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Stunting and flower buds deficiency of *Lilium sp.*: a new phytoplasma associated disease

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Abstract

The presence of phytoplasmas in *Lilium sp.* showing severely stunted growth, leaf malformation and flower buds deficiency was demonstrated for the first time using polymerase chain reaction assays with primers amplifying phytoplasma 16S rDNA regions. These phytoplasmas were found in leaves as well as roots and bulb scales of symptomatic and CMV and/or LSV affected and asymptomatic virus-free lilies.

Introduction

The *Lilium sp.* is one of the most important flower bulbs in the world. Several viruses have been reported infecting lilies naturaly, out of which lily symptomless (LSV), lily mottle (LMoV) and cucumber mosaic viruses (CMV), all aphid-transmitted, are the most frequently reported (Asjes *et al.* 1973, Derks *et al.* 1994, Kamińska, 1996).

In 1982 in Italy, Bertaccini and Marani described flower and leaf malformation and discoloration in lily hybrid 'Pink Perfection' associated with multiple infection of LMoV (TBV), LSV and the presence of mycoplasma-like bodies (phytoplasmas). Similar symptoms were reported by Verhoeven and Horvat (1972), which they attributed to the synergical effect of LSV and CMV infection.

In spring of 1997, oriental lily hybrids 'Casablanca' growing in a commercial greenhouse in Poland from imported bulbs, demonstrated severe symptoms of unknown etiology.

The objective of the present study was to describe the disease symptoms and determine the etiology of pathogenic agents associated with them by DAS-ELISA and molecular genetic techniques.

Materials and methods

Plant samples

Samples of leaves from two asymptomatic lilies 'Casablanca' and from five others showing stunting, leaf malformation and flower buds deficiency were taken at the end of June 1997. In July samples from roots and bulb scales of the same plants were collected. In addition, leaf samples from *Gladiolus* and *Helichrysum* plants affected with American aster yellows (Kamińska *et al.*1996) were also included for comparison.

Virus testing

Samples of lily leaves were tested for the presence of LSV, CMV, LMoV and lily X virus (LXV) using DAS-ELISA. Immunoglobulines (IgG) and alkaline phosphate conjugate of LSV, LXV and LMoV were obtained from the Bulb Research Center, Holland. IgG and conjugate of CMV were of our own production (Korbin and Kamińska, in press). The results of ELISA tests were checked by mechanical inoculation to *Chenopodium quinoa* Willd., *Nicotiana glutinosa* L. and *N. tabacum* L. 'Samsun' plants as described previously (Kamińska 1996).

DNA isolation

Total nucleic acid was extracted from fresh plant tissue according to Ahrens and Seemüller (1992).

PCR conditions

PCR was carried out in 50 µl volume containing 5 µl of isolated DNA, 1 unit of Taq-DNA polymerase (Biometra), 125 µM the four dNTP and 0.5-1.0 µM each primers. Universal for phytoplasma primers rA and fA, complementary to 558 bp fragment of 16S rDNA (Ahrens and Seemüller 1992, Schneider *et al.*1993) were used in these studies.

The prepared mixture was subjected to 25 cycles as follows: 1 minute (2 min. in first cycle) denaturation step at 95 °C, annealing for 1 minute at 55 °C and extension for 1 minute (10 min. in final cycle) at 72 °C.

PCR products (5 µl) were analyzed by electrophoresis through a 1% agarose gel followed by staining with ethidium bromide. DNA bands were visualized using UV transilluminator.

Restriction enzyme analyses

PCR product (15-20 µl) was digested with *Alu*I (Promega, USA) according to manufacturer procedure at 37 °C for 4 h. The restriction products were separated by electrophoresis through 5% polyacrylamide gel, stained with ethidium bromide and then visualized in UV-light.

Results

Symptoms

In the oriental lily hybrid 'Casablanca', the first symptoms were observed 3-4 weeks after bulb planting. They concerned inhibited root formation, severe stunting and leaf chlorosis (Fig. 1A). The leaves were narrowed and the leaf midribs enlarged (Fig. 1B). The most heavily affected plants showed shortening of internodes and deficiency of flower buds. Some plants yielded flowers of very bad quality - inflected, with floral parts degenerated and partially transformed into leaf structures. They grew on strongly elongated and branched stems (Fig. 1C,D).

In lilies grown in commercial greenhouse, more than 50 % of 21,000 plants showed severe symptoms of the disease including stunting, leaf malformation and deficiency of flower buds. Many other plants (20 %) seemed to grow normally, however, they showed abortion of flower buds and produced a strongly reduced number of malformed flowers.

Virus detection

The studies revealed that tested lilies were naturally infected with LSV or LSV and CMV. None of the tested lilies was affected by LMoV or LXV. We failed to transmit any virus from tested lilies to herbaceous test plants.

Phytoplasma detection

After enzymatic amplification of DNA isolated from symptomatic and asymptomatic lilies with primers rA and fA, specific DNA bands (~ 560 bp) were obtained. These bands were observed in all samples of DNA isolated from diseased plants (*Lilium, Gladiolus, Helichrysum*) and one asymptomatic lily. No amplification was observed in nucleic acid extracts from the other asymptomatic lily (Fig. 2).

The specifity of this PCR product was confirmed by RFLP analysis with *Alu*I digestion. The pattern of three DNA bands (240, 190 and 70 bp) was obtained (Fig. 3). The restriction profile was similar to those described by Ahrens and Seemüller (1992) for some phytoplasmas of group I - American aster yellows (AAY).

Fig. 1. Lily 'Casablanca' naturally infected with phytoplasma showing : A - severe stunting, chlorosis and leaf malformation; B - narrowed leaves and deficiency of flower buds; malformed flowers (C, D) growing on elongated, leafless stem (D).

A	**B**
C	**D**

Fig. 2. PCR products of 16S rDNA derived from symptomatic (1,2) and asymptomatic *Lilium* (3), *Gladiolus* (4) and *Helichrysum* (5), M - DNA markers

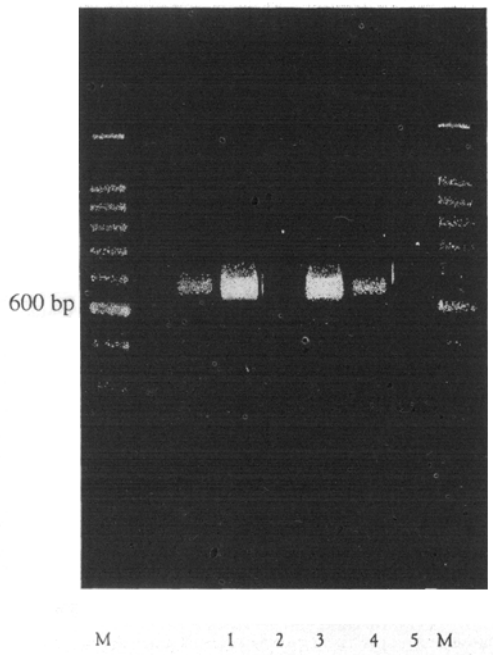
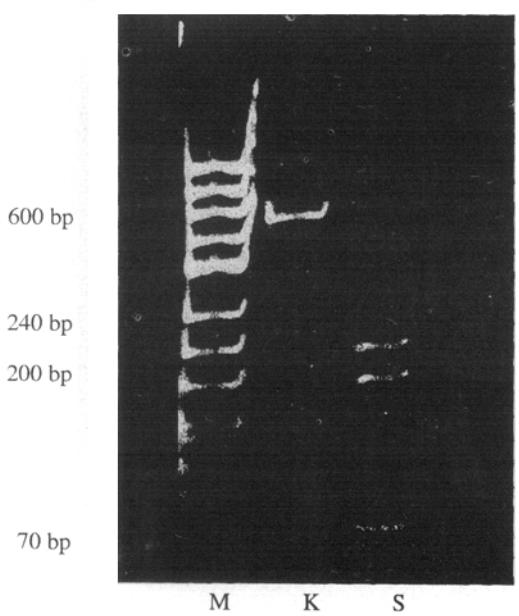


Fig. 3. *Alu* I restriction profile of PCR - amplified 16S rDNA of symptomatic lily (S), K - undigested product, M - DNA markers



Discussion

The results of this preliminary study revealed for the first time the presence of phytoplasmas in lily plants. The data obtained by PCR amplification and RFLP analysis suggest that the phytoplasma recovered from lilies can be included in group I, American aster yellows (AAY) described by Ahrens and Seemüller (1992).

In Europe, natural infection by phytoplasmas were detected in several plant species. Recently, AY related phytoplasmas were identified on the basis of 16S rDNA-RFLP studies in Italy in *Brassica*, *Hydrangea* and *Chrysanthemum* (Bertaccini *et al.*1992), *Gladiolus* (Bertaccini *et al.* 1994), *Anemone* (Vibio *et al.*1995), *Alstroemeria* (Bertaccini *et al.*1996a) and in Poland in annual ornamental plants (Kamińska *et al.* 1996).

As regards the symptoms observed in 'Casablanca', to our knowledge the stunting and flower buds deficiency or abortion were not described. However, some of them are similar to leaf and flower discoloration and malformation, associated with the presence of MLOs and LMoV (Bertaccini and Marani 1982).

The results of the research indicate that the incidence of symptoms in lily plants is interacted with the infection of virus and phytoplasma although it has been difficult to associate them; phytoplasmas were stated using PCR technique in virus infected symptomatic as well as asymptomatic plants. In order to verify this hypothesis it will be necessary to study larger samples and to evaluate the relationship between the occurrence of symptoms and phytoplasma and virus/viruses infection.

The presence of phytoplasma in asymptomatic lily plants could be explained by a delay between infection and symptoms expression or the phenomenon of tolerance. The presence of phytoplasma in symptomless hosts, is valid and important for understanding epidemics of phytoplasma associated diseases, and for disease control. Phytoplasmas were detected in symptomless apricot (Kirpatrick *et al.* 1990) and almond trees (Uyemoto *et al.*1992), in asymptomatic and declining *Alnus* spp. (Lederer and Seemüller 1991), *Fraxinus velutina* Torr. (Sin-

clair *et al.*1994) and in *Celtis australis* L. (Bertaccini *et al.*1996).

Extensive investigations are necessary to further characterize and classify the phytoplasma examined and to determine the real incidence of the disease on lily production and its presence in other lily species and hybrids in countries where lilies are reproduced and are of serious economic importance.

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