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Hibiscus witches' broom disease associated with different phytoplasma taxa in Brazil

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Abstract

Stolbur group phytoplasmas were detected in symptomatic hibiscus plants from Brazil showing witches' broom symptoms while in other symptomatic samples of the same locations '*Candidatus* Phytoplasma brasiliense' was identified. This finding confirms that diverse phytoplasmas can be associated with this disease in hibiscus. This is the first report of identification of a 16SrXII phytoplasma in this genus worldwide.

Key words: Brazil, '*Candidatus* Phytoplasma brasiliense', China rose, stolbur phytoplasma.

Introduction

Hibiscus rosa-sinensis (Malvaceae) make an excellent foundation planting, being widely used in landscaping (Lorenzi, 2008). In Brazil, due to hibiscus witches broom disease, the adoption of hibiscus plants is under restriction, mainly in the state of Rio de Janeiro. Symptoms of the disease are characteristic of witches' broom syndrome, such as leaf yellowing, short internodes, proliferation of shoots, and in some cases, premature flower dropping. Diseased plants may die in case of severe infection (Vicente *et al.*, 1974, Kitajima, 1994). The identity of the phytoplasma associated with hibiscus witches' broom disease in Brazil was demonstrated and the phytoplasma, designated '*Candidatus* Phytoplasma brasiliense', was affiliated to the group 16SrXV, subgroup A (Montano *et al*., 2001).

To evaluate epidemiological spreading of this disease in hibiscus further research to verify the phytoplasma presence in symptomatic plants, from different areas in Brazil were carried out.

Materials and methods

Eight hibiscus samples exhibiting shoot proliferation, short internodes and reduced leaf size (figure 1) were collected in two locations, in the quarter of Barra da Tijuca, in the city of Rio de Janeiro, Brazil. Six of the samples (1 to 6) were collected in the location known as Península, and two of them (7 and 8) in Barra Shopping Mall. One gram of phloem tissue scraped from twigs of symptomatic plant was submitted to nucleic acid extraction with a chloroform/phenol protocol (Prince *et al*., 1993). Nucleic acid was employed at the concentration of 20 ng in direct PCR with universal primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995), followed by nested PCR with primers F1/B6 (Davis and Lee, 1993; Padovan *et al*., 1995), and by a second nested PCR with R16F2/R2 or R16(I)F1/R1 (Lee *et al.*, 1995).

Figure 1. *Hibiscus rosa-sinensis* with proliferation, short internodes and reduced leaf size.

RFLP analyses were carried out and with *Tru*I, *Taq*I and *Alu*I (Fermentas, Vilnius, Lithuania) restriction enzymes and patterns were compared with phytoplasma reference strains (Bertaccini *et al.,* 2000). Amplicons obtained after R16(I)F1/R1 were further selected and purified with "PCR Clean-Up" (Macherey–Nagel, Germany) for sequencing. The obtained sequences were aligned by using Clustal W and BioEdit (Hall, 1999) softwares, and compared with those of phytoplasma sequences deposited in GenBank.

Results

On the basis of phytoplasma-specific DNA amplification in PCR phytoplasmas were detected in all symptomatic hibiscus plants. In particular RFLP analyses with *Alu*I, *Taq*I and *Tru*I restriction enzymes on R16F2/R2 fragments allowed indentification of phytoplasmas belonging to ribosomal group 16SrXV only in the Península 4 sample (figure 2). Further nested PCR with R16(I)F1/R1 allowed detection of phytoplasma presence in all symptomatic samples, and RFLP analyses with *Tru*I indicated a 16SrXII phytoplasma in the majority of samples. However a profile different from all those already reported, was detected in samples Península 4 and 5 (figure 2).

Sequencing of selected amplicons from each profiles confirmed the presence of '*Ca*. P. brasiliense' in the samples with different RFLP profiles, while the sequencing of samples having the 16SrXII profile was not readable with several double peaks indicating possible presence of mixed phytoplasma infection.

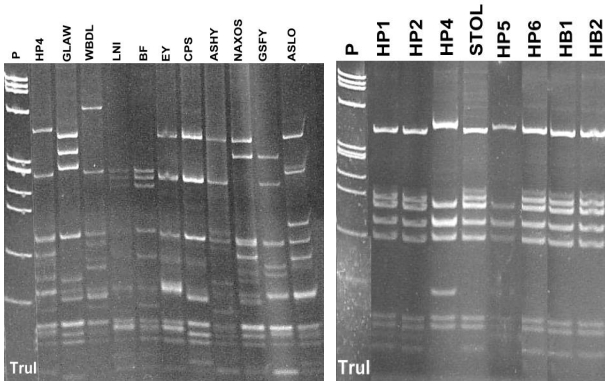

Figure 2. RFLP profiles of R16F2/R2 (left) and R16(I)F1/R1 (right) amplicons after *Tru*I digest. Acronyms: HP, Hibiscus Península; HB, Hibiscus Barra; GLAW, gladiolus witches' broom (16SrI-B); WBDL, witches' broom disease of lime (16SrII-B); LNI, plum leptonecrosis (16SrII-B); BF, peach X disease (16SrIII-A); EY, elm yellows (16SrV-A); CPS, crotalaria phyllody (16SrVI-C); ASHY, ash yellows (16SrVII-A); Naxos, periwinkle virescence from Sicily (16SrIX-C); GSFY, German stone fruit yellows (16SrX-B); ASLO, aster yellows from Slovenia (16SrXII-A); STOL, stolbur from pepper from Serbia (16SrXII-A); P, marker ΦX174 *Hae*III digested.

Discussion

Detection of stolbur group phytoplasmas in symptomatic hibiscus plants from Brazil indicates that witches' broom symptoms can be associated with the presence of a phytoplasma other than '*Ca.* P. brasiliense'. The work carried out also indicated that the primers 16Sr(I)F1/R1 are more general than reported amplifying also the group 16SrXV, besides groups 16SrI, -II, and -XII. The finding of phytoplasmas distinct from 16SrXV-A, as previously reported to be associated with hibiscus witches' broom (Montano *et al*., 2001), confirms that diverse phytoplasmas can be associated with this disease in hibiscus, and this is a common feature for phytoplasma-associated diseases worldwide. Moreover, this is the first report of identification of a 16SrXII phytoplasma in this genus worldwide; there is only a report of an unidentified phytoplasma in Australia associated with a witches broom disease of *Hibiscus heterophyllus* (Hiruki, 1987).

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