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Detection and identification of aster yellows and stolbur phytoplasmas in various crops in Spain

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Abstract

In this study '*Candidatus* Phytoplasma asteris' (subgroups 16SrI-B) and stolbur phytoplasma (subgroup 16SrXII-A) were sporadically identified in several horticultural crops in Spain by nested-PCR and RFLP analyses. One parsnip sample was infected with stolbur, and '*Ca.* P. asteris' was detected in lettuce and chicory plants. However, these two phytoplasmas were able to infect also carrot, celery and radish. This work extends the knowledge of phytoplasma diversity affecting horticultural crops in Spain.

Key words: phytoplasma, nested-PCR, horticultural crops, RFLP, 16S rDNA.

Introduction

Phytoplasmas are a group of pathogenic phloem-restricted plant wall-less prokaryotes (class Mollicutes) naturally transmitted by phloem-sap feeding insects specifically leafhoppers, planthoppers and psyllids (Lee *et al.,* 2000). Plants infected by phytoplasmas exhibit symptoms that suggest profound disturbances in the normal balance of plant hormones or growth regulators (Lee *et al.,* 2000; Bertaccini and Duduk, 2009). The development of polymerase chain reaction (PCR) techniques with universal primers pairs for general detection of various phytoplasmas and restriction fragment length polymorphism (RFLP) analysis of the 16S rDNA fragment enables phytoplasma detection, differentiation and classification (Lee *et al.,* 1993; Lee *et al.,* 1998; Marzachì, 2004). To date, 19 RFLP groups and more than 40 sub-groups have been identified based on 16S rDNA sequences (Bertaccini and Duduk, 2009). The main objective of the present work was to determine phytoplasmas presence in some affected horticultural Spanish crops which showed symptoms referable to phytoplasma presence, and identify the specific phytoplasmas groups and strains present in those samples.

Materials and methods

Samples of different horticultural crops with phytoplasmas-like symptoms were collected in different years (table 1). Healthy samples of each plant species and positive samples to stolbur and '*Ca.* P. asteris' were also included in the assay as negative and positive controls, respectively. Total DNA was extracted as described Green and Thompson, (1999). A nested-PCR assay was performed using the universal phytoplasma primers P1/P7 (Deng and Hiruki, 1991; Schneider *et al*., 1995) in the first amplification followed by R16F2n/R16R2 (Gundersen and Lee, 1996) in the second amplification to detect phytoplasmas in the affected plants. The PCR products were analysed on 1.2% agarose in TAE buffer gels, stained in ethidium bromide and visualized with a UV transilluminator. Restriction fragment length polymorphism (RFLP) analysis of the nested-PCR products (1.2 kb 16S rDNA fragments) was used for identification of the phytoplasmas detected (Lee *et al.,* 1998) with *Mse*I and *Hha*I endonucleases (MBI Fermentas, Vilnius, Lithuania).

Results and discussion

Fragments of the expected size (1.2 kb) were only amplified from the symptomatic samples and positive controls, but were not produced from healthy samples or water used as negative controls. The RFLP profiles when compared with control phytoplasma profile and with profiles of other phytoplasma 16S rRNA groups described by Lee *et al.* (1998) indicated that the phytoplasmas present in the different plant species belong to the 16SrI-B ('*Ca.* P. asteris') and 16SXII-A (stolbur) subgroups (table 1). In some horticultural crops only one phytoplasma was detected, as for example in lettuce where just '*Ca.* P. asteris' was identified. However, in carrot, celery or radish both phytoplasmas were found. Phytoplasmas have been described in all the analyzed hosts in previous works worldwide. In some cases the phytoplasmas reported were the same as in this study such as in lettuce (Zhang *et al.,* 2004). However in other crops the phytoplasmas detected were different, as for example, celery which was reported to be infected by tomato big bud phytoplasma (subgroups 16SrI-A) in Australia (Tran-Nguyen *et al.,* 2003) or stolbur (subgroups 16SrXII-A) in Czech Republic (Navratil *et al.,* 2009) and parsnip which was infected by aster yellows phytoplasma (subgroups 16SrI) in Canada (Kadhair and Evans, 2000). To our knowledge, this work represents the first report of phytoplasmas detected in chicory, radish, parsnip and celery in Spain.

Table 1. Samples of different horticultural crops collected in different regions of Spain showing phytoplasma-like symptoms and characterization of the phytoplasmas by RFLP analyses with endonucleases *Mse*I and *Hha*I.

Host	Sample code	Year of Collection	Region	Phytoplasmas group	Phytoplasmas subgroup
Carrot	Car-97	1997	Canarias	Stolbur	16SrXII-A
	Car-05	2005	La Rioja	Stolbur	16SrXII-A
	Car-09-1	2009	Alicante	'*Ca.* P. asteris'	16SrI-B
	Car-09-2	2009	Alicante	'*Ca.* P. asteris'	16SrI-B
	Car-10-1	2010	Albacete	'*Ca.* P. asteris'	16SrI-B
	Car-10-2	2010	Alicante	'*Ca.* P. asteris'	16SrI-B
	Car-10-3	2010	Valladolid	'*Ca.* P. asteris'	16SrI-B
Celery	Cel-07	2007	Alicante	Stolbur	16SrXII-A
	Cel-08	2008	Alicante	'*Ca.* P. asteris'	16SrI-B
	Cel-09	2009	Alicante	'*Ca.* P. asteris'	16SrI-B
	Cel-10-1	2010	Alicante	'*Ca.* P. asteris'	16SrI-B
	Cel-10-2	2010	Alicante	'*Ca.* P. asteris'	16SrI-B
Radish	Rad-10-1	2010	Valencia	'*Ca.* P. asteris'	16SrI-B
	Rad-10-2	2010	Valencia	Stolbur	16SrXII-A
Lettuce	Let-08-1	2008	Castellón	'*Ca.* P. asteris'	16SrI-B
	Let-08-2	2008	Castellón	'*Ca.* P. asteris'	16SrI-B
	Let-09	2010	Castellón	'*Ca.* P. asteris'	16SrI-B
Parsnip	Par-09	2009	Alicante	Stolbur	16SrXII-A
Chicory	Chi-99	1999	Murcia	'*Ca.* P. asteris'	16SrI-B

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References

BERTACCINI A., DUDUK B., 2009.- Phytoplasma and phytoplasma diseases: a new review of recent research.- *Phytopathologia Mediterranea*, 48: 355-378.

DENG S., HIRUKI C., 1991.- Genetic relatedness between two non-culturable micoplasma-like organisms revealed by nucleic acid hybridization and polymerase chain reaction.- *Phytopathology*, 81: 1475-1479.

GREEN M. J., THOMPSON D. A., 1999.- Easy and efficient DNA extraction from woody plants for the detection of phytoplamas by polymerase chain reaction.- *Plant Disease*, 83:482-485.

GUNDERSEN D. E., LEE I-M., SCHAFF D. A., HARRISON N.A., CHANG C. J., DAVIS R. E., KINSBURY D. T., 1996.- Genomic diversity among phytoplasma strains in 16S rRNA Group I (aster yellows and related phytoplasmas) and III (X-disease and related phytoplasmas).- *International Journal of Systematic Bacteriology*, 46: 64-75.

KADHAIR A. H., EVANS I. R., 2000.- Molecular and microscopical detection of aster yellows phytoplasma associated with infected parsnip.- *Microbiological Research,* 155: 53-57.

LEE I-M., HAMMOND R. W., DAVIS R. E., GUNDERSEN D. E., 1993.- Universal amplification analysis of pathogen 16S rDNA for classification and identification of mycoplasma-like organisms.- *Phytopathology*, 83: 834-842.

LEE I-M., GUNDERSEN-RINDAL D. E., DAVIS R. E., BARTOSZYK I. M., 1998.- Revised classification scheme of phytoplasmas based on RFLP analyses of 16S rRNA and ribosomal protein genes sequences.- *International Journal of Systematic Bacteriology*, 48: 1153-1169.

LEE I-M., DAVIS R. E., GUNDERSEN-RINDAL D. E., 2000.- Phytoplasma: phytopathogenic mollicutes.- A*nnual Review of Microbiology*, 54: 221-255.

MARZACHÌ C., 2004.- Molecular diagnosis phytoplasmas.- *Phytopathologia Mediterranea*, 43: 228-231.

NAVRATIL M., VALOVA P., FIALOVA R., LAUTERER P., SAFAROVA D., STARY M. 2009.- The incidence of stolbur disease associated yield losses in vegetable crops in South Moravia (Czech Republic).- *Crop Protection,* 28: 898-904.

SCHNEIDER B., SEEMÜLLER E., SMART C. D., KIRKPATRICK B. C., 1995.- Phylogenetic classification of plant pathogenic mycoplasma-like organisms or phytoplasmas, pp. 369-380. In: *Molecular and Diagnostic Procedures in Mycoplasmology, Vol. I* (RAZIN S., TULLY, J. G., Eds).- Academic Press, San Diego, CA, USA.

TRAN-NGUYEN L. T. T., PERSLEY D. M., GIBBS K. S., 2003.- First report of phytoplasma diseases in capsicum, celery and parsnip in Queensland, Australia.- *Australasian Plant Pathology,* 32: 559-560.

ZHANG J., HOGENHOUT S. A., NAULT L. R., HOY C. W., MILLER S. A., 2004.- Molecular and symptom analyses of phytoplasma strains from lettuce reveal a diverse population.- *Phytopathology,* 94: 842-849.

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