Reactions of Bahamian Hot Chile to Single and Double Infections with Tobacco Mosaic Virus and Potato Virus Y

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ABSTRACT

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Field-grown Bahamian hot chile (*Capsicum annuum*) from Bixby, OK, showed mild stunting and leaves with mild mosaic or severe stunting and leaves severely chlorotic to necrotic. Tobacco mosaic virus (TMV) or potato virus Y (PVY) was found in plants with milder symptoms. TMV and PVY were found in plants with severe symptoms. Bahamian hot chile plants inoculated with TMV and/or PVY showed symptoms similar to those found in the field. TMV or PVY inoculated singly caused stunting and mosaic. TMV and PVY inoculated together caused severe stunting and systemic necroses. Thus, a synergistic interaction between TMV and PVY in Bahamian hot chile is indicated.

There are numerous reports of virus diseases of many cultivars of pepper (*Capsicum annuum* L.), but information on the reaction of the cultivar Bahamian hot chile to virus infection is lacking (12). Bahamian hot chile production is a multimillion dollar industry in the United States with about $0.5 million of production in Oklahoma. Virus disease can be a limiting factor in pepper production in the southwestern United States (12). Each year since 1980, field-grown Bahamian hot chile in propagation areas at the Vegetable Research Station at Bixby in eastern Oklahoma have shown either mild stunting and leaves with mild mosaic or severe stunting and leaves severely chlorotic to necrotic. Severe symptoms have also been observed in commercial production fields in Caddo County in west central Oklahoma. Plants showing the milder symptoms had reduced fruit production, and plants with necrosis had no marketable fruit. In 1984 and 1985, samples were collected from affected plants at Bixby. Initial mechanical inoculations to *Nicotiana tabacum* L. cv. Xanthi-nc from affected material with mild stunting and leaves with mild mosaic resulted in necrotic local lesions or a systemic mosaic. Initial mechanical inoculations to *N. tabacum* cv. Xanthi-nc from affected material with severe stunting and leaves severely chlorotic to necrotic resulted in necrotic lesions and a systemic mosaic. The purpose of this work was to identify the virus(es) from the affected plants and determine the etiology of the two distinct symptoms on Bahamian hot chile.

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MATERIALS AND METHODS

Material was prepared for mechanical inoculations by grinding in a mortar and pestle in 0.01 M phosphate buffer, pH 7.0, with 0.02 M 2-mercaptoethanol. Inoculations were made with small four-ply gauze pads saturated with inoculum and rubbed lightly on leaves previously dusted with 225-μm corundum (no. 225 optical powder, Semi-Tech, Inc., Dallas, TX). Inoculated leaves were immediately covered for 6–12 hr with damp paper. Plants were grown in 10.2-cm pots in a greenhouse maintained at about 27 C.

For protein-A immunosorbent electron microscopy (PA-ISEM) (10), grids were prepared by coating with protein-A (0.1 mg/ml, Sigma Chemical Co., St. Louis, MO) in 0.1 M sodium phosphate buffer, pH 7.0, for 5 min and draining onto filter paper. Grids were then incubated in antiserum (diluted 1:50 in phosphate buffer) for 10 min, rinsed in water, and drained. A 1:10 dilution of virus extract in phosphate buffer was placed on the grids for 1 hr; the grids were rinsed in water and drained. Virions were decorated with antiserum by incubation in a 1:50 dilution of antiserum as above before viewing by electron microscopy at 80 kV. Particle morphology was determined with uranyl acetate negative-stained material as previously reported (9).

Agar gel double-diffusion tests for tobacco mosaic virus (TMV) were carried out in 0.75% Ionagar No. 2 (Consolidated Laboratories, Chicago Heights, IL) in phosphate-buffered saline (PBS) with 0.25% sodium azide. Antiserum had previously been prepared to the common strain of TMV. A female New Zealand rabbit received intramuscular infections twice a week of 1 mg of virus with Freund's complete adjuvant for 5 wk. Antiserum had a microprecipitin titer against purified TMV of 1,024. Plant material was ground in PBS to a final dilution of 1:10 (w/v) before addition to wells. Agar gel double-diffusion tests for potato virus Y (PVY) were carried out in 0.6% agarose in 0.1 M Tris-HCl, pH 9.0, with 0.2% sodium dodecyl sulfate (SDS), 0.7% NaCl, and 0.1% sodium azide (7). Plant material was ground in Tris-HCl buffer with SDS, NaCl, and sodium azide to a final dilution of 1:10 (w/v) before addition to wells.

The PAS-ELISA (protein-A sandwich enzyme-linked immunosorbent assay) was conducted after Edwards and Cooper (4). ELISA plates (Plastic Injectors, Spartanburg, SC) were rinsed with water, and protein-A (1 μg/ml) in 0.05 M sodium carbonate buffer, pH 9.6, was added and incubated for 2 hr at 30 C. Plates were rinsed with PBS and 0.05% Tween (PBS-Tween), and antiserum was added at a 1:1,000 dilution in PBS-Tween. Plates were incubated for 2 hr at 30 C and rinsed with PBS-Tween, then virus samples in PBS-Tween (1:5 w:v) were added followed by incubation overnight at 5 C. Plates were rinsed and antiserum was added as before. After a 2-hr incubation at 30 C, protein-A-alkaline phosphatase (Sigma) diluted 1:1,000 in PBS-Tween was added and incubated 2 hr at 30 C. Plates were then rinsed in PBS-Tween and the *p*-nitrophenylphosphate substrate added. After plates were incubated for about 2 hr at room temperature, the absorbance at 405 nm was read on a Dynatech Minireader II (Dynatech Laboratories, Inc., Chantilly, VA). There were seven wells per virus sample, and wells containing PBS-Tween were used to calibrate the instrument.

The virus isolate identified as PVY was purified by method 2 of Reddick and Barnett (8). Antiserum to the PVY isolate was prepared by an intradermal injection of 1 mg of purified PVY into a female New Zealand rabbit followed by two sets of intradermal and intramuscular injections (1 mg of PVY each) 2 wk apart.

Rabbits were bled 1 wk after the third injection, and the serum was separated by centrifugation.

The Wisconsin isolate of PVY and antiserum to this isolate were provided by S. A. Slack, University of Wisconsin. TEV was provided by O. W. Barnett, Clemson University. Antiserum to TEV had previously been made as outlined for PVY. Pepper mottle virus (PMV) and antiserum to PMV were provided by M. R. Nelson, University of Arizona.

RESULTS AND DISCUSSION

One virus isolate from Bahamian hot chile (Bixby, OK) was identified as TMV based on host range, electron microscopy, and reaction to antiserum to the common strain of TMV in agar gel double-diffusion tests. The isolate produced necrotic localized lesions on *N. tabacum* cv. Xanthi-nc and *Chenopodium amaranticolor* Coste & Reyn. and produced a systemic mosaic in *N. tabacum* cv. Samsun and *N. sylvestris* Speg. & Comes. Negatively stained virions from leaf dips had an average size of 18 × 300 nm (n = 25). In agar gel double-diffusion tests, the isolate reacted with antiserum to the common strain of TMV without spur formation when run in wells adjacent to the common strain of TMV. All these criteria indicate the isolate was TMV (13).

Another virus isolate was identified as PVY based on host range, PA-ISEM, PAS-ELISA, and agar gel double-diffusion tests. Symptoms were not observed on inoculated *Datura stramonium* L. or *C. frutescens* L. cv. Agronomico-8 nor could the virus be reisolated. *D. meteloides* L. had a symptomless reaction to inoculation by the isolate, but *D. metel* L. had a severe reaction to the virus. The isolate produced necrotic local lesions on *C. amaranticolor*, a systemic mosaic in *N. tabacum* cv. Xanthi-nc, a systemic mosaic and necrosis in *Physalis floridana* L., and a severe mosaic in *C. frutescens* cv. Tabasco. The isolate did not cause wilting and death in the cultivar Tabasco as does tobacco etch virus (TEV) (6).

Negatively stained purified virions had an average size of 12× 724 nm (n= 50). In PA-ISEM, virions reacted with rabbit serum made to the isolate and to antiserum to the Wisconsin isolate of PVY. The PVY isolate from Bahamian hot chile did not react with antiserum to TEV in PA-ISEM.

In PAS-ELISA, the isolate reacted to antiserum made to that isolate but did not react significantly to antiserum to TEV or to antiserum to PMV (Table 1). In agar gel double-diffusion tests, the PVY isolate from Bahamian hot chile reacted with antiserum made to that isolate and to antiserum to the Wisconsin isolate of PVY. The PVY isolate from Bahamian hot chile did not react with antiserum to TEV. All these criteria indicate the isolate was PVY (3,6).

To determine the etiology of the two types of symptoms observed on Bahamian hot chile in the field, seedlings of Bahamian hot chile were inoculated with either TMV, PVY, or TMV and PVY recovered from Bahamian hot chile. Seedlings of *C. annuum* cv. Lady Bell Hybrid and *C. annum* cv. KS-1 Paprika, cultivars of pepper also grown in Oklahoma, were also inoculated with TMV, PVY, or TMV and PVY. Bahamian hot chile seedlings inoculated with either TMV or PVY showed some stunting and a mild mosaic. Seedlings inoculated with both TMV and PVY showed severe stunting and systemic chlorosis and necrosis (Fig. 1). Seedlings of cultivar Lady Bell Hybrid inoculated with TMV and/or PVY showed systemic necrosis and mosaic when inoculated with PVY alone. Seedlings of cultivar KS-1 Paprika showed mosaic when inoculated with TMV, PVY, or TMV and PVY.

There have been numerous reports of TMV and PVY infecting pepper (1,2,13). In some cases, mixed infections of PVY and TMV in some *Capsicum* cultivars have been recorded, although a synergistic interaction was not noted (11). Information on the reaction of Bahamian hot chile to infection by plant viruses has been lacking. Synergism between different plant viruses can be dependent on the host in which the viruses are present (5). This appears to be the case with TMV and PVY. The presence of both viruses in Bahamian hot chile leads to severe stunting, chlorosis, and necrosis, which can result in severe yield reductions of this host. Other cultivars of pepper grown in Oklahoma do not respond to TMV and PVY in a similar fashion.

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Table 1. Reaction of potato virus Y (PVY) from Bahamian hot chile to antisera to PVY, tobacco etch virus (TEV), and pepper mottle virus (PMV) in PAS-ELISA[a]

Sample	Mean absorbance at 405 nm from immunoglobulin shown[b]		
	PVY	TEV	PMV
PVY	1.56 ± 0.09	0.05 + 0.01	0.05 ± 0.01
TEV	0.04 ± 0.01	1.16 ± 0.13	0.03 ± 0.01
PMV	0.07 ± 0.01	0.00	0.76 ± 0.01
Healthy sap	0.00	0.00	0.02 ± 0.00
PBS-Tween	0.00	0.00	0.02 ± 0.01

[a] Plates coated with protein-A at 1 μg/ml, capture and probe antibody added at a 1:1,000 dilution, sample added at a 1:5 dilution, and protein-A-alkaline phosphatase added at a 1:1,000 dilution.
[b] Absorbance mean and standard deviation of seven wells.

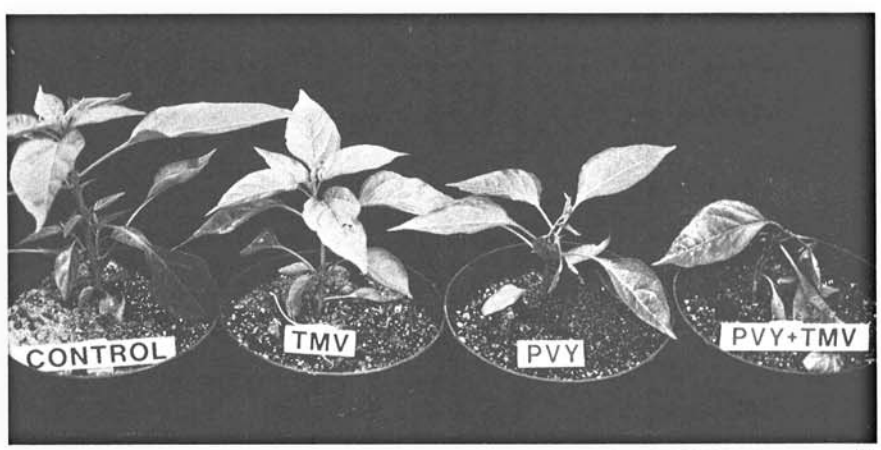

Fig. 1. Reaction of Bahamian hot chile to inoculation with potato virus Y (PVY), tobacco mosaic virus (TMV), or PVY and TMV.

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