Bulletin of Insectology ***64*** *(Supplement): S99-S100, 2011*
ISSN 1721-8861

Turnera ulmifolia, a new phytoplasma host species

Helena G. MONTANO[1], Nicoletta CONTALDO[2], João P. PIMENTEL[1], Jadier O. CUNHA JUNIOR[1], Samanta PALTRINIERI[2], Assunta BERTACCINI[2]
[1]*DEnF, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, Estado do Rio de Janeiro, Brazil*
[2]*Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale,* Alma Mater Studiorum-*University of Bologna, Bologna, Italy*

Abstract

Turnera ulmifolia L., the yellow alder, is a widely distributed species in Brazil where, besides being an ornamental, it is used as a tea for the treatment of gastric diseases. In plants showing yellowing and witches' broom symptoms, a phytoplasma was detected by molecular analyses; its characterization by RFLP analyses of 16S rDNA gene allowed preliminary classification of this phytoplasma into the 16SrXIII ribosomal group. This is the first time that a phytoplasma from the 16SrXIII group has been reported in Brazil.

Key words: Brazil, chanana, witches' broom, yellow alder, phytoplasma.

Introduction

Turnera ulmifolia L. (Turneraceae), the yellow alder, is a perennial, dense, compact shrub native to tropical America. The species is widely distributed in Brazil, where it is popularly known as turnera, chanana and flor-do-guarujá. With showy yellow flowers that blossom year-round, turnera is adopted as an ornamental plant, being used as foundation, border, mass planting and ground cover (Lorenzi, 2008). In Brazilian folk medicine, turnera is also used as a tea for the treatment of diseases related mainly to gastric dysfunction. Research has produced data indicating that the plant extract has a significant antiulcerogenic effect (Gracioso *et al*., 2002). Plants of *T. ulmifolia* exhibiting witches' broom growths (figure 1) and yellowing that are symptoms typically induced by phytoplasmas, have been observed in the state of Rio de Janeiro. The aim of the present work was to verify phytoplasma association with the *Turnera ulmifolia* witches' broom disease in Brazil and to molecularly identify detected phytoplasmas.

Materials and methods

Samples from *T. ulmifolia* exhibiting shoot proliferation and yellowing (figure 1) were collected in the location of Penedo, state of Rio de Janeiro, in 2003. DNA extraction procedure followed that of Montano *et al*. (2000). Reference phytoplasma strains in periwinkle were employed as control (figure 2) and a strain of erigeron witches' broom phytoplasmas [16SrVII-B, (Barros *et al*., 2002)] from naturally infected plant from Brazil was also employed as a positive control. Universal primer pairs P1/P7 (Deng and Hiruki, 1991; Schneider *et al.,* 1995) and R16F2/R2 (Lee *et al*., 1995) were used to prime amplification of phytoplasma 16S rDNA sequences in nested PCR assays. Further nested PCR assays were carried out with primers F1/B6 (Davis and Lee, 1993; Padovan *et al*., 1995). RFLP analyses were carried out with *Tru*I on F1/B6 and R16F2/R2 amplicons and with *Taq*I, and *Alu*I restriction enzymes (Fermentas, Vilnius, Lithuania) on R16F2/R2 amplicons. Obtained patterns were compared with those of phytoplasma reference strains (Bertaccini *et al.,* 2000) on the same size amplicons. Further amplification for molecular characterization of detected phytoplasmas were carried out using rpF(I)1/rp(I)R1A primers to amplify the rplV (rpl22) and rpsC (rps3) genes; obtained amplicons were subjected to RFLP analyses with *Tru*I under described conditions (Martini *et al*., 2007).

Figure 1. *Turnera ulmifolia* asymptomatic (left and right) and with yellowing and witches' broom (arrow). (In colour at www.bulletinofinsectology.org)

Results

Phytoplasmas were detected in turnera plants exhibiting symptoms of witches' broom disease in direct, as well as in nested PCR tests. RFLP analyses with *Alu*I, *Taq*I and *Tru*I restriction enzymes on P1/P7, F1/B6 and on R16F2/R2 amplicons (figure 2 and Lee *et al*., 1998) allowed the tentative phytoplasma affiliation to ribosomal subgroup 16SrXIII. The amplification of the rpl22-rps3 gene resulted in the expected 1.2 kb amplicons and the RFLP profile obtained after *Tru*I digestion was clearly different from any of those available in the literature for the same gene.

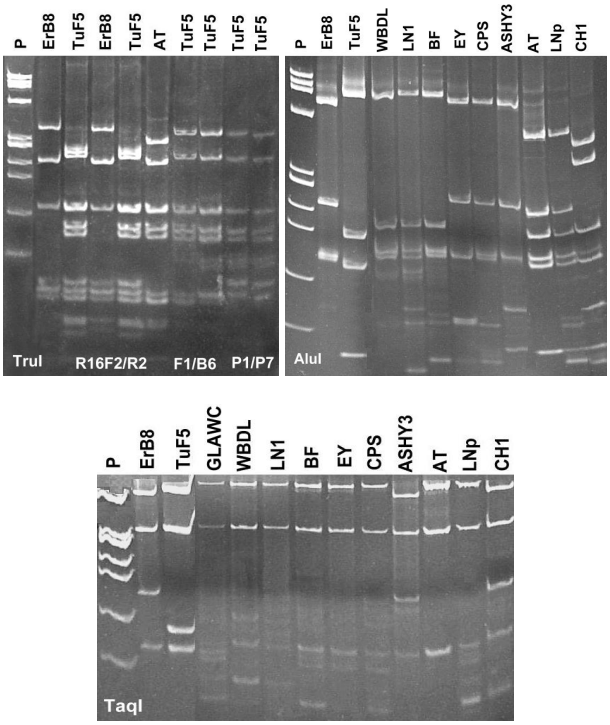

Figure 2. RFLP profiles with *Tru*I of the three types of amplicons produced (top left) and with *Alu*I (top right) and *Taq*I (bottom) of R16F2/R2 amplicons. Acronyms: ErB8, erigeron witches' broom; TuF5, turnera; GLAWC, 16SrI-B; WBDL 16SrII-B; LN1, 16SrIII-B; BF, 16SrIII-A; EY, 16SrV-A; CPS, 16SrVI-C; ASHY3, 16SrVII-A; AT, 16SrX-A; LNp, 16SrX-B; CH1, 16SrXII; P, marker ΦX174 *Hae*III digested.

Discussion

The PCR/RFLP results demonstrate that a phytoplasma is associated with *Turnera ulmifolia* and this is the first report of phytoplasma infection in the family Turneraceae family. The RFLP profiles obtained are referable to subgroup 16SrXIII (Lee *et al*., 1998) for wich *rp* gene profiles are not available in literature, further characterization of the phytoplasma is in progress.

In Brazil, the diseases associated with phytoplasmas have been reported in a wide range of families (Montano *et al.*, 2007), however 16SrXIII group phytoplasmas were not reported, although phytoplasmas genetically related with this group (98% homology on 16S rDNA) are listed in GenBank as associated with a papaya apical curl necrosis disease (EU719111).

Acknowledgements

Helena Guglielmi Montano acknowledges the National Research Council of Brazil (CNPq) for research grant (2007-2009). The work was carried out in the frame of COST action FA0807 'Integrated Management of Phytoplasma Epidemics in Different Crop Systems'.

References

BARROS T. S. L., DAVIS R. E., RESENDE R. O., DALLY E. L., 2002.- Erigeron witches' broom phytoplasma in Brazil represents new subgroup VII-B in 16S rRNA gene group VII, the Ash Yellows phytoplasma group.- *Plant Disease*, 86(10): 1142-1148.

BERTACCINI A., CARRARO L., DAVIES D., LAIMER DA CAMARA MACHADO M., MARTINI M., PALTRINIERI S., SEEMÜLLER E., 2000.- Micropropagation of a collection of phytoplasma strains in periwinkle and other host plants, p. 101. In: *13th International Congress of IOM*.- July 14-19, Fukuoka, Japan.

DAVIS R. E., LEE I-M., 1993.- Cluster-specific polymerase chain reaction amplification of 16S rDNA sequences for detection and identification of mycoplasmalike organisms.- *Phytopathology,* 83: 1008-1001.

DENG S., HIRUKI C., 1991.- Amplification of 16S rRNA genes from culturable and nonculturable mollicutes.- *Journal of Microbiological Methods,* 14: 53-61.

HALL T. A., 1999.- Bio Edit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT.- *Nucleic Acids Symposium Serie,* 41: 95-98.

GRACIOSO J. L. , VILÉGAS W., HIRUMA-LIMA C. A., BRITO A. R. M. S., 2002.- Effect of tea from *Turnera ulmifolia* L. on mouse gastric mucosa support the Turneraceae as a new source of antiulcerogenic drugs.- *Biological & Pharmaceutical Bulletin,* 25: 487-491.

LEE I-M., BERTACCINI A., VIBIO M., GUNDERSEN D. E., 1995.- Detection of multiple phytoplasmas in perennial fruit trees with decline symptoms in Italy.- *Phytopathology*, 85: 728-735.

LEE I-M., GUNDERSEN-RINDAL D., DAVIS R. E., BARTOSZYK I., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein gene sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

LORENZI H., 2008.- *Plantas ornamentais no Brasil: arbustivas, herbáceas e trepadeiras*.- Nova Odessa, São Paulo: Instituto Plantarum.

MARTINI M., LEE I-M., BOTTNER K. D., ZHAO Y., BOTTI S., BERTACCINI A., HARRISON N., CARRARO L., MARCONE C., KHAN A. J., OSLER R., 2007.- Ribosomal protein gene-based phylogeny for differentiation and classification of phytoplasmas.- *International Journal of Systematic and Evolutionary Microbiology*, 57: 2037-2051.

MONTANO H. G., DAVIS R. E., DALLY E. L., PIMENTEL J. P., BRIOSO P. S. T., 2000.- Identification and phylogenetic analysis of a new phytoplasma from diseased chayote in Brazil.- *Plant Disease,* 84: 429-436.

MONTANO H. G., BRIOSO P. S. T., PIMENTEL J. P., 2007.- List of phytoplasma hosts in Brazil.- *Bulletin of Insectology*, 60: 129-130.

PADOVAN A. C., GIBB K. S., BERTACCINI A., VIBIO M., BONFIGLIOLI R. E., MAGAREY P. A., SEARS B. B., 1995.- Molecular detection of the Australian grapevine yellows phytoplasma and comparison with a grapevine yellows phytoplasma from Emilia-Romagna in Italy.- *Australian Journal of Grape Wine Research*, 1: 25-31.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I* (RAZIN S., TULLY, J. G., Eds).- Academic Press, San Diego, CA, USA.

Corresponding author: Helena G. MONTANO (e-mail: hgmontano@yahoo.com.br), DEnF, Universidade Federal Rural do Rio de Janeiro (UFRRJ), Seropédica, RJ, Brazil.