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Verification of phytoplasma presence in certified fruit tree material in Emilia Romagna region

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Abstract

CAV (Centro Attività Vivaistiche) is one of the Italian centres for conservation and premultiplication of fruit tree material since 1984. In CAV the source material is maintained under insect-proof screen-houses and is periodically tested for virus and virus-like diseases. Total DNA was extracted from plant material and PCR assays were performed to verify the presence of phytoplasmas associated to economic important diseases affecting fruit tree plants. All the plants tested resulted phytoplasma free.

Key words: Phytoplasmas, certification, fruit trees, PCR.

Introduction

CAV is a professional group of nurserymen associations founded in order to get an improvement of nursery material and horticultural productions. The main goal of CAV is the genetic and phytosanitary certification of nursery productions. It was recognized as nuclear and propagation centre by the regional government of Emilia Romagna for its regional certification scheme in 1987 and as nuclear and propagation stock by the Italian Ministry of Agriculture for the National Certification Service in 1998.

The activity of CAV is essentially devoted to find nursery "source" material with the best genetic and sanitary quality and to conserve it in the nuclear and propagation stock centre located in Tebano in order to supply to the multiplication centres that join CAV basic material (cuttings, rootstocks, plants) certified according to national and European community rules.

For this purpose CAV manages a laboratory equipped for the detection of the pathogens (viruses, fungi, bacteria, phytoplasmas, viroids) causing the most dangerous infectious diseases of fruit plants, grapevine, strawberry and vegetables. The most economically important phytoplasma diseases affecting fruit trees are pear decline (PD), European stone fruit yellows (ESFY) and apple proliferation (AP), all belonging to group 16SrX (IRPCM, 2004). The 793 fruit tree accessions (nuclear and propagation stock) maintained under screen-houses in the Centre of CAV are periodically retested to verify their health status according to D.M. 14th April 1997. All the plants resulting infected by phytoplasmas are excluded from the certification program according to the Plant Protection Service rules.

Materials and methods

In the last four years (2004-2007) during spring, samples from 459 of the 793 fruit tree accessions (table 1) were collected and analysed for phytoplasma infection. Total DNA was extracted from 1g of mid-vein leaf tissues using DNAeasy Plant Mini Kit (Qiagen). One to 3 µl of the extracted DNA were used in direct PCR using 16S rRNA universal primer pair P1/P7 (Deng and Hiruki, 1991; Schneider *et al.*, 1995) followed by nested PCR using R16(X)F1/R1 (Lee *et al*., 1995) specific for phytoplasmas belonging to group 16SrX. Nested PCR assays were performed using as template 1 µl of P1/P7 product diluted 1: 30 in sterile water. Tubes with the reaction mixture devoid of DNA template and a phytoplasma infected periwinkle isolate (PD) were used respectively as negative and positive controls in all PCR assays. Twenty-five µl reaction mixture for PCR amplification contained 200 µM each dNTP, 0.4 µM each primer, 1.5 mM $MgCl_2$ and 2U GoTaq DNA polymerase (Promega). Thirty-five cycles were conducted in an automated thermocycler (Biorad, MyCycler) at the following conditions: 94 °C for 1 min, 55 °C for 2min and 72 °C for 3 min (extended to 10 min for final cycle). Five-ten µl of PCR products were analysed by electrophoresis on a 1% agarose gel followed by staining with ethidium bromide and visualization of the DNA bands with an UV transilluminator.

Results

In three years of survey, 820 fruit tree plants (192 pome fruits, 629 stone fruits) have been tested which were maintained in the screenhouse, which were always asymptomatic and which resulted healthy after biological indexing on indicator plants. After PCR using phytoplasma primer pairs amplifying a portion of the 16SrRNA gene, the specific band was obtained only from positive control samples both in direct (1,800 bp band) and in nested PCR (1,100 bp band). No amplification products were obtained from any of the collected samples and from negative PCR controls confirming the healthy status of the conserved plants.

Table 1. Results of phytoplasma detection by PCR on CAV's accession during the period 2004 -2007.

Fruit trees	2004			2005			2006			2007		
	N° tested accessions	N° tested plants	PCR results	N° tested accessions	N° tested plants	PCR results	N° tested accessions	N° tested plants	PCR results	N° tested accessions	N° tested plants	PCR results
Pome fruit Source Material	7	24	-	16	55	-	7	22	-	1	3	-
Stone fruit Source Material	40	68	-	29	83	-	45	52	-	26	95	-
Apple - Nuclear Stock	8	19	-	1	2	-	1	5	-	1	19	-
Pear - Nuclear Stock	1	1	-	33	42	-	0	0	-	0	0	-
Peach - Nuclear Stock	48	67	-	138	148	-	10	32	-	4	9	-
Apricot- Nuclear Stock	0	0	-	21	35	-	4	8	-	5	7	-
Plum - Nuclear Stock	3	4	-	2	2	-	1	3	-	0	0	-
Rootstocks - Nuclear Stock	6	11	-	1	4	-	0	0	-	0	0	-
Total tested plants	113	194		241	371		68	122		37	133	

Discussion

In the last 20 years nurserymen are showing a growing interest in genetic-sanitary certification programs to guarantee, qualify and make the propagation material more and more competitive in national and international markets. The periodical evaluation of the health status of the nuclear and propagation stock is one of the main tools to achieve this aim.

Phytoplasma detection in source material is really important to prevent the spreading of phytoplasma associated diseases, as one of the main ways of transmission of these pathogens is through the propagation material.

The source plants are tested before entering the certification program, and then they are maintained in insect-proof screen-houses to prevent the transmission by insect vectors. They are periodically retested to confirm the absence of latent infections detectable only after some years of incubation.

Molecular techniques, in particular PCR, have revolutionized the phytopathological diagnosis representing an important support to the traditional biological diagnostic methods (indexing) for their high reliability, sensitivity and low time consuming. The high costs of these tests make them non-applicable on a large scale. However, testing relatively few plants of the nuclear and propagation stocks with high sensitive tools can efficiently protect the subsequent propagated material and the Italian fruit-growing.

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