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A severe disease induced by '*Candidatus* Phytoplasma asteris' in *Digitalis lanata*

Maria Grazia BELLARDI, Alessandro BENNI, Samanta PALTRINIERI, Assunta BERTACCINI
Dipartimento di Scienze e Tecnologie Agroambientali, Patologia vegetale, University of Bologna, Bologna, Italy

Abstract

In spring of 2007, a severe disease was observed in *Digitalis lanata* plants cultivated in an herb garden. Stunting of the whole plants, size reduction and reddish colour of leaf lamina, axillary bud proliferation (witches' broom), virescence and rosetting of flower spikes were observed. '*Candidatus* Phytoplasma asteris'-related phytoplasmas were identified by direct and nested PCR assays followed by RFLP analyses on 16S ribosomal gene. RFLP analyses on rpL22, elongation factor EF-Tu, aminoacid kinase plus ribosome recycling factors and *Amp* genes showed that phytoplasmas infecting *D. lanata* are undistinguishable from representative phytoplasmas enclosed in ribosomal subgroup 16SrI-B. This is the first report of phytoplasma presence in this species.

Key words: *Digitalis lanata*, woolly foxglove, virescence, rosette disease, aster yellows.

Introduction

Digitalis lanata Ehrh. (woolly floxglove or Grecian floxglove; *Scrophulariaceae* family) is an herbaceous plant indigenous to western and central Europe: under Italian environments the plant is an evergreen flowering from April to June. Like some other floxglove species (*D. purpurea* L. and *D. lutea* L.), *D. lanata* is toxic in all its parts. Floxgloves are commonly cultivated as ornamental, even if some *Digitalis* species are also important herb employed in pharmaceutical manufacture. *Digitalis* glycosides, normally used to treat congestive heart failure or atrial fibrillation, are difficult to synthesize, so it is easier to extract them from floxglove. Digoxin, the main drug used to treat some heart diseases is extracted only from the leaves of *D. lanata.*

D. lanata is a natural host of *Broad bean wilt virus* and *Turnip mosaic virus*; both these two aphid-born viruses, alone or in mixed infection, were reported in Italy (Rubies-Autonell and Bellardi, 1999; Bellardi and Bertaccini, 2005).

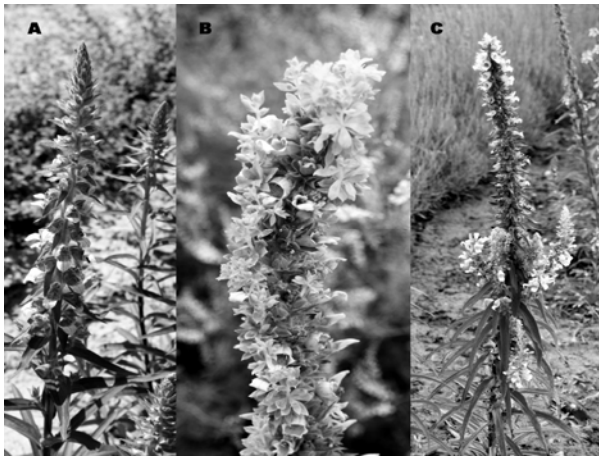

Figure 1. *Digitalis lanata*: A, healthy plants; B, C, virescent and rosetted phytoplasma infected flowers; axillary shoots are also visible (C).

During spring 2007 a phytoplasma-like disease was observed in a cultivation of *D. lanata* located at the herb garden "Augusto Rinaldi Ceroni" of Casola Valsenio (Ravenna; Emilia Romagna region, northern Italy). After first symptoms observation in April an increasing percentage of symptomatic plants was found in the following months. Affected plants showed reduction of leaf size, reddish lamina, stunting, axillary bud proliferations (witches' broom), virescence, phyllody (development of floral parts into leaf-like structures) and partially or complete rosetting of flower spikes (figure 1). The aim of this study was to verify phytoplasma presence and identity in woolly floxglove.

Materials and methods

Samples from symptomatic plants were collected and tested by direct PCR with primers P1/P7 followed by nested PCR with primers F1/B6 (Duduk *et al*., 2004). RFLP analyses were performed on both amplicons with *Tru*I and *Tsp509*I for 16 hours at 65 °C. Further PCR analyses were carried out with primers fTufu/rTufu (Schneider *et al*., 1997), BB88F1/R1 (Gundersen *et al*., 1996), and Amp-N1/C1 (Kakizawa *et al.*, 2003). PCR conditions were as described by Schaff *et al*. (1992). Phytoplasma stains representative of 16SrI-B ribosomal subgroup employed as reference were: AYW, AY2192, CCH, EAY, KAY; of 16SrI-A: CHRY; of 16SrI-C: GY, PPT; of 16SrI-F: CVB and of 16SrI-L: BGWL. ASLO phytoplasmas belonging to ribosomal subgroup 16SrXII-A (Bertaccini *et al*., 2000) were also employed. Analyses with selected restriction enzymes were performed on all the above described amplicons (figure 2b).

Results and discussion

Both direct and nested PCR as well as RFLP analyses on 16Sr DNA gene confirmed that in all the symptomatic samples examined phytoplasmas identified belong

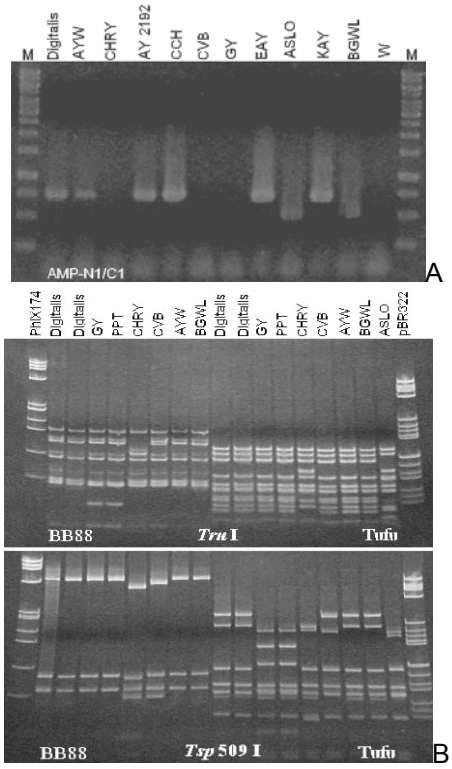

Figure 2. PCR amplification of *Amp* gene (A) and RFLP results on *tuf* and aminoacid kinase plus ribosome recycling factor genes.

to ribosomal subgroup 16SrI-B. Samples from asymptomatic plants gave always negative results in all PCR assays. *D. lanata* samples as well as all phytoplasma reference strains were amplified with all primers employed except Amp-N1/C1. In this latter amplification the expected 702 bp products was observed only from 16SrI-B phytoplasma-infected samples, 16SrI-A, I-C and I-F phytoplasmas did not amplify, while 16SrI-L and 16SrXII-A phytoplasmas produce an amplification product of about 500 bp (figure 2a). Collective RFLP profiles on the other amplicons distinguished among phytoplasmas belonging to diverse 16SrI subgroups as reported (Botti and Bertaccini, 2003) (figure 2b).

Discussion

Aster yellows ribosomal group (16SrI) phytoplasmas are associated with a number of economically important diseases worldwide and represent one of the most diverse and widespread phytoplasma groups. Nevertheless this is the first report of a phytoplasma infection in *D. lanata.* Study of phytoplasma infection in Italian herb crops started in recent years, however diverse phytoplasma infections were observed in various cultivated medicinal plants such as *Galega officinalis* L., *Hyssopus officinalis* L., *Spartium junceum* L., and *Parietaria* spp. (Bellardi and Bertaccini, 2005). About 10 years ago 16SrI-B phytoplasmas were identified in *Digitalis lutea* L. (yellow floxglove) cultivated in the same herb garden (Bellardi *et al*., 1999). *D. lanata* is seed propagated therefore the epidemic could be consequence of leafhopper vectors, weeds and/or other infected plant species presence in the same area. The clear selective detection of 16SrI-B phytoplasmas using Amp-N1/C1 primers could be very useful in further ecological and epidemiological studies of aster yellows-related phytoplasmas (16SrI-B).

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Corresponding author: Maria Grazia BELLARDI (e-mail: mariagrazia.bellardi@unibo.it), DiSTA - Patologia Vegetale, viale G. Fanin 42, 40127 Bologna, Italy.