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First report of group 16SrIII phytoplasma in loofah (*Luffa cylindrica*)

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Abstract

Loofah (*Luffa cylindrica*) is grown in several states of Brazil, but is found more frequently as spontaneous plant and in small plantings, as in the State of Rio de Janeiro. In different locations in the State of Rio de Janeiro loofah plants were observed exhibiting symptoms characteristic of diseases caused by phytoplasmas. Symptomatic plants were tested for the presence of phytoplasmas, using phytoplasma-specific DNA amplification in PCR. Sequences of 16S rRNA phytoplasma were amplified in nested PCR primed by P1/P7 and R16F2n/R2. PCR assays primed by R16(III)F2/R1 yielded an amplified product of approximately 0.8 kb. The results demonstrated the presence of a phytoplasma associated with loofah witches' broom for the first time in Brazil, and revealed that the phytoplasma is affiliated to 16SrIII group.

Key words: witches' broom, *Cucurbitaceae*, vegetable sponge, Brazil.

Introduction

Loofah [*Luffa cylindrica* (L.) Roemer] is a cucurbit known as vegetable sponge or sponge gourd. In Brazil, the commercial growth of loofah is still of small-scale, it is grown mainly in the states of Espírito Santo, Minas Gerais, Rio de Janeiro and São Paulo. So far, loofah is found frequently as spontaneous plant in several Brazilian regions growing in backyards and in small plantings. In different locations in the State of Rio de Janeiro, naturally diseased loofah plants were observed exhibiting symptoms of witches' broom, generalized stunting and yellowing. In addition, other symptoms included fruit malformation and aborted seeds.

In the country, the association of phytoplasmas with cucurbits was demonstrated to chayote witches' broom disease and to witches' broom disease of *Momordica charantia*, which was termed ChWBIII phytoplasma and classified as belonging to group 16SrIII, subgroup J (Montano *et al*., 2000). Also, a phytoplasma associated to pumpkim yellows, in Brazil, was assigned to group 16SrIII, subgroup J (Montano *et al*., 2006). The present work aimed at demonstrating, for the first time, the presence of phytoplasma associated to loofah witches' broom disease in Brazil.

Materials and methods

Symptomatic leaves of loofah were collected from ten naturally diseased plants exhibiting symptoms, in the location of INCRA, Seropédica, 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/R16(III)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 *Alu*I, *Rsa*I, *Kpn*I, *Dde*I, *Eco*RI, *Hae*III, *Hpa*I, *Hpa*II, *Sau*3AI and *Hha*I (Invitrogen). The products of restriction 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 *Hae*III 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 (data no shown), phytoplasmas were detected in all of the ten loofah plants 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 (data not shown). 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 *Alu*I, *Rsa*I, *Kpn*I, *Dde*I, *Eco*RI, *Hae*III, *Hpa*I, *Hpa*II, *Sau*3AI and *Hha*I RFLP patterns of 16S rDNA, the phytoplasma in loofah plants could not be distinguished from one another. Furthermore, the collective RFLP patterns were indistinguishable from those reported previously for the chayote witches' broom phy-

toplasma (ChWBIII) (Montano *et al.*, 2000) (figure 1). Therefore, we classified the phytoplasma in loofah as a group 16SrIII strain.

Discussion

The results of the present study demonstrate that a phytoplasma is associated with diseased loofah in Brazil and that it belongs to group 16SrIII. The collective RFLP patterns of 16S rDNA phytoplasma from loofah were indistinguishable from those reported previously for the ChWBIII phytoplasma (Montano *et al.*, 2000). Loofah (*Luffa* spp.) has previously been reported as a phytoplasma host from Taiwan, where a group 16SrVIII (loofah witches' broom phytoplasma group) (Lee *et al*., 1994) was the causal agent, similarly a 16SrI-B subgroup phytoplasma has been reported in naturally infected chayote plants, in Costa Rica (Villalobos *et al*., 2002). These findings suggest that cucurbits may be good hosts to different groups of phytoplasmas. To our knowledge, this is the first report of a group 16SrIII phytoplasma in loofah.

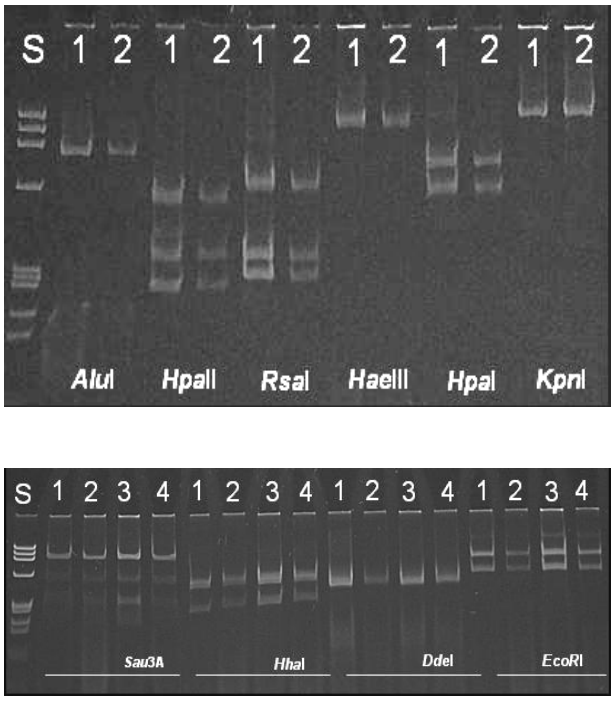

Figure 1. Restriction fragment length polymorphism (RFLP) analysis of 16S rDNAs amplified in PCRs from naturally diseased plants of loofah (*Luffa cylindrica*) in Brazil. Lane S: fragment size standard, PhiX174 RF *Hae*III digest. 1: digest of DNA from reference phytoplasma chayote witches' broom, ChWBIII. 2, 3, 4: digests of DNAs amplified from diseased plants of loofah.

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