Parallel Identification of Five Luteoviruses That Cause Barley Yellow Dwarf

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ABSTRACT

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A modified, indirect enzyme-linked immunosorbent assay (EIA) was tested for SGV to permit simultaneous use with direct EIA for four other luteoviruses (RPV, RMV, PAV, and MAV) that also cause barley yellow dwarf. Specificity and reliability of the method were shown during 15 mo in tests of both clarified and purified preparations of the five viruses and of samples of grains and grasses from the field. The comparative assay was especially useful in study of five SGV-like viruses from Idaho that had a range of biological properties. For the sixth consecutive year, most samples collected in New York contained viruses similar to PAV, and none had virus similar to MAV.

Additional key words: aphid vectors, monoclonal antibodies

For some years, we have identified luteoviruses of small grains by using four antisera in direct enzyme-linked immunosorbent assays (EIA). This procedure provided a homologous test for four of the viruses we encounter most often in field samples. We have identified a fifth virus, SGV, transmitted specifically by the greenbug (*Schizaphis graminum* (Rondani)), serologically only by weak heterologous reactions in tests with immunoglobulins against PAV and MAV, two viruses related to SGV (5,6,12). This procedure has usually been satisfactory, especially when parallel aphid transmission tests were also done (8,10,13), but we have been seeking a homologous serological test for SGV to make our procedures more complete and to permit parallel assays in one EIA plate for all five of the viruses we study.

On the basis of a range of properties, the five viruses fall into two rather distinct groups: RPV and RMV in one and PAV, MAV, and SGV in the other (5). We refer to these viruses here and elsewhere as luteoviruses or as isolates of barley yellow dwarf virus (BYDV) for reasons previously discussed (7). We think these designations are the most simple, straightforward way to identify the viruses at present and to recognize the unsettled, developing state of plant virus nomenclature at this level. When relationships among more luteoviruses are better understood, improved designations may evolve (1).

Recent production of an antiserum against SGV by use of hen eggs (5) provided useful immunoglobulins for indirect EIA of SGV, but the immunoglobulins were not active enough in the coating step of direct EIA for routine use. Now we have used a modified, indirect EIA for SGV, based on use of combinations of antibodies, that permits parallel tests for five luteoviruses commonly found in small grains. This paper describes the modified, indirect EIA for SGV and shows its reliability and specificity in tests of clarified and purified preparations of the five luteoviruses, in assays of samples from Idaho in the fall of 1984, and in tests of field samples collected in New York in 1985 or sent from other locations.

MATERIALS AND METHODS

All EIA were carried out according to the 2-day schedule previously described for parallel tests with four immunoglobulins in direct (double-sandwich) tests with virus-specific immunoglobulins from rabbit (7,8). We used Immulon I polystyrene substrate plates (Dynatech Laboratories Inc., Chantilly, VA) with 100 µl of liquid per round-bottom well. The coating step (immunoglobulin at 10 µg/ml) was at 37 C for about 6 hr, antigens were incubated at 4 C overnight, conjugated antibodies (diluted from 1:800 to 1:3,200) were incubated at 37 C for about 5 hr, and the alkaline phosphatase-mediated production of *p*-nitrophenol was measured colorimetrically after 45 min at room temperature with a Dynatech model MR-580 Microelisa Reader at 405 nm.

In the modified, indirect EIA for SGV, we coated with mAb-MAV4 (diluted 1:1,000), a monoclonal antibody that reacts with SGV and MAV but not with the other viruses tested (4). The special feature of the test was incubation of trapped virus with a mixture of anti-SGV immunoglobulin precipitated from hen egg yolks (final concentration of 2 µg/ml) together with a commercial conjugate of antichicken IgG (Kirkegaard and Perry Laboratories, Gaithersburg, MD) diluted 1:335 (5). This mixture was used in parallel with conjugates for each of the four other immunoglobulins. A reaction was usually considered positive only if the absorbance was at least 0.1, a value within the visible range. This detection threshold was much higher than the twice-background range of "healthy" controls often used in such tests. Compared with previous tests and other lots of immunoglobulin, background readings for anti-RMV conjugate (up to 0.04) were often higher than usual (8–10).

Biological identifications were made in transmission tests with four aphid species: *Rhopalosiphum padi* (L.), *R. maidis* (Fitch), *Sitobion* (= *Macrosiphum*) *avenae* (Fabricius), and *Schizaphis graminum*. Tests were based on a 2-day acquisition feeding and a 5-day inoculation test feeding as previously described (8,10). The test plant was Coast Black oats (*Avena byzantina* K. Koch). In every test, some aphids fed on healthy tissue for use as controls.

In both biological and serological tests, viruses recovered from the field-collected samples were compared with five characterized isolates of BYDV (7,9,11,12): RPV, transmitted specifically by *R. padi*; RMV, transmitted specifically by *R. maidis*; MAV, transmitted specifically by *S. avenae*; SGV, transmitted specifically by *S. graminum*; and PAV, transmitted nonspecifically by *R. padi*

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and *S. avenae*. *S. graminum* also transmits RPV and PAV but less consistently than does *R. padi*. Additional aphid transmission tests were often made of infected test plants, because initial virus transmission results from a field sample are not usually adequate for thorough identification of these viruses (9,10). In most of the additional tests, EIA was again done in parallel with aphid transmission tests.

The 30 samples collected in New York in July 1985 that included oats, wheat, and barley were from test plots maintained by M. E. Sorrells in Tompkins County near Ithaca. The seven samples collected in Idaho in October 1984 were mostly wheat (one barley) from different locations in the Rockland Valley, where large populations of *R. padi* and *S. graminum* were observed during and before the time of collection. Each sample was handled as previously described (8,13). Usually, adjacent leaves were used in the aphid transmission test; the remaining tissue was used to prepare 3 g of finely chopped sample for extraction with a PT-20 probe of a Polytron Homogenizer (Brinkmann Instruments, Westbury, NY). All samples for EIA were clarified with chloroform (12). Partially purified preparations of all five characterized BYDV viruses were also used in these studies as controls (11).

Other samples, sent by various cooperators, included a range of small grains and grasses in conditions that varied from fresh and turgid through partly rotted to freeze-dried. These were tested only by EIA.

RESULTS

During development of the modified, indirect test for SGV we evaluated factors that affected its use. When we compared mAb-MAV4 and anti-MAV immunoglobulins from rabbits in the coating step, we found the monoclonal antibody two to three times more effective than the polyclonal ones for SGV; the polyclonal antibodies were slightly more sensitive for MAV. In all other tests, we used mAb-MAV4 for coating. We also compared various proportions of the antibodies used in reactions with trapped virus. Final reactions had about a twofold difference over a range of onefold to threefold proportions of the two components. The amount of conjugated antichicken antibody appeared to be more important than the amount of the anti-SGV immunoglobulin. In all other tests, we used amounts of each reactant described. Use of crude anti-SGV immunoglobulin in the mixture, in place of precipitated immunoglobulin (5), was not acceptable because of high background readings.

Our preliminary tests showed, as expected, that MAV also reacted in the modified, indirect test for SGV. This presented no problem because the parallel reaction with anti-MAV immunoglobulins allowed clear separation of MAV and SGV. In fact, this MAV reaction was an advantage because it provided another confirming test for MAV. Mixtures of MAV and SGV, however, would be difficult to identify in these combinations of tests. We have identified such mixtures by first absorbing a portion of the sample with a monoclonal antibody (mAb-MAV1) that reacts only with MAV (4) before use in the EIA.

In many tests during a period of about a year, we assayed all five luteoviruses in clarified preparations to evaluate specificity and reproducibility of the parallel assays. Although absorbance values in tests for SGV were usually lower than those of homologous tests for the other viruses, reactions were clear and consistent (Table 1). Results of the five tests summarized in Table 1 also show how reactions in tests for MAV allow distinction between SGV and MAV. In addition to tests of clarified preparations made from fresh tissue, as shown in Table 1, we obtained similar results in tests of various partially purified preparations of the five viruses and in tests of clarified preparations made from tissue previously dried over desiccant at 4 C and stored at about −20 C.

Reliability of the modified, indirect test for SGV was observed especially in assays of wheat and barley from Idaho (Table 2). In initial tests of seven samples, made only by EIA, four samples were found to contain SGV-like virus. When we retested all seven samples both by EIA and aphid transmission, we found an additional sample with SGV-like virus. The small size of plants made it impossible for us to use a single plant for both kinds of tests. No virus was detected in two of the wheat samples. Because initial aphid transmission tests suggested that some of these SGV-like viruses had vector specificities different from SGV, we made additional tests, by both methods, of plants from serial transmissions during a 15-mo period. In each of 73 EIA tests for the five viruses, the SGV-like nature of these isolates was confirmed (Table 2). Some of the five isolates appeared to differ in their vector specificity, a variation noted by Gill for similar isolates from Manitoba in 1969 (3). All were transmitted most efficiently by instars of *S. graminum* (Table 2). In tests of isolates 1 and 3, one or two transmissions by *R. padi* and/or *S. avenae* also occurred in almost all of the eight serial passages. Transmission by these two vectors was rare in tests of the other three isolates, a pattern more like that of SGV (6,12), which was studied in parallel as a control. Isolate 5 consistently caused more severe symptoms than did the others. No transmission of any of these isolates occurred in tests with *R. maidis*. Despite apparent biological

Table 1. Specificity of enzyme-linked immunosorbent assays (EIA) for five cereal luteoviruses in clarified preparations

Virus	Mean absorbance at 405 nm from five tests with immunoglobulin shown[a]				
	RPV	MAV	PAV	RMV	SGV
RPV	0.504	0.010	0.007	0.034	0.014
MAV	0.007	0.727	0.092	0.024	0.288
PAV	0.012	0.167	0.680	0.027	0.010
RMV	0.014	0.013	0.014	0.232	0.007
SGV	0.011	0.044	0.049	0.029	0.259
Healthy	0.011	0.012	0.011	0.026	0.006

[a] All tests were direct EIA with immunoglobulin shown, except for SGV, which was done in the modified, indirect assay described in the text. Only values ≥0.1 were usually considered positive. Coast Black oats were used in all tests.

Table 2. Results from eight serial transmissions of five SGV-like isolates assayed by serological and biological methods

Idaho isolate and controls	Mean absorbance at 405 nm in eight tests with antiserum for isolate shown[a]					Transmission in tests with aphid species shown[b]		
	RPV	MAV	PAV	RMV	SGV	*Rhopalosiphum padi*	*Sitobion avenae*	*Schizaphis graminum*
1	0.011	0.079	0.071	0.039	0.537	9/24	10/24	23/24
2	0.012	0.087	0.085	0.052	0.410	2/24	2/24	21/24
3	0.012	0.063	0.055	0.043	0.349	8/24	8/24	24/24
4	0.013	0.084	0.077	0.040	0.446	1/24	4/24	22/24
5	0.010	0.056	0.049	0.054	0.229	0/24	1/24	19/24
SGV	0.012	0.046	0.048	0.042	0.204	0/24	1/24	20/24
Healthy	0.009	0.014	0.011	0.039	0.016	0/24	0/24	0/24
50 ng virus	0.573	0.784	0.743	0.267	0.245	...	...	...

[a] All tests were direct enzyme-linked immunosorbent assays with immunoglobulin shown, except for SGV, which was done in the modified, indirect assay described in text. Virus controls were dilutions of preparations of homologous virus. Coast Black oats were used in all cases. Only values ≥0.1 were usually considered positive.
[b] Number of Coast Black oat plants that became infected per number infested with about 10 aphids for a 5-day inoculation test feeding after an acquisition feeding for 2 days on detached leaves at 15 C.

differences among these SGV-like isolates, all reacted similarly in the new EIA for SGV (Table 2).

We also made comparative five-way tests with SGV and another SGV-like isolate obtained in 1978 from Idaho (2). We kept this 1978 isolate, because it consistently gave higher readings than SGV in EIA tests with MAV- and PAV-immunoglobulins. In one series of six comparisons, for example, mean values for tests with MAV-immunoglobulins were 0.045 and 0.088, respectively, for SGV and the 1978 isolate. Corresponding values for tests with PAV-immunoglobulins were 0.045 and 0.089, respectively. We have noticed a similar difference in reactions for the modified, indirect test for SGV; mean values in the six tests were 0.204 for SGV and 0.387 for the 1978 Idaho isolate, but again, the EIA for SGV always gave clear results.

During 1985, we made parallel tests for the five viruses in field samples collected in New York. No viruses similar to MAV or SGV were detected in any of 30 samples assayed both by EIA and aphid transmission tests (Table 3). Of the 28 virus isolates identified from 26 plants, 22 were similar to PAV, four were similar to RPV, and two resembled RMV. Two of the plants were infected by a mixture of viruses, a finding confirmed by additional serological and aphid transmission tests. These two cases were detected by EIA but not by aphids (Table 3, groups E and F). The RMV-like isolate (Table 3, group D) may also have occurred in a mixed infection with PAV-like virus, but we prefer the conservative conclusion because only RMV-like virus was detected in subsequent tests of the two infected test plants. None of the four samples negative for BYDV luteovirus (Table 3, group B) had symptoms typical of barley yellow dwarf. Two of them were wheat with symptoms of wheat spindle streak mosaic (15).

These data show that for the sixth consecutive year, no MAV-like virus was detected in samples from New York (10,13). Virus similar to PAV again was the most common. This pattern contrasts sharply with that of earlier years (1957 through 1963), when MAV-like virus predominated. One of the PAV-like viruses (Table 3, group A) was transmitted from the field sample by *S. avenae* but not by *R. padi*, a pattern associated with MAV. Thus, the isolate could have been identified incorrectly as similar to MAV if we had not made additional aphid transmission tests and if we had lacked parallel EIA data. Isolates similar to SGV have always been rare in New York (6,9). Use of the modified, indirect assay for SGV provided additional evidence for this rarity and showed that the tests with a range of field-collected material did not produce false positives. Parallel work with aphids and with known amounts of virus (50 ng/well) also confirmed reliability of the new test for SGV (Tables 2 and 3).

We made additional tests only by EIA for all five viruses on 123 samples of various grains and grasses from five states and four foreign countries. Forty-five of the samples were found infected with viruses similar to RPV, RMV, MAV, or PAV alone or in mixed infections. Only one SGV-like virus was detected (in a sample from William Brown, Colorado State University). In all these tests, infected controls for all five viruses were always positive. Again, the new test for SGV was reliable for a range of different kinds of samples.

DISCUSSION

Although the modified, indirect test for SGV is not quite as specific as the direct EIA for the other four viruses, the procedure provides a simple, easy way to assay SGV and similar isolates under a range of conditions. A possible disadvantage is failure of the test to detect mixtures of SGV and MAV. This would be a problem only under rare circumstances and can be overcome by preabsorbing with mAb-MAV1, which reacts only with MAV (4). Crude antibody preparations and precipitated immunoglobulins from hen eggs are not useful in the coating step for direct EIA of SGV. Although purified immunoglobulin was active in some tests (5), we have found its sensitivity too low for use in virus identification.

A possible way to do direct EIA for SGV would be to use monoclonal antibodies. We processed some 13 kg of infected tissue and obtained 540 μg of purified SGV for this purpose. We did obtain some SGV-specific antibodies, but they were antibodies for cryptotopes of the capsid protein and were not active against intact SGV. This pattern resulted from the fact that we had difficulties screening for anti-SGV antibodies by using heterologous rabbit immunoglobulins (prepared against MAV) to trap SGV. When we changed to a direct coating of plates with partially purified SGV, the alkaline pH of the coating buffer resulted in disruption of the virus particles (14) and hence the selection for cryptotopes. In our previous screening for monoclonal antibodies against RPV and MAV, we had homologous rabbit immunoglobulins to use for the coating step, and thus we were able to select antibodies for surface epitopes of intact virions (4).

The common occurrence of SGV-like virus in Idaho contrasts sharply with other areas, a contrast somewhat clouded by lack of tests for SGV in many studies. We have tested at least a few samples from Idaho in each of seven recent years. In five of those years, we detected SGV-like virus, a total of 14 isolates. An SGV-like virus was also found in a sample from Colorado. We have not found such

Table 3. Results of 30 parallel tests of New York small grains for luteoviruses by serological and biological methods in 1985

	Enzyme-linked immunosorbent assays						Virus transmission tests			
		Absorbance at 405 nm[b]					Transmission with aphid species shown[c]			
Group	No. plants infected[a]	RPV	MAV	PAV	RMV	SGV	No. plants infected[a]	*Rhopalosiphum padi*	*Sitobion avenae*	*R. maidis*	*Schizaphis graminum*
A	20 PAV	0.011	0.135	1.033	0.046	0.009	20 PAV	56/60	43/60	1/60	40/60
B	4 None	0.007	0.016	0.020	0.043	0.010	4 None	0/12	0/12	0/12	0/12
C	3 RPV	0.982	0.012	0.010	0.051	0.008	3 RPV	9/9	1/9	0/9	4/9
D	1 RMV	0.027	0.025	0.104	0.161	0.004	1 RMV	0/3	0/3	1/3	1/3
E	1 PAV + RMV	0.026	0.158	0.814	0.220	0.015	1 None	0/3	0/3	0/3	0/3
F	1 PAV + RPV	0.710	0.202	1.270	0.034	0.013	1 PAV	3/3	1/3	0/3	0/3
Healthy controls		0.006	0.010	0.010	0.036	0.008	Aphid controls	0/6	0/6	0/6	0/6
50 ng homologous virus		0.553	0.742	0.807	0.217	0.299					

[a] With isolates similar to those shown: PAV transmitted nonspecifically by *R. padi* and *S. avenae*, RMV transmitted specifically by *R. maidis*, RPV transmitted specifically by *R. padi*, MAV transmitted specifically by *S.*(= *Macrosiphum*) *avenae*, and SGV transmitted specifically by *Schizaphis graminum*.

[b] Using antiserum for isolate shown. Values are means of absorbance for number of individual plants shown at left in each case, usually two EIA wells per plant. Only values ≥ 0.1 were usually considered positive. Coast Black oats were used as controls.

[c] Number of plants infected per number of plants infested with about 10 aphids of species indicated. Tests were made in parallel with four aphid species shown. Data are only for original test of field-collected sample.

isolates in samples from other locations or from those collected in New York except in three of 29 yr (9,13).

Although viruses similar to all five known BYDV isolates have been confirmed in Idaho since 1978, SGV-like ones tend to predominate in the dryland wheat area of southeastern Idaho. This area is characterized by 38–46 cm of annual precipitation and many high-elevation (1,500–2,100 m) valleys isolated by mountain ranges. The only cultivated crop hosts of BYDV produced there are wheat and barley. The source of inoculum for the BYDV epidemics in this area is unknown, but is believed to be local weed grasses. Population dynamics of the greenbug vector in this area are also poorly understood. This aphid is commonly found infesting young wheat plants in the fall, but it has never been detected in early spring. We do not know if the greenbug is endemic or migrates over long distances.

Absence of MAV-like virus in New York completes a major shift in predominating isolates over a long period (9,13). Despite the relatively low numbers of samples tested in some years, we are confident that the change to dominance of PAV-like isolates is real. One of us is also pleased that the Cornell-USDA-ARS luteovirus research program on vector specificity began in 1955 rather than in 1985!

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