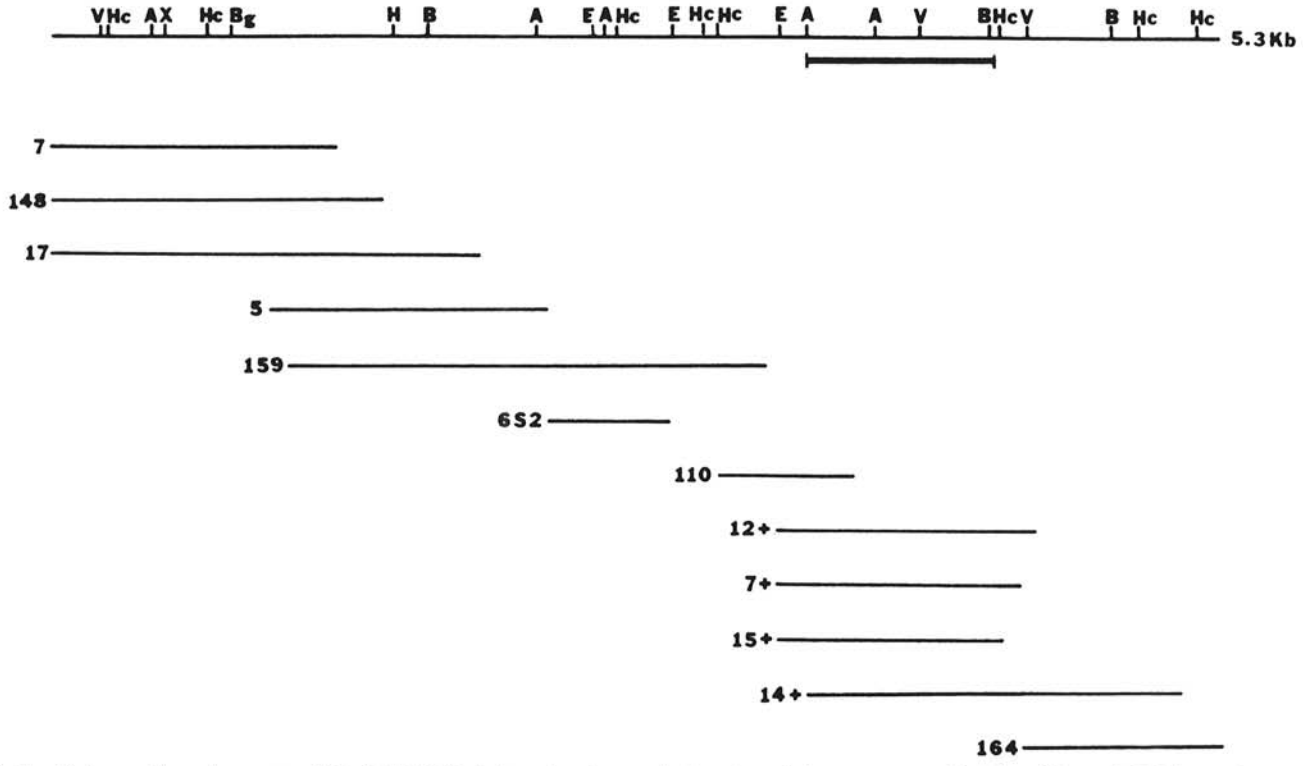

Fig. 1. Restriction endonuclease map of the MAV-PS1 isolate showing restriction sites of the enzymes used and positions of cDNAs used as probes in dot blot hybridization experiments. Enzymes used were as follows: A = *Acc*I; B = *Bam*HI; E = *Eco*RI; Bg = *Bgl*II; Hc = *Hin*cII; H = *Hin*dIII; V = *Eco*RV; X = *Xba*I. ⊢—⊣ = immunogenic regions determined as described in the text.

as an expression vector, which allowed clones containing those sequences coding for viral antigens ("+" clones) to be identified serologically (2). Selected inserts prepared from plate stocks of λgt11 (13) were subcloned into pUC18 (24) for further study and use as hybridization probes.

Clones for the genomes of both P-PAV and NY-RPV were synthesized by the same procedures used with MAV-PS1; however, in these cases libraries were constructed in both λgt11 and pUC18. Lambda clones that reacted with virus antisera (polyclonal antisera made in rabbits to virus preparations) were used as probes to identify the corresponding sequences in the pUC18 libraries. Restriction enzyme maps were deduced for each genome (Figs. 1–3), based on sites determined from single and double restriction enzyme digests and by cross-hybridization of clones.

Hybridization probes were made by nick translation of plasmid DNA (14), usually to a specific activity of about 2×10^8 cpm/μg DNA. The prefixes pPP, pMP, and pRP (from the initial letters of the virus acronyms and Purdue) refer to plasmid DNAs derived from P-PAV, MAV-PS1, and NY-RPV, respectively. For hybridizations to purified virus, a preparation equivalent to 2×10^6 cpm of probe was used, whereas for hybridizations to plant tissue extracts, 5×10^6 cpm of the probe preparation was used.

Sample preparation and dot blot hybridization. Purified virus samples (of concentrations estimated spectrophotometrically), tRNA, and pUC18 plasmid DNA, were diluted to 1 μg/ml in 0.1 M sodium phosphate, pH 6.5, and a series of twofold dilutions for each was prepared in the phosphate buffer, as required. Healthy and infected oat leaf samples were usually stored at −20 C. To prepare aqueous extracts, the tissue was pulverized to powder in a mortar in liquid nitrogen, then similarly ground in 0.1 M sodium phosphate buffer, pH 6.5, at 1 g of tissue per 2 ml of buffer. The extracts were either directly expressed through cheesecloth and briefly clarified by a 2-min centrifugation in a microfuge, or, more commonly, clarified by shaking with an equal volume of chloroform before centrifugation to separate the aqueous phase. Chloroform clarification and dilution both usually improved reaction signals (11), presumably by removing or diluting proteinaceous material interfering with RNA-cDNA probe interactions. A series of twofold dilutions was then prepared in phosphate buffer.

To prepare nucleic acid extracts, tissue powder was ground in 50 mM Tris-HCl, pH 7.4, containing 2% SDS, at a 2:1 ratio

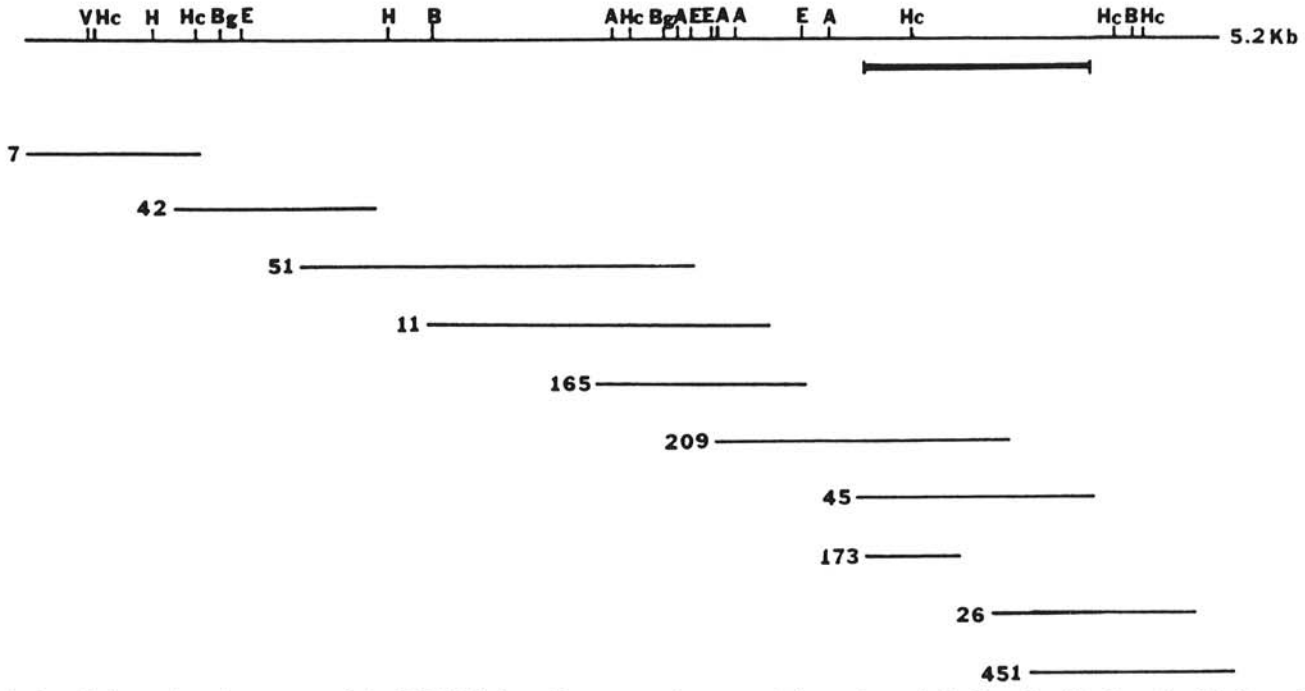

Fig. 2. Restriction endonuclease map of the P-PAV isolate. Enzymes used were as follows: A = *Acc*I; B = *Bam*HI; E = *Eco*RI; Bg = *Bgl*II; Hc = *Hin*cII; H = *Hin*dIII; V = *Eco*RV; X = *Xba*I. ⊢—⊣ = immunogenic regions determined as described in the text.

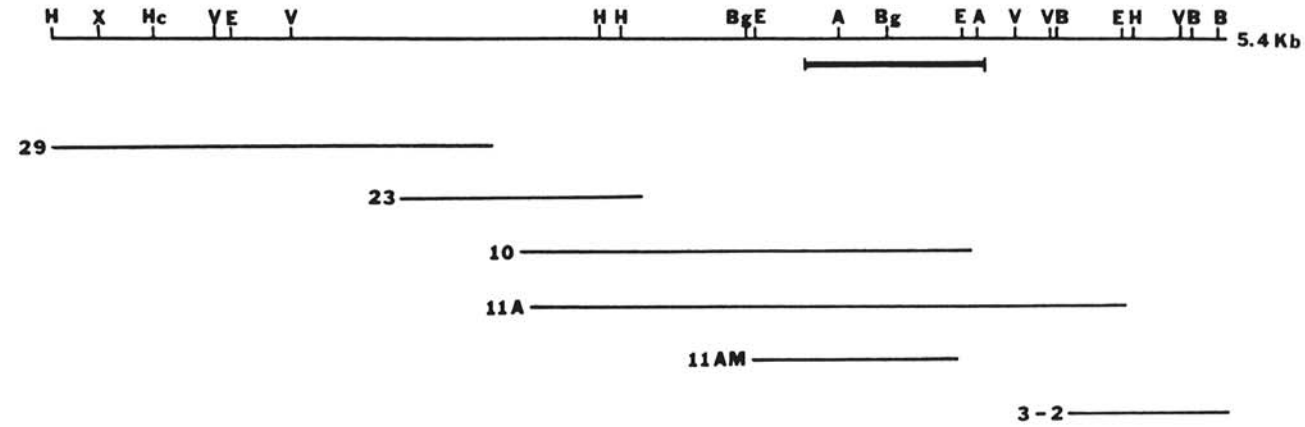

Fig. 3. Restriction endonuclease map of the NY-RPV isolate. Enzymes used were as follows: A = *Acc*I; B = *Bam*HI; E = *Eco*RI; Bg = *Bgl*II; Hc = *Hin*cII; H = *Hin*dIII; V = *Eco*RV; X = *Xba*I. ⊢—⊣ = immunogenic regions determined as described in the text.

(1 g of leaf per 2 ml of buffer). An equal volume of water-saturated phenol was added, and the mixture agitated for 15 min. After centrifugation at 4,000 *g* for 15 min at 4 C, the aqueous phase was collected. Each sample was then shaken with an equal volume of chloroform/amylalcohol (24:1, v/v) and centrifuged for 15 min as above, removing the aqueous phase. Nucleic acid was precipitated by adding a one-tenth volume of 3 M sodium acetate followed by 2.5 volumes of chilled ethanol, and then incubating in an ice bath for 20 min before centrifugation at 4,000 *g* for 30 min at 4 C. Pellets were washed in 70% ethanol, dried in a stream of air, resuspended in 0.1 M EDTA (100 μg/g starting material), and stored at −20 C until used. To denature nucleic acid, 10 μl of sample made as above was mixed with 30 μl of formaldehyde (37% formaldehyde, w/v, plus an equal volume of 20× SSC), incubated at 65 C for 15 min, and cooled in an ice bath. (1× SSC is 0.015 M sodium citrate, 0.15 M sodium chloride).

Aqueous samples of 50 μl each, or their equivalent of nucleic acid extracts, were applied with the aid of a Hybri-Dot manifold (BRL) onto a nitrocellulose membrane filter that had been wetted in distilled water followed by 2× SSC. After sample application, all wells were washed with 200 μl of 0.1 M sodium phosphate, pH 6.5. The nitrocellulose filters were then placed between two sheets of 3-mm Whatman filter paper and baked for 2 hr at 80 C under vacuum.

Prehybridization was for 20 hr at 68 C, as described by Barbara et al (2), in 5× SSC, 50 mM sodium phosphate, pH 6.5, 250 μg/ml calf thymus DNA, 0.1% SDS, and 5× Denhardt's solution, in a heat-sealable polyethylene bag with a buffer amount of 0.1 ml/cm^2 per filter. Prehybridized blots containing samples of purified virus were hybridized for 24 hr at 68 C in 5× SSC, 20 mM sodium phosphate, pH 6.5, 100 μl/ml calf thymus DNA, 0.1% SDS, 1× Denhardt's solution, and 5% dextran sulfate (2). They were washed twice at room temperature for 10 min and twice at 68 C for 10 min, in 1× SSC, 0.1% SDS. Blots were then exposed to X-ray film at −80 C for 1–4 days (usually 1 day) with intensifying screens. Where leaf tissue extracts were used, hybridization conditions were essentially the same except that the hybridization buffer contained 1× SSC instead of 5× SSC, and the blots were washed twice for 20 min at room temperature and then twice at 68 C for 30 min in 0.1× SSC, 0.1% SDS. Exposure of these blots to the X-ray film at −80 C was for 1–4 days with intensifying screens. For comparative purposes, some hybridizations (see Results section) were also done in 50% formamide at 42 C, as used by Waterhouse et al (25).

RESULTS

Hybridization reactions of probes with BYDV isolates. Examples of the results of dot blot hybridizations of BYDV probes

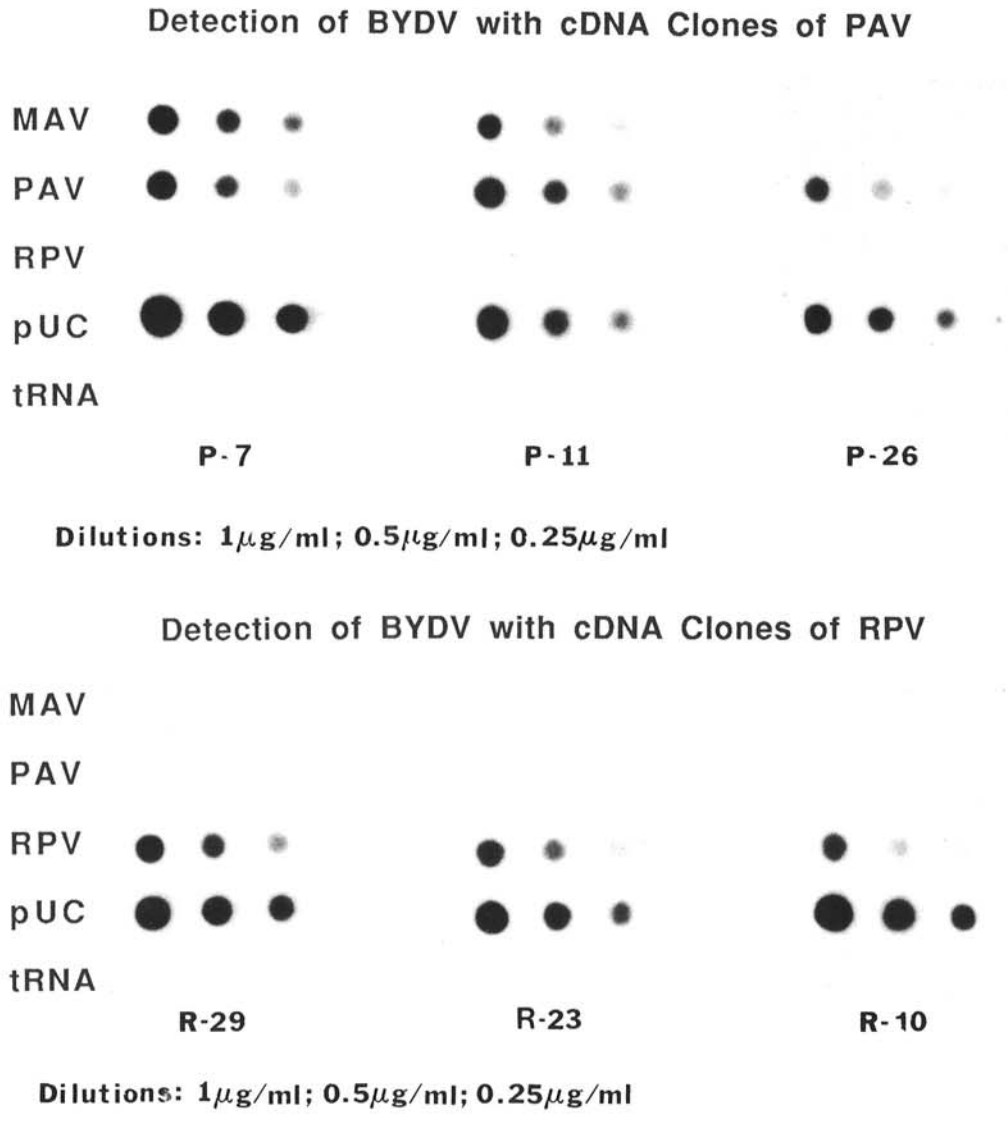

Fig. 4. Results of dot blot hybridizations with representative cDNA probes and preparations indicated. Top, probes P-7, P-11, and P-26 of P-PAV (PAV) with MAV-PS1 (MAV), P-PAV, NY-RPV (RPV), pUC18 (pUC), and tRNA, at 1 μg/ml, 0.5 μg/ml, and 0.25 μg/ml. Bottom, probes R-29, R-23, and R-10 of NY-RPV with the same preparations.

with homologous and heterologous BYDV isolates are presented in Figure 4, and these experiments are summarized overall in Table 2. As in earlier work (2), the sensitivity of detection was proportional to the size of the cDNA insert for all probes tested, under equivalent conditions, and sensitivity was at best to a dilution end point of 1/256 of sap extract, and to about 1.4 μg/ml of virus (70 pg in a 50-μl sample). Though hybridization reaction signals were stronger with purified virus preparations, the indications of cross-hybridization reactions in tests with them were in complete agreement with the results for chloroform-clarified plant extracts.

Regardless of whether purified virus or chloroform-clarified extracts were used, all dot blot hybridizations with probes representing the genomes of the MAV-PS1 and P-PAV isolates (both in Subgroup 1) agreed in indicating a high degree of conservation among genomic regions other than that encoding immunogenicity. Thus, probes pMP 7, 17, 5, and 6s2, and probes pPP 7, 42, 51, 11, and 165, each representing sequences 5′ of the immunogenic regions of the respective genomes (Figs. 1 and 2), readily detected the heterologous virus as well as homologous virus. In contrast, however, tests with probes representing the immunogenic regions of these viral genomes showed a high degree of specificity and hybridized readily with the homologous virus but far less readily, if at all, with the heterologous virus.

Probes pMP 6s2 and pMP 5 were mapped to central regions and toward the 5′-end of the genome, respectively (Fig. 1). They hybridized to the NY-SGV isolate (also in Subgroup 1), though very weakly. This was not the case with probes pMP 12+, or 15+, which mapped as including immunogenic regions. No P-PAV or MAV-PS1 probes tested hybridized to either the Subgroup 2 isolate NY-RPV (as pure virus or chloroform-clarified extracts) or to the RMV isolates (as chloroform-clarified extracts), also classified in Subgroup 2. Similarly, no NY-RPV probe tested hybridized to purified virus or chloroform-clarified extracts of P-PAV, MAV-PS1, or to chloroform-clarified extracts of SGV isolates. No probes derived from any portion of the NY-RPV genome detected RMV in chloroform-clarified leaf extracts.

Probe specificity under various conditions. Specificity was not affected with probes pRP 29, 23, 10, and 11A when representative hybridizations were attempted at 42 C using 50% formamide and 5× SSC in the hybridization buffer and no dextran sulfate (e.g., 25). All these probes hybridized only to the homologous NY-RPV and not to the MAV-PS1 or P-PAV isolates, whether tested as chloroform-clarified extracts or purified virus. Indeed, the intensity of the signals was much reduced as compared to that obtained when hybridization was performed at 68 C in aqueous media (Fig. 5).

In the same set of experiments, total nucleic acid preparations and denatured preparations from infected leaf were also probed. In this case, formaldehyde-denaturation of the preparations apparently enhanced hybridization in aqueous media. However, specificity was maintained, except for what seemed likely to be background reactions, which did not occur with purified virus preparations or with extracts from noninfected tissue (Fig. 5). Although these signals may have indicated a degree of sequence homology, similar ones occurred in several experiments involving hybridization tests of probes with nucleic acid preparations from infected leaf, and sometimes even with such preparations from noninfected leaf. For example, in one experiment, when the hybridization specificity of probes pRP 29, pMP 5, and pPP 51 was checked with nucleic acid preparations from oat leaf infected with MAV-PS1, P-PAV, NY-RPV, NY-SGV, or NY-RMV, some nonspecific background reactions occurred with preparations from both infected and noninfected sources. However, such reactions did not occur when standard precautions against RNase degradation were strictly followed, including heating glassware and treating all buffers, reagents, and glassware with diethyl pyrocarbonate solution (3). When this was done, the results corresponded to those for chloroform-clarified extracts not treated with phenol. The NY-RPV probe pRP 29 hybridized only homologously; both P-PAV and MAV-PS1 probes (pPP 51 and pMP 5) hybridized to MAV-PS1 and P-PAV but not to NY-RPV or RMV preparations. A very faint signal to the NY-SGV preparation was detected with the MAV probe pMP 5.

Hybridizations of probes with other viruses. Dot blot hybridizations were attempted with selected cDNAs and chloroform-clarified preparations from leaf samples infected with a variety of luteoviruses or with PEMV, as provided by various colleagues (Table 1). PEMV was included as it is luteoviruslike in some respects, though not presently classified as such. The cDNA probes used in these experiments were representative of the immunogenic and nonimmunogenic regions of the MAV-PS1, P-PAV, and NY-RPV isolates (i.e., pMP 7+, pMP 5, pPP 26, pPP 51, pRP 11 AM, pRP 23, and pRP 29; Figs. 1–3). Each test was done at least twice, and the BYDV isolates were included for comparison. Apart from the cross-hybridizations within BYDV listed in Table 2, no hybridizations were detected in these experiments other than with the appropriate positive control samples, which reacted at least as strongly as those in Figure 4.

TABLE 2. Results of dot blot hybridizations of barley yellow dwarf virus (BYDV) isolates with BYDV cDNA clones as hybridization probes

	Clone no.[b]	Size (kb)	Hybridization with isolates indicated[a]				
			MAV-PS1	P-PAV	NY-RPV	"SGV"[c]	"RMV"[c]
MAV-PS1	5	1.4	+	+	−	tr[d]	−
	7	1.3	+	+	−	−	−
	6s2	0.3	+	+	−	tr[d]	−
	17	2.2	+	+	−	0	0
	12+	1.8	+	tr	−	−	−
	15+	1.6	+	tr	−	−	−
	7+	1.2	+	tr	−	0	0
P-PAV	7	0.8	+	+	−	0	0
	42	1.2	+	+	−	0	0
	51	1.3	+	+	−	0	0
	11	2.2	+	+	−	0	0
	165	0.5	+	+	−	0	0
	45	1.3	−	+	−	0	0
	26	1.2	−	+	−	0	0
	173	0.8	−	+	−	0	0
NY-RPV	29	2.3	−	−	+	−	−
	23	1.4	−	−	+	−	−
	10	2.3	−	−	+	−	−
	11A	2.5	−	−	+	0	0
	11AM	1.0	−	−	+	0	0

[a] + = positive reaction, − = no reaction, tr = trace reaction, and 0 = no test was done.
[b] For genomic locations, see maps in Figures 1–3.
[c] "SGV" includes NY-SGV and I-SGV, and "RMV" includes NY-RMV and I-RMV (Table 1).
[d] Reactions were obtained with NY-SGV, and not with I-SGV.

DISCUSSION

The availability of mapped clones from cDNA libraries to the P-PAV, MAV-PS1, and NY-RPV genomes allowed us to conduct dot blot hybridization analyses to compare the genomes of these and other isolates of BYDV in relation to homologies within specific genomic regions. Similarly, we have also examined BYDV relatedness with some other viruses. Such molecular hybridizations can provide information relevant to the classification and interrelationships of viruses.

Thus, from the results of the dot blot hybridizations with either the MAV-PS1 or the P-PAV probes, it is clear that those derived from the immunogenic region of the genome showed a high degree of specificity, and hybridize readily to the homologous virus, but far less readily, if at all, with the heterologous virus. This evidence of heterogeneity between genomic regions encoding immunogenicity is consistent with the serological differences between these isolates (17,19,20). In contrast, probes derived from the nonantigenic region of their genomes hybridized heterologously in reciprocal tests between the MAV-PS1 and P-PAV isolates. This finding is in accord with similarities in other characteristics of such isolates. Both the MAV and PAV of Rochow are similar enough in several respects to be classified in Subgroup 1 of BYDV

(6,19). The hybridization results indicate that P-PAV and MAV-PS1 represent closely related BYDV types that probably differ more significantly in capsid properties than in other respects. SGV is also classified in BYDV Subgroup 1, and cross-reacts with MAV antisera but not with PAV antisera (20). Thus, it was of special interest that in our experiments two of the MAV-PS1 probes, pMP 5 and pMP 6s2, detected the NY-SGV isolate consistently, but weakly. These results suggest that the SGV type differs more significantly from the MAV and PAV types than these do from each other and that the genomic similarity between

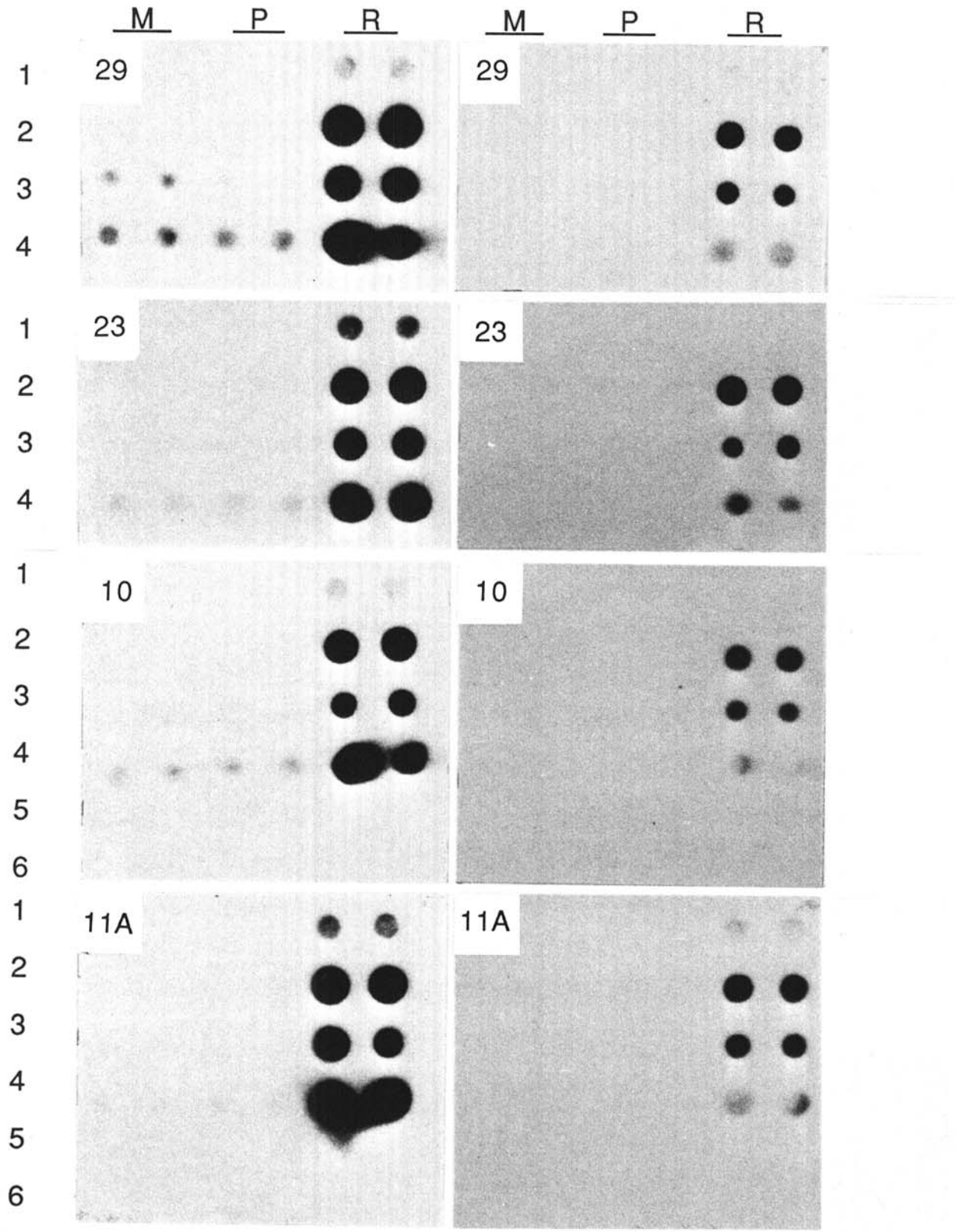

Fig. 5. Hybridization of representative NY-RPV cDNA clones (numbered as in Fig. 3) when used as probes for the MAV-PS1 (M), P-PAV (P), and NY-RPV (R) isolates of barley yellow dwarf virus. Left panels = hybridizations at 68 C under aqueous conditions; right panels are hybridizations of identical reagents at 42 C under nonaqueous (formamide) conditions. Preparations probed were, by rows: 1, chloroform-clarified extract from infected leaf; 2, purified virus at 0.1 mg/ml; 3, nucleic acid extract from infected leaf; 4, formaldehyde-denatured nucleic acid extract from infected leaf; 5, chloroform-clarified extract from noninfected leaf; 6, nucleic acid extract from noninfected leaf. Virus isolates and clone numbers are indicated.

MAV and SGV indicated by their serological relationship is insufficient to allow efficient hybridization in the conditions used here.

The RMV and RPV types represent a second subgroup of BYDV (6,19). In our experiments, no P-PAV or MAV-PS1 probes hybridized to the RMV or RPV isolates used, indicating no significant sequence homology between the MAV-PS1 and P-PAV genomes and the genomes of the members belonging to BYDV Subgroup 2. These findings are consistent with the degree of difference in other properties between members of BYDV Subgroups 1 and 2.

Although RMV has been reported to show serological relatedness to RPV (19), no probes derived from any portion of the NY-RPV genome detected RMV in our experiments. However, the serological relationship reported was weak, and in reciprocal DAS ELISA tests (12) conducted in our laboratory with polyclonal antisera prepared to NY-RPV and NY-RMV, no cross-reactions were observed.

Summarizing, the results indicate that the NY-RPV genome has less sequence homology with those of MAV-PS1 or P-PAV than these do with each other. In fact, these results are in agreement with sequencing analysis of the three viral genomes ongoing in this laboratory. Further, the findings for MAV-PS1 probe activity are in agreement with those of Barbara et al (2). However, they differ from those reported by Waterhouse et al (25), who found that probes from a PAV-like isolate from Australia reacted heterologously with an RPV-like isolate, and were, moreover, capable of detecting other luteoviruses. This disparity is unlikely to result from the differing conditions used for hybridization in our experiments as compared to those used by Waterhouse et al (25) (i.e., 68 C in an aqueous buffer environment versus 42 C in 50% formamide), for the hybridization specificity of the NY-RPV probes in the two environments was not affected. In both sets of conditions, the probes reacted only to the homologous viruses, although the reactions in the aqueous conditions gave stronger signals. The finding of similar hybridization specificity but differing sensitivity in these two conditions of stringency is in agreement with observations reported by Maule et al (15).

Although Waterhouse et al (25) reported "broad range probes" reacting with luteoviruses other than their PAV-like isolate, use of representative MAV-PS1, P-PAV, and NY-RPV clones from our libraries as probes did not detect any of the other viruses tested, including various isolates of BWYV, PLRV, SMYEV, and PEMV (Table 1). Perhaps the PAV-like isolate of Waterhouse et al (25) differs significantly in nucleotide sequence from our P-PAV isolate. Conceivably, the parts of the MAV, PAV, or RPV genomes not represented in the cDNA probes we used may show sequence homology with other luteoviruses. However, these can represent only about 14–18% of the MAV-PS1 and P-PAV genomes, and 10% of the NY-RPV genome. In this regard, it is of interest that results of Valverde et al (23) are in accord with ours. These workers found that one of the Waterhouse et al (25) probes reported to detect BWYV and PLRV hybridized only to MAV and PAV, but not to other luteoviruses.

Our data are substantiated by internal consistencies, by consistency with accepted relationships among the luteoviruses, and by our ability, through mapping, to examine hybridization behavior for cDNAs representing defined parts of each viral genome examined. However, care is needed in interpreting dot blot hybridizations. For example, our results with nucleic acid preparations from infected leaf indicate that their use for testing relationships requires precautions to reduce nuclease activity. Many other factors also affect dot blot hybridization results (e.g., 10). At best, such tests only indicate whether complementarities between probes and viral RNA are adequate for successful hybridizations under the conditions used. It remains possible that probes comprising certain sequences might cross-hybridize under appropriate conditions. This possibility is supported by recent indications of protein and nucleic acid homologies between isolates in Subgroups 1 and 2 in immunological (5) and nucleotide sequencing studies (J. R. Vincent, *personal communication*), respectively. Definitive answers on the question of luteovirus interrelationships at the genomic level must obviously await detailed comparisons of nucleotide sequence analyses of various BYDV isolates and other luteoviruses.

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