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GENETIC DIFFERENCES BETWEEN ISOLATES OF *PHYTOPHTHORA CITRICOLA* OBTAINED FROM ORNAMENTAL NURSERIES IN POLAND

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Abstract

Genetic study was carried out among 76 isolates of *Phytophthora citricola* obtained from trees and shrubs belonging to 12 different plant genera grown in more than 10 nurseries. DNA was extracted from pure pathogen cultures and amplified by the PCR technique using ISSR and RAPD primers. The isolates clustered in two main groups. In one of them two subgroups were present. One of those subgroups contained isolates mostly from the family *Ericaceae* and the second mostly from coniferous plants. Close similarity between isolates from the same nursery was not a rule.

Key words: *Phytophthora citricola*, genetic similarity, RAPD-PCR, ISSR-PCR

Introduction

Phytophthora citricola Sawada is a multi-host, soil-borne pathogen posing an increasing threat to trees and shrubs (Erwin and Ribeiro 1996). Its harmfulness to horticultural and forest nurseries as well as to the natural environment has increased together with the general introduction of industrial technology to nursery production and with the growth of an unrestricted cross-border market. This pathogen has been reported as one of the most often isolated pathogens in Polish ornamental nurseries (Orlikowski and Szkuta 2002) and is known to be genetically highly diverse (Brasier and Hansen 1992). It has been proposed that the reason for its high diversity could be dispersion in ancient times, isolation of biological species and hybridization between this and other *Phytophthora* species. We would like to know if there is specialization for pathogenesis of different hosts. As the first step, we have been analysing genetic similarity on the basis of DNA polymorphism.

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Material and methods

76 isolates of *P. citricola* were obtained from symptomatic plants (listed in Table 1) in the time frame 1995–2004. Systematic classification was made on the basis of PCR amplifications with specific primers CITR1 and CITR2 (Schubert et al. 1999). DNA was extracted from pure cultures using the method of Aljanabi and Martinez (1997), modified by Wiejacha et al. (2002). Eight arbitrary primers, which produced rich profiles containing polymorphic and reproducible bands, were chosen for analyses (Table 2). Amplifications were performed by Gene*Amp* PCR System 9700 (PE Applied Biosystems) in 13 μl volumes. The thermal profile for RAPD amplifications was performed in 45 cycles: 94°C for 15 s, 36°C for 30 s, 72°C for 74 s. The thermal profile for ISSR was performed in 43 cycles: 95°C for 30 s, 55°C for 30 s, 72°C for 90 s. Reaction mixtures were those given by Wiejacha et al. (2002). The PCR products were electrophoresed in a 1.4% agarose gel at 4 V/cm of gel and were stained with ethidium bromide. Each of the PCR reactions was carried out at least twice. Only the bands with sizes from 300 to 2300 bp were analyzed. For a statistical analysis, the bands were coded in the binary form, 1 for the presence or 0 for the absence, for each isolate and primer. To determine genetic similarities (GS), after a visual examination for the bands' presence/absence, the PHYLIP 3.61 Package (Felsenstein 2004) was used. GS was analyzed by the distance-based method introduced by Nei and Li (1979). The distance matrices produced by PHYLIP RESTDIST were used as input in the PHYLIP NEIGHBOR program to construct UPGMA-based phylogenetic trees. The UPGMA option constructs a tree

Table 1

Isolates of *Phytophthora citricola* obtained from nurseries (numbers 71 and 76 are referential isolates)

No. of isolate	Host plants	
	family	species
71	*Taxaceae*	*Taxus baccata* L.
76	*Rutaceae*	*Citrus sinensis* (L.) Osbeck
41, 51, 57, 58, 63, 97–99, 102, 113, 156	*Cupressaceae*	*Chamaecyparis lawsoniana* (Murr.) Parl
42, 44, 48, 50		*Thuja occidentalis* L.
46		*Thuja plicata* Donn ex D. Don
43, 45, 47, 52–55, 64, 83–89, 91, 93–96, 110, 117, 123–129, 131, 132, 141–143, 147, 148, 169, 175, 181–184, 209–212, 215, 216	*Ericaceae*	*Rhododendron* sp.
225		*Azalea* sp.
135, 150, 165		*Vaccinium vitis-idaea* L.
49, 59	*Pinaceae*	*Abies concolor* Gord. & Glend.
60		*Picea omorica* Pancic.
109	*Fagaceae*	*Fagus sylvatic*a L.
56	*Oleaceae*	*Fraxinus* sp.

Table 2

Nucleotide sequences of primers, the number of scorable bands and the number and percentage of polymorphic bands obtained in PCR reactions with templates of *Phytophthora citricola*

Primers		No. of scorable bands	Polymorphic bands	
code	nucleotide sequence 5' to 3'		No.	%
C92	AGG CAC CCT TCG	10	10	100
AL4	DBD $GA_{(7)}$	6	6	100
AL6	$AC_{(8)}$ G	8	7	87
AL8	DBD $AC_{(7)}$	7	5	71
808	$AG_{(8)}$ C	7	6	85
842	$GA_{(7)}$ G	5	5	100
889	DBD $AC_{(7)}$	8	6	75
890	VHV $GT_{(7)}$	13	11	85
Total		64	56	
Average		8	7	88

by successive (agglomerative) clustering using an average-linkage method of clustering. The tree was drawn using the program TREEVIEW (Page 1996).

Results

The number of reproducible bands analyzed was 3206. Separate primers produced from 5 to 13 bands (Table 2). The percentage of polymorphic bands constituted from 71 to 100% (Table 2).

The isolates were distributed within two clusters (Fig. 1). The majority of isolates were in cluster I. This cluster was divided into two sub-clusters, A and B. Sub-cluster A contained about 80% of the isolates obtained from plants belonging to the family *Ericaceae* and only single isolates from *Fraxinus*, *Fagus* and *Abies*, as well as reference isolates from *Taxus* and *Citrus*, although they were mostly different from the rest. Sub-cluster B included isolates obtained mostly from coniferous plants – *Chamaecyparis*, *Thuja*, *Abies* and *Picea*. Isolates from ericaceous plants represented only 32%. In cluster II, which was significantly different in the level of genetic similarity, there were six isolates from *Rhododendron* sp. and *C. lawsoniana*.

Discussion

Our previous results obtained from a smaller representation (24) of *P. citricola* isolates (Wiejacha et al. in press) and those presented in this paper confirm the high genetic diversity within this species, which was reported earlier on the basis

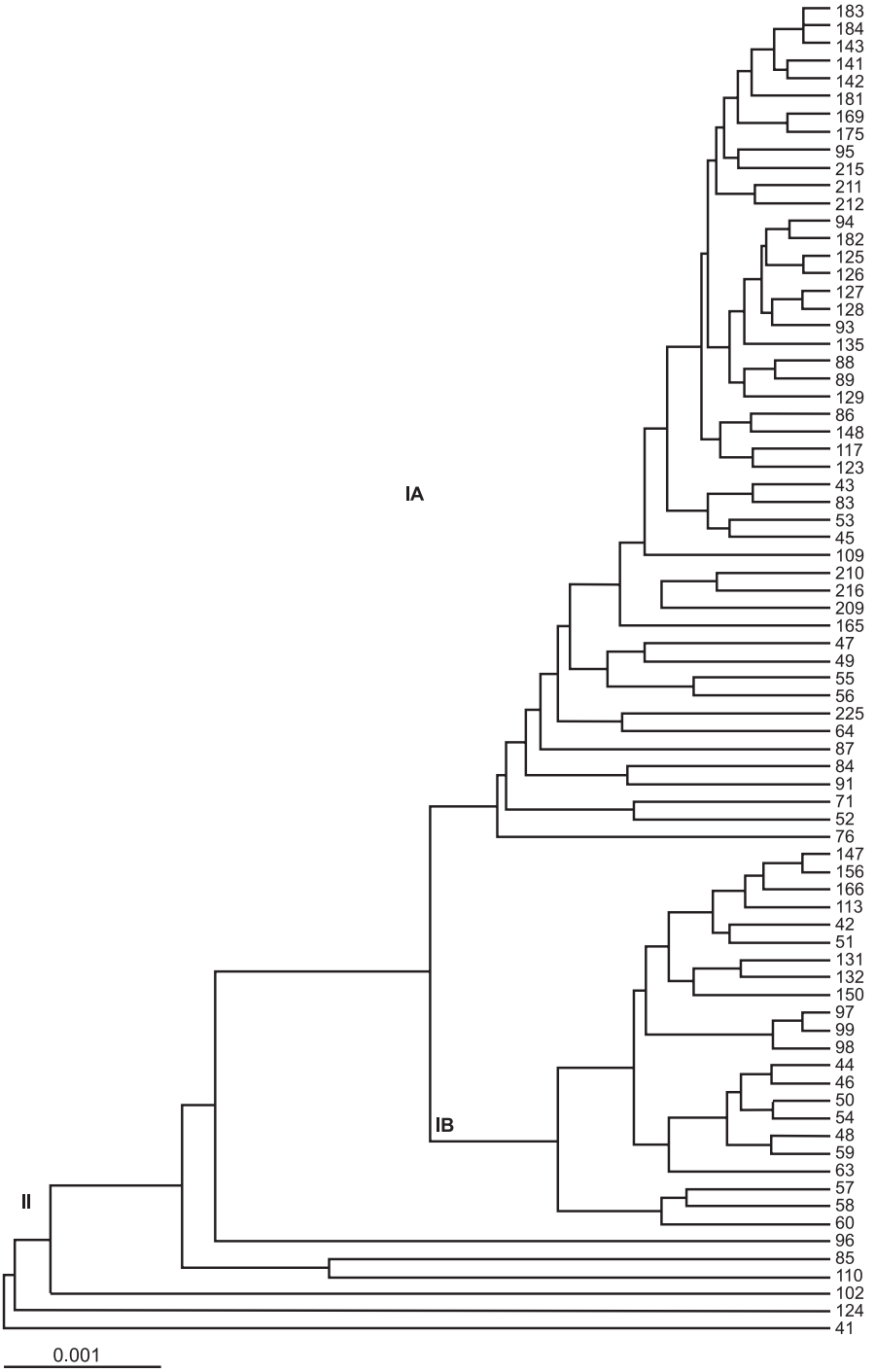

Fig. 1. Phylogram of genetic relationships between 75 isolates of *Phytophthora citricola*. Numbers represent isolates listed in Table 1

of isoenzymes (Oudemans and Coffey 1988) and mtDNA polymorphism (Förster et al. 1990, Förster and Coffey 1991). Depending on the host plant and climatic zone, similarity levels were between 0.32–0.96 (Förster and Coffey 1991). Oudemans and Coffey (1988) found four groups in their isolate representation, referring to geographical regions. One of the groups was connected with avocado from California, while another referred to tree crops from California and citrus crops from Australia. All isolates analyzed here were obtained from hosts of our climatic zone, to which the pathogen has adapted very well, although some geographical specificity can not be excluded, as at least a part was imported with plant material from different European countries. Such a high DNA polymorphism suggests a possibility of specification in pathogenesis against main groups of nursery plants, for example ericaceous and coniferous. This knowledge is important in rational nursery organization. We plan to study a level of virulence of isolates from different clusters and groups of plants with the goal to know more about interaction between hosts listed here and the pathogen.

Literature

Aljanabi S.M., Martinez I., 1997: Universal and rapid salt-extraction of high quality genomic DNA for PCR-based techniques. Nucl. Acids Res. 25: 4692–4693.

Brasier C.M., Hansen E.M., 1992: Evolutionary biology of *Phytophthora*. Part II. Phylogeny, speciation and population structure. Annu. Rev. Phytopathol. 30: 173–200.

Erwin D.C., Ribeiro O.K., 1996: *Phytophthora* diseases worldwide. APS Press, St. Paul, Minnesota.

Felsenstein J., 2004: PHYLIP (Phylogeny Inference Package) version 3.6. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle.

Förster H., Coffey M.D., 1991: Approaches to the taxonomy of *Phytophthora* using polymorphisms in mitochondrial and nuclear DNA. In: *Phytophthora*: Symposium of British Mycological Society, British Society for Plant Pathology and the Society of Irish Plant Pathologists Held. Eds J.A. Lucas, R.C. Shattock, D.S. Shaw, L.R. Cooke. Cambridge University Press, Cambridge: 164–183.

Förster H., Oudemans P., Coffey M.D., 1990: Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. Exp. Mycol. 14: 18–31.

Nei M., Li W.H., 1979: Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA 74: 5267–5273.

Orlikowski L.B., Szkuta G., 2002: Fytoftorozy w szkółkach roślin ozdobnych w Polsce. Pr. Inst. Bad. Leśn. Ser. A 2: 134–144.

Oudemans P., Coffey M.D., 1988: *Phytophthora citricola* – advances in our understanding of the disease. In: Summary of avocado research. Avocado Research Advisory Committee, University of California, Riverside: 16–24.

Page R.D.M., 1996: TREEVIEW: an application to display phylogenetic trees on personal computers. Comput. Applic. Biosci. 12: 357–358.

Schubert R., Bahnweg G., Nechwatal J., Jung T., Cooke D.E.L., Duncan J.M., Müller-Starck G., Langebartels C., Sandermann H. Jr., Oßwald W., 1999: Detection and quantification of *Phytophthora* species which are associated with root-rot diseases in European deciduous forests by species-specific polymerase chain reaction. Eur. J. For. Pathol. 29: 169–187.

Wiejacha K., Szkuta G., Orlikowska T., 2002: Optimization of DNA isolation procedure as the first step in identification of *Phytophthora* spp. Bull. Pol. Acad. Sci. Biol. Sci. 50: 165–171.

Wiejacha K., Trzewik A., Orlikowski L.B., Szkuta G., Orlikowska T., in press: Genotypic differences of isolates of *Phytophthora cinnamomi* and *P. citricola* obtained from ten nursery plant species. J. Plant Prot.

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