

Detection and Characterization of an Isolate of Cucumber Mosaic Virus (CMV) Infecting Borage (*Borago officinalis*) in Spain

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ABSTRACT

Luis-Arteaga, M., Rodriguez-Cerezo, E., Maestro, C., and Garcia-Arenal, F. 1988. Detection and characterization of an isolate of cucumber mosaic virus (CMV) infecting borage (*Borago officinalis* L.) in Spain. *Plant Disease* 72:265-267.

Cucumber mosaic virus (CMV) was shown to be the causal agent of a borage (*Borago officinalis*) disease in northeastern Spain characterized by stunting of the plant, leaf deformation, and mosaic. The borage isolate, Bo-CMV, was characterized biologically by the symptoms induced in 17 indicator plants and serologically by its reaction with antiserum of LQ-CMV. Bo-CMV encapsidates RNAs 1, 2, 3, and 4, no satellite-RNA being found. In Northern blot analysis, it hybridized with cDNA made against Fny-CMV (in the WT hybridization group of CMV isolates) but not to cDNA made against WL-CMV (in the S group). This is in agreement with the biological and serological data, suggesting that Bo-CMV belongs to the DTL-WT group of CMV. In the greenhouse, Bo-CMV was nonpersistently transmitted by *Myzus persicae*.

Borage (*Borago officinalis* L.), grown for consumption of leaves and petioles, is an important crop in the Ebro Valley of northeastern Spain. During the past few years, growers drew our attention to a new disease characterized by stunting, reduction of the leaf surface, and mosaic. The disease affected borage production severely, and a possible viral etiology was investigated. This paper presents data showing cucumber mosaic virus (CMV) as the causal agent of this disease and presents characteristics of Bo-CMV, the isolate found in diseased borage plants. This is, to our knowledge, the first report of CMV causing an important disease in borage as well as the first report of CMV infecting plants of the genus *Borago*.

MATERIALS AND METHODS

Viral isolates and biological assays. Borage plants showing disease symptoms were collected from commercial plots in the Huesca and Zaragoza provinces. Sap from diseased borage plants was used to inoculate *Vigna unguiculata* (L.) Walp. 'Black Local', *Nicotiana tabacum* L. 'Xanthi-nc', *Cucumis melo* L. 'Doublon', *C. sativus* L. 'Marketer', *Chenopodium amaranticolor* Coste & Reyn., and *C. quinoa* Willd. by Marrou's method (10). A wider range of indicator plants (Table 1) was inoculated with sap from these

plants. For cloning the virus, three single-lesion passages were made in *V. unguiculata*. The cloned virus was multiplied in *N. tabacum* 'Xanthi-nc' or in *N. clevelandii* L. 'Gray.' All plants were kept in a greenhouse with temperatures ranging from 15 to 25 C. Fny-CMV and WL-CMV, belonging to the WT and the S hybridization groups, respectively (14), were supplied by P. Palukaitis of Cornell University.

Serology. Immunodiffusion tests in the presence of sodium dodecyl sulfate (15) were done with sap extracted from 1 g of leaf tissue ground in 2 ml of water clarified by 15 min of low-speed centrifugation. Samples from field-infected borage plants as well as from experimentally infected cucumber, melon, tobacco, and *Physalis floridana* Rydb. were tested. An antiserum against LQ-CMV (belonging to the DTL serological group of CMV isolates and supplied by H. Lot of the Institut National de la Recherche Agronomique, Montferret, France) (2) was used.

Aphid transmission. *Myzus persicae* Sulz., after a 1-hr fasting period, were allowed 3-min access to a squash (*Cucurbita pepo* L.) plant inoculated with borage sap. Ten aphids per plant were then transferred to four muskmelon and four healthy borage plants and allowed to feed for 1 hr before the plants were sprayed with pirimicarb.

Nucleic acid analysis. The virus was prepared from infected *N. tabacum* or *N. clevelandii* following the procedure of

Lot et al (8), and the encapsidated RNA was obtained by phenol extraction of virions and ethanol precipitation. Electrophoretic analyses were made in 1.2% agarose gels in MAE buffer (1× MAE = 20 mM MOPS [3-(*N*-morpholino)propanesulphonic acid]-NaOH pH 7.0, 5 mM sodium acetate, 1 mM EDTA) after denaturation in (50% deionized formamide, 6% formaldehyde, 1×MAE) for 5 min at 65 C. A bidirectional transfer to nitrocellulose was made from these gels, and blots were probed (12) with cDNA made against different CMV isolate RNAs by random priming (7).

RESULTS

Biological characterization. The symptoms that developed in different indicator plants are shown in Table 1. Sap from seven field borage plants produced the same symptoms in all the indicator plants, but only *Ocimum basilicum* L. and *Datura stramonium* L. showed a heterogeneous response even against the same field isolate. In these two indicator plants, some plants had no symptoms while others had systemic mosaic. The range of symptoms observed agreed with those reported for CMV (4,11). Healthy borage plants mechanically inoculated with sap from infected indicator plants showed diminished growth and stunting, with dark, sometimes blisterlike spots on the leaves and deformations of the leaf lamina and margins (Fig. 1). These symptoms, which appeared 2 wk after inoculation, matched those occurring in field-infected plants.

M. persicae transmitted the virus nonpersistently from infected *C. pepo* to all tested *C. melo* plants, which showed symptoms 8 days after aphid inoculation. Aphid-inoculated borage plants, on the other hand, did not show symptoms, even 45 days after inoculation.

Serology. The antiserum to LQ-CMV reacted positively with the sap from field-infected borage plants as well as with the sap from experimentally inoculated muskmelon, cucumber, squash, tobacco, and *P. floridana* plants. No precipitation line was observed with sap from noninoculated controls (Fig. 2).

Table 1. Reactions of test plants inoculated with crude extracts from seven samples of borage

| Family Species | Local reactions | Systemic reactions |
|---|---|--|
| Boraginaceae <i>Borago officinalis</i> L. 'Flor Blanca' | None | Mosaic, stunt, leaf deformation, leaf narrowing |
| Chenopodiaceae <i>Chenopodium amaranticolor</i> Coste & Reyn. <i>C. quinoa</i> Willd. | Necrotic local lesions Necrotic local lesions | None None |
| Cucurbitaceae <i>Cucumis melo</i> L. 'Doublon' <i>C. sativus</i> L. 'Marketer' <i>Cucurbita pepo</i> L. F ₁ 'Diamante' | None Chlorotic spots None | Mosaic Mosaic Mosaic, leaf deformation |
| Labiatae <i>Ocimum basilicum</i> L. | None | None or mosaic |
| Leguminosae <i>Phaseolus aureus</i> Roxb. <i>Vigna unguiculata</i> (L.) Walp. 'Black Local' | Necrotic ring spots Necrotic local lesions | None None |
| Solanaceae <i>Datura stramonium</i> L. <i>Capsicum annuum</i> L. 'Doux de Landes' <i>C. annuum</i> 'Yolo Wonder' <i>Nicotiana glutinosa</i> L. <i>N. tabacum</i> L. 'Xanthi-nc' <i>N. tabacum</i> 'Samsun' <i>N. tabacum</i> 'Paraguay' <i>Physalis floridana</i> Rydb. | None or chlorotic spots None None None None None None None None | None or mosaic Mosaic Mosaic Mosaic, leaf deformation Mosaic Mosaic Mosaic Mosaic Mosaic, leaf narrowing |



Fig. 1. Symptoms induced in *Borago officinalis* 2 mo after mechanical inoculation with sap from *Cucurbita pepo* inoculated with sap from field-infected borage. (Left) Healthy control and (right) inoculated plants.

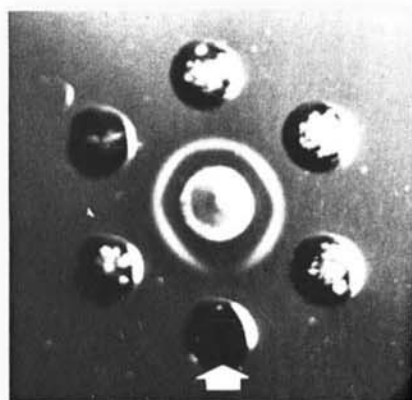


Fig. 2. Reactions in immunodiffusion tests of crude extracts from *Cucurbita pepo* inoculated with sap from different field-infected borage plants, or mock-inoculated (arrow), to antiserum to LQ-CMV (center well).

infected with CMV. The presence of CMV was further confirmed by the positive reaction of the sap from infected plants with an antiserum made to LQ-CMV and by the presence of CMV particles in this sap as shown by negative-stained preparations at the electron microscope (data not shown). We have called this CMV isolate from borage Bo-CMV. When healthy borage plants were inoculated with Bo-CMV, disease symptoms were similar to those observed in field-infected plants, and we concluded that CMV was the causal agent of the disease.

Bo-CMV was characterized at the biological, serological, and RNA levels. *M. persicae* transmitted Bo-CMV nonpersistently to *C. melo* but not to borage; this may be attributable to the morphology of borage leaves, which are covered by long, rigid epidermal hairs, or to the experimental conditions. More data on this subject are needed to ascertain how the virus is transmitted in the field. Based on the symptoms induced in 17 indicator species in six families, and specifically in *N. tabacum* 'Xanthi-nc' and *P. floridana*, Bo-CMV should be included in group C of CMV isolates (11). This is consistent with positive reactions shown by antibodies made to LQ-CMV, an isolate belonging to the serological group DTL (2) in which isolates from biological group C are included (3). Piazzolla et al (14) have divided CMV isolates into two hybridization groups, S and WT, corresponding, respectively, to Devergne and Cardin's (2) serological groups ToRS and DTL. Our cDNA probe to WL-CMV, in the S group, did not hybridize to Bo-CMV RNAs, whereas cDNA to Fny-CMV, in the WT group, did hybridize with Bo-CMV RNA, further confirming that Bo-CMV belongs to the DTL-WT group of CMV isolates. CMV has been found in

Nucleic acid analysis. When virion-extracted RNA was run in a 1.5% agarose gel in MAE buffer after denaturation, four RNA species were seen, comigrating with RNAs 1, 2, 3, and 4 of Fny-CMV. Neither RNA 4a nor RNA 5 of the size of the CMV-sat RNAs was found (Fig. 3A) When RNAs from gels were transferred to nitrocellulose membranes and probed with cDNA made to WL-CMV or to Fny-CMV, RNA from Bo-CMV hybridized only to Fny-CMV (Fig. 3C).

DISCUSSION

Diseased borage plants were collected from commercial field plots during an epidemic in 1985 of an undescribed disease, and their sap was used to inoculate a range of indicator plants, whose reactions showed the borage plants to be

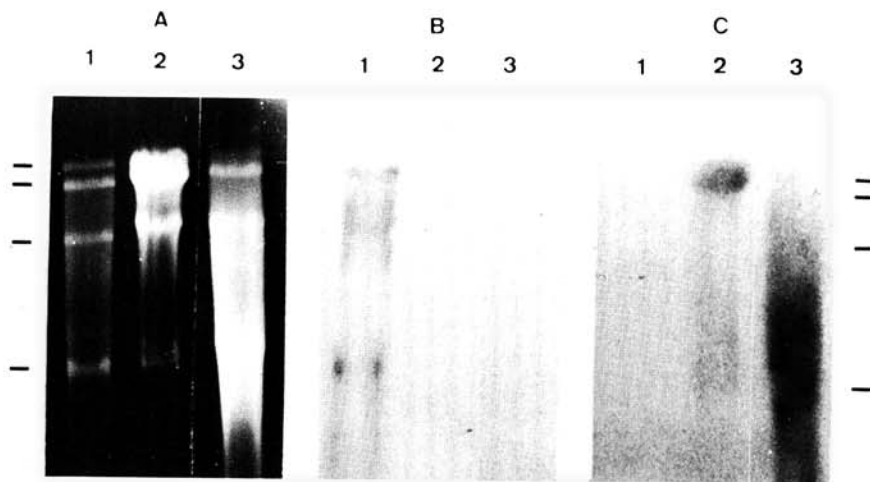


Fig. 3. Gel photograph and autoradiograms of RNA blots. RNAs were (A) electrophoresed under denaturing conditions in horizontal 1.5% agarose-MAE gels, stained with ethidium bromide, and photographed, then blotted to nitrocellulose and hybridized to cDNA of (B) WL-CMV RNAs or (C) Fny-CMV RNAs. 1 = WL-CMV RNAs, 2 = Bo-CMV RNAs, 3 = Fny-CMV RNAs. Lines on both sides of figure represent RNAs 1-4 of WL-CMV.

Spain in a wide range of horticultural crops (1,5,9,13), and all isolates reported so far belong to the DTL group (5).

No sat-RNA was found in RNA preparations from Bo-CMV virions, even after several passages through *N. tabacum* 'Xanthi-nc,' a host that supports sat-RNA replication. These results do not agree with those of García-Luque et al (6), in which sat-RNA was found among CMV isolates obtained from the same area.

This is, to our knowledge, the first report of CMV infecting the genus *Borago* and causing an important disease in borage crops. Work on aphid transmission to borage and on the possibility of seed transmission, related to field epidemiology, is currently in progress.

ACKNOWLEDGMENT

This work was partially financed by grant CCB 8402017 of Comité Conjunto Hispano Norteamericano para la Investigación Científica y Tecnológica.

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