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Phytoplasma strains detected in ornamental plants in Lithuania

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Abstract

During recent years, increasing attention has been devoted to the development of field floriculture in Lithuania. Since the quality of ornamental plants is adversely affected by diseases attributed to phytoplasmas, we surveyed plant collections in botanical gardens and floriculture farms in Lithuania for phytoplasmal diseases. 37 ornamental species belonging to 18 plant families exhibiting disease symptoms including general yellowing and stunting, proliferation of shoots, phyllody, virescence and reduced size of flowers, and reddening of leaves were collected in commercial floriculture farms and botanical gardens. Analysis of phytoplasmal 16S rRNA gene sequences amplified in the polymerase chain reaction revealed that the plants were infected by phytoplasmas belonging to five distinct subgroups (16SrI-A, 16SrI-B, 16SrI-C, 16SrI-L, and 16SrI-M) of group 16SrI (aster yellows phytoplasma group) and two subgroups (16SrIII-B, 16SrIII-F) of group 16SrIII (X-disease phytoplasma group).

Key words: 16S rDNA, PCR, RFLP, phytoplasma, ornamental plants.

Introduction

During recent years, increasing attention has been devoted to the development of field floriculture in Lithuania, particularly for benefit of small farming businesses that produce seedlings of perennial ornamental plants for the domestic market and for export to neighboring countries. Like other segments of agricultural economies, this industry is threatened by plant diseases, including those attributed to infection by phytoplasmas. Diseases attributed to phytoplasmas have been reported in plant species belonging to more than 90 families worldwide. Based on analysis of 16S rRNA gene sequences, phytoplasmas have been classified into 18 major RFLP groups and over 38 subgroups (Lee *et al*., 1998; Marcone *et al*., 2000). Thus far, phytoplasmas belonging to ten major 16S rRNA gene groups (groups 16SrI – 16SrIII, 16SrV, 16SrVII, 16SrIX – 16SrXII and 16SrXIV) have been reported in Europe (Bertaccini, 2007). Phytoplasmas belonging to eleven subgroups of groups 16SrI, 16SrIII, and 16SrV have been found in monocotyedonous, and dicotyledonous plants in Lithuania (Jomantiene *et al*., 2002; Valiūnas, 2003). This paper summarizes results from the survey of phytoplasmal diseases of ornamental plant species in Lithuania.

Materials and methods

Plant samples, PCR conditions, and PCR primers

Samples of leaf tissue were collected from naturally infected ornamental plants during periodic inspections of plant collections at commercial floriculture farms and botanical gardens in Lithuania. Nucleic acid for use as template in the polymerase chain reaction (PCR) was extracted from tissues using a Genomic DNA Purification Kit (MBI Fermentas, Vilnius, Lithuania) according to manufacturer's instructions.

Ribosomal (r) DNA was amplified in nested PCRs using phytoplasma-specific primer pairs P1/P7 and R16F2n/R16R2 (Gundersen and Lee, 1996) as described by Jomantiene *et al*. (1998).

RFLP analyses of amplified phytoplasma DNA

Products from the nested PCR primed by R16F2n/R16R2 were analysed by single enzyme digestion with restriction endonucleases: *Alu*I, *Mse*I, *Kpn*I, *Hha*I, *Hae*III, *Hpa*II, *Rsa*I, *Hinf*I, *Sau*3AI, and *Taq*I, according to manufacturer's instructions (MBI Fermentas). RFLP profiles were analysed by electrophoresis of digested DNA through 5% polyacrylamide gel, staining with ethidium bromide, and visualisation using an UV transilluminator.

Results

Detection of phytoplasmas

Symptoms of general yellowing and stunting of plants, proliferation of shoots, phyllody, virescence and reduced size of flowers, and reddening of leaves were observed in diverse ornamental plants in botanical gardens and various floriculture farms in Lithuania. Nested PCRs primed by primer pair R16F2n/R16R2 yielded phytoplasma characteristic 1.2 kbp 16S rDNA PCR products from templates derived from 37 species belonging to 18 botanical families of naturally infected plants, indicating that the plants were infected by phytoplasma.

Comparison of RFLP patterns of amplified 16S rDNAs with those previously published for 16S rDNA of other phytoplasmas (Lee *et al*., 1998; Marcone *et al*., 2000) revealed that phytoplasmas detected in the ornamental plant species belonged to group 16SrI (aster yellows phytoplasma group) represented by five distinct subgroups (16SrI-A, 16SrI-B, 16SrI-C, 16SrI-L, 16SrI-M) and 16SrIII (X-disease phytoplasma group) represented by two subgroups (16SrIII-B and 16SrIII-F) (table 1).

Table 1. Phytoplasma subgroups detected in ornamental plants in Lithuania.

Phytoplasma subgroup	Host plant family	Species
16SrI-A	Asteraceae	*Bellis perennis*
		Gaillardia pulchella
		Rudbeckia hirta
		Helenium autumnale
	Buxaceae	*Pachysandra terminalis*
	Fumariaceae	*Dicentra formosa*
	Hyacinthaceae	*Hyacinthus orientalis*
	Iridaceae	*Gladiolus* sp.
	Plumbaginaceae	*Limonium sinuatum*
	Ranunculaceae	*Aconitum napellus*
		Consolida ajacis
		Helleborus lividus
		Trollius chinensis
	Rosaceae	*Geum coccineum*
16SrI-B	Caryophyllaceae	*Lychnis chalcedonica*
		Silene orientalis
	Crassulaceae	*Sedum spectabile*
	Ranunculaceae	*Delphinium cultorum*
	Solanaceae	*Schizanthus pinnatus*
16SrI-C	Asteraceae	*Gaillardia aristata*
16SrI-L	Asteraceae	*Grosheimia macrocephala*
	Brassicaceae	*Lunaria annua*
	Hyacinthaceae	*Hyacinthus orientalis*
	Plumbaginaceae	*Armeria alliaceae*
	Ranunculaceae	*Aconitum napellus*
		Aquilegia vulgaris
		Thalictrum sp.
	Scrophulariaceae	*Veronica teucrium*
16SrI-M	Amaranthaceae	*Celosia argentea*
	Apiaceae	*Eryngium alpinum*
	Asteraceae	*Callistephus chinensis*
		Helenium hybridum
	Caryophyllaceae	*Lychnis chalcedonica*
	Hyacinthaceae	*Muscari armeniacum*
	Liliaceae	*Tulipa* sp.
	Plumbaginaceae	*Limonium sinuatum*
	Palemoniaceae	*Phlox drummondii*
	Ranunculaceae	*Aquilegia vulgaris*
		Nigella damascena
	Scrophulariaceae	*Verbascum densiflorum*
	Solanaceae	*Schizanthus pinnatus*
16SrIII-B	Asteraceae	*Gaillardia* sp.
16SrIII-F	Rutaceae	*Dictamnus albus*

Discussion

Results presented in this study indicate that ornamental plants in Lithuania are predominantly infected by phytoplasma strains of group 16SrI. The prevalent subgroups (16SrI-A, -B, -L, and -M) have a wide host range and may also occur in the same host. Subgroup I-A phytoplasmas once appeared to be rare in Europe and prevalent in North America. Later, phytoplasmas of subgroup I-A were reported in Belgium, Germany, Italy, and other European countries (Marcone *et al*., 2000). In Lithuania, phytoplasmas of this subgroup were detected in a wide range of host plants (Valiūnas, 2003). Among the group 16SrI phytoplasmas in Europe, the strains of subgroup I-B have the widest host range, including ornamentals (Marcone *et al*., 2000, Lee *et al*., 2004). After intensive screening of plants for phytoplasma infection in recent years, strains belonging to subgroups I-L and I-M seem to be more frequently detected and widespread in Lithuania than strains belonging to subgroup I-B. The results also indicated that strains in subgroups I-L and I-M are closely related to strains in subgroup I-B.

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