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Virescence of tenweeks stock associated to phytoplasma infection in Sicily

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Abstract

In April 2007, a severe disease occurred in Sicily (Italy) in a glasshouse cultivation of tenweeks stock belonging to the cultivar White-Beach. Plants were stunted and rosetted, and the flowers were of small size and characterized by virescence symptoms. Phytoplasma presence and identity was detected by applying PCR/RFLP techniques and sequencing of 16S ribosomal gene. Phytoplasmas were identified as belonging to ribosomal subgroup 16SrII-A, never reported before in Italy and showed 99% of homology with '*Candidatus* Phytoplasma aurantifolia' and related phytoplasmas. This is the first report of a phytoplasma disease of tenweeks stock. Considering that this *Brassicaceae* ornamental species is widely grown in Italy, it could play an important role in spreading these phytoplasmas, new for Italy.

Key words: Tenweeks stock, virescence, phytoplasma, PCR, RFLP, epidemiology.

Introduction

Tenweeks stock (*Matthiola incana* R. Br.; *Brassicaceae*) is considered one the most common herbaceous ornamental species cultivated in Sicily (southern Italy) for late winter and early spring flower production. This plant is usually susceptible to attacks by several pathogens. In Italy, protection measures against *Peronospora matthiolae*, *Xanthomonas campestris* pv. *incanae*, *Turnip mosaic virus*, alone or in mixed infection to *Cauliflower mosaic virus* (Alioto *et al.*, 1994) are applied.

In April 2007, a severe disease occurred in Sicily (southern Italy) in a glasshouse cultivation of tenweeks stock belonging to the cultivar White-Beach. Plants were stunted and rosetted, but the main symptoms, appearing at the flowering stage, were malformation of white flowers and virescence (figure 1). The incidence of symptomatic plants was about 65%. To verify phytoplasma presence, PCR (polymerase chain reaction), RFLP (restriction fragment length polymorphism) and sequencing were carried out on 16S ribosomal gene.

Figure 1. Virescence and flower malformation on tenweeks stock flowers.

Materials and methods

Nucleic acids were extracted from 1 g of flower and leaf tissues from both symptomatic and asymptomatic tenweeks stock plants using a chloroform/phenol procedure (Prince *et al.*, 1993). Nucleic acid (0.8 µL), suspended in TE buffer and diluted to obtain a final concentration of 20 ng/µL, was added to a PCR reaction mixture (25 µL total volume) containing 200 µmol of dNTP, 0.8 U of Taq polymerase (Polymed, Florence, Italy), 0.4 µmol of each primer, 10 mM TRIS buffer (pH 8.3), 50 mM HCl, 1.5 mM $MgCl_2$ and 0.001% (w/v) gelatine as PCR buffer. The primer pairs employed were P1/P7 followed in nested PCR by F1/B6 under PCR conditions described (Duduk *et al*., 2004). The PCR products were detected by 1% agarose gel electrophoresis followed by ethidium bromide staining and UV observation.

In RFLP analyses, 100-200 ng of F1/B6 products were digested with *Tru*I, *Taq*I, and *Tai*I restriction enzymes at 65 °C for 16 h. The restriction patterns were compared with those of selected phytoplasma control strains (Bertaccini *et al*., 2000) after electrophoresis on a 5% polyacrylamide gel, ethidium bromide staining, and photographed under UV using a transilluminator.

F1/B6 amplified product of sample TS1 (figure 2) was purified using Qiagen PCR Purification Kit and then sequenced in both directions. The sequences were then aligned using BLAST engine for local alignment (version Blast N 2.2.12). The gene sequence (1,379 bp) obtained was deposited in the NCBI, Bethesda, MD, USA under accession No. EF570134.

Results

No amplification was obtained after direct PCR from *M. incana* samples, only after nested PCR and only samples from symptomatic flowers showed expected amplification bands of about 1,300 bp. RFLP analyses with

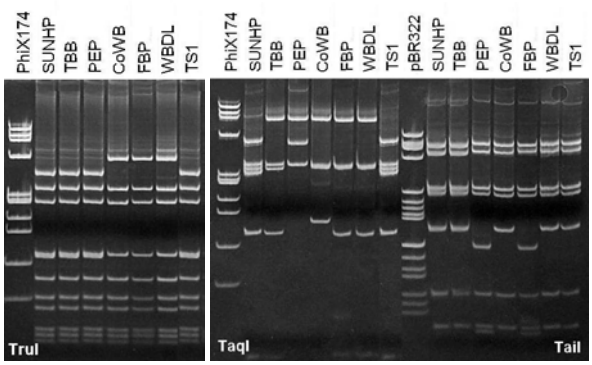

Figure 2. RFLP patterns on polyacrylamide gels of F1/B6 amplicons from *M. incana* and selected phytoplasma reference strains.

3 restriction enzymes revealed profiles indistinguishable from control sample belonging to 16SrII-A ribosomal subgroup (SUNHP, *Crotalaria juncea* witches' broom from Thailand) (figure 2). The asymptomatic samples gave negative results as well as the leaf samples from symptomatic plants in which flowers resulted positive.

Phylogenetic comparison of the 16S rRNA gene of TS1 sample confirmed that the phytoplasmas detected are very closely related to '*Ca.* P. aurantifolia'. The homology search with blastn on 16S sequence plus spacer region showed homology values of 99% with several phytoplasmas belonging to 16SrII ribosomal group.

Discussion

Phytoplasmas are responsible for several hundred of disease in numerous plant species worldwide and also in Italy. Their spreading can cause severe losses in many economical crops and cultivations, especially ornamentals. Among *Brassicaceae*, several species of *B. oleracea* L. (*Botrytis* group and *Italica* group) were described in Italy to be affected by virescence symptoms and dwarfing of the inflorescence (Bertaccini *et al*., 1983). Moreover, cabbage virescence and phyllody were described in a high percentage of plants (30-60%). The molecular characterization of this phytoplasma revealed that it belonged to 16SrI-B ribosomal subgroup (Bertaccini *et al*., 1990; 1992). Later on similar symptoms on winter oil-seed rape were observed in the Czech Republic and the molecular identification of involved phytoplasmas confirmed again association with 16SrI-B ribosomal subgroup (Bertaccini *et al.*, 1998).

Phytoplasmas infecting virescent tenweeks stocks appear to belong to a ribosomal subgroup (16SrII-A) never described before in Italy or in Europe and never detected in *Brassicaceae* family plants. No other flower and/or ornamental cultivations were present in the Sicilian area where *M. incana* plants were growing; asymptomatic crops of tomato, pepper and eggplants surrounding the tenweeks stock greenhouse showed no leafhopper presence and no leafhoppers were observed inside this greenhouse. *M. incana* cultivation was grown from seeds, 5 cm high plantlets were transplanted in the greenhouse. Therefore, it is unlikely that the phytoplasma infection was originated from the environment. Considering that *M. incana* is an ornamental species widely cultivated in Italy, it could play an important role in spreading these phytoplasmas, new for Italy.

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