

## Phytoplasma infection in the four o'clock flower (*Mirabilis jalapa*)

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### Abstract

The four o'clock flower (*Mirabilis jalapa* L.), a native of tropical South America, has been naturalized as an ornamental garden plant in many parts of the world, including Israel. Plants are multi-branched perennials that produce fragrant colorful flowers over the course of a few months. Plants with small yellow leaves and petite, distorted flowers, resembling phytoplasma-like symptoms, were observed in a home garden in the north of Israel. Using a nested polymerase chain reaction assay (PCR) with phytoplasma universal primers a product of 860 bp was obtained. Sequence analysis of the PCR product associated with infected *M. jalapa* clustered within a major group of phytoplasmas - 16SrII, peanut witches' broom - and showed a 99% similarity with alfalfa witches' broom, a member of this group. To the best of our knowledge this is the first report of phytoplasma infection in the four o'clock flower.

**Key words:** *Mirabilis jalapa*, phytoplasma, symptoms.

### Introduction

*Mirabilis jalapa* L. is an ornamental plant commonly known as the 'four o'clock flower' because its flowers open from late afternoon and onwards. Plants produce fragrant flowers in a range of colours from white to red over the course of a few months. It is a multi-branched perennial plant in southern and warm western regions, and an annual in cooler northern regions, of its native tropical South America. It has been naturalized in many parts of the world, including Israel.

*M. jalapa* has long been cultivated for scientific purposes: in the late 1800's it was the subject of trials in Mendelian genetics (Engels *et al.*, 1975). It is known to have an anti-viral protein whose mode of action is ribosome-inactivation (Ikeda *et al.*, 1987; Ago *et al.*, 1994). Because of its antiviral activity, it is not surprising that it hosts very few pathogens. The only known host of *Phytophthora mirabilis* is *M. jalapa*. This fungus infects only foliage and other above-ground plant parts (Goodwin and Fry, 1994). *M. jalapa* is known to be host to two viruses: *Mirabilis* mosaic virus (family *Caulimoviridae*) (Brunt *et al.*, 1973) and parietaria mottle virus (Parrella, 2002). Finally, it is known to be infected by a bacterial blight, due to *Xanthomonas campestris* (Koike and Azad, 2003).

Plants of *M. jalapa* with small yellow leaves, short internodes and small-sized flowers were observed in a private garden in northern Israel (Kibbutz Lavi). The possibility of viral or phytoplasma infection was explored, and here we report the observation, for the first time, of phytoplasma in *M. jalapa*.

### Materials and methods

Plants with obvious symptoms were transferred from the garden to a screenhouse at the Volcani Center and maintained for further studies.

Testing for phytoplasma was done by PCR analysis. DNA was extracted from plant tissue using a DNeasy Plant Mini Kit (Qiagen, CA, USA) according to the manufacturer's instructions. DNA fragments were amplified, employing a nested-PCR system. The universal primers P1/P7 (Deng and Hiruki, 1991) were used for direct PCR and the universal primers fU5/rU3 (Lorenz *et al.*, 1995) for the nested reaction. DNA extracted from asymptomatic plants served as a negative control. Amplified products were analyzed by electrophoresis and DNA bands were visualized by a UV transilluminator after staining with ethidium bromide.

The 860 bp PCR product was cloned into a pGEM-T Easy Vector (Promega, Madison, WI, USA). The nucleotide sequence of the insert was determined using an ABI Prism 3700 DNA analyzer (Hy Laboratories, Rehovot, Israel). Three clones were analyzed independently from both directions. Sequence homology analysis with other phytoplasmas in GenBank database was done using BLAST (Altschul *et al.*, 1997).

To determine if the symptom expressing plants involved a virus infection, a bioassay was employed. Samples from symptomatic leaves were ground in 1% K<sub>2</sub>HPO<sub>4</sub> and inoculated mechanically onto leaves of 17 different herbaceous indicator plants and healthy seedlings of *M. jalapa*. Inoculated plants were maintained in the greenhouse (22-24 °C) for up to 30 days and observed for symptom development.

### Results

*M. jalapa* plants had typical phytoplasma-like symptoms (figure 1): small yellow leaves with very short internodes and small-sized flowers. Nested PCR analysis, using the universal primer pair fU5/rU3 revealed an 860 bp amplified product supporting the presence of a phytoplasma in these plants. Nucleotide sequence analysis of DNA amplified products from affected *M.*

*M. jalapa* clustered within the 16SrII peanut witches' broom phytoplasma group and has shown a 99% homology with alfalfa witches' broom and *Scaevola* witches' broom.

The possibility of a virus infection involved in the disease symptoms described above was ruled out on the basis of mechanical inoculation results. None of the 17 indicator plants inoculated developed any symptoms during a post inoculation period of one month.

## Discussion

Since *M. jalapa* is known to be a natural host to two viruses, we first checked the possibility of a virus infection. Based on mechanical transmission results this possibility was ruled out. PCR analysis of DNA confirmed the involvement of a phytoplasma with the symptoms described above.

Sequence analysis of amplification products from infected *M. jalapa* places it in the 16SrII peanut witches' broom group. Phytoplasmas in this group have been found in the Middle East (Choueiri *et al.*, 2005; Livingston *et al.*, 2006), Mediterranean region (Tolu *et al.*, 2006) and in Israel (Gera *et al.*, 2006; Weintraub *et al.*, 2007). This is the first report of a phytoplasma infecting *M. jalapa*.

Although no leafhoppers, planthoppers or psyllids were observed on the plants, it is presumed that one or more of these species transmitted the phytoplasma to the plant (Weintraub and Beanland, 2006). Because of the presence of phytoplasma in *M. jalapa* plants, it would be advantageous to use seeds rather than vegetative propagation to avoid spreading the phytoplasma to other plants and areas.

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**Figure 1.** *Mirabilis jalapa*: left side leaves symptom-free; right side leaves, phytoplasma-infected. (In colour at [www.bulletinofinsectology.org](http://www.bulletinofinsectology.org)).

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