Partial Characterization of a Colorado Isolate of Agropyron Mosaic Virus

DALLAS L. SEIFERS, Plant Pathologist, Fort Hays Branch Agricultural Experiment Station, Hays, KS 67601

ABSTRACT

Seifers, D. L. 1992. Partial characterization of a Colorado isolate of Agropyron mosaic virus. Plant Dis. 76:564-569.

Wheat collected in Colorado was found to be infected with both wheat streak mosaic virus (WSMV) and Agropyron mosaic virus (AMV). The viruses were identified by means of host range tests, serology, and electrophoretic comparisons of coat proteins. The AMV isolates (the Colorado isolate and PV 75) were further characterized by partial chemical proteolysis of capsid proteins and electrophoretic analysis of cleavage products and by comparison of single-stranded and double-stranded RNAs. This is the first report of AMV in Colorado.

Several viruses have been documented as infecting wheat (*Triticum aestivum* L.) in the Great Plains region of the United States, including wheat streak mosaic virus (WSMV), which is a serious threat to wheat production (36). During the verification of WSMV isolates collected from Kansas and adjoining states, an additional virus was observed in volunteer wheat collected near Loveland, Colorado.

This paper provides evidence for the identification of Agropyron mosaic virus (AMV) in wheat, hitherto unreported in Colorado, including the partial characterization of capsid and single-stranded and double-stranded RNA and the response of wheat cultivars to infection by AMV isolates.

MATERIALS AND METHODS

Virus source and maintenance. AMV (PV 75), brome mosaic virus (BMV, PV 47), and WSMV (PV 57 and PV 91) were obtained from the American Type Culture Collection. The WSMV isolate (H81) was collected at Hays, Kansas, in 1981; the OSU WSMV was obtained from Emil Sebesta (Oklahoma State University, Stillwater); the Timian WSMV (alternate source of PV 57) was obtained from M. K. Brakke (USDA-ARS, Lincoln, Nebraska); and the Sidney WSMV was obtained from W. G. Langenberg (USDA-ARS, Lincoln, Nebraska). The Colorado isolate (AMV and WSMV) was obtained from virus-infected wheat collected near Loveland, Colorado, in 1985.

Inoculum was prepared by grinding the infected tissue in a mortar and pestle at a 1:20 (w/v) dilution in 0.02 M potassium phosphate buffer (pH 7). The homogenate was filtered through cheesecloth, an abrasive (Crystolon flour B, 600 mesh) was added at 1 g/100 ml of inoculum, and the mixture was inoculated onto wheat cv. Arkan seedlings planted 7 days previously. The seedlings infected with different virus isolates were maintained in separate growth chambers at 20 C, both before and after inoculation, with 12 hr of illumination (500 $\mu E \cdot s^{-1} \cdot m^{-2}$) per day.

Host range tests. Infected Arkan seedlings were harvested 10 days postinoculation and used for inoculum production as described above. The first leaf of seedlings of quackgrass (*Agropyron repens* (L.) P. Beauv., oat (*Avena sativa* L. 'Lodi'), sweet corn (*Zea mays* L. 'Early Golden Giant'), and foxtail barley (*Hordeum jubatum* L.) were inoculated. Quackgrass and oats have been established as indicator plants to differentiate WSMV and AMV (31). Early Golden Giant sweet corn was used as an indicator plant for the detection of BMV, which also infects quackgrass but kills this corn cultivar (2,30). Foxtail barley was used to detect Hordeum mosaic virus (31). Indicator plants were maintained in the same growth chambers as the isolate with which they were inoculated. Virus isolates shown to be free of other contaminating viruses were maintained in Arkan wheat using procedures previously described (25).

Virus purification. The method of Lane (16) was used for minipurification of infected plant tissue for initial determination of viral proteins. However, virus used for characterization of capsid and RNA was purified by grinding virus-infected Arkan wheat (inoculated 14 days previously) in a mortar and pestle in grinding buffer (0.1 M diammonium citrate, 0.25% [v/v] 2-mercaptoethanol, 0.1% [v/v] sodium diethyldithiocarbamate, and 1.0% [w/v] polyvinylpyrrolidone, estimated molecular weight 40,000, pH 6.5), filtering the homogenate through cheesecloth, and adding stock 33% Triton X-100 (v/v) to give a 4% (v/v) concentration. The extract was centrifuged at 23,700 *g* for 15 min at 5 C, and the supernatant was pipetted into 50 Ti centrifuge tubes and underlaid with 2 ml of 0.988 M sucrose (made in grinding buffer). The virus extract was centrifuged for 2 hr at 85,000 *g* at 5 C. The liquid was decanted from the tubes, and the pellets were resuspended in 3.5 ml of grinding buffer. The resuspended virus then was centrifuged for 10 min at 31,000 *g*, transferred to SW 50.1 centrifuge tubes, and underlaid with 1.0 ml of 2.33 M and 0.5 ml of 3.297 M CsCl. The tubes then were centrifuged for 3 hr at 139,000 *g* at 5 C. After centrifugation, a syringe with an attached bent needle was used to collect the banded virus. The collected virus was made to 5 ml with grinding buffer and pelleted by centrifugation for 1 hr at 85,000 *g* at 5 C. Virus purified in this manner was used as a source for coat protein and viral nucleic acid analysis. Relative centrifugal forces were calculated using R_{max}.

Analysis and SDS-PAGE of viral coat proteins. Purified virus was suspended in 400 μl of sodium dodecyl sulfate (SDS) treatment buffer (0.125 M Tris-Cl [pH 6.8], 4% [w/v] SDS, 20% glycerol [v/v], and 10% [v/v] 2-mercaptoethanol), heated for 4 min at 100 C, reduced, and alkylated (15); then, 20 μl was loaded into a well for the respective sample. Polyacrylamide gel electrophoresis (PAGE) was conducted according to the procedure of Laemmli (14). The gels were stained with Coomassie blue R-250 or with silver according to the procedure by Wray et al (37) for coat proteins. All electrophoretic analyses were repeated three times with extracts derived from three different sets of infected plant tissue.

Peptide mapping. Procedures for partial chemical cleavage of coat protein in polyacrylamide gel slices were as described previously (19,23,32). Cyanogen bromide (CNBr) cleaves at methionine, *N*-chlorosuccinimide at tryptophanyl, and formic acid at aspartyl-prolyl residues. The CNBr cleavage (20 mg/ml) was conducted at room temperature for 1 hr, *N*-chlorosuccinimide (0.15 M) at room temperature for 30 min, and formic acid (75%, w/v) for 8 hr (4 hr at room temperature and 4 hr at 37 C). Virus (4 μg) of each isolate (virus adjusted to 1 mg/ml with an absorbance of $A_{280} = 1$ mg/ml of protein) was first loaded onto and separated in an SDS-10% PAGE. After staining with Coomassie blue R-250, the gel band containing the capsid proteins was excised and rinsed with distilled water. The gel slice was then subjected

Contribution No. 89-389-J from the Kansas Agricultural Experiment Station, Manhattan.

Accepted for publication 19 December 1991 (submitted for electronic processing).

to chemical cleavage in a test tube filled with cleavage reagents. All gel slices were incubated in cracking buffer for 30 min at room temperature before being analyzed in a second SDS-10% PAGE.

Viral nucleic acid analysis. Virus pellets were resuspended in 300 μl of 0.1 M ammonium carbonate buffer (8) containing 2% SDS, 1 mM EDTA (pH 9.0), 500 μg/ml of bentonite (10), and 1 mM aurintricarboxylic acid (1). The resuspended virus was incubated overnight at 4 C. An equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v) was added to the virus solution. The solution was centrifuged for 5 min at 16,000 *g* at room temperature in an Eppendorf microcentrifuge, the aqueous phase was withdrawn, and an equal volume of phenol/chloroform/isoamyl alcohol (25:24:1, v/v) was added, vortexed, and centrifuged. The process was repeated until no material was visible at the aqueous-organic interface. Then, 0.1 vol of 3 M sodium acetate was added to the aqueous phase, followed by 2.5 vol of −20 C absolute ethanol. After being incubated overnight at −20 C, the solution containing the viral nucleic acid was centrifuged for 5 min at 16,000 *g*. The supernatant was discarded, and the pellets were washed consecutively with 1 ml of 70% ethanol and absolute ethanol, then dried under vacuum for 5 min at room temperature.

Glyoxal modification of viral RNA, electrophoresis, and silver staining were done as described by Skopp and Lane (27), with slight modification of resuspension volumes and tracking dyes. Vacuum-dried pellets were dissolved in 20 μl of glyoxal modification buffer (1 M glyoxal, 75 mM *N*-ethylmorpholine, 50 mM phosphoric acid, and 50% [v/v] dimethylsulfoxide), incubated at 70 C for 10 min, and cooled; 1–2 μl of tracking dye was then added (24).

The gel was prepared by heating 1% (w/v) agarose (Sigma A 3768) in 75 mM *N*-ethylmorpholine, 50 mM phosphoric acid, and 0.1% (w/v) SDS; cooled to 50 C; and made to 8 mM with iodoacetic acid. The agarose was poured to a depth of 5 mm (65 ml) onto a 11 × 14 cm GelBond film (Hoefer Scientific Instruments, San Francisco, CA).

The 20-μl sample (representing 30 g of tissue) was loaded into a well (2 mm thick × 4 mm wide), and electrophoresis was conducted for 5 hr at constant voltage (60 V) with buffer recirculation. The buffer was cooled by passage through coils of tubing immersed in a ice bath. An RNA ladder of 0.3- to 9.5-kb fragments was used as RNA standards (2-μg loads).

After electrophoresis, gels were immersed in 200 ml of fixative (17.3 g of sulfosalicylic acid, 25 g of trichloroacetic acid, 25 g of zinc sulfate, and distilled water to 500 ml) for 15 min and placed in 200 ml of distilled water overnight. Gels were dried under a stream of hot air, silver-stained, and photographed.

The nature of the nucleic acid was determined by digestion with RNase at 2 μg/ml (Sigma type I-A) or DNase (RNase-free, 1 μg/ml) in enzyme buffer (50 mM Tris-Cl [pH 7.5], 10 mM $MgCl_2$, and 10 mM 2-mercaptoethanol) for 30 min at 37 C before electrophoresis. An RNA ladder (0.3–9.5 kb) and *Hin*dIII digested lambda DNA (Bethesda Research Laboratories, Gaithersburg, MD) were used as controls. The relative molecular mass (M_r) of the viral nucleic acid was established by linear regression analysis of the distance migrated in the gel and the log of the molecular weight of the RNA markers.

Double-stranded RNA extraction and electrophoresis. The procedure described by Valverde (33) was used for extraction of dsRNA from 10 g of AMV-infected (inoculated 14 days previously) Arkan wheat. Pellets of dsRNA were resuspended in 100 μl of buffer (0.04 M Tris-Cl, 0.02 M sodium acetate, 1 mM EDTA [pH 7.8], 20% glycerol, and 0.01% bromophenol blue). Samples of 100 μl were loaded into individual wells of 6% polyacrylamide gels (1.0 mm thick × 14 cm wide × 16 cm long, 40:1 acrylamide/bisacrylamide) in a vertical slab gel apparatus and electrophoresis was in a mixture of 40 mM Tris-Cl, 20 mM sodium acetate, and 1 mM EDTA (pH 7.8) at constant voltage (100 V) for 3 hr. Gels were stained with 2.5 μg/ml of ethidium bromide, visualized by exposing the gel to 302 nm of ultraviolet light, and photographed.

Some stained gels were soaked in low salt (45 mM NaCl and 4.5 mM trisodium citrate, pH 7.0) and high salt (450 mM NaCl and 45 mM trisodium citrate, pH 7.0) buffer with or without 1 μg/ml of RNase (Sigma type I-A) or in 5 mM $MgCl_2$, pH 7.0, with or without 1 μg/ml DNase. RNA and DNA controls were as described above.

Reference dsRNAs of known M_r included several (2.2, 2.0, 1.5, and 0.6 × 10^6) (12) from BMV-infected Arkan wheat plants (inoculated 14 days previously) and one (8.0×10^6) isolated from *Phaseolus vulgaris* L. 'Black Turtle' (34,35); leaf tissue from Black Turtle was harvested 15 days after planting. The M_rs of AMV were estimated by the method of Bozarth and Harley (4). In addition, a sample of dsRNA (from 10 g of healthy and 10 g of AMV-infected wheat) was sent to R. F. Bozarth, who determined the M_r of the CO 85 AMV isolate against viral dsRNA standards from *Helminthosporium maydis* Nisik. (5.7 × 10^6) (3), *Penicillium chrysogenum* Thom (2.0 × 10^6) (22), and *P. stoloniferum* Thom (1.0, 0.51, and 0.46 × 10^6) (5).

Enzyme-linked immunosorbent assay (ELISA). The processing of WSMV serum and conditions used for the double-antibody sandwich ELISA were as previously described (25). Concentration of IgG for coating and conjugates was at 2.5 μg/ml. A dilution series was made from tissue infected by each isolate to test for reaction against WSMV serum. The tissue was ground (1:5, w/v) in PBS in a mortar and pestle, and four fivefold dilutions were made by transferring 50 μl from the first well (containing 250 μl of the 1:5 dilution) to the next (containing 200 μl of PBS) until the dilution series was completed. Absorbance was measured at 405 nm, and values were compared against equivalent healthy tissue extracts. ELISA values twice those of healthy checks were considered positive.

Antiserum to AMV, provided by W. G. Langenberg, was cross-absorbed using proteins from healthy, 14-day-old Arkan wheat plants. First, 3 g was ground in a mortar and pestle in a 1:3 dilution of grinding buffer (5 mM Tris, 1 mM KCl, 0.1 mM Na EDTA, 1 mM $MgCl_2$, 10% glycerol, 1% Triton X-100, and 0.1% 2-mercaptoethanol) and filtered through cheesecloth, the pH was adjusted to 6.0 with 1 N acetic acid, and the extract was placed in a 40 C water bath for 1 hr. The extract was centrifuged for 10 min (17,400 *g*), the pH was adjusted to 8.0 with 1 N NaOH, and the extract was centrifuged again as previously described. The supernatant then was centrifuged for 2 hr at 85,000 *g*. The pellets were resuspended in 2 ml of the AMV IgG ($A_{280nm} = 1.0$), incubated for 2 hr in a 37 C water bath and overnight at room temperature, and then centrifuged for 10 min at 12,400 *g*. The IgG treated in this manner had no reaction to a 1:20 dilution of extract from healthy wheat.

Sera to AMV, BMV (PVAS 178, ATCC), and WSMV were used in an indirect ELISA against extracts of the type specimen of AMV (PV 75), the Colorado AMV isolate (CO 85), BMV (PV 47), WSMV isolate H81, and healthy wheat. Infected Arkan wheat (14 days after inoculation with CO 85, PV 75, and H81) was ground at a 1:20 dilution in coating buffer (9), and 0.25 ml was placed in appropriate wells on ELISA plates. Extracts (0.25 ml of a 1:20 dilution) containing BMV were made from wheat tissue that had been dried at 4 C, then stored in tubes with a desiccant at −20 C. After the plates containing the extracts were incubated for 4 hr at 37 C and washed, 0.25 ml of a 1:200 dilution (in PBS Tween buffer [9] containing 2.0 g/L of ovalbumin as the blocking agent) of each IgG was put into the wells, so that each virus was reacted with each serum, and incubated at 37 C for 4 hr. The plates were washed, 0.25 ml of a 1:1,000 dilution of alkaline phosphatase–labeled goat-antirabbit IgG was added to each well, and the plates were incubated for 4 hr at 37 C. The plates were washed again, and 0.3 ml of *p*-nitro-

phenyl phosphate (0.7 mg/ml) was added to each well and allowed to react for 30 min at room temperature. The reaction was terminated by the addition of 0.05 ml of 3 M NaOH to each well. The absorbance (405 nm) was assessed as previously described. The 1:200 serum dilution was used because it gave the largest difference between AMV-infected and healthy wheat in preliminary indirect ELISA.

Symptom rating and dry weight analysis. Seeds of wheat cultivars Triumph 64, Arkan, Centurk, and Siouxland were sieved to uniform size and planted at 20 per row into 20 × 30 cm soil-filled (3.178 kg) metal flats, which were maintained in a greenhouse at 23 ± 5 C under natural lighting. At 7 days after planting (two-leaf stage), each plant was inoculated on the first leaf with a 1:10 dilution of inoculum (prepared from Arkan source plants inoculated 14 days previously) of the appropriate virus (PV 75 or CO 85). After inoculation, the flats were randomized (block) and maintained in a greenhouse. At 7 days after inoculation, plants not showing symptoms were removed, and rows were thinned to a uniform number of plants. At 14 and 21 days after inoculation, each flat was watered with 100 ml of nutrient solution (11.7 g of a 20:20:20 N-P-K mix in 1 L of distilled water). At 28 days after inoculation, plants were rated for symptom severity (whole plant) on a 1–6 scale, where 1 = no mosaic, 2 = light green streaks, 3 = mostly light green streaks and a few yellow streaks, 4 = mixed green and yellow streaks, 5 = all yellow streaks, and 6 = severe yellow streaks. Symptoms were rated independently by two observers, who did not know the identities of the cultivars and virus isolates. At 28 days after inoculation, the plants were harvested at the soil line, bulked, and weighed, then placed into preweighed paper bags and dried to a constant weight in a forced-air oven at 80 C. Control plants for each wheat cultivar were treated in a like manner. Temperature during the study ranged from 21 to 36 C. Cultivars were chosen on the basis of reaction to WSMV, another mite-transmitted virus (25).

The experiment was repeated twice with three replications per experiment. Data were analyzed by ANOVA in a randomized complete block. When significant treatment effects were observed, means were compared with the Student-Newman-Keuls multiple range test ($P < 0.05$).

RESULTS

Extracts from all isolates reacted positively with WSMV serum in double-antibody ELISA tests at all dilutions, indicating that WSMV was present in all tissue sources.

The extract from the Colorado sample (CO 85) infected quackgrass, indicating the presence of AMV, whereas the other WSMV isolates and type specimens of WSMV infected oats but not quackgrass. Leaves from quackgrass and oats systemically infected by the CO 85 isolate were ground separately, and each extract was used to inoculate Arkan wheat, quackgrass, and oats. The CO 85 isolate from successive transfers through oats failed to infect quackgrass, and the CO 85 isolate from successive transfers through quackgrass failed to infect oats. Following this, the extracts from oats infected by CO 85 reacted with WSMV serum, but the extracts from quackgrass infected by CO 85 failed to react with the same serum. The 1:5 dilutions of healthy quackgrass, oats, or wheat typically had values of 0.039, 0.031, or 0.033, respectively, against WSMV serum. The 1:5 dilutions of AMV-infected quackgrass and wheat had values of 0.039 and 0.035, respectively. A 1:5 dilution from the WSMV isolates always had an ELISA A_{405nm} value in excess of 1.000. The corn did not die, indicating the absence of BMV. No symptoms were evident on foxtail barley inoculated by all isolates. The type specimen of AMV infected quackgrass but not oats.

To further confirm the identity of viruses from the CO 85 material, the

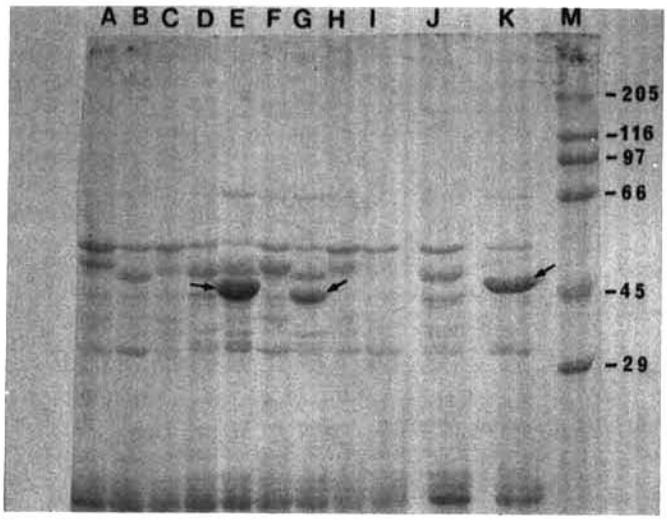

Fig. 1. Wheat streak mosaic virus (WSMV) and Agropyron mosaic virus (AMV) concentrated by minipurification, separated under SDS-PAGE, and stained with Coomassie blue (0.125% Coomassie blue R-250, 50% methanol, and 10% acetic acid). Lane A, Timian WSMV (alternate source of PV 57); lane B, H 81 WSMV; lane C, OSU WSMV; lane D, Sidney WSMV; lane E, CO 85 WSMV and AMV from original sample; lane F, PV 57 WSMV; lane G, CO 85 WSMV and AMV in Arkan wheat after inoculation from original tissue; lane H, PV 91 WSMV; lane I, healthy extract; lane J, CO 85 WSMV after successive transfers through oats; lane K, AMV type specimen PV 75; lane M, molecular weight standards: myosin (205 kDa), β-galactosidase (116 kDa), phosphorylase B (97 kDa), bovine serum albumin (66 kDA), egg albumin (45 kDa), and carbonic anhydrase (29 kDa). Numbers indicate the molecular masses (in kilodaltons) of protein markers. Electrophoresis was in a 12% gel 0.75 mm thick. Duration of electrophoresis was 3 hr at 15 mA, with starting and ending voltages of 100 and 320, respectively. Arrows indicate position of AMV capsid.

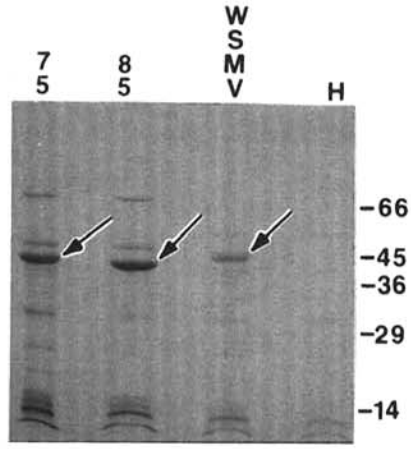

Fig. 2. Agropyron mosaic virus (AMV) and wheat streak mosaic virus (WSMV) from purified preparations separated under SDS-PAGE and stained with Coomassie blue R-250. Lane 75, AMV type specimen PV 75; lane 85, Colorado isolate of AMV; lane WSMV, isolate H81 from Hays, Kansas; lane H, extract from equivalent healthy wheat. Numbers (kilodaltons) at right indicate positions of standard proteins, and arrows indicate position of virus capsid.

Table 1. Response of Agropyron mosaic virus (AMV) Colorado (CO 85) and type specimen (PV 75) isolates in indirect ELISA to AMV, brome mosaic virus (BMV), and wheat streak mosaic virus (WSMV) sera

	Antiserum		
Isolate	**AMV**	**BMV**	**WSMV**
CO 85	0.215[z]	0.022	0.021
PV 75	0.209	0.020	0.021
BMV	0.004	2.000	0.025
WSMV	0.004	0.021	1.485
Healthy wheat	0.004	0.022	0.021

[z] Average values from three assays (1:20 dilution) read at 405 nm.

capsid proteins of several WSMV isolates and CO 85 AMV (obtained from the Colorado sample after successive transfers through quackgrass) and CO 85 WSMV (obtained from successive transfers through oats) were compared with type specimens of both viruses (Fig. 1). The apparent size of the CO AMV and the AMV type specimen capsid proteins were 41 and 43 kDa, respectively. The extracts from successive transfers of the CO 85 isolate through oats (Fig. 1, lane J) had no band present corresponding to AMV, whereas an extract from the original wheat tissue (Fig. 1, lane E) and from Arkan wheat mechanically infected with extracts from the original tissue (Fig. 1, lane G) showed bands corresponding to both WSMV and AMV. Equivalent healthy tissue processed under identical conditions (Fig. 1, lane I) had no bands corresponding to WSMV or AMV. The apparent size of the WSMV capsid from the isolates varied slightly, with PV 57, PV 91, and AC 29 isolates at approximately 48 kDa and the remaining isolates at 46 kDa. Like values were obtained for the capsid protein from purified AMV PV 75, CO 85, and WSMV H81 (Fig. 2).

The samples (both AMV and WSMV) also contained a band of approximately 66 kDa that was present in all samples from tissue infected with AMV (Fig. 1, lanes E, G, and K) and with WSMV PV 57 (Fig. 1, lane F) but not in the control (Fig. 1, lane I). Stained bands representing proteins smaller than the capsid proteins were present in all samples from virus-infected tissue but not in extracts from equivalent healthy tissue. The smaller proteins were also present in preparations from highly purified AMV and WSMV (Fig. 2). The 66 kDa bands were present in preparations from highly purified AMV but absent from WSMV H81 (Fig. 2).

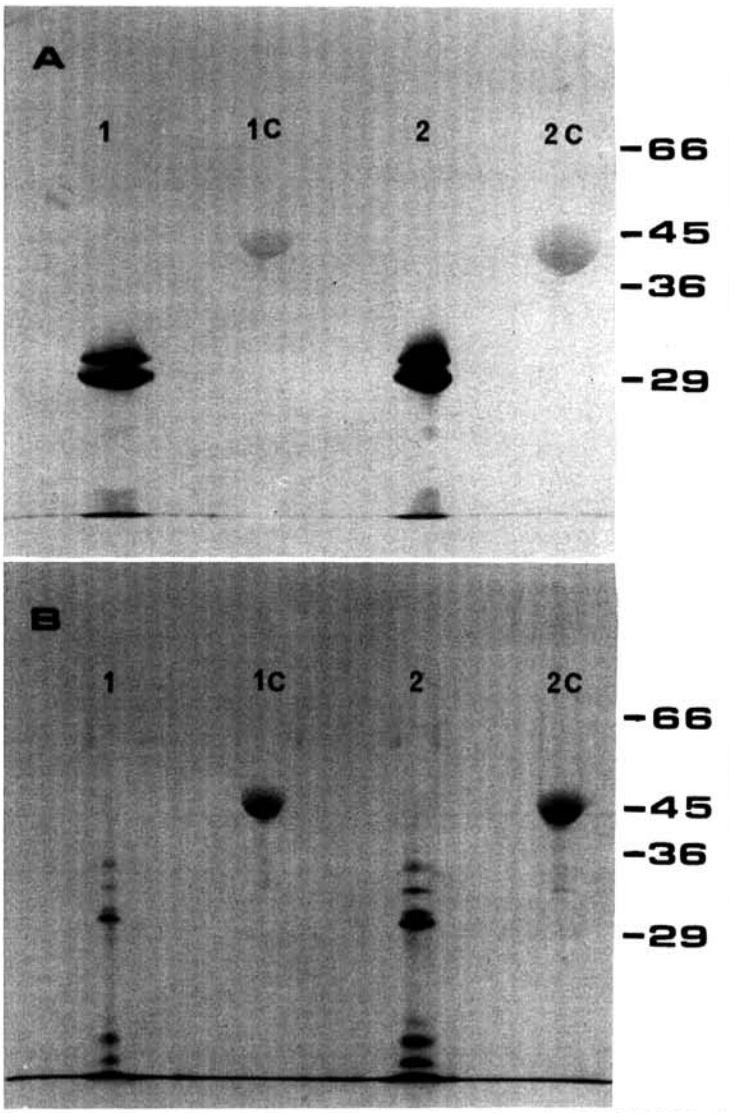

Fig. 3. Patterns of peptide bands produced after electrophoresis (SDS-PAGE) of chemical digests of coat proteins of isolates of Agropyron mosaic virus (AMV). Lane 1, AMV type specimen PV 75; lane 1C, PV 75 control; lane 2, Colorado isolate of AMV; lane 2C, Colorado AMV control. **(A)** *N*-chlorosuccinimide and **(B)** cyanogen bromide cleavage products. Numbers (kilodaltons) at right indicate positions of standard proteins.

The average values (1:20 dilution of antigen) from three indirect ELISAs for the AMV isolates against different serum are shown in Table 1. The AMV isolates reacted to the AMV serum but not to the WSMV or BMV serum or to healthy controls. The WSMV and BMV sera did not react to the AMV isolates or viruses (except the virus to which the serum was prepared) or to extracts from healthy control plants.

Partial proteolysis of capsid protein of AMV PV 75 and the CO 85 isolate with CNBr and *N*-chlorosuccinimide resulted in identical protein banding within each cleavage system (Fig. 3). The CNBr system resulted in seven bands and the *N*-chlorosuccinimide, two bands. The formic acid system did not cleave the capsid.

The nucleic acid from the purified virus migrated as a single band with an M_r of 3×10^6 Da (Fig. 4). The nucleic acid was RNA, because it was degraded by RNase but stable to DNase.

Profiles of virus-specific dsRNA isolated from AMV-infected wheat showed one major and two minor classes of dsRNA (Fig. 5). A heavily stained dsRNA band that migrated to identical positions on gels was obtained for both AMV isolates. The M_r of this band was 5.7×10^6 when run on gels using the Hm 9 dsRNA standard (Fig. 6) and 6×10^6 when the 8×10^6 dsRNA marker from cv. Black Turtle was used in conjunction with the BMV standards (Fig. 5). Lightly stained bands (Fig. 5, arrows) were observed consistently and varied slightly between the isolates (M_r 1.1 and 1.15, 1.15 and 1.20×10^6, determined using BT and BMV dsRNA standards, for PV 75 and CO 85, respectively). No bands were observed in any of 10 extractions from equivalent healthy wheat tissue.

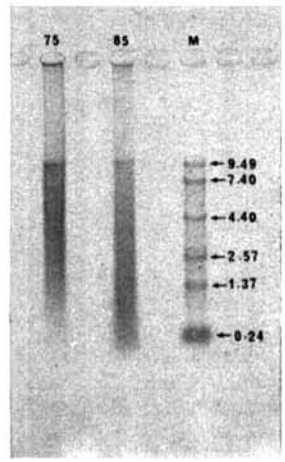

Fig. 4. Glyoxal-denatured RNAs separated by electrophoresis on horizontal 1% agarose gel stained with silver. Lane 75, Agropyron mosaic virus (AMV) type specimen PV 75; lane 85, Colorado isolate of AMV. RNA ladder is standard used to determine apparent size of viral RNA; numbers are in kilobases.

The double-stranded nature of the RNAs was verified by treatment with DNase and RNase (*data not shown*). The ethidium bromide–staining bands were not digested when subjected to DNase treatment but were degraded by RNase I-A only when incubated in low salt buffer.

Infection of wheat by the two AMV isolates resulted in significantly different responses in dry weight accumulation and symptom severity (Table 2). In all instances, the CO 85 isolate was associated with a greater reduction in dry matter accumulation and with more severe symptoms. The PV 75 isolate did not cause a significant reduction in dry matter for wheat cultivars Siouxland and Arkan compared with controls.

DISCUSSION

The viruses in the infected wheat sample from Colorado were determined to be WSMV and AMV. This is the first report of AMV in Colorado, although the virus has been identified in Montana (26), South Dakota (28), Iowa and Maryland (21), and Virginia (20). Also, this occurrence is distinctive because it documents the presence of AMV in wheat rather than in quackgrass (21,26).

Little basic information is available concerning AMV, except the modal length and vector (30). To obtain further basic information for comparison of the AMV type specimen and the CO 85 isolate, tests were conducted on the coat protein (apparent size and peptide analysis), viral genomic nucleic acid (type and size), and presence of dsRNA (number of species and size). In our study, the size of the CO 85 and AMV capsids differed by 2 kDa; no other reports are available on the size of the AMV capsid protein for comparison. The apparent sizes of the WSMV isolates agree with previously reported values of 45–47 kDa (7), 45 kDa (17), and 48 kDa (18). The slight difference in values for WSMV in those studies and ours probably results from the gel concentrations used, because the apparent molecular weight can vary with the gel concentration in SDS gel electrophoresis (6).

Both AMV and WSMV had protein bands (although not identical) between 31 and 40 kDa (Figs. 1 and 2). These bands may be the result of degradation of the capsid by proteases during purification. Similar banding patterns were observed for WSMV by Brakke et al (7), who showed that these proteins reacted in western blots to antiserum prepared to WSMV capsid protein. Several sources are possible for the proteins of approximately 66 kDa for AMV (Figs. 1 and 2) and for some WSMV isolates (Fig. 1). The proteins may be of host origin or may be encoded inclusion proteins. AMV is considered a potyvirus (11), and potyviruses have inclusion proteins ranging from 67 to 70 kDa (11). Another possibility is that the 66-kDa and 50-kDa proteins (Fig. 2) resulted from aggregation of intact and partially degraded viral protein. This is most feasible for the 50 kDa protein, because it was not present in minipurification preparations (Fig. 1).

Peptide analysis detected no difference between the AMV isolates. No information exists concerning analysis of peptide fragments of AMV for comparative purposes.

AMV is mite transmitted (30) and is considered as a possible member of the potyvirus group (11). The M_r of the RNA obtained from purified virions of both isolates was approximately 3×10^6 Da and is within the stated range for potyviruses (13). The relative mass of the RNA of this virus has not been determined previously, so no comparative values are available.

The major dsRNA from AMV-infected wheat had a M_r of approximately 5.7×10^6 Da (using Hm 9 fungal dsRNA standard) to 6×10^6 Da (using *P. vulgaris* dsRNA), which would be predicted based on the 3×10^6 Da value determined for the genomic RNA of this virus in this study. The difference in values obtained under the two systems probably results from minor errors in measurements from gels when using the cv. Black Turtle (8×10^6) and BMV markers. Because the major dsRNA comigrated with the Hm 9 band (the M_r of which has been determined by electron microscopy [3]), the 5.7×10^6 value probably reflects the more precise estimate of the size of the AMV dsRNA. Albeit only limited information is available concerning dsRNA of potyviruses, the results of Valverde et al (34) are similar to those of this study. Valverde et al (34) observed few dsRNA species and a major dsRNA of approximately 6.5×10^6 Da for four potyviruses. No previous information exists for comparison of dsRNA of AMV.

The results of the dry weight and symptom experiments indicate that the two AMV isolates are not identical. The CO 85 isolate caused more severe symptoms and a greater reduction in dry matter accumulation of wheat. Differences in virulence have been reported for Canadian AMV isolates (29) but not for isolates from the United States.

This study documents the presence of

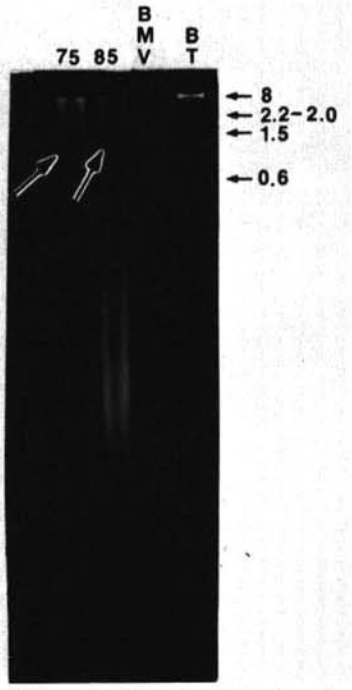

Fig. 5. Polyacrylamide gel electrophoresis (6%) of double-stranded RNA (dsRNA). Lane 75, type specimen PV 75 of Agropyron mosaic virus (AMV); lane 85, Colorado isolate of AMV isolate; lane BMV, dsRNA standard from brome mosaic virus; lane BT, dsRNA from *Phaseolus vulgaris* 'Black Turtle'. Numbers at right represent relative mass of standard designations ($\times 10^6$) of dsRNA in lanes BMV and BT. Arrows indicate position of subgenomic dsRNAs.

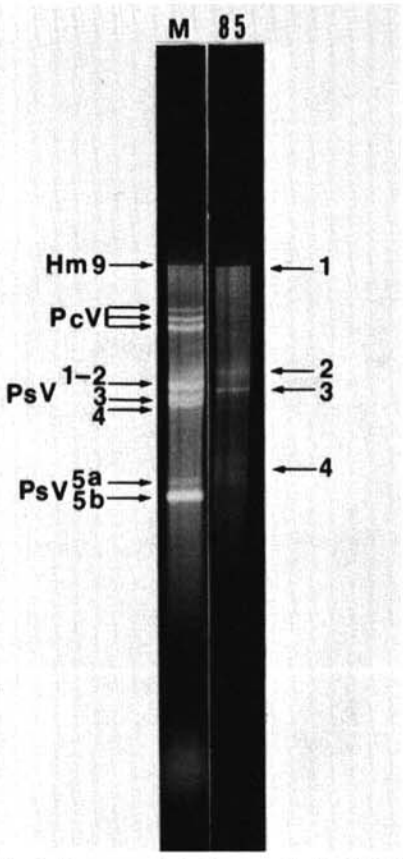

Fig. 6. Agarose gel electrophoresis (1.4%, 72 V, 2 hr) of double-stranded RNA (dsRNA). Lane M, mixture of dsRNA of mycoviruses from *Helminthosporium maydis* (Hm 9) at 5.7×10^6 Da; *Penicillium chrysogenum* (PcV), three segments with an average of 2.0×10^6 Da; and *P. stoloniferum* (PsV), three segments (1–4) with an average of 1.0×10^6 Da and one segment (5a) at 0.51 Da and one (5b) at 0.46×10^6 Da. Lane 85, Colorado isolate of Agropyron mosaic virus; arrows indicate location of dsRNA (bands at arrows 1–3 were consistently observed, whereas faint bands at arrow 4 were rarely observed).

Table 2. Dry weight and symptom severity of wheat cultivars infected by Agropyron mosaic virus isolates PV 75 and CO 85[y]

Cultivar × isolate	Dry weight (g)	Symptom severity[z]	
		Rater 1	Rater 2
Triumph 64 × PV 75	7.1 bc	2.1 d	2.0 d
Triumph 64 × CO 85	6.0 d–f	3.0 c	2.5 c
Centurk × PV 75	6.4 c–e	2.8 c	1.8 d
Centurk × CO 85	4.4 g	4.8 b	3.6 b
Siouxland × PV 75	5.7 ef	5.2 b	4.2 ab
Siouxland × CO 85	4.5 g	6.0 a	4.7 a
Arkan × PV 75	6.8 b–d	4.8 b	3.8 b
Arkan × CO 85	5.3 f	5.3 b	4.7 a
Healthy Triumph 64	8.2 a	1.0 e	1.0 e
Healthy Centurk	7.6 ab	1.0 e	1.0 e
Healthy Siouxland	6.5 c–e	1.0 e	1.0 e
Healthy Arkan	7.0 bc	1.0 e	1.0 e

[y]Results of two experiments combined for ANOVA.
[z]Two independent observers rated symptoms on a scale of 1–6, where 1 = no mosaic, 2 = light green streaks, 3 = mostly light green streaks and a few yellow streaks, 4 = mixed green and yellow streaks, 5 = all yellow streaks, and 6 = severe yellow streaks.

AMV on wheat in Colorado and provides new information concerning the relative molecular weight and peptide analysis of the capsid protein, molecular weight of genomic RNA, and presence and molecular weights of dsRNA of the AMV type specimen PV 75 and the CO 85 isolate. Additional information is presented concerning response of wheat cultivars to infection by these virus isolates.

ACKNOWLEDGMENTS

I thank W. G. Langenberg for the gift of AMV serum, Anis Khimani and R. F. Bozarth for aid in determining the relative mass of dsRNA, Ramon Jordan for advice concerning dsRNA standards, Rodrigo Valverde for advice and the gift of CMV dsRNA markers used in a part of this study, and Anthony Cole for technical assistance.

LITERATURE CITED

1. Baker, S. S., Rugh, C. L., and Kamalay, J. C. 1990. RNA and DNA isolation from recalcitrant plant tissues. BioTechniques 9:268-272.
2. Bancroft, J. B. 1970. Brome mosaic virus. No. 3 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England. 4 pp.
3. Bozarth, R. F. 1977. Biophysical and biochemical characterization of viruslike particles containing a high molecular weight dsRNA from *Helminthosporium maydis*. Virology 80:149-157.
4. Bozarth, R. F., and Harley, E. H. 1976. The electrophoretic mobility of double-stranded RNA in polyacrylamide gels as a function of molecular weight. Biochim. Biophys. Acta 432:329-335.
5. Bozarth, R. F., Wood, H. A., and Mandlebrot, A. 1971. The *Penicillium stoloniferum* virus complex: Two similar virus-like particles in a single cell. Virology 45:516-523.
6. Brakke, M. K. 1987. Virus diseases of wheat. Pages 585-624 in: Wheat and Wheat Improvement. Agron. Monogr. 13. 2nd ed. E. G. Heyne, ed. American Society of Agronomy, Madison, WI.
7. Brakke, M. K., Skopp, R. N., and Lane, L. C. 1990. Degradation of wheat streak mosaic virus capsid protein during leaf senescence. Phytopathology 80:1401-1405.
8. Brakke, M. K., and Van Pelt, N. 1970. Properties of infectious ribonucleic acid from wheat streak mosaic virus. Virology 42:699-706.
9. Clark, M. F., and Adams, A. N. 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34:475-483.
10. Dunn, D. B., and Hitchborn, J. H. 1965. The use of bentonite in the purification of plant viruses. Virology 25:171-192.
11. Francki, R. I. B., Milne, R. G., and Hatta, T. 1985. Potyvirus group. Pages 183-217 in: Atlas of Plant Viruses. Vol. 2. CRC Press, Boca Raton, FL.
12. Gildow, F. E., Ballinger, M. E., and Rochow, W. F. 1983. Identification of double-stranded RNAs associated with barley yellow dwarf infection of oats. Phytopathology 73:1570-1572.
13. Hollings, M., and Brunt, A. A. 1981. Potyvirus group. No. 245 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./ Assoc. Appl. Biol., Kew, England. 5 pp.
14. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of head of bacteriophage T4. Nature 227:680-685.
15. Lane, L. C. 1978. A simple method for stabilizing protein-sulfhydryl groups during SDS-gel electrophoresis. Anal. Biochem. 86:655-664.
16. Lane, L. C. 1978. Virus diagnosis by purification and SDS gel electrophoresis. Phytopathol. News 12:198.
17. Lane, L. C., and Skoop, R. 1983. The coat protein of wheat streak mosaic virus. Phytopathology 73:791.
18. Langenberg, W. G. 1986. Soil-borne wheat mosaic virus protein interactions with wheat streak mosaic virus cylindrical inclusions. J. Ultrastruct. Mol. Struct. Res. 94:161-169.
19. Lischwe, M. A., and Ochs, D. 1982. A new method for partial peptide mapping using *N*-chlorosuccinimide/urea and peptide silver staining in sodium dodecyl sulfate-polyacrylamide gels. Anal. Biochem. 127:453-457.
20. McKinney, H. H. 1937. Mosaic diseases of wheat and related cereals. U.S. Dep. Agric. Circ. 442. 22 pp.
21. McKinney, H. H. 1953. Virus diseases of cereal crops. Pages 350-360 in: Plant Diseases, Yearbook of Agriculture. U.S. Department of Agriculture, Washington, DC.
22. Nash, C. H., Douthart, R. J., Ellis, L. F., Van Frank, R. M., Burnett, J. P., and Lemke, P. A. 1973. On the mycophage of *Penicillium chrysogenum*. Can. J. Microbiol. 19:97-103.
23. Nikodem, V., and Fresco, J. R. 1979. Protein fingerprinting by SDS-gel electrophoresis after partial fragmentation with CNBr. Anal. Biochem. 97:382-386.
24. Rosen, K. M., and Villa-Komaroff, L. 1990. An alternative method for the visualization of RNA in formaldehyde agarose gels. Focus 12:1, 23-24.
25. Seifers, D. L., and Martin, T. J. 1988. Correlation of low level wheat streak mosaic virus resistance in Triumph 64 wheat with low virus titer. Phytopathology 78:703-707.
26. Shepard, J. F. 1968. Occurrence of Agropyron mosaic virus in Montana. Plant Dis. Rep. 52:139-141.
27. Skopp, R. N., and Lane, L. C. 1988. Agarose gel electrophoresis of denatured RNA with silver staining. Anal. Biochem. 169:132-137.
28. Slykhuis, J. T. 1953. Wheat streak mosaic in Alberta and factors related to its spread. Can. J. Agric. Sci. 33:195-197.
29. Slykhuis, J. T. 1962. Agropyron mosaic as a disease of wheat in Canada. Can. J. Bot. 40:1439-1447.
30. Slykhuis, J. T. 1973. Agropyron mosaic virus. No. 118 in: Descriptions of Plant Viruses. Commonw. Mycol. Inst./Assoc. Appl. Biol., Kew, England. 4 pp.
31. Slykhuis, J. T., and Bell, W. 1966. Differentiation of Agropyron mosaic, wheat streak mosaic, and a hitherto unrecognized Hordeum mosaic virus in Canada. Can. J. Bot. 44:1191-1208.
32. Sonderegger, P., Jaussi, R., Gehring, H., Brunschweiler, K., and Christen, P. 1982. Peptide mapping of protein bands from polyacrylamide gel electrophoresis by chemical cleavage in gel pieces and re-electrophoresis. Anal. Biochem. 122:298-301.
33. Valverde, R. A. 1990. Analysis of double-stranded RNA for plant virus diagnosis. Plant Dis. 74:255-258.
34. Valverde, R. A., Dodds, J. A., and Heick, J. A. 1986. Double-stranded ribonucleic acid from plants infected with viruses having elongated particles and undivided genomes. Phytopathology 76:459-465.
35. Wakarchuk, D. A., and Hamilton, R. I. 1985. Cellular double-stranded RNA in *Phaseolus vulgaris*. Plant Mol. Biol. 5:55-63.
36. Wiese, M. V. 1977. Wheat streak mosaic. Pages 75-77 in: Compendium of Wheat Diseases. American Phytopathological Society, St. Paul, MN.
37. Wray, W., Boulikas, T., Wray, V. P., and Hancock, R. 1981. Silver staining of proteins in polyacrylamide gels. Anal. Biochem. 118:197-203.