Serological Identification of Luteoviruses of Small Grains in China

GUANG-HE ZHOU, ZHUO-MIN CHENG, YOU-TING QIAN, and XIANG-CAI ZHANG, Plant Protection Institute, Chinese Academy of Agricultural Sciences, Beijing, People's Republic of China; and W. F. ROCHOW, Agricultural Research Service, U.S. Department of Agriculture, Department of Plant Pathology, Cornell University, Ithaca, NY 14853

ABSTRACT

Zhou, G.-H., Cheng, Z.-M., Qian, Y.-T., Zhang, X.-C., and Rochow, W. F. 1984. Serological identification of luteoviruses of small grains in China. Plant Disease 68:710-713.

Samples of 307 plants of wheat or oats collected from six provinces in China were dried and sent to Ithaca, NY, for serological assays. Each sample was tested by enzyme-linked immunosorbent assays with four virus-specific antisera to compare Chinese isolates with four luteoviruses previously characterized in New York. Results permitted division of the samples into five groups. One group (99 isolates) appeared similar to MAV (transmitted specifically by *Sitobion avenae*), a second group (17 isolates) appeared similar to RPV (transmitted specifically by *Rhopalosiphum padi*), and a third group (3 isolates) appeared similar to RMV (transmitted specifically by *R. maidis*). A fourth group (45 isolates) was more similar to PAV (transmitted nonspecifically by *R. padi* and *S. avenae*) than to any of the others, but identity of these isolates was not clear. The remaining 143 samples did not react with any of the virus-specific antisera, a result that might merely reflect presence of luteoviruses serologically distinct from the four we used. When results for all samples were summarized according to location of each collection, a pattern emerged that was in agreement with occurrence of specific aphid vectors in the areas involved.

Luteoviruses that cause barley yellow dwarf of small grains are very important pathogens in northern and northwestern China. Losses of 20–30% have been observed in many years, and even greater losses were measured in 1966, 1970, 1973, and 1978 in some provinces. Most research on barley yellow dwarf in China has stressed epidemiology, disease control, and identification of aphid vectors. Viruses that cause this widespread disease were probably described at least 25 yr ago (12), but their characterization in China has received attention only in recent years (1,13). We were especially interested in the serological identity of barley yellow dwarf viruses (BYDV) that occur in China in order to develop better control methods.

The purpose of this study was to test the feasibility of identifying luteoviruses in samples of small grains collected in China and shipped to the United States for assay based on comparison with BYDV characterized in New York (5,6,10). We used enzyme-linked immunosorbent assay (EIA) to test 307 samples of small grains collected from six provinces in the People's Republic of China in 1982 with four virus-specific antisera. A preliminary report has been published (14).

Cooperative investigation, Chinese Academy of Agricultural Sciences, Agricultural Research Service, U.S. Department of Agriculture, and Cornell University Agricultural Experiment Station. Supported in part by NSF grant PCM8007852 and by Rockefeller Foundation grant 80019 to Cornell University.

Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply approval to the exclusion of other products that may also be suitable.

Accepted for publication 21 February 1984.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

This article is in the public domain and not copyrightable. It may be freely reprinted with customary crediting of the source. The American Phytopathological Society, 1984.

MATERIALS AND METHODS

Individual plants of wheat or oats with symptoms that resembled barley yellow dwarf were collected from 10 counties of six provinces during the growing season of 1982. Plants were chopped very finely with a razor blade, and 3 g of tissue from each was dried over calcium chloride in the cold. The dried samples were then packaged individually and shipped in two separate lots to Ithaca, NY, for testing. In addition to samples collected in the field, some samples of healthy and infected wheat from the greenhouse in Beijing were included as controls. A preliminary test in Ithaca showed that such dry samples reacted as did fresh ones in EIA tests with the four globulins we used.

When samples were received in Ithaca, they were stored at −20 C and processed during a period of several months in lots of about 20 each. Each sample was placed in a test tube with 9 ml of 0.02 M phosphate-buffered saline, pH 7.4, containing 0.05% Tween 20. The tissue was ground for 6 sec in the PT-20 probe of a Brinkmann Polytron Homogenizer. Each sample was then shaken by hand with 9 ml of chloroform, centrifuged at low speed to break the emulsion, and stored at 4 C for 1–2 days before use.

Each sample was tested by double-sandwich EIA as described previously (8–10). Immulon substrate plates (Dynatech Laboratories, Inc.) with 200 μl of liquid per well were used for all tests. Wells were first coated with virus-specific globulin (10 μg/ml) for about 6 hr at 37 C; antigen samples were incubated overnight at 4 C. Alkaline phosphatase-conjugated globulin, usually at a dilution of 1:800 of stock, was incubated for about 5 hr at 37 C, and finally, after 45 min at room temperature, the substrate reaction was measured at 405 nm with a Dynatech Micro ELISA Reader Model 2-580. Each sample was tested in parallel with four BYDV-specific globulins (9,10). The antisera were those made against the RPV isolate, transmitted specifically by *Rhopalosiphum padi* (L.); the MAV isolate, transmitted specifically by *Sitobion* (= *Macrosiphum*) *avenae* (F.); the PAV isolate, transmitted nonspecifically by both *R. padi* and *S. avenae*, and the RMV isolate, transmitted specifically by *R. maidis* (Fitch). These viruses, characterized previously, have provided the basis for many comparative tests in the past (7,9). Each EIA plate included healthy controls that were samples of wheat from China and/or of oats from the greenhouse in Ithaca, together with virus controls, which were 3-g samples of infected plants from the greenhouse in Ithaca as well as known amounts (100–500 ng) of virus from purified preparations. A reaction (except for heterologous ones) was usually considered positive only if the absorbance was about 0.1, a value well within the visible range of color. Because the mean absorbance of controls was less than 0.01, this criterion was a conservative one.

In additional tests, we used two recently described monoclonal antibodies (4) to study samples of some MAV-like viruses that had been stored in a freezer after the original tests. Equal portions of individual samples (or sometimes combinations) were mixed with one of four reactants. These were buffered saline as control, anti-PAV globulin at 0.1 mg/ml, and 1:300 dilutions of ascites fluid for two monoclonal antibodies. One monoclonal antibody (MAV-1) reacts only with MAV; the other (MAV-3) reacts with both MAV and PAV (4). Mixtures were incubated at 37 C for 30 min, kept overnight at 4 C, and assayed in

double-sandwich EIA tests with MAV-specific globulin to estimate the amount of unabsorbed (unreacted) virus. Parallel tests were done with clarified preparations of MAV-infected tissue.

RESULTS

Each of the 307 samples fell into one of five groups (Table 1). For 143 samples, results were negative with all four virus-specific globulins. These negative samples were especially striking because they occurred in plates where other samples were clearly positive. A second group of 99 samples reacted very strongly with MAV-globulin, weakly with PAV-globulin and not at all with the other two globulins (Table 1). This reaction pattern was similar to that of MAV and of MAV-like isolates identified in many samples collected in the United States (8,9). For many of the Chinese isolates, however, the relative strength of the heterologous reaction with PAV globulin was very low. For example, the ratio of the PAV/MAV globulin reaction for 56 of the 99 isolates was between 0.01 and 0.05. For only 10 of the Chinese isolates was the PAV/MAV reaction ratio greater than 0.10. In contrast, we usually observe a PAV/MAV reaction ratio for MAV around 0.10. In 50 assays of MAV used as controls here and in related experiments done during the same months, the PAV/MAV reaction ratio was greater than 0.10 in 35 cases. We are not sure that such differences are significant, but we think these results mean that many of the Chinese MAV-like isolates are somewhat different from the characterized MAV isolate.

A third group of 45 isolates reacted more strongly at a moderate level with PAV-globulin than with MAV-globulin, a result that indicates these isolates might be similar to PAV (Table 1). Compared with the other four groups, however, this relationship was less clear. The PAV isolate, and many PAV-like isolates we have detected in other tests (7–9), always gave a strong reaction with PAV-globulin and a weak one with MAV-globulin. With some of the Chinese isolates in this group, however, the reaction levels with these two globulins were nearly equal, a pattern similar to heterologous reactions with SGV, a fifth characterized BYDV isolate (4,9). We do not yet have a homologous antiserum for SGV and identify SGV-like isolates serologically only on the basis of relatively weak reactions with MAV and PAV globulins, but such reactions with SGV are normally stronger for MAV than for PAV globulins. Thus, if these 45 isolates are similar to PAV or SGV, they are different from viruses encountered in past tests (8,9).

The fourth group of 17 isolates all reacted only with RPV-globulin. These results were clouded slightly by the relatively weak reactions in these tests compared with results with isolates similar to RPV that we have identified in other collections (8,9). The fifth group of isolates from China included three that appear similar to RMV, the vector-specific isolate transmitted by *R. maidis* (Table 1).

Table 1. Results of enzyme-linked immunosorbent assays of 307 samples of wheat or oats collected in six provinces of the People's Republic of China in 1982

No. of plants infected with isolate similar to that shown	Absorbance at 405 nm in tests with antiserum for characterized isolate of barley yellow dwarf virus shown[a]			
	RPV	MAV	PAV	RMV
143 None	0.019	0.003	−0.005	0.011
99 MAV	−0.011	0.870	0.042	0.000
45 PAV	0.013	0.194	0.320	0.014
17 RPV	0.128	0.009	0.004	0.012
3 RMV	0.011	0.038	0.037	0.348
35 Healthy controls	0.003	0.008	−0.001	0.003
6 RPV controls	1.248	0.004	0.003	0.014
6 MAV controls	0.017	1.385	0.154	0.040
3 PAV controls	0.004	0.246	1.338	0.005
6 RMV controls	0.017	0.008	0.004	0.618
8 SGV controls	0.003	0.110	0.071	0.023
31 Homologous virus controls (500 ng)	1.101	0.774	1.011	0.712

[a] Values are means for the number of samples shown at left of each line after a 45-min reaction at room temperature.

Although the data in Table 1 are based only on results of readings of single wells for each of the four globulins with each sample, we confirmed the consistency of the data by making additional tests of some samples in separate plates. Sometimes the samples were kept at 4 C for a week or two before the second test; sometimes they were stored in a freezer for several months before retesting. In all cases, results of a second test agreed with those of the first. Reactions for MAV-specific globulin were generally equal in strength for first and second tests, but reactions with RPV-specific globulins were sometimes lower the second time than the first. Negative samples always remained negative in subsequent tests.

When we studied some of the MAV-like isolates further in absorption tests with monoclonal antibodies (4), we found additional evidence that some Chinese isolates were not identical to MAV. Previously absorbed samples assayed by EIA reacted in a similar way in tests with PAV-globulin and with the monoclonal antibody specific for MAV (MAV-1). Both MAV and the Chinese isolates were completely absorbed by MAV-1 (negative EIA reaction) and partly by PAV globulin (partial EIA reaction) (Table 2). In most tests with MAV-3, however, no reaction occurred with the Chinese isolates; EIA reactions were equal or higher than saline controls. In contrast, MAV-3 consistently absorbed MAV partially; EIA values were 10–54% of controls (Table 2). Further tests are needed to evaluate these results, but the differences seemed consistent enough to be of possible importance.

Table 2. Comparison of MAV with MAV-like isolates from China in enzyme immunosorbent assays after previous absorption with each of two monoclonal antibodies

Antiserum used for absorption	Absorbance at 405 nm in tests with Chinese MAV-like isolates and MAV after absorption with antibodies shown[a]	
	Chinese	MAV
Monoclonal MAV-3	0.331	0.193
Monoclonal MAV-1	0.002	0.010
Polyclonal PAV	0.055	0.124
Saline control	0.275	0.682

[a] Data are means of reactions of seven Chinese isolates and seven samples of MAV from different source plants. Absorptions were carried out at 37 C for 30 min; mixtures were kept at 4 C and used the next day in double-sandwich enzyme immunosorbent assays with anti-MAV globulin. Monoclonal MAV-3 reacted with both MAV and PAV; MAV-1 reacted only with MAV (4).

When we summarized the data for all 307 samples according to location where each was collected, we found a pattern that was consistent with conditions and observations in the areas involved (Table 3). For example, most of the MAV-like isolates came from four counties of three provinces that are characterized by higher elevations than other collection sites. Moreover, in the provinces of Shanxi, Gansu, and Nei Menggu, the predominating aphid vector was often *Sitobion avenae*, the aphid species that transmits such virus isolates. In contrast, most of the BYDV isolates we have tentatively identified as similar to PAV were found in plants collected in Henan and Shaanxi provinces. In these lower-lying areas, the predominating aphid vector species is often *Schizaphis graminum* (Rondani), an aphid that would be expected to transmit PAV- and SGV-like isolates. This observation underscores the possibility discussed before that these isolates could be related to SGV, a luteovirus transmitted specifically by *S. graminum* (5). It is also interesting that 55 of the negative samples

came from Zhangye County in Gansu Province, an area where barley yellow dwarf is usually very severe and where *S. graminum* is also usually the predominating vector species. Perhaps these samples appeared negative only because they were infected by a luteovirus unrelated to the four virus-specific globulins we used.

DISCUSSION

Comparing viruses that cause disease in one part of the world with those that occur in other locations is always a problem because of hazards of transferring active viruses from one country to another. These results show the feasibility of serological identifications of luteoviruses collected in one area and identified by serological tests in another. Although such data are limited by the absence of parallel biological data, we think this study shows that the serological results themselves can be useful.

Our data support the results of Zhang et al (13) that indicate the presence of a diverse range of luteoviruses that cause barley yellow dwarf in China. Because our comparisons were based only on four virus-specific antisera, actual variation among luteoviruses in nature in China may be much greater than our limited tests show. It is clear, however, that a variety of barley yellow dwarf viruses cause the disease in China, as in other parts of the world where studies have been made (3,7,11).

Some of the virus isolates we identified from China appear similar to those characterized in the United States, but others seem different. We are especially interested in the 143 samples that gave negative results. It is possible that these plants did not contain luteoviruses and that the symptoms observed by collectors in the field had other causes, such as aster yellows pathogens (11). Physiological conditions can produce symptoms easily confused with barley yellow dwarf. Perhaps these samples contained luteoviruses that did not withstand shipment. But we think it is just as likely that these plants were infected with luteoviruses serologically unrelated to the four used as our basis for comparison. In the future, we will explore this possibility.

Nearly a third of the samples tested proved to be infected with luteovirus similar to MAV. These isolates seemed to represent a range of variation that included some that may be nearly identical with MAV but also others that appear less closely related. Differences in heterologous reactions with PAV-antiserum and variations in reactions with a monoclonal antibody indicated such a range. Whether these differences merely represent variations in the precision of our assays or variations in sample preparation, or whether they represent differences that have some role in epidemiology, remains to be seen.

The relationship of the 45 isolates in another group to isolates previously characterized in New York is not clear. We have tentatively identified these isolates as similar to PAV, but they are different from the PAV-like isolates we have identified over the years from material collected in the United States. The strength of the homologous reaction with PAV-globulin was low and the strength of the heterologous reaction with MAV-globulin was high compared with tests with PAV. For some time, we thought these isolates were probably some type of SGV-like virus (14), especially when we observed that most of the samples were collected in areas in China where *S. graminum* is the predominating aphid species. Although the identity of isolates in this group is uncertain, it is clear that the 45 isolates in this group differ from those of the other four groups.

Tests of the 17 isolates similar to RPV gave clear results, except the reaction with RPV-globulin was weaker than that usually observed in tests of other samples. This probably means that these isolates are merely similar, not identical, to RPV or that the isolates were simply at lower concentrations in the dried tissue than usually observed. Identity of the RMV-like isolates seemed straightforward, but only three such isolates were encountered in the tests.

It is difficult to compare results of these serological tests with a recent report of a study of aphid transmission tests in China (13); the virus isolates that Zhang et al identify as DAV could be similar to the isolates we found related to MAV. The single leaf from a DAV-infected plant sent to Ithaca for testing by J. H. Tsai gave very clear results for MAV-like virus in tests with four virus-specific globulins. The isolates Zhang et al (13) considered similar to RPV also may be the same as those we identified as similar to RPV in serological tests. In both studies, such isolates represented a relatively small proportion of those encountered. We do not understand, however, what Zhang et al (13) meant by the comment that RPV "is related to the PAV-like isolate of BYDV in the United States by means of enzyme-linked immunosorbent assay." The RPV and PAV isolates are distinct on the basis of a range of tests (2,3,10). The isolates Zhang et al (13) called GPDAV do not appear similar to any we detected, but this might reflect the fact that mixed infections may have been involved in the aphid transmission work.

It is not clear how the GPV isolates of Zhang et al (13) are related to any we identified. Perhaps such isolates in our studies were among those that gave negative reactions because they are unrelated serologically to the four viruses we used for comparison. Perhaps these GPV-like viruses are related to those we identified as similar to PAV. Our serological reactions with MAV- and PAV-specific globulins may have been a heterologous reaction with GPV-like virus. We have difficulty in relating these GPV isolates to any we detected, especially because the leaf of GPV-infected tissue sent to Ithaca by J. H. Tsai for EIA assay reacted the same as the DAV-sample and the same as the isolates from China that are similar to MAV (Table 1).

Our difficulties in comparing results of this study with those of Zhang et al (13) illustrate the need for parallel biological and serological identifications of luteoviruses (8,9,11). We used the EIA procedure with four virus-specific globulins because it is the best single method available. During a period of 5 yr in New York, for example, parallel biological and serological tests were made of 437 samples from the field. In 351 of these tests, both methods were in agreement. But in 77 tests, more information was provided by EIA assays than by four aphid species used in transmission tests. In only four cases did we learn more by using aphids. In five cases, results from the two procedures were different; we could not evaluate which procedure provided more reliable data. Despite advantages of using EIA in such tests, however, results are limited by the numbers of virus-specific antisera available. The negative results in our tests may illustrate this fact. Some specific limitations of EIA assays have been discussed elsewhere (11). Use of EIA has

Table 3. Distribution of variants of barley yellow dwarf virus (BYDV) among 10 counties where samples were collected

		No. of samples similar to BYDV isolate shown[a]				
Province	**County**	**MAV**	**PAV**	**RPV**	**RMV**	**None**
Shanxi	Yongji	1	0	0	1	18
Shanxi	Linfen	4	1	3	0	12
Shanxi	Shuoxian	22	3	6	1	10
Henan	Linbao	1	12	0	1	6
Shaanxi	Weinan	0	29	2	0	9
Gansu	Gangu	19	0	2	0	21
Gansu	Inst. Pl. Prot.	12	0	1	0	5
Gansu	Zhangye	3	0	2	0	55
Nei Menggu	Fengzhen	37	0	1	0	4
Beijing	···	0	0	0	0	3

[a] All samples were winter wheat except for those from Shuoxian and Fengzhen counties, which were spring wheat or oats. Data for isolate identifications are in Table 1.

additional advantages in that results are based on properties of the virus itself, it avoids quarantine problems, and relatively small samples of dried tissue are needed. It is also easier to detect mixed infections of viruses in EIA assays than it is with vectors (8,9). The study by Zhang et al (13) with vectors is complicated by unknown variations in the aphid vectors as well as variations in their interaction with viruses. For example, the report of transmission of GPV and DAV within as little as 1 min and lack of a relation between longer feeding times and increased rates of transmission indicates that a virus different from luteovirus may have been involved (13).

In attempts to resolve some of these questions, we plan future cooperative experiments that will be based on comparative aphid transmission tests in China, preparation of tissue samples from specific test plants in the greenhouse in China, and assay of such samples in comparative serological tests in the United States. Until both kinds of tests can be done in parallel in China, we think this approach will be most helpful.

ACKNOWLEDGMENTS

We thank Irmgard Muller, Laura Tufford, and Dawn Smith for excellent technical assistance in Ithaca and Shu-xiang Zhang in Beijing.

LITERATURE CITED

1. Chen, Z., Chen, J., Fu, C., and Li, Z. 1982. Purification and diagnosis of barley yellow dwarf virus in wheat. Acta Phytopathol. Sin. 12:58-60.
2. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf virus infection of oats. Phytopathology 73:1570-1572.
3. Gill, C. C., and Chong, J. 1979. Cytopathological evidence for the division of barley yellow dwarf virus isolates into two subgroups. Virology 95:59-69.
4. Hsu, H. T., Aebig, J., and Rochow, W. F. 1984. Differences among monoclonal antibodies to barley yellow dwarf viruses. Phytopathology 74:600-605.
5. Johnson, R. A., and Rochow, W. F. 1972. An isolate of barley yellow dwarf virus transmitted specifically by *Schizaphis graminum*. Phytopathology 62:921-925.
6. Rochow, W. F. 1969. Biological properties of four isolates of barley yellow dwarf virus. Phytopathology 59:1580-1589.
7. Rochow, W. F. 1979. Field variants of barley yellow dwarf virus: Detection and fluctuation during twenty years. Phytopathology 69:655-660.
8. Rochow, W. F. 1979. Comparative diagnosis of barley yellow dwarf by serological and aphid transmission tests. Plant Dis. Rep. 63:426-430.
9. Rochow, W. F. 1982. Identification of barley yellow dwarf viruses: Comparison of biological and serological methods. Plant Dis. 66:381-384.
10. Rochow, W. F., and Carmichael, L. E. 1979. Specificity among barley yellow dwarf viruses in enzyme immunosorbent assays. Virology 95:415-420.
11. Rochow, W. F., and Duffus, J. E. 1981. Luteoviruses and yellows diseases. Pages 147-170 in: Handbook of Plant Virus Infections. E. Kurstak, ed. Elsevier/North Holland, Amsterdam.
12. Yu, T. F., Pei, M. Y., and Hsu, H. K. 1957. Studies on the red-leaf disease of the foxtail millet (*Setaria italica*). 1. Red-leaf, a new virus disease of the foxtail millet, transmissible by aphids. Acta Phytopathol. Sin. 3:1-18.
13. Zhang, Q. F., Guan, W. N., Ren, Z. Y., Zhu, X. S., and Tsai, J. H. 1983. Transmission of barley yellow dwarf virus strains from northwestern China by four aphid species. Plant Dis. 67:895-899.
14. Zhou, G.-H., Cheng, Z.-M., Qian, Y.-T., Zhang, X.-C., and Rochow, W. F. 1983. Enzyme-linked immunosorbent assays for luteoviruses of small grains in China. (Abstr.) Phytopathology 73:378.