European Journal of Plant Pathology **102:** 201–203, 1996.

Short communication

Evidence from nucleic acid hybridization tests for geminivirus infection of ornamental crotons in India

S. K. Raj, K. M. Srivastava and B. P. Singh
Plant Virus Laboratory, National Botanical Research Institute, Lucknow 226 001, India

Accepted 25 May 1995

Key words: Codiaeum variegatum, geminivirus detection, nucleic acid probe

Abstract

Reduced growth of plants and excessive generalised and spotty yellowing of leaves occurred in ornamental crotons that were infected with a geminivirus, which was detected by spot hybridization tests using a cloned probe derived from DNA-A of acalypha yellow mosaic geminivirus. The croton virus was transmitted by the whitefly, *Bemisia tabaci*, to *Nicotiana tabacum* and *Acalypha indica*, in which it caused leaf curl and yellow mosaic, respectively, but it was not transmitted to five other species. Infected crotons may therefore serve as reservoirs of a geminivirus that is able to infect a crop species.

Codiaeum variegatum (L.) Jussien var. pictum, popularly known as ornamental croton, is a house plant of the family Euphorbiaceae. Several variants having vivid variegated foliage are grown in gardens all over the world. Their ornamental value is due to yellow veins, yellow spots, bright yellowing of the leaves and spots of various colours on a darkgreen background. Similar effects, especially yellowing and yellow spots, are also produced in several plants by infection with whitefly transmitted germiniviruses [Harrison, 1985]. It may be difficult to distinguish between such virus infections and expension of inherited yellow veins and yellow spots on crotons grown in gardens. Our observations from 1991 to 1993 on these plants indicated that a pathogen is involved in deterioration of plant growth, reduction of leaf size and excessive yellowing in crotons. Preliminary studies failed to detect a fungal/bacterial pathogen or a nematode as the causal agent (data not included). In this communication, we provide evidence using nucleic acid probes that crotons which grow poorly and display excessive yellow spotting or yellowing of leaves are infected with a geminivirus.

Leaf samples from various varieties of croton showing reduced growth or abnormal yellowing of the leaves along with some apparently healthy looking plants were collected from the gardens of the National Botanical Research Institute, Lucknow. Twenty four samples were collected, including four apparently healthy ones. Total DNA was extracted from 1 g leaf tissue as described earlier [Dellaporta *et al.*, 1983]. Briefly, 1 g tissue was powdered in liquid nitrogen, suspended in extraction buffer (containing 0.1M tris, pH 8.0, 0.05 M ethylene diamine tetra acetic acid, 0.5 M NaCl and 1% 2-hydroxyethyl mercaptan), treated with 1% SDS at 65 °C for 10 min, centrifuged at 10,000 g for 15 min and DNA precipitated by isopropanol. Further, DNA was bound to CTAB and precipitated several times with ethanol. The DNA equivalent to 50 or 100 mg of fresh tissue was directly blotted on to Zeta probe membrane (Bio Rad, USA) by a dot blot apparatus (Bio Rad, USA) and immobilized by heating in a vacuum oven at 80 °C.

A cloned DNA sequence, derived from acalypha yellow mosaic virus (AYMV) a geminivirus, was used a probe. The fragment contains the complete DNA-A except the intergenic region and the contiguous part of the ALI gene (unpublished data). This probe was selected because *Acalypha indica*, a weed host of AYMV, and croton belong to the same family, Euphorbiaceae. To prepare radiolabelled probe, the pBS II KS+ vector containing the 1.8 kb EcoRI frag-

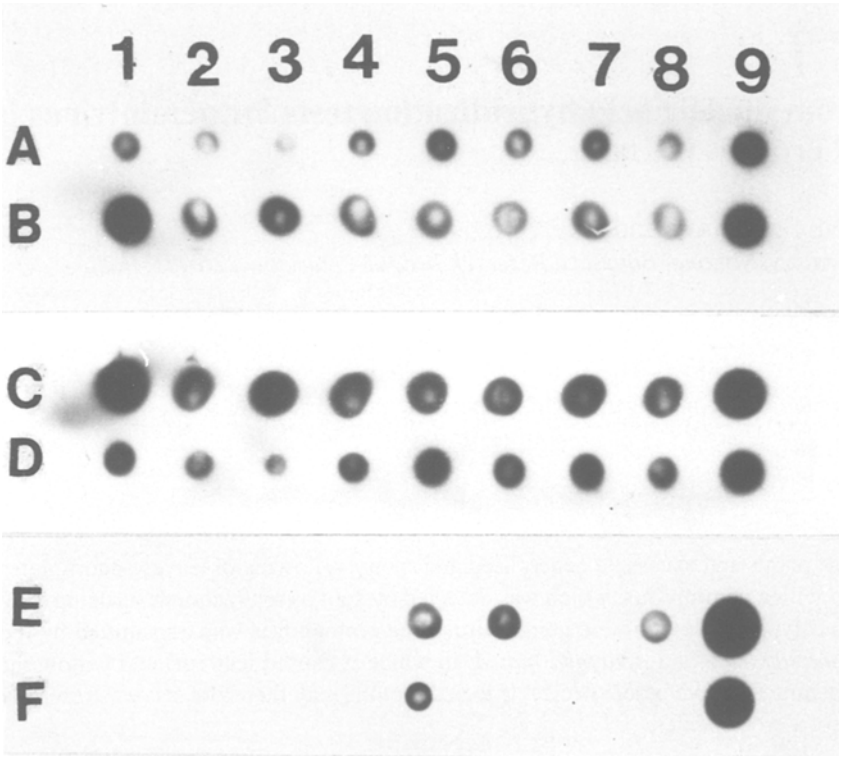

Fig. 1. Dot blot hybridization of samples from suspected croton plants – total DNA equivalent to that isolated from 50 mg/100 mg of the leaf tissue was blotted on Zeta probe membrane (lanes 1–8 of A,D,F and B,C,E, respectively). Lanes 9 of A,D,F and B,C,E contain 100 & 200 ng cloned probe specific DNA respectively. Samples E7, F6, F7 and F8 gave very weak signals. Lanes 1–4 of E and F represent DNA from apparently healthy samples collected from vigorously growing plants.

ment of DNA-A of AYMV was digested with EcoRI, electrophoresed in low melting point agarose and the desired DNA fragment eluted from the gel as described by Sambrook *et al.* [1989]. The DNA was labelled by the random primer extension method [Feinberg and Vogelstein, 1983] using hexanucleotide primers and Klenow fragment (Pharmacia).

Prehybridization and hybridization were performed in a solution containing 50% formamide, 0.12M Na_2HPO_4, pH 7.2, 0.25M NaCl, 7% SDS and 1mM EDTA. The hybridization was done at 42 °C for 1 h. After hybridization, Zeta probe membrane were washed with 2 × SSC, 2 × SSC + 0.1% SDS, 0.5 × SSC + 0.1% SDS each for 20 min at room temperature and finally with 0.1 × SSC + 0.1% SDS at 65 °C for 20 min. They were then exposed to X-ray film for 12 h.

Dot blot experiments showed the presence of geminivirus-like DNA in all except a few (lane E7, F6, F7 & F8, Fig. 1) samples of croton suspected to be infected. Thus, bright yellow golden spots/yellow veins on mutant crotons and similar pathological effects induced by geminivirus infection might not be differentiated visually. However, decreased plant growth or leaf size, or excessive yellowing may be a possible indication of the involvement of a geminivirus.

We tried to ascertain whether the croton virus infects other species known to be hosts of whitefly transmitted geminiviruses, viz.: *Abelmoschus esculentus, Acalypha indica, Carica papaya, Croton bonplandianum, Lycopersicon esculentum, Nicotiana glutinosa, N. tabacum* cv. *Samsun NN, N. tabacum* cv. *White Burley* and *Vigna mungo*.

Non-viruliferous whiteflies, *Bemisia tabaci*, reared on *Clitoria ternatea* and handled as described [Srivastava *et al.*, 1977] were given an acquisition access period of 24 h on croton plants followed by an inoculation access period of 24 h on 5 test plants of each of the above species (>50 whiteflies/test plant). Only *Acalypha indica* (4/5) and *N. tabacum* cv. White Burley (5/5) developed symptoms. Infected *N. tabacum* plants

showed stunting with extreme reduction and curling of leaves similar to that reported for tobacco leaf curl virus infection on tobacco [Pal and Tandon, 1937] while *A. indica* plants developed bright yellow mosaic symptoms similar to those observed in nature [Chenulu and Phatak, 1965]. Virus could not be detected in the symptomless plants of the other species by using a radiolabelled probe prepared from total DNA of infected croton by a strategy described earlier [Srivastava *et al.*, 1992].

The above results suggest that the virus in infected croton plants may serve as a geminivirus reservoir for infection of *N. tabacum and A. indica*. The same might be true for ornamental crotons. Efforts are in progress to identify the geminivirus found in croton and to determine its relationship to tobacco leaf curl and acalypha yellow mosaic viruses.

Acknowledgements

We acknowledge partial funding from Department of Biotechnology, Govt. of India for carrying out this work. Thanks are also due to Dr. P.V. Sane, for his keen interest in the work and providing facilities.

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