Results of patch-grafting of tissue infected by 'Candidatus *Phytoplasma pyri*' or by 'Candidatus *Phytoplasma prunorum*', respectively on pear and apricot plants cultivated in pot

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Abstract

Molecular analyses carried out either on the pear varieties 'Conference', 'Comice' and 'William' grafted on different rootstocks or on sixty-eight apricot varieties grafted on Myrobalan, showed the susceptibility of the tested combinations to 'Candidatus *Phytoplasma pyri*', transmitted by *Cacopsylla pyri*, and to 'Candidatus *Phytoplasma prunorum*', transmitted by *Empoasca decedens*, respectively. In order to find pear and/or apricot combinations immune to the associated Phytoplasma, several varieties grafted on new rootstock were tested in the period 2002-2008. 68 pear plants belonging to seven variety/rootstock combinations and 76 apricot plants belonging to seven combinations, all cultivated in pot, in greenhouse covered by anti-aphid tissue, were grafted with patches of infected tissues containing the specific phytoplasmas. Young healthy potted plants belonging to the pear combination 'Comice'/*P. communis* and to the apricot combination 'Palummella'/Myrobalan, both susceptible in open field to the associated phytoplasmas transmitted by the specific vectors, were also used and patch-grafted. Molecular analyses, carried out on nucleic acids extracted from leaf samples, to detect the presence of the pathogens, showed the pear variety 'William' grafted on *Pyrus betulaefolia* to be susceptible to 'Candidatus *Phytoplasma pyri*'. Neither the pear combination 'Comice'/*P. communis* nor the apricot 'Palummella'/Myrobalan 29 C, susceptible, in open field, to the associated phytoplasmas, became infected after patch-grafting under greenhouse conditions. Thus the results show that patch-grafting cannot be utilized in young potted plants for artificial transmission of these two phytoplasmas.

Keywords: Phytoplasmas, source of immunity, variety/rootstock combination, molecular tests, insect proof green-house

Introduction

Previous researches showed that pear varieties 'Conference', 'Comice' and 'William', grafted on different rootstocks, were susceptible to 'Candidatus *Phytoplasma pyri*' transmitted by Cacopsylla pyri (Pastore et al., 1998), and that sixty-eight apricot varieties showed different susceptibility to 'Candidatus *Phytoplasma prunorum*' (Pastore et al., 1995). In order to find a source of immunity to the two phytoplasmas we tested seven new combinations varieties/rootstock of pear and seven new combinations varieties/rootstock of apricot that were patch-grafted by infected tissues containing the specific phytoplasmas were tested. The pear combination 'William'/*Pyrus betulaefolia* and apricot combination 'Palummella'/Myrobalan both susceptible to the specific phytoplasma in open field were also tested.

Materials and methods

Variety/rootstock combinations: The list of pear combinations is reported in Table 1, while the one of apricot combinations is showed in Table 2.

Time schedule and schemes of grafting: The first patch-grafting trial was carried out in Locorotondo (Bari) from July to September 2002 on two pear and six apricot variety/rootstock combinations on young plants cultivated in pots and maintained in a screen-house covered by anti-aphid tissue. The experimental scheme consisted on five plants without graft-inoculation as control, and ten plants for each combination, grafted with patches of shoots derived from infected plants tested by PCR/RFLP analyses.

In the year 2007 the plants were transferred to Caserta, in another greenhouse, covered by aphid-proof tissue, where six pear variety/rootstock combinations and two apricot variety/rootstock combinations were added to the experiment. From June to October 2007 the patch-grafting was carried out on the majority of the plants already grafted in Locorotondo, and on eight out of ten plants belonging to the new combinations (see Table 1 and Table 2).

Tab. 1 Results of patch-grafting of tissues infected by '*Ca.* P. pyri' on pear variety/rootstock combinations.

Variety/rootstock	Grafted July and September 2002	Plants resulted infected 05/03	09/03	10/05	Grafted in June, July and September 2007	Plants resulted infected 09-10/07	07-09/08
Conference/*P. communis*	10	0/10	0/10	0/10	5	Not tested	0/5
Comice/*P. communis* (positive in field 1995)	10	0/10	0/10	0/10	8	Not tested	0/8
Comice/*P. betulaefolia*	-				8	0/8	0/8
Comice/Quince BA 29	-				8	0/8	0/8
William/*P. betulaefolia*	-				8	1/8	1/8
Conference/*P. betulaefolia*	-				8	0/8	0/8
William/Quince BA 29	-				8	0/8	0/8
Conference/Quince BA 29	-				8	0/8	0/8

- not grafted

Tab. 2 Results of patch-grafting of tissues infected by 'Ca. *P. prunorum*' on apricot variety/rootstock combinations.

Variety/Rootstock in pots	Grafted in July and September 2002	Plants resulted infected 05/03	09/03	10/05	Grafted in October 2007	Plants infected in September 08
Tyrinthos/ Myrobalan 29 C	10	0/10	0/10	0/10	5	0/5
Cafona/ Myrobalan 29 C	10	0/10	0/10	0/10	7	0/7
Monaco Bello/ Myrobalan	10	0/10	0/10	0/10	3	0/3
Monaco Bello/MRS 2/5	10	0/10	0/10	0/10	2	0/2
Palummella/Myrobalan 29 C	10	0/10	0/10	0/10	2	0/2
Palummella/ Myrobalan (positive in field 1995)	10	0/10	0/10	0/10	6	0/6
Sancastrese/ Myrobalan	-				8	0/8
Monaco Bello/ Myrobalan	-				8	0/8

-, not grafted

Source of plant tissue infected by phytoplasmas: The pear plant used as source of 'Ca. *P. pyri*' belongs to 'William' grafted on a selection of *P. communis*. It became infected in 1995 by *Cacopsylla pyri* and it is still positive in PCR/RFLP analyses.

The apricot shoots used as source of 'Ca. *P. prunorum'*, in 2002, belonged to 'Harogem', infected by patch-grafting of plum infected tissue (Pastore et al., 2001), while for tests of 2007, shoots were kindly provided by Department of 'Biologia applicata alla difesa delle piante' of the University of Udine.

Molecular tests for phytoplasma detection: To detect the phytoplasmas, molecular tests were carried out on leaf samples collected in May and September 2003, October 2005, from September to October 2007, and from July to September 2008.

Nucleic acids were extracted from leaf samples according to Bosco et al. (2002), while PCR experiments were carried out according to Schaff et al. (1992). Nested PCR reactions were performed under the same conditions using as template the amplicons of the previous reaction diluted 2: 30 with sterile distilled water. Ribosomal general primers P1/P7 (Deng and Hiruki, 1991; Schneider et al., 1995), for direct PCR amplification, primer pair R16F2/R2 (Lee *et al*., 1995), for the first nested PCR reaction, and primer pair R16(X)F1/R1 (Lee et al., 1995), for the second nested PCR amplification, were used. Alternatively primers f01/r01 (Lorenz et al., 1995), for nested PCR, were used after direct amplification with P1/P7 primers. Samples with the reaction mixture devoid of DNA templates were included in each experiment as negative controls. Six µl of each PCR product were subjected to electrophoresis in a 1% agarose gel and visualized by staining with ethidium bromide and UV illumination. Three µl of PCR products amplified after first or second nested PCR reaction were digested using endonucleases *Ssp*I and *Rsa*I at 37°C for at least 16 hours following the instructions of the manufacturer (Fermentas, Vilnius, Lithuania). The restriction patterns were then compared with those of reference strains after electrophoresis through a 5% poliacrylamide gel in 1X TBE buffer followed by staining with ethidium bromide and visualization under an UV transilluminator.

Results and discussion

Only one plant belonging to the combination 'William'/*P. betulaefolia*, sampled after the second grafting, resulted positive in nested PCR reaction with primers f01/r01, but it did not show symptoms of the disease. RFLP analyses of PCR products with restriction enzymes *Ssp*I and *Rsa*I confirmed that phytoplasma infecting this pear belonged to 16SrRNA subgroup X-C, 'Ca. *P. pyri*'. All the other samples were negative independently either from the number of grafting or from the PCR detection system utilized, including the combinations 'Comice'/*P. communis* and

'Palummella'/Myrobalan 29 that in open field resulted susceptible to 'Ca. *P. pyri*' and 'Ca. *P. prunorum*' respectively (see Table 1 and Table 2).

Thus we conclude that the presented experimental approach, the patch-grafting, efficient for transmission of phytoplasma-microorganisms to apricot (*Prunus armeniaca* L.) and Japanese plum (*Prunus salicina* LINDL) trees cultivated in field (Pastore et al., 2001) is not efficient when used on young pear and apricot plants cultivated in pots.

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