Characterization and Field Studies of a Cucumber Mosaic Virus Isolate from Spinach in the Winter Garden Area of Texas

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ABSTRACT


An isolate of cucumber mosaic virus (CMV) was identified from spinach in the Winter Garden area of Texas. The isolate was very closely related serologically to strain S of CMV and is designated the Texas spinach isolate of CMV-S. The virus infected 39 species of crop plants and wild hosts in 12 of 13 families tested. The green peach aphid efficiently transmitted the virus experimentally. The isolate had a sedimentation coefficient of 91.8 ± 0.1 S as determined by analytical ultracentrifugal analysis. Virions with a mean diameter of 28.9 ± 0.3 nm were found in purified preparations with electron microscopy. A single protein subunit with a mean molecular weight of 35,000 ± 235 daltons was found by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Four separate RNA species were resolved by agarose-gel electrophoresis under denaturing conditions. The mean molecular weight distribution of the viral genome was 1.22, 1.09, 0.77, and 0.36 ± 10^6 daltons. CARM 5 was not jointly encapsidated with the viral genome. In the field studies, planting date and plant age at inoculation had no significant effect on crop yield, although CMV infection was highly significant. The isolate significantly affected yields of three spinach cultivars, with reductions ranging from 23.8 to 47.4%.

Additional key words: spinach blight, Spinacia oleracea

Spinach (Spinacia oleracea L.) was the third most economically important vegetable crop for fresh market in Texas for 1981. About 22,000 t of fresh-market spinach were produced in Texas in 1981 with a market value exceeding $18 million (11). The two major production areas in the state, the Winter Garden and the Lower Rio Grande Valley, account for about 45% of the total fresh-market spinach acreage harvested in the United States (1).

Five viruses are presently known to contribute consistently to yield losses of spinach each year in Texas. They are beet curly top virus, cucumber mosaic virus (CMV), spinach yellow dwarf virus, tobacco ringspot virus (9), and a newly described isometric virus, as yet unnamed, which has severely affected spinach production in southern Texas (5). In a survey during the 1979-1980 spinach growing season, a new virus isolate was obtained. Infected spinach plants not only showed the familiar spinach blight symptoms most commonly associated with CMV but also showed systemic mottling, acute vein distortion, or severe leaf narrowing, distortion, and leaf curling. The range and variability of symptoms expressed in different spinach cultivars were examined, and the effect of the virus on yields of selected spinach cultivars was determined under field conditions.

In this paper, we characterize the spinach blight virus with host range, vector transmission studies, analytical ultracentrifugal analysis, electron microscopy, serology, and electrophoresis. Furthermore, we demonstrate yield losses of commercial spinach cultivars attributed to the CMV isolate.

MATERIALS AND METHODS

Virus isolate source. The isolate characterized in this paper was obtained in February 1980 from naturally infected spinach plants. The virus was collected within the Winter Garden area in Zavala County, TX, and maintained on Nicotiana tabacum L. 'Samsun NN.' For purification purposes, the virus was propagated in tobacco cultivar Xanthi NN.

Host range and aphid transmission. Test plants were selected from host ranges of spinach viruses and from weed species common around spinach fields. All plants were grown in 25-cm pots in the greenhouse. Mechanical inoculations utilized sap from apical leaves of the maintenance host buffered with 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1% 2-mercaptoethanol and 600-mesh Carborundum. Control plants were maintained and recovery tests were made on Cucurbita maxima Duchs. 'Zucuco' to detect latent infections in hosts that remained symptomless after inoculation.

Green peach aphids (Myzus persicae Sulz.) reared on spinach were held for a 1-hr preacquisition starvation period, then transferred to strongly symptomatic and caged spinach plants infected with the spinach isolate. After 1-min to 3-hr acquisition feeding periods, the aphids were placed on six healthy caged spinach plants (10-15 cm high) overnight. The test plants were observed 2 wk for symptom development.

Purification, analytical ultracentrifugation, and electron microscopy. The spinach isolate was purified using a modification of the procedure described by Lot et al (8). Triton X-100 was added to the extraction buffer to 2% at the beginning of the procedure rather than after clarification with chloroform extraction (13). In addition, the virus was finally resuspended in 5 mM sodium borate buffer, pH 9.0, with sodium metaphosphate added to 0.5% to reverse aggregation.

Sedimentation analyses were carried out with purified virus fixed with formaldehyde (4), dialyzed against 5 mM borate resuspension buffer to equilibrium, and analyzed using a Spino Co-A-D rotor run at 32,000 rpm and at 23.4 C.

Virus morphology was determined with a Hitachi HS-75 electron microscope. Purified virus particles were fixed with formaldehyde, freeze-dried on a Parlodion-coated EM grid, and stained with 1% uranyl acetate in 95% ethanol. Mean virion diameter was determined from measurements of 100 virus particles.

Antiserum production and serology. Antisera against purified and formaldehyde-fixed CMV were obtained by intramuscular and subcutaneous injections of rabbits, 0.5 mg of virus per injection, at weekly intervals. The preparations were emulsified with an equal volume of Freund's complete adjuvant (Difco).

Antiserum prepared against the spinach isolate and CMV-S antiserum (ATCC PVAS 242a) was tested against purified and formaldehyde-treated antigens of the spinach isolate and CMV-Collumella (ATCC PV 30) to compare homology between the antigens.

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Analysis of virion protein and nucleic acid. CMV capsid protein molecular weights were determined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (6). Molecular weight marker proteins, 14,400-92,500 daltons, were obtained from Bio-Rad.

The viral genomic RNAs were analyzed by electrophoresis in 1.4% agarose gels under denaturing conditions (7). Three markers, including the genomic RNAs of tobacco mosaic virus, brome mosaic virus, and Hind III restriction endonuclease DNA fragments of phage λ, were used as molecular weight standards.

RNA was extracted from purified virus preparations following the protocol of McMaster and Carmichael (10). The molecular weights of viral RNA's were determined by plotting the logarithms of the molecular weights of marker RNAs and DNAs against migration distances.

Field studies. Two field studies were conducted to determine the effect of planting date, plant age, and cultivar selection on plant density and final crop yield of CMV-infected spinach plants. Four smooth-leaf spinach cultivars from Del Monte, designated A, B, C, and D, were tested in each study. In study I, the cultivars were planted three times during the growing season and inoculated at the four-leaf stage. In study 2, the cultivars were planted at the beginning of the growing season and inoculated at different stages of growth. Three field plots 46.9 × 13.4 m were used as replicates for each study. Three doubled-rowed plots, 0.97 m wide, were planted for each cultivar per plot, and border rows were planted to encompass each plot. Each row was divided into six 3.66-m sections, each separated by a 3-m section of unplanted vacant row, except for border rows, which were planted solid. Disulfoton (Di-Syston) and diazinon (Diazinon) were applied to the test plots to minimize vector transmission of the virus.

The virus inoculum was prepared by blending CMV-infected tobacco leaf tissue with 0.05 M potassium phosphate buffer, pH 7.0, containing 0.1% thiglycolic acid, at a ratio of 4 ml of buffer per gram fresh weight of tissue. The extract was filtered twice through cheesecloth, and 600-mesh Carborundum was added to 1% (w/v) to enhance inoculation. The inoculum was applied with a 250-ml DeViibiss paint sprayer and propelled with CO₂ at 45-50 psi of tank pressure. The sprayer was calibrated to a flow rate of 258 ± 28.9 ml/min. A spray nozzle-to-leaf distance of 5 cm was maintained. For each inoculation, a 3.66-m row section of each cultivar was spray-treated. The cultivars were arranged randomly in a diagonal pattern across each field plot. One bed of each row section was spray-inoculated, and the other bed was sprayed with extraction buffer containing only 1% Carborundum. Double rows of untreated plants on each side of the sprayed rows served as additional controls.

RESULTS


The green peach aphid efficiently transmitted the spinach isolate to spinach plants at each acquisition feeding period. In every case, all test plants developed symptoms and eventually blighted within 30 days of inoculation.

Analytical ultracentrifugation and electron microscopy. The sedimentation coefficient curve obtained for the virus isolate using polynomial least-squares regression analysis very closely fit the data (R² = 0.9999) and is expressed by the function: S20,w = 91.80 + 0.165C - 0.271C²), where C is the virus concentration in milligrams per milliter. Linear regression analysis did not fit the data as closely (R² = 0.9577).

Small isometric particles were found in purified virus preparations with electron microscopy. The mean diameter of the virions, determined from measurements of 100 particles, was 28.9 ± 0.3 nm.

Antiserum production and serology. Precipitin titters of all antisera prepared against the spinach isolates did not exceed a dilution end point of 1/32. Antigen fixed by dialysis with formaldehyde before injection was not significantly more immunogenic than unfixed antigen.

Homology tests between purified antigens of the spinach isolate and known CMV-Commelina at the same concentration were compared using antiserum prepared against the spinach isolate and CMV-S antiserum (PVAS 242a). No spur formation was observed between antigens of the spinach isolate and CMV-Commelina when antisera against the spinach isolate was used (Fig. 1A). However, spur formation did occur between the spinach isolate and known antigens when CMV-S antiserum was used (Fig. 1B).

Fig. 1. Serological comparison of the Texas spinach isolate of CMV-S with CMV-Commelina in agarose-gel double-diffusion tests. Well H contained healthy tobacco sap and the unlabeled well was blank. The remaining wells contained 1.28 mg/ml of purified virus. Wells C and M represent two separate isolations of the Texas spinach isolate that were found to be identical. Well K contained purified virions of CMV-Commelina (ATCC PV 30). Central wells contain antisera to (A) the Texas spinach isolate and (B) CMV-S (ATCC PVAS 242a).

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Analysis of virion protein and nucleic acid. The viral capsid consisted of a single protein subunit. The mean subunit molecular weight estimate of eight determinations was calculated to be about 25,300 ± 255 daltons.

Four separate RNA components (RNAs 1-4) of the viral genome were resolved. The mean molecular weight distribution of the viral genome, estimated from four determinations, was 1,223 ± 0.016, 1,086 ± 0.018, 0.774 ± 0.010, and 0.356 ± 0.008 × 10^6 daltons. The fifth RNA component, CARN 5, was not detected in the RNA preparations.

Field studies. Symptoms of CMV-infected spinach in the field ranged from blight of seedlings to mottling, stunting, upward leaf cupping, narrowing of leaves, and mild veinal distortion of spinach at intermediate growth stages to severe veinial distortion with no noticeable stunting or chlorosis of mature plants. Most plants developing inoculation symptoms at the four-leaf stage did not show the severe symptoms associated with the seedling and intermediate growth stages, although they did eventually develop the veinial distortion symptom associated with mature plants. Plants usually developed the severe symptoms associated with the intermediate growth stages only when they were inoculated at those stages. Visual detection of diseased plants increased with plant age and approached 100% in fully mature plants. The relatively low detection rate of <10% for seedlings was due to the rapid decomposition of blighted plants and the low frequency of observations.

Three planting dates were used to determine the effect of planting date, plant age, and cultivar selection on final crop yield after inoculation. In study 1, plants were inoculated at the four-leaf stage after consecutive plantings on 29 September, 27 October, and 30 December 1982, respectively. In study 2, all plants were planted on 29 September and inoculated at different growth stages on the same inoculation dates selected for study 1. Comparisons between yield of inoculated and control plants in plantings 1 and 2 indicated that planting date, plant age, and cultivar selection had no significant effect on final crop yield. However, CMV-infection did significantly affect yield (*P < 0.001). CMV-infected plants had significantly lower yield (1.9 kg) compared with about 2.8 kg/m of row in untreated and Carborundum-treated controls (P = 0.05). The third planting was not compared with plantings 1 and 2 because it was harvested before maturity because of incipient bolting tendency.

Table 1. Effect of CMV-S infection on yield of each spinach cultivar in plantings 1 and 2 on September 27 and October 1982

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>df</th>
<th>Mean square</th>
<th>F value</th>
<th>GLM** (PR &gt; F)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A'</td>
<td>2</td>
<td>1,205</td>
<td>1.73</td>
<td>0.1965</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>11,148</td>
<td>10.77**</td>
<td>0.0004</td>
</tr>
<tr>
<td>C</td>
<td>2</td>
<td>5,669</td>
<td>5.30*</td>
<td>0.0445</td>
</tr>
<tr>
<td>D</td>
<td>2</td>
<td>10,123</td>
<td>25.77**</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

*Degrees of freedom.
**Dependent variable (yield) measured in kilograms.
***Significant at P = 0.005 and **Significant at P = 0.01.

Table 2. Reductions in mean yield of spinach cultivars resulting from CMV-S infections in plantings 1 and 2 on September 27 and October 1982

<table>
<thead>
<tr>
<th>Planting</th>
<th>Cultivar</th>
<th>Mean yield</th>
<th>Difference</th>
<th>(S)</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Inoculated</td>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>A'</td>
<td>2.482</td>
<td>-17.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.877</td>
<td>-42.1*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>1.967</td>
<td>-40.4*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>1.392</td>
<td>-47.4*</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>2.368</td>
<td>-5.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>1.634</td>
<td>-38.5*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>2.126</td>
<td>-23.8*</td>
<td></td>
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<tr>
<td></td>
<td>D</td>
<td>1.756</td>
<td>-27.5*</td>
<td></td>
</tr>
</tbody>
</table>

*Kilograms per 1.65 m row sections sampled.
**Percent difference in yield followed with an asterisk are significant at P = 0.05.

Table 3. Effects of cultivar selection on yield of CMV-S-infected spinach in planting 3 on 30 December 1982

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Mean square</th>
<th>F value</th>
<th>ANOVA (PR &gt; F)</th>
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</thead>
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<td>Model</td>
<td>7</td>
<td>299</td>
<td>7.22</td>
<td>0.0001</td>
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<tr>
<td>Cultivar</td>
<td>3</td>
<td>1,154</td>
<td>9.33*</td>
<td>0.0002</td>
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<tr>
<td>Treatment</td>
<td>2</td>
<td>504</td>
<td>6.11*</td>
<td>0.0063</td>
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<tr>
<td>Error</td>
<td>28</td>
<td>41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Degrees of freedom.
**Dependent variable (yield) measured in kilograms.
***Significant at P = 0.01.

Analysis of variance.
reacted very strongly to both antiserum. Since the spinach isolate was strongly related serologically to strain S of CMV, we place the isolate in the ToRS serotype group as defined by Devergne and Cardin (2,3). The development of necrotic local lesions on tobacco cultivar Xanthi NN in addition to systemic symptoms provided further confirmation that the isolate belongs to this group. On the basis of serological results, morphology, and physical characteristics, we conclude that the spinach isolate is very closely related to strain S of CMV and we designate it the Texas spinach isolate of CMV-S.

Field evaluations of symptomatology with experimentally inoculated spinach provided evidence that symptom severity was age-dependent. Symptom severity appeared to be inversely proportional to plant age. Since symptoms were more severe when plants were inoculated at earlier stages of growth, disease detection in younger plants was often difficult because the blighted plants decomposed rapidly. Symptoms of mature plants were generally milder with no noticeable stunting or chlorosis, yet the conspicuous veinal distortion symptom was most developed and obvious in mature plants. Planting date and plant age at inoculation had no significant effect on final crop yield. The effects of planting date on yield were compared with mean daily temperature highs during the studies, since temperature is known to be a major factor affecting resistance to CMV (12). Pound and Cheel (12) demonstrated that under experimental conditions, blight resistance of spinach cultivar Virginia Savy failed at air temperatures of 28 C and higher. For all three plantings, the mean daily temperature highs during the studies were 28.3 C. The lack of significant yield differences for these planting dates suggests that the critical temperature for breakdown of resistance in the field may be higher than 28 C.

Plant age at inoculation did not significantly affect yield of three cultivars. This is contrary to the observation that symptom severity is age-dependent. The discrepancy may be explained as mortality increases, competition between plants for available resources, including space, decreases. Therefore, high mortality at the seeding stage may be offset by increased growth of surviving plants. This is a major reason why the virus effects on plant density do not necessarily result in reduced yield. Significant differences between yield of the cultivars tested were found only in planting 3 when the crop was harvested before maturity.

LITERATURE CITED