Wheat Streak Mosaic Virus: Increased Yields of Purified Virus from Corn

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ABSTRACT

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A wheat streak mosaic virus isolate from corn (WSMV-C) and another from wheat (WSMV-W) were easily purified and yielded more virus particles from infected corn (*Zea mays*) than from infected wheat (*Triticum aestivum*) plants. Tissue was triturated in phosphate buffer containing 2-mercaptoethanol, and the extract was clarified with chloroform and given differential centrifugation. Partially purified preparations were further subjected to sucrose density-gradient centrifugation or zone electrophoresis. Greater yields of both isolates were obtained consistently from corn, as evidenced by relative sizes of virus bands in density-gradient columns and by differences in numbers of virus particles observed by electron microscopy. Antiserum prepared to WSMV-C and tested in enzyme-linked immunosorbent assay (ELISA) was reactive with homologous virus, with crude preparations containing WSMV type strain, and with other wheat and corn isolates.

In Kansas, wheat streak mosaic virus (WSMV) has caused annual crop losses of 3.5 t (13×10^6 bu) (7). At present, virus infection is diagnosed by symptoms and/or bioassays. During the early stages, however, WSMV infection is difficult to distinguish from symptoms caused by nitrogen deficiency, cold temperature injury, chemical damage, and other plant disease problems. Also, bioassays require greenhouse space for indicator plants and time, eg, 7–21 days of incubation for symptoms to appear. Serology is the method of choice by virologists for detecting and identifying plant viruses and diseases they cause. Routine seroassays for WSMV have not been possible, however, because of the paucity of virus-specific antiserum.

Antiserum production requires large quantities of purified virus particles. Although published methods for purifying WSMV reportedly gave consistent results, the procedures involved several time-consuming steps and virus yields were low, ie, 0.4 mg/100 g of tissue (1,2). We have devised an alternative procedure for WSMV purification. The procedure and data comparing purified virus yields and WSMV enzyme-linked immunosorbent assay (ELISA) tests are reported here.

MATERIALS AND METHODS

Isolates of WSMV were obtained from naturally infected field corn (*Zea mays* L.) (WSMV-C) and wheat (*Triticum aestivum* L.) (WSMV-W). Each isolate was increased on both N28Ht inbred corn and Parker wheat seedlings for 14 days. Infected plants were cut off above the soil line and cut into 4-cm lengths. Tissue was triturated in a Waring Blendor with phosphate buffer (0.1 M potassium phosphate, pH 7.0, containing 0.5% 2-mercaptoethanol, 1:3, w/v). After filtration through cheesecloth, the extract was mixed with 0.5 vol chloroform and shaken mechanically for 15 min; the emulsion was broken by centrifugation (5,000 *g*) for 5 min. Virus was sedimented twice at 78,000 *g* for 60 min. The first high-speed pellets were resuspended in 0.02 M phosphate buffer and the second, in borate buffer (0.0125 M borate, pH 7.5, and 0.024 M NaCl). These preparations, respectively, were layered onto a sucrose density-gradient column (10–40% sucrose in 0.02 M phosphate buffer and centrifuged at 80,000 *g* for 2 hr) or zone electrophoresed (11) for 12–15 hr at 200 V and 15 mA in a sucrose gradient borate-NaCl system.

Partially purified preparations were stained with 2% sodium phosphotungstate, pH 7.0, and examined in a Phillips 200 electron microscope.

Purified virus (1 mg/ml) was emulsified with Freund's incomplete adjuvant (2:1, v/v), and a rabbit was given a series of subcutaneous and intravenous (without adjuvant) injections. Antiserum collected on day 95 after the initial injection was used. Although nonreactive with plant proteins, antiserum was absorbed with host antigens (8) before being used in ELISA. Test samples and anti-WSMV immunoglobulin were prepared as described previously (10).

RESULTS

Initially, all purification trials were attempted with WSMV-C infected corn plants. Tissue (25–50 g) was triturated with different molarities (0.5, 0.1, and 0.05 M) of phosphate buffer, pH 7.5, and citrate buffer, pH 7.5 (0.1 M), clarified with chloroform, and concentrated by centrifugation. Resuspended pellets (equivalent to 25 g of tissue) were layered onto sucrose gradient columns, centrifuged, and analyzed with an ISCO density-gradient fractionator. No virus band was detected in extracts made in 0.5 M phosphate or 0.1 M citrate. Extracts in 0.1 or 0.05 M phosphate, however, produced infectious bands of comparable size in the gradient column. The higher molarity (0.1 M) was selected for further tests with phosphate solutions buffered at pH 6.5, 7.0, 7.5, and 8.0. All preparations yielded virus, but neutral pH buffer produced the largest and sharpest virus band in gradient columns. Because yields of virus in corn infected 10–19 days were similar, plants were used routinely at 14 days.

When purified from corn, both isolates consistently produced a virus band in sucrose gradient columns (Fig. 1), but those from wheat did so only occasionally. Although WSMV-wheat preparations were infectious, WSMV-corn preparations contained many more virus

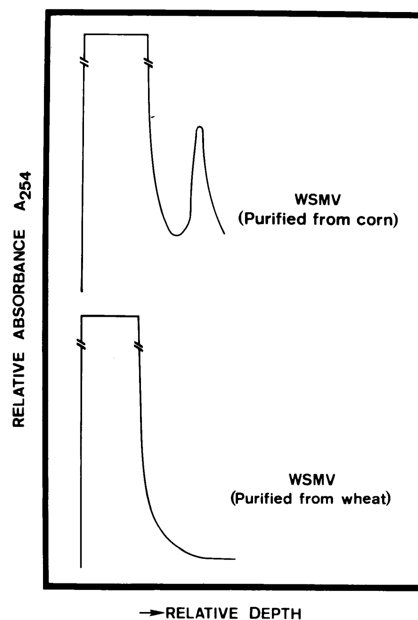

Fig. 1. Sedimentation profiles of density gradient columns layered with wheat streak mosaic virus preparations from corn and wheat. Gradients were centrifuged for 2 hr at 83,000 *g* and scanned at 254 nm. A virus peak was detected routinely only in corn preparations. Sedimentation was from left to right.

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particles, as determined by electron microscopy (Fig. 2). An average yield of virus from corn, after zone electrophoresis and assuming A_{254nm} of 1.2 = 0.4 mg (1), was about 3.3 mg/100 g of tissue.

In zone electrophoresis with NaCl dissolved in phosphate or tris-HCl buffers, the slower migrating (toward the anode) component always precipitated. This did not occur in the borate-NaCl buffer, where the infectious band was opalescent and homogeneous throughout.

The chloroform procedure was compared with the acid-heat method described by Brakke and Ball (2). All clarification procedures produced infectious preparations. However, virus yields from the acid-heat treatment for wheat or chloroform emulsification for corn were greater than for acid-heat with corn or chloroform with wheat. This suggested that, depending on the infected host plant source, different clarification methods may be necessary to realize reasonable yields of purified WSMV particles.

In microprecipitin tests, antiserum to WSMV-C showed virus-specific reactivity to 1/64 dilution. In ELISA with 10 μg/ml concentration of unlabeled and enzyme-labeled anti-WSMV immunoglobulins, the absorbancies at 405_{nm} of purified WSMV-C adjusted to OD_{260nm} of 0.5 and 0.1 were 1.28 and 1.23, respectively. In later ELISA tests, other WSMV isolates (type strain and a corn isolate from M. K. Brakke, University of Nebraska) were increased on wheat and corn seedlings and tested. The results are shown in Table 1. Plant extracts were prepared in 0.01 M phosphate buffer, pH 7.0, 0.8% sodium chloride, 0.05% Tween 20, and 2% polyvinylpyrrolidone (mol wt 44,000); each sample was tested in duplicate and absorbance values were averaged. Although WSMV was identified in all extracts (except WSMV type strain in N28Ht corn, an inbred line resistant to type strain) up to a 1/100 dilution, wheat extracts gave higher absorbance averages than comparable dilutions of corn sap.

DISCUSSION

Although WSMV susceptibility in corn is documented (3–5), use of this host for virus increase and purification has not been reported. Based on results obtained with our purification procedure, N28Ht corn is superior to Parker wheat for consistently high yields of purified WSMV. The propensity of WSMV to precipitate in certain buffers, as reported by Brakke and Ball (2), also occurred in our experiments. Both phosphate-NaCl and tris-HCl-NaCl buffers caused excessive precipitation of virus in the electrophoresis gradient column. That problem was circumvented with a borate-NaCl buffer, in which the virus clearly migrated away from host components and maintained an even opalescence during overnight electrophoresis.

In contrast to the high titer WSMV antisera (ie, 1/256–1,024) reported by others (1,6), ours were quite low (1/64), suggesting that WSMV-C was only moderately immunogenic. Treatment of purified preparations with an aldehyde (9) to help stabilize the integrity of virus particles may increase response of immunized rabbits, and we now are testing this possiblity.

In ELISA, infected wheat sap showed higher absorbance values than corn, suggesting that wheat contained more

Table 1. ELISA absorbance readings for wheat streak mosaic virus isolates in plant sap[a]

		Plant sap dilution[c]			
Virus isolate[b]	**Host plant**	**1/10**	**1/50**	**1/100**	**1/1,000**
WSMV-C	Corn	0.75	0.52	0.36	0.11
	Wheat	0.94	0.87	0.67	0.12
WSMV-W	Corn	0.67	0.48	0.37	0.13
	Wheat	0.85	0.59	0.46	0.11
WSMV-T	Corn[d]	0.19	0.09	0.05	0.03
	Wheat	0.71	0.46	0.32	0.10
WSMV-N	Corn	0.53	0.33	0.24	0.08
	Wheat	0.93	0.92	0.87	0.22
	Healthy corn	0.09	0.08	0.06	0.03
	Healthy wheat	0.03	0.01	0.01	0.01

[a] Unlabeled and enzyme-labeled anti-WSMV-C immunoglobulins were used at 10 μg/ml each. Sources of test antigens were N28Ht corn and Parker wheat infected for 16 days.
[b] -C = corn, -W = wheat, -T = type strain, -N = Nebraska corn isolate.
[c] Absorbance at 405 nm; values are averages derived from duplicate tests.
[d] N28Ht corn is not susceptible to WSMV-T.

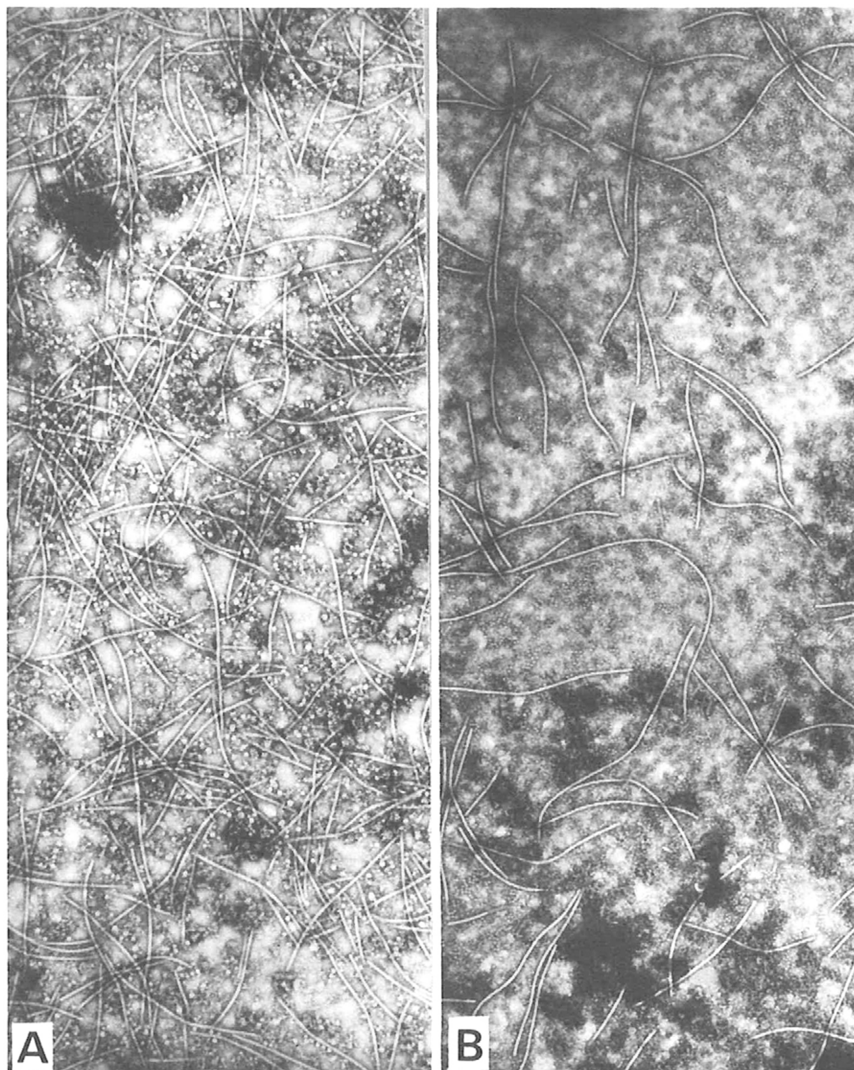

Fig. 2. Electron micrographs of partially purified wheat streak mosaic virus in extracts of corn **(A)** and wheat **(B)**. Particles were stained with 2% sodium phosphotungstate, pH 7.0.

viral coat protein. Even so, corn, in comparative experiments, afforded more extractable virus particles (ie, eight times more than previously reported where wheat and acid-heat treatment were used) with the purification procedure adopted here. The chloroform procedure was also simpler to perform and required fewer steps than the acid-heat method.

ELISA tests have been used to detect and differentiate virus serotypes (10). In our WSMV-ELISA tests with relatively low titer antiserum, however, the ease of detecting different virus isolates (even though WSMV type strain was a distinct biotype) as well as a number of wheat isolates recently collected in the field (J. K. Uyemoto, *unpublished*), suggests that the isolates are closely related antigenically. Hence, in contrast to virus groups that contain known serotypes and require antiserum with broad cross-reactivity, a single source of WSMV antiserum apparently can be used for routine virus diagnosis.

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