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A new disease causing stunting and shoot proliferation in *Gypsophila* is associated with phytoplasma

Abdullah GERA[1], Phyllis G. WEINTRAUB[2], Ludmila MASLENIN[1], Sara SPIEGEL[1], Mohammad ZEIDAN[3]
[1]*Department of Virology, Agricultural Research Organization, The Volcani Center, Bet Dagan, Israel*
[2]*Dept. of Entomology, Gilat Research Station, D.N. Negev, Israel*
[3]*The Plant Protection and Inspection Services, Ministry of Agriculture, Bet Dagan, Israel*

Abstract

Phytoplasma diseases have been identified in Israel in numerous species of various botanical families. The disease occurs in ornamentals, vegetables, field crops and fruit trees. In cut flowers, the disease was identified in *Ranunculus spp*., *Celosia*, *Anemone* and *Limonium*. In all these cases the disease was sporadic with no serious economical losses. In 2003, symptoms typical of a phytoplasma infection were observed in a large number of *Gypsophila paniculata* L. (i.e. baby's breath) plants grown in commercial fields in Israel. The symptoms include leaf yellowing, production of abundant long and narrow leaves, stunting, shoot proliferation, poor flower set and consequently reduce the yield of flowers by up to 80%. Examination of ultrathin sections of samples from diseased plants by electron microscopy revealed the presence of pleomorphic membrane-bound bodies in the phloem cells. Total nucleic acid was extracted from asymptomatic and symptomatic *Gypsophila* leaves. All leaf samples from twenty symptomatic plants consistently tested positive using a polymerase chain reaction assay (PCR) with phytoplasma universal primers (P1/P7) that amplify a 1.8-kb phytoplasma rDNA product and followed by nested PCR with R16F2n/R16R2 primers yielding a product of 1.2 kb. No PCR products were evident when DNA extracted from healthy plants was used as a template. Sequence analysis of the PCR products obtained from numerous preparations, associated with infected *Gypsophila,* clustered within one ribosomal group of phytoplasmas (16SrII), peanut witches' broom. This is the first published record of these phytoplasmas in *Gypsophila* in Israel. The present paper reports the outbreak of phytoplasma in *Gypsophila* grown in commercial fields in Israel, survey of potential insect vector(s) of phytoplasma and possible control strategies using screen barriers.

Key words: Ornamental plants, *Gypsophila*, peanut witches' broom, control.

Introduction

Phytoplasma diseases have been identified, in Israel, in cultivated ornamentals, vegetables, field crops, fruit trees and in wild crops. In cut flowers, the disease was identified in *Anemone* and *Ranunculus,* and *Matthiola* spp. In these cases the disease was sporadic with no serious economical losses. However, in grapevines and carrots the disease is causing losses of great economic significance (Orenstein *et al*., 1999). In the summer of 1998, the disease was identified in *Cosmos* (Cohen *et al*., 1999), *Verbesina* (Cohen *et al*., 2000) and *Celosia* (Tanne *et al*., 2000), causing general yellowing of leaves, stunting, witches' broom growth of auxiliary shoots and flower malformation (phyllody).

In October 2000, the disease was identified in *Limonium* spp. in the Arava Valley (Gera *et al*., 2004). Recently, phytoplasma-like symptoms were observed in *Gypsophila*. The present paper reports the outbreak of phytoplasma in *Gypsophila* grown in commercial fields in Israel, molecular identification and phylogenetic relationships of diverse phytoplasmas associated with the disease.

Materials and methods

Electron microscopy

For ultrathin sections, pieces of petioles and/or leaf veins excised from healthy and symptomatic leaves of *Gypsophila* were fixed in 2.5%, embedded in an Epon-Araldite resin mixture, and polymerized as described by Orion and Franck (1990). Sectioned material was stained with uranyl acetate followed by lead nitrate and examined by electron microscopy.

Polymerase chain reaction and sequence analysis

DNA was prepared from leaf midribs and petioles as described by (Gera *et al*., 2004). DNA fragments were amplified using the universal phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995). The product was further amplified by nested PCR with the R16F2/ R16R2 and fU3-rU5 (Lee *et al*., 1995; Lorenz *et al*., 1995), and used for PCR amplification. Amplified products from several plant samples were sequenced and compared to the sequences in the Gene Bank by BlastN procedure. Their Taxonomic relatedness was determined from the dendrogram presented by the NCBI Web BLAST service.

Leafhopper survey

Yellow sticky traps (14 x 20 cm) were placed vertically just above the canopy and replaced weekly throughout the year. Leafhoppers were carefully removed from the traps, washed in two changes of technical grade hexanes to remove the glue, sorted to species, and then stored in 95% ethanol at room temperature for further analysis.

Results

Symptoms typical of a phytoplasma infection were observed on a large number of *Gypsophila* plants. Symptoms included: small, narrow basal leaves, often yellow

in color; shoot proliferation, excessive leaf growth (witches' broom or 'asparagus fern') and poor flower set (figure 1). Over the course of the growing season up to 80% of the *Gypsophila* plants grown in the Arava valley were affected.

Electron microscope observations of ultrathin sections of infected leaves from *Gypsophila* revealed the presence of pleomorphic membrane-bound bodies in the phloem cells. No other types of bodies were observed. Similar bodies were not observed in samples of healthy *Gypsophila* plants.

Amplification of phytoplasma related sequences using the universal primers P1/P7 followed by a nested PCR with internal primers indicated that all plants expressing symptoms were infected by phytoplasma, while symptom less plants were phytoplasma-free. The PCR fragments were directly sequenced. Nucleotide sequencing of DNA amplified products from different *Gypsophila* shared between 95.5% and 98.6 % identity with members of the peanut witches' broom and clustered within this group.

Three known leafhopper vectors of phytoplasmas [*Orosius orientalis* (Matsumura), *Circulifer haematoceps* (Mulsant and Rey) and *Circulifer tenellus* (Baker)] were captured on sticky traps during the course of the survey; no planthoppers were caught.

Discussion

In October 2000, phytoplasma disease was identified in *Limonium* spp. in the Arava Valley (Gera *et al*., 2001). Three years later, in October 2003, the disease was identified in *Gypsophila* grown in the Arava Valley. Symptoms included stunting, excessive branching, witches' broom growth of auxiliary shoots and leaf yellowing. Sequence analysis of amplification products from infected *Gypsophila* is consistent with peanut witches' broom phytoplasmas (PnWB) classified into the groups of 16SrII according to Lee *et al*. (1998).

Phytoplasmas are transmitted by leafhoppers and planthoppers. In our survey, leafhoppers were trapped throughout the year, except mid-summer. The Arava has a plant-free period in mid-summer when temperatures are about 40 ºC daily, and all annual plants are removed; the combined effects of intense heat and reduced host plant numbers severely reduces insect populations.

Since potential leafhopper vectors [*O. orientalis*, *C. haematoceps*, *C. tenellus* and *Exitianus exitiosus* (Uhler)] were already present in the Arava area, it is not surprising that the disease rapidly spread in *Gypsophila*. Now that insect vectors of phytoplasma have been identified, research into possible management strategies for the insects and the pathogen is underway.

To date, no successful therapy for yellow diseases has been established, though screen barriers (1.80 cm in height) were found to be effective against vector movement and phytoplasma infection in the field.

Figure 1. Symptoms of phytoplasma in *Gypsophila*.

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Corresponding author: Abed GERA (e-mail: abedgera@volcani.agri.gov.il), Department of Virology, Agricultural Research Organization, The Volcani Center, Bet Dagan 50250, Israel.