

Sicana odorifera (Cucurbitaceae) a new phytoplasma host

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Abstract

Sicana odorifera also known as cassabanana, sikana or musk cucumber, is naturally grown from Mexico to Brazil and the west Indies. Fruits are edible, the pulp and the seeds have a medicinal use, and the whole plant is used as an ornamental. In the State of Rio de Janeiro, Brazil, naturally diseased sikana plants were observed, for the first time, with witches' broom growths and other symptoms characteristic of plant diseases caused by phytoplasmas. The present work aimed at detecting and classifying the phytoplasma that may be the causal agent of the disease. Phytoplasma was discovered in sikana affected by witches' broom, on the basis of phytoplasma-specific DNA amplification in PCR. The phytoplasma found in sikana belongs to group 16SrIII. The disease was named sikana witches' broom.

Key words: witches' broom, sikana, 16SrIII, Brazil, phytoplasma.

Introduction

Sicana odorifera Naud is a cucurbit known as cassabanana, sikana or musk cucumber. It is believed native to Brazil, but it has been spread throughout Mexico, Guatemala, El Salvador, Nicaragua, Costa Rica, Puerto Rico, Cuba, Panama, Venezuela, Colombia, Peru and Bolivia. The vine is perennial and herbaceous. Fruit is smooth, glossy, cylindrical, hard-shelled, orange-red, maroon, or black. Fruit flesh is pale yellow to orange, juicy. Seeds are oval, light-brown bordered with a dark-brown stripe. Sikana is used as edible fruit, and as medicinal and ornamental plant (Morton, 1987). In Brazil, the plant is used as an ornamental, and seed infusion is taken as a febrifuge. Sikana is naturally found in Brazil, in the states of Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia, Paraná, São Paulo, Minas Gerais and Rio de Janeiro.

In 2006, naturally diseased sikana plants were observed for the first time in the State of Rio de Janeiro. Symptoms exhibited by diseased plants included witches' broom growths, generalized stunting and yellowing suggestive of phytoplasma disease. The aim of the present work was to demonstrate the presence of a phytoplasma that may be the cause of sikana witches' broom disease in Brazil.

Materials and methods

Symptomatic leaves of sikana were collected from six naturally diseased plants exhibiting symptoms, in the location of Ponte Coberta, Paracambi, in the state of Rio de Janeiro. DNA extraction and PCR conditions followed Montano *et al.* (2000). Universal primer pairs P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) and R16F2n/R2 (Gundersen and Lee, 1996) were used to prime amplification of phytoplasma 16S rDNA sequences in nested PCR assays. DNA fragment size standard was 1 kb ladder (Invitrogen). Negative controls consisted of reaction mixtures devoid of templates. PCR

products were analyzed by electrophoresis through 1% agarose gel, staining with ethidium bromide, and visualization of DNA bands using a UV transilluminator. P1/P7 diluted products were used as template for reamplification in nested PCR, primed by group-specific primer pair R16(III)F2/R1 (specific for group 16SrIII, X-disease phytoplasma group) (Lee *et al.*, 1994). Analyses of amplified products were carried out as previously described.

Products from nested PCR primed by R16F2n/R2 were analyzed by single restriction endonuclease digestion with *AluI*, *MseI*, *RsaI*, *HpaII*, *HaeIII* and *KpnI* (Invitrogen). The products of digestion were analyzed by electrophoresis through a 5% polyacrylamide gel followed by staining with ethidium bromide and visualization of DNA bands with UV transilluminator. DNA fragment size standard used was PhiX174 RF *HaeIII* digest (Invitrogen). The RFLP patterns of phytoplasma DNAs were compared with the RFLP patterns previously published (Lee *et al.*, 1994, Montano *et al.*, 2000; Montano *et al.*, 2001).

Results

On the basis of phytoplasma-specific DNA amplification in PCR, phytoplasmas were detected in all of the six sikana plants tested exhibiting symptoms of witches' broom disease. PCR assays primed by primer pair R16 (III)F2/R1 yielded an amplified product of approximately 0.8 kb (figure 1b). Phytoplasma was identified by RFLP analysis of 16S rDNA amplified in PCR primed by F2n/R2, and phytoplasma classification was done according to Lee *et al.* (1998). On the basis of *AluI*, *HpaII*, *HaeIII*, *KpnI* (figure 1a), *MseI* and *RsaI* (data not shown), the collective RFLP patterns of phytoplasma in sikana were indistinguishable from those reported previously for the chayote witches' broom phytoplasma (ChWBIII) (Montano *et al.*, 2000). Therefore, the phytoplasma in sikana was classified in ribosomal group 16SrIII, and named the disease as sikana witches' broom.

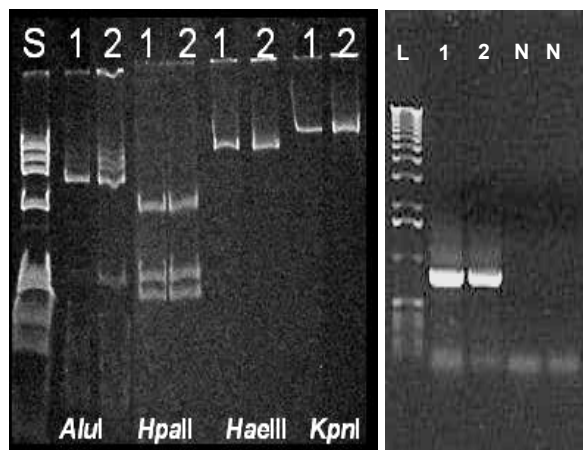


Figure 1. (A). Restriction fragment length polymorphism (RFLP) analysis of 16S rDNAs amplified in PCRs from naturally diseased plants of sikana (*Sicana odorifera*) in Brazil. Lane S: fragment size standard, PhiX174 RF *Hae*III digest. 1: digest of DNA from reference phytoplasma chayote witches' broom, ChWBII. 2: digest of DNA amplified from diseased sikana. (B). Nested PCR primed by group-specific primer pair R16(III)F2/R1. Lane L: fragment size standard 1 kb ladder. 1: ChWBIII, positive control. 2: sikana phytoplasma. N: reaction controls devoid of DNA template, negative controls.

Discussion

The findings presented here demonstrate that a phytoplasma is associated with sikana plants in Brazil, and the sikana-infecting phytoplasma belongs to group 16SrIII. The collective RFLP patterns of 16S rDNA phytoplasma from sikana were indistinguishable from those reported previously for the ChWBIII phytoplasma, with strains causing disease in other two cucurbits, chayote (*Sechium edule*) and *Momordica charantia* (Montano *et al.*, 2000). Sikana is indigenous to Brazil and it is distributed in several states of the country. As it happens to *M. charantia*, a natural reservoir of the chayote witches' broom disease agent (Montano *et al.*, 2000), sikana could be a potential source of phytoplasma inoculum for infection of commercial cucurbits, like pumpkin (*Cucurbita moschata*) (Montano *et al.*, 2006) and chayote (Montano *et al.*, 2000). To our knowledge, this is the first report of phytoplasma in *S. odorifera*.

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