

Identification of a Virus Causing a Mosaic on Coleus

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ABSTRACT

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Viruslike symptoms of mosaic, oak-leaf line patterns, ring spots, and color fading were observed on landscape plantings of coleus (*Coleus scutellarioides* 'Alabama') and two unidentified coleus cultivars. Stocks of Alabama with similar symptoms were found commonly in a survey of local garden centers and landscape plantings. The suspected virus was transmitted mechanically to cowpea and back to coleus, on which it produced symptoms similar to those originally observed. The virus was identified as a strain of cucumber mosaic virus based on gel electrophoresis of dsRNA, serology, particle morphology, and host reaction.

Coleus (*Coleus scutellarioides* (L.) Benth.) is widely cultivated as a bedding plant for its brightly colored and variegated foliage (4) and was listed 10th in importance in the United States in 1982 (10). The cultivar Alabama, a seedling selection from Bellingrath Gardens (Theodore, AL), is very popular in Baton Rouge and other areas in the South because it is considered more heat tolerant than many other cultivars.

In August 1989, viruslike symptoms were observed in the form of chlorotic line patterns, ring spots, and mosaic on cv. Alabama and several unidentified cultivars in landscape plantings on the Louisiana State University campus in Baton Rouge. A 1945 report (1) described a mosaic disease of coleus that produced symptoms of oak-leaf patterns, ring spots, and mosaic that was observed on 40 different coleus cultivars. The disease agent was transmitted only by grafting, but the presumed causal virus was not identified. The natural occurrence of identified virus(es) in coleus has not been reported. Experimental transmission studies, however, have shown coleus to be susceptible to tobacco necrosis, tobacco ringspot, tomato ringspot, cucumber mosaic, and alfalfa mosaic viruses (3,6). Descriptions of symptoms produced on coleus, if they occurred, were not included in these reports (3,6). Recently, a viroid has been reported to occur in coleus (7), although no diagnostic symptoms were associated with infection.

Because a preliminary test revealed the presence of dsRNA in cv. Alabama

showing viruslike symptoms, efforts were initiated to further characterize the disease. We report here the identification of an isolate of cucumber mosaic virus causing a disease in landscape plantings and commercial stocks of vegetatively propagated coleus.

MATERIALS AND METHODS

Plant materials. Plant materials were produced and maintained in a greenhouse in which temperatures ranged from 16 to 34 C (June–November). *Coleus* cuttings from cv. Alabama and two unidentified coleus cultivars showing viruslike symptoms were collected from landscape plantings and local garden centers and propagated in the greenhouse by rooting them in a commercial potting mix in polystyrene flats. *Coleus* plants were also propagated from seed of cv. Alabama and from a commercial seed

mix (Crosman Co. *Coleus* Mixed Colors No. 102). Eight named coleus accessions (Table 1) maintained as vegetative stocks were obtained from the USDA Plant Introduction Station at Ames, IA. Leaf samples of cv. Alabama were obtained from stock plants from Bellingrath Gardens. Plants propagated from seed were checked for the presence of virus by dsRNA analysis and then used as sources of virus-free plants for virus transmission studies. Rooted plants and seedlings were grown in clay pots in a 3:2:1 soil/peat/sand mix that had been steam pasteurized.

Double-stranded RNA extraction and analysis. Leaf tissue (3.5 g) was used for dsRNA analysis. dsRNA was extracted using a modification of the CF-11 cellulose column chromatography described by Morris and Dodds (5,8). dsRNA was analyzed in 6% polyacrylamide gels at 100 V for 2.5 hr. An identified isolate of cucumber mosaic virus (CMV) from *Apios americana* Medik. (wild groundnut) was included as a control in representative tests (9).

Serology and electron microscopy. Agar double-diffusion tests were performed in plastic petri dishes using 0.9% sodium chloride, 0.5% Ionagar No. 2 (Oxoid), and 0.02% sodium azide per 100 ml of deionized water. Sap extracts of virus-infected and healthy coleus samples

Table 1. Occurrence of cucumber mosaic virus (CMV) in vegetatively propagated coleus cultivars from different sources

Cultivar	Source	CMV ^a
Alabama	LSU grounds ^b	+
Alabama	Retail store 1 ^c	+
Alabama	Retail store 2	–
Alabama	Retail store 3	+
Alabama	Retail store 4	+
Alabama	Retail store 5	+
Alabama	Local private garden	+
Alabama	Bellingrath Gardens ^d	–
Unknown 1	LSU grounds	+
Unknown 2	LSU grounds	+
Beauty	PI 249771 ^e	–
Beckwith's Gem	PI 249772	–
Cristata	PI 249778	–
Freckles	PI 249783	–
Harlequin	PI 249786	–
Pegasus	PI 249791	–
Pineapple Beauty	PI 249793	–
Tapestry	PI 249803	–

^a Presence of CMV determined by dsRNA analysis.

^b Louisiana State University campus, Baton Rouge, LA.

^c Retail stores located in Baton Rouge, LA.

^d Located at Theodore, AL.

^e Plant introductions (PI) from USDA Plant Introduction Station, Ames, IA.

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were diluted with 2 ml of sodium phosphate buffer (0.01 M, pH 7.0) per gram of tissue and placed in peripheral wells. CMV antiserum (ATCC PVAS 88) was placed in center wells. Brome mosaic virus (BMV) antiserum (ATCC 178) was also used in serology tests. Extracts from coleus plants showing virus symptoms were negatively stained with 2% uranyl acetate (pH 6.8) and viewed with a JEOL 100 CX transmission electron microscope to determine virus particle morphology.

Virus transmission studies. Coleus leaf tissues showing viruslike symptoms were ground in a mortar in cold 0.01 M sodium phosphate buffer, pH 7.0, plus 0.01 M sodium diethyl dithiocarbamate (DIECA) to which a small amount of

600-mesh silicon carbide had been added. Plant sap inoculum was rubbed on leaves of test plants with the end of a pestle and the inoculated leaves rinsed with tap water. Inoculated plants were maintained in the greenhouse and observed for symptom development.

RESULTS

A preliminary assay by gel electrophoresis of a cv. Alabama sample showing disease symptoms (Fig. 1) revealed dsRNA bands that resembled those obtained from plants infected with CMV. Coleus samples from different locations were then collected and analyzed for the presence of dsRNA and compared with a CMV isolate from wild

groundnut (9). Six of eight Alabama sources tested positive for the presence of virus (Table 1). One Alabama sample from Florida was positive, and the samples from Bellingrath Gardens (origin of this cultivar) were negative for the virus. Two different unidentified coleus cultivars, from LSU landscape plantings, were also positive for the presence of CMV.

Double-stranded RNA analysis. All gels of dsRNA extracts from symptomatic coleus plants showed similar dsRNA patterns (Fig. 2). The three upper bands from coleus matched those for the wild groundnut strain of CMV. The seedling coleus sources (cv. Alabama and six Crosman color types) tested negative for dsRNA. All isolates contained a low molecular weight dsRNA that comigrated with the satellite RNA of CMV (lowest bands in Fig. 2). Virus isolates from coleus that were mechanically transmitted to cowpea and coleus, then assayed for dsRNA, produced gel bands that were identical to those obtained from the original virus-infected coleus plants.

Serology and electron microscopy. In agar double-diffusion tests, specific precipitin bands developed between wells containing CMV antiserum and extracts from infected coleus samples. Serology tests for the presence of BMV were negative. Virus-infected coleus samples prepared for electron microscopy revealed the presence of spherical particles approximately 25 nm in diameter.

Virus transmission. A selected virus isolate (from coleus cv. Alabama from LSU) was mechanically transmitted to several herbaceous plants that are hosts of CMV. Symptoms produced by the coleus virus isolate on *Vigna unguiculata* (L.) Walp. 'California No. 5' and 'TVu 612' included local chlorotic spots and systemic mosaic. *Nicotiana tabacum* L. 'Turkish' reacted with a mild systemic mosaic to the coleus isolate, and *Chenopodium quinoa* Willd. developed only local necrotic lesions. *Datura stramonium* L. and *Cucumis sativus* L. 'Poinsett' were inoculated with the virus isolate but did not produce symptoms nor was virus recovered from them.

Transmission of the coleus virus isolate back to coleus seedlings of cv. Alabama and to Crosman seedlings of one color type was also successful. Cowpea was usually used as a source of the virus in these tests. In three separate tests, 11 Alabama plants (propagated from virus-free seedlings), two plants of an unidentified virus-free coleus cultivar, and five Crosman seedling plants developed symptoms after inoculation with the virus isolate. Inoculated Alabama plants developed the full range of symptoms seen on naturally infected plants, whereas the inoculated Crosman seedlings developed only mosaic and color fading symptoms. Results of

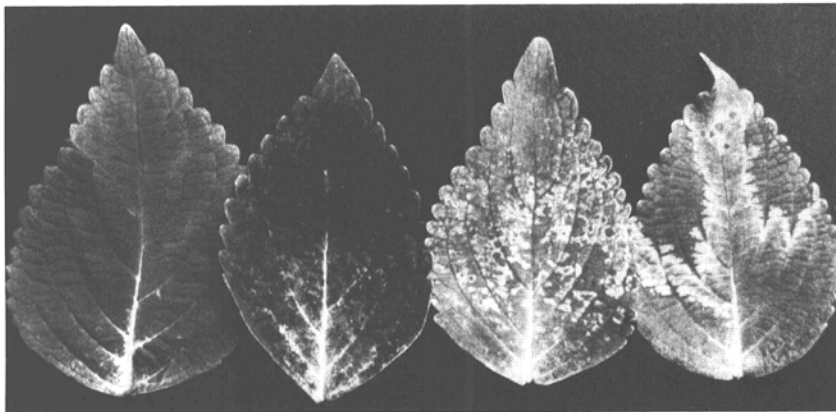


Fig. 1. Symptoms induced by a cucumber mosaic virus isolate from coleus on coleus cv. Alabama. Leaves from left to right are: healthy coleus, virus-infected showing only mosaic, virus-infected showing chlorotic ring spots, and virus-infected showing chlorotic line pattern.

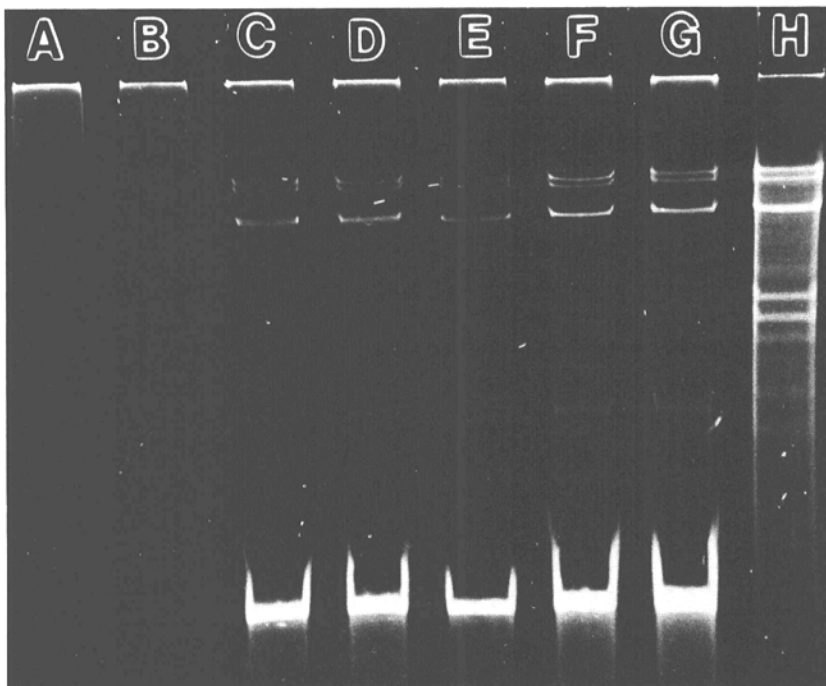


Fig. 2. Polyacrylamide gel (6%) electrophoresis patterns of dsRNAs extracted from: (A) healthy coleus cv. Alabama; cv. Alabama from (B) retail store no. 2, (C) retail store no. 3, (D) retail store no. 4, (E) retail store no. 5, and (F) a private garden; (G) unidentified coleus cv. from LSU landscape planting; and (H) tobacco cv. Turkish infected with an isolate of cucumber mosaic virus from wild groundnut. Gel was run at 100 V for 2.5 hr.

positive transmission tests with the virus were confirmed by dsRNA analysis.

DISCUSSION

Based on dsRNA similarities, serological tests, particle morphology, and assay host reactions, we conclude that the coleus virus isolate is an isolate of CMV (2). Because the symptoms we observed on coleus cultivars were the same as those described by Creager (1), it is possible that the disease he described was also caused by CMV. Two vegetatively propagated cultivars, Beauty and Beckwith's Gem, found infected by Creager were found virus-free in our tests. Because named coleus cultivars would have to be propagated vegetatively, they would likely remain diseased once they became infected with virus.

The virus symptoms observed in coleus were not the same in all cultivars and they varied during the growing season. One of the unidentified cultivars showed only moderate to severe mosaic throughout the year. Landscape plantings of Alabama showed symptoms of severe chlorotic line patterns and ring spots in early spring and in the fall, with amelioration of these symptoms during the hot summer months. This pattern was more noticeable under greenhouse

growing conditions where symptoms often disappeared in cv. Alabama but reappeared in the fall when cooler temperatures returned. Symptoms on inoculated coleus plants developed slowly under hot greenhouse temperatures during summer months. There was no obvious growth reduction in virus-infected cv. Alabama plants in comparison with virus-free cv. Alabama plants. Landscape plants that showed severe line pattern and ring spot symptoms showed no apparent ill effects from virus infection.

Our surveys of coleus in private and commercial landscape plantings and from retail garden stores indicate that CMV is widespread in cv. Alabama and two unidentified cultivars in Baton Rouge and southern Louisiana. Local retail stores obtained their stocks from several propagators located in south Louisiana. The absence of virus in cv. Alabama samples from Bellingrath Gardens suggests that our source(s) of this cultivar became infected with CMV, possibly by aphid transmission of the virus. Because most coleus is propagated annually from seed, it is not likely that this virus disease will become a problem, although seed transmission of CMV is known in some hosts (2). This is believed to be the first report of an identified virus occurring naturally in coleus.

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