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Phytoplasma in "fava d'anta" tree (*Dimorphandra gardneriana*) in Brazil

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Abstract

Dimorphandra gardneriana, known as "fava d'anta" and "faveiro", is naturally found in South America, in Brazil and in Bolivia. Fruits of fava d'anta are source of rutin, a flavonoid that strengththens capillaries. In the State of Maranhão, Brazil, naturally diseased "fava d'anta" trees, with witches' broom growths and other symptoms characteristic of plant diseases caused by phytoplasmas were observed. The aim of the present work was to detect the presence of a phytoplasma that may be the causal agent of the disease. Phytoplasma was discovered in "fava d'anta" trees affected by witches' broom, on the basis of phytoplasma-specific DNA amplification in PCR.

Key words: *Dimorphandra gardneriana, Dimorphandra mollis,* "fava d'anta", "faveiro", witches' broom, rutin.

Introduction

Dimorphandra gardneriana Tul., known as "fava d'anta" and "faveiro", is a Brazilian native leguminous tree, naturally found in the states of Maranhão, Piauí, Ceará, Pernambuco, Bahia, Pará, Goiás, Mato Grosso and Minas Gerais. The seed pod of "fava d'anta" is one of the sources for the extraction in industrial scale of rutin. Rutin belongs to an important class of flavonoids, vital in their ability to increase the strength of the capillaries and to regulate their permeability. Besides *D. gardneriana,* another "fava d'anta" native species, *Dimorphandra mollis* Benth, is used for extraction of the rutin.

In the last ten years, "fava d'anta" trees of the species *D. gardneriana* exhibiting witches' broom growths, reduced leaf and yellowing have been observed in the locations of the states of Ceará and Maranhão; the same has been seen for *D. mollis.* The aim of the present work was to demonstrate whether a phytoplasma could be the cause of "fava d'anta" witches' broom disease in Brazil.

Materials and methods

Eight "fava d'anta" samples (from *D. gardneriana*) exhibiting shoot proliferation, reduced leaf size and yellowing (figure 1a) were collected in Barra do Corda, State of Maranhão, in 2004 and in 2005, and sent to the Laboratório Oficial de Diagnóstico Fitossanitário/ UFRRJ. 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 PhiX174 RF *Hae*III digest (Invitrogen). Samples from asymptomatic trees (figure 1b) were collected and assayed for the presence of phytoplasma. 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.

Products from nested PCR primed by R16F2n/R2 were analyzed by single restriction endonuclease digestion with *Alu*I, *Mse*I, *RsaI*, *Hpa*II, *Hae*III and *Kpn*I (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 *Hae*III digest (Invitrogen). The RFLP patterns of phytoplasma DNAs were compared with the RFLP patterns previously published (Barros *et al.*, 2002; 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 "fava d'anta" plants tested exhibiting symptoms of witches' broom disease. Figure 1c shows amplification products of four tested symptomatic samples. Asymptomatic sample yielded no amplification product. Initial trials to identify the phytoplasma associated with diseased trees of "fava d'anta" were carried through RFLP analysis of 16S rDNA amplified in PCR primed by F2n/R2 (Lee *et al*., 1998).

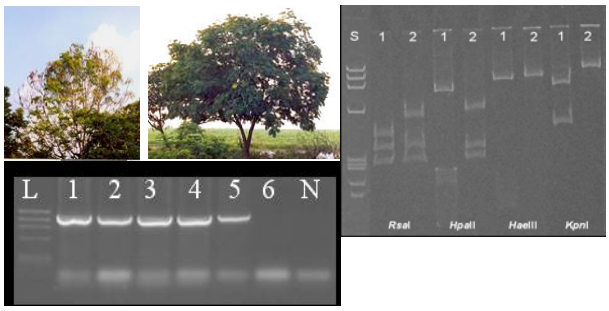

Figure 1. Upper left (a): "Fava d'anta" with symptoms; upper center (b): Asymptomatic "fava d'anta"; low left (c): Nested PCR primed by universal primer pairs P1/P7 and R16F2n/R2. Lane L: PhiX174 RF *Hae*III digest fragment. 1: ChWBIII, positive control. 2, 3, 4, 5: "fava d'anta" phytoplasma. 6: asymptomatic "fava d'anta". N: reaction control devoid of DNA template, negative control; upper right (d): Restriction fragment length polymorphism (RFLP) analysis of 16S rDNAs amplified in PCRs from naturally diseased plants of "fava d'anta" (*D. gardneriana*) in Brazil. Lane S: fragment size standard, PhiX174 RF *Hae*III digest. 1: digest of DNA amplified from diseased "fava d'anta". 2: digest of DNA from reference phytoplasma chayote witches' broom, ChWBIII.

On the basis of *Rsa*I, *Hpa*II, *Hae*III, *Kpn*I (figure 1d), *Alu*I and PhiX174 RF *Hae*III digest *Mse*I (data not shown), the collective RFLP patterns of phytoplasma in "fava d'anta" were different from those observed for the phytoplasmas previously discovered in Brazil, that were fully characterized *sensu* Lee *et al*. (1998), and associated with chayote witches' broom (ChWBIII) (Montano *et al.*, 2000), hibiscus witches' broom (HibWB) (Montano *et al.*, 2001), and erigeron witches'-broom (EriWB) (Barros *et al*., 2002).

Discussion

Observation of symptoms and the PCR results demonstrate that a phytoplasma is associated with "fava d'anta" in Brazil. The collective RFLP patterns of 16S rDNA phytoplasma from "fava d'anta" observed in this study are preliminary, but they suggest that the phytoplasma in "fava d'anta" may be affiliated to a 16Sr group, distinct from those already identified in the country. The use of "fava d'anta" seed pods for industrial extraction of rutin as a therapeutic product is very promising, as reported for the species *D. mollis* (Valois, Oliveira, 2005; Pedriali, 2005). In Brazil, the presence of phytoplasmas in association with diseases of cultivated and spontaneous leguminous plants have been reported (Kitajima, 1994). To our knowledge, this is the first report of phytoplasma in "fava d'anta" trees (*Dimorphandra* sp.).

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