
Hyacinthus orientalis is the host for a new phytoplasma exhibiting ribosomal interoperon sequence heterogeneity

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Hyacinth (*Hyacinthus orientalis*) exhibiting symptoms of phytoplasma infection (vi-rescens and phyllody of flowers) was observed in the field (Vilnius region). Analysis of phytoplasmal 16S rDNA amplified from diseased hyacinth revealed a new member of 16SrI group. The genome of the phytoplasma detected exhibited a ribosomal interoperon sequence heterogeneity.

Key words: hyacinth, phytoplasma, rRNA, PCR

INTRODUCTION

Phytoplasmal diseases are spread worldwide. The symptoms of these diseases are yellowing or reddening of leaves, virescence, stunting, fruit abortion, proliferation, phyllody and others. The development of such unusual specific symptoms in infected plants is related with the hormone disbalance caused by cell wall-less plant pathogenic bacteria named by the term of phytoplasmas. Phytoplasmas are transmitted from plant to plant by insect vectors, mostly leafhoppers that feed on the phloem [1]. The inability to isolate and cultivate phytoplasmas in artificial medium hindered the progress of their investigation, and for a long time phytoplasmas were investigated only by the use of electron microscopy. However, since 1990 the application of molecular methods for the investigation of these unculturable organisms has been very successful. On the basis of the analysis of 16S rDNA, phytoplasmas were identified and classified into at least 14 groups and 38 subgroups [2].

In the literature there are no molecular data about the spread of phytoplasmas in the region of Baltic States. The aim of this investigation was to detect, identify and classify phytoplasma in the symptomatic hyacinth exhibiting symptoms of virescence (Fig. 1). The obtained data are of importance for evaluating the spread of phytoplasmal infections in Lithuania.



Fig. 1. Virescence and phyllody symptoms of infected *Hyacinthus orientalis*

MATERIALS AND METHODS

Hyacinth (*Hyacinthus orientalis*) plants exhibiting symptoms specific to phytoplasma infection (vires-

cence and phyllody of flowers) were collected in spring from the field, Vilnius region. Template DNA was extracted from the tissues by a previously described method [3] and used in a nested polymerase chain reaction (PCR) for amplification of sequences from phytoplasmal ribosomal (r) RNA operons. In nested PCR, the first reaction was primed by phytoplasma-universal primer pair P1/P7 [4, 5]. Products obtained in the first PCR were diluted 1:50 and used as template in the second (nested) PCR primed by primer pair R16F2n/R2 (F2n/R2) [4]. Both amplifications were conducted under the same conditions (94° for 1 min, 55° for 2 min, 72° for 3 min) for 35 cycles, in Perkin Elmer PCR buffer, 0.4 mM dNTPs and 1 unit of recombinant Tag polymerase per 50 µl of reaction mixture.

Products (1.2 kbp) of the nested PCR, primed by primer pair F2n/R2, were subjected to enzymatic RFLP (Restriction Fragments Length Polymorphism) analysis using restriction endonucleases: *AluI*, *HhaI*, *HpaI*, *HpaII*, *HinfI*, *KpnI*, *MseI*, *RsaI*, *Sau3AI*, and *TagI* (MBI Fermentas, Vilnius, Lithuania) and electrophoresis through 5% acrylamide gel. DNA bands were stained with etidium bromide and visualized using a UV transilluminator.

RESULTS AND DISCUSSION

In nested PCR, 16S rDNA was amplified from several symptomatic hyacinth plants, confirming that they were infected by phytoplasma (data not shown). RFLP analysis of nested PCR product indicated that the phytoplasma infecting hyacinth belongs to 16SrI group. *AluI*, *MseI*, *KpnI*, *RsaI*, *HpaII*, *HhaI* and *Sau3AI* profiles were very similar to those of already known phytoplasmas placed into group 16SrI [2]. However, *HinfI* and *HpaI* patterns distinguished the study phytoplasma from the known phytoplasma strains. The DNA fragments in the *HinfI* pattern were approximately 0.8, 0.4, and 0.35 kbp in size (Fig. 2), which totalled a size larger than that expected for the product of PCR primed by F2n/R2. We interpreted this pattern as a composite of RFLP patterns derived from two sequence heterogeneous rRNA operons, *rrnA* and *rrnB*, in the genome of this new phytoplasma. The other RFLP pattern, *HpaI*, also consisted of DNA fragments of two heterogeneous rDNA sequences (Fig. 2). The ribosomal interoperon sequence heterogeneity is an already known phenomenon in the genome of phytoplasmas [6]. Since the analysis of 16S rDNA of phytoplasmas is still the main tool for classification of these unculturable organisms, ribosomal interoperon sequence heterogeneity may hinder the usual identification. According to the classification system ba-

sed on RFLP analysis of 16S rDNA, either a missing or a new restriction site in 16S rDNA indicates a new subgroup [2]. In case of sequence heterogeneity between two ribosomal operons in the same genome, we should classify phytoplasmas on the basis of a new RFLP pattern of 16S rDNA. RFLP analysis of 16S rDNA from the phytoplasma infecting hyacinth revealed two new (*HinfI* and *HpaI*) patterns. This result indicated a new phytoplasma strain which was named HyacVir .

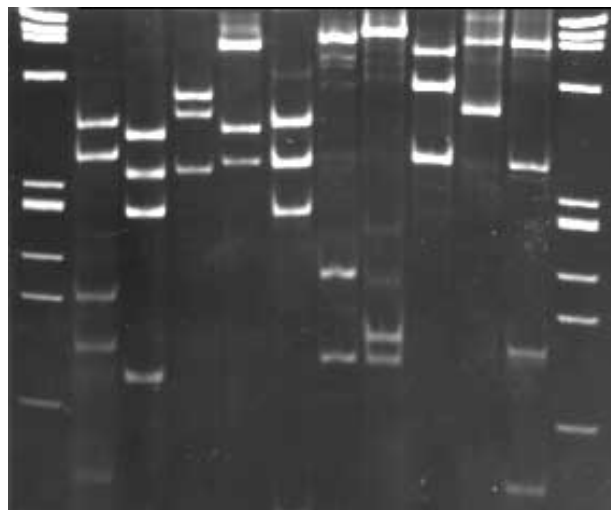


Fig. 2. RFLP analysis of phytoplasmal 16S rDNA amplified from infected hyacinth.

Lanes: 1. ϕ X 174 DNA HaeIII digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72; 2 - *AluI*; 3 - *MseI*; 4 - *KpnI*; 5 - *HinfI*; 6 - *RsaI*; 7 - *HpaII*; 8 - *HhaI*; 9 - *HpaI*; 10 - *Sau3AI*; 11 - *TagI*; 12 - ϕ X 174 DNA HaeIII digest, fragment sizes (bp) from top to bottom: 1353, 1078, 872, 603, 310, 281, 271, 234, 194, 118, 72

Most symptomatic herbaceous plants, also ornamental plants grown in Lithuania, were infected by phytoplasmas belonging to aster yellows (AY) group described by Marcone et al. [7]. This finding [8] indicates that insect vectors spread in Vilnius region carry phytoplasmas of 16SrI group. The phytoplasma strain HyacVir detected in hyacinth is a unique phytoplasma strain found only in Lithuania. Molecular characterization of phytoplasmas in the region of Baltic states is an important contribution to the information about the diversity, spread, hosts, and ecological niches of these still poorly learned plant pathogenic bacteria.

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**HYACINTHUS ORIENTALIS YRA NAUJOS
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S a n t r a u k a

Hiacintas (*Hyacinthus orientalis*), pasižymintis fitoplazminėmis ligoms būdingais simptomais (žiedyno filodija ir pažaliavimu), buvo aptiktas Vilniaus apylinkėse. Fitoplazminės 16S rDNA, amplifikuotos nuo simptomatinio augalo, analizės pagrindu aptikta fitoplazma buvo identifikuota ir klasifikuota. Rezultatai parodė, kad tai yra nauja, unikali, iki šiol nežinoma fitoplazma.