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High tolerance of European plum varieties to plum leptonecrosis

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Abstract

A 13 year comparative study was carried out on the behaviour of four European and two Japanese plum varieties grown in adjacent rows in an area of northern Italy where plum leptonecrosis is epidemic. Within seven years, 100% of the Japanese plum trees became symptomatically infected. Nine years after planting, five trees of each of the European cvs, which were asymptomatic, were top-grafted with healthy buds of the cv Ozark Premier, which is an indicator for plum leptonecrosis. Based on the results of PCR analysis, DAPI staining and on the reaction of the top-grafted Ozark Premier indicators, 50% of the European plum trees, despite their healthy appearance, were shown to be infected with plum leptonecrosis. The detectable presence and graft transmissibility of the plum leptonecrosis phytoplasma in the asymptomatic European plum trees means that the European plum trees are not resistant to the infection but that they are tolerant. The active presence of a still unknown vector/s in the investigated area is stressed as well as the important role of *Prunus domestica* L. played in the conservation and spread of plum leptonecrosis.

Abbreviations: DAPI – 4′, 6-diamidino-2-phenyl indole; PCR – Polymerase Chain Reaction; PLN – plum leptonecrosis.

Introduction

Plum leptonecrosis was first described in the Emilia-Romagna region of Italy by Goidanich (1933) on Japanese plum (*Prunus salicina* Lindl). More recently the disease was reported to occur in many other fruit growing areas of Italy and it is becoming more economically important because of the increased usage of new cultivars of Japanese plums (Marcone et al., 1996). A phytoplasma is considered the causal agent of PLN disease (Giunchedi et al., 1978; Lee et al., 1995).

A similar disease has been reported to occur in various other species of stone fruit trees in France (Desvignes et al., 1982), Spain (Sanchez-Capucino et al., 1973), Greece (Syrgianidis et al., 1976), Romania (Ionica, 1985) and Germany (Lederer and Seemüller, 1992). In 1982 Giunchedi et al., demonstrated that the causal agent of PLN can also induce typical PLN-like symptoms in apricot, peach, almond and sweet cherry and proved the tolerance to PLN of one European plum tree cv Stanley. Recently, using molecular techniques, it has been demonstrated that a strict correlation exists among the phytoplasma diseases affecting Japanese plum, apricot, peach and flowering cherry (Ahrens et al., 1993; Marcone et al., 1996). In France the European plum (*Prunus domestica* L.) is regarded as tolerant or resistant to Apricot chlorotic leaf roll (Desvignes, 1982), a disease that is considered to have the same etiology as PLN.

Based on surveys carried out in several regions of northern Italy during the last ten years, healthy looking European plum trees were commonly observed to grow in the vicinity of obviously diseased Japanese plums. Among the European cvs we noticed several plants of the cv Susina di Dro with the typical symptoms of the disease. In subsequent work this variety was shown to contain phytoplasmas of the apple proliferation cluster (Poggi Pollini et al., 1995).

The precise aim of the present work was to carry out a comparative study of the behaviour of four European and two Japanese plum varieties grown in adjacent rows in an area of northeastern Italy where PLN is epidemic. Nine years after planting, from each European cv five trees were top-grafted with healthy buds of the cv Ozark Premier indicator. The reaction of the plum trees either simply exposed to natural infections or top-grafted is described.

Materials and methods

Natural spread of PLN and susceptibility to the phytoplasma agent of PLN of six plum varieties

In 1983 an experimental plum orchard was planted in Gemona, an area of the Friuli-Venezia Giulia region in northeastern Italy where PLN is epidemic and a high rate of infection is observed (Carraro et al., 1992). The four European cvs investigated were Ruth Gerstetter, Bluefree, President and Stanley. The compared Japanese plum cvs were Ozark Premier and Shiro. The common rootstock was Myrobalan B. Thirty virus-free trees were used for each plum cv. All 180 trees were regularly checked for symptom appearance up to 1996. The macroscopic symptoms considered in the observations were: early bud burst, development of leaves before flowering, small chlorotic rolled leaves, yellowing and/or reddening of the veins, deformed fruits, phloem necrosis and decline of the plants.

Top-grafting of European plum trees using Japanese plum buds as indicator

In August of 1992 five trees of each of the four European cvs were top-grafted (on opposing branches) with two healthy buds of the Japanese cv Ozark Premier. The grafted buds were derived from healthy Ozark Premier trees grown inside an insect-proof screenhouse. Both the European plum trees and the sprouts of the Ozark Premier indicator were periodically checked for symptom expression up to 1996. In 1995 the 20 top-grafted European plum trees and the same number of the top-grafted Ozark Premier indicators were tested for the presence of phytoplasmas by using DAPI staining (Seemüller, 1976). PCR was also used to test three European plum trees for each of the four cvs grafted with the Ozark Premier indicator: two of them bearing PLN symptomatic grafts and one asymptomatic.

Fluorescence microscopy observation

Specimens (3–6 $\times$ 10–15mm) of new buds were fixed in 3% glutaraldehyde (0.1 M phosphate buffer, pH 7) and stored at 4 °C for at least one day. After rinsing in buffer, they were cut longitudinally with a freezing microtome (Jung CM 1500). The 20 μ thick sections were treated with DAPI (1 μg/ml in 0.1 M phosphate buffer) and examined with a fluorescence microscope (Leitz Orthoplan with pleomopack 2.1). Five symptomatic trees of Ozark Premier and Shiro cvs were used as positive controls and the same number of healthy plants, grown in the screenhouse, as negative controls.

DNA amplification

DNA was isolated from approximately 1.5 g of leaf petiol and midrib tissues using a modification of the phytoplasma enrichment procedure developed by Kirkpatrick (Malisano et al., 1996). The presence of phytoplasmas was determined by polymerase chain reaction using the ribosomal primers AP3 and AP5. The PCR assay used is specific for a group of related phytoplasmas including plum leptonecrosis, apple proliferation and pear decline (Firrao et al., 1994).

Results

Natural spread of PLN and susceptibility to the phytoplasma of the different plum varieties

Among the six varieties of plum trees planted in the experimental orchard of Gemona, only the two Japanese cvs Ozark Premier and Shiro showed typical symptoms of PLN. In seven years, from 1985 to 1991, all the 30 trees of these cvs became infected. From 1986 to 1995, 77% of Ozark Premier trees died because a complete phloem necrosis. The Myrobalan B rootstock survived the death of the scion, since it generally showed small leaves but no bark necrosis. None of the 30 infected Shiro plants showed bark necrosis nor died. Among the 120 plants of the four European plum cvs observed, none developed visible symptoms attributable to PLN or to any other phytoplasma infections.

Table 1. Results of DAPI staining and PCR analyses (1) of both European plum trees (2) grown in a plum leptonecrosis-infested area and of top-grafted Ozark Premier indicators (3)

Cultivar	European plums			Top-grafted Japanese plum indicators		
	Plant n°	DAPI	PCR	Symptoms	DAPI	PCR
Ruth Gerstetter	1	+	+	+	+	+
	2	+	+	+	+	+
	3	-	-	-	-	-
	4	-	°	-	-	°
	5	-	°	-	-	°
Bluefree	1	+	+	+	+	+
	2	+	+	+	+	+
	3	-	-	-	-	-
	4	-	°	-	-	°
	5	-	°	-	-	°
President	1	+	+	+	+	+
	2	-	+	+	+	+
	3	-	-	-	-	-
	4	+	°	+	+	°
	5	-	°	-	-	°
Stanley	1	+	+	+	+	+
	2	-	+	+	+	+
	3	-	-	-	-	-
	4	+	°	+	+	°
	5	-	°	-	-	°

(1) + =positive reaction; - =negative reaction; ° =test not done.
(2) None of the European plum trees showed typical symptoms of the disease.
(3) Healthy scions of Ozark Premier plums were top-grafted to five trees of each of the four European plum varieties studied.

Top-grafting of Ozark Premier indicators on symptomless European plum trees

Ozark Premier indicator buds grafted satisfactorily onto branches of European plum trees; at least one bud survived on each European tree and developed good sprouts. In October 1993 seven of the twenty top-grafted European plum trees developed symptomatic grafts and three more were seen in the spring of 1994. In 1995 and 1996 no further symptomatic indicator plants were noticed. Altogether 10 out of the 20 top-grafted European plum trees were found to bear Ozark Premier scions showing obvious symptoms of PLN i.e. two trees of Ruth Gerstetter, two of Blue Free and three of both President and Stanley.

Fluorescence microscope observations

The DAPI test was positive on all the 10 symptomatic Ozark Premier scions top-grafted on the four European plum cvs (Table 1). Vice versa none of the asymptomatic Ozark Premier scions was positive to the test.

Eight of the ten European plum trees bearing symptomatic indicator grafts reacted positively to the DAPI staining (Table 1).

In the sections of the symptomatic Ozark Premier the phloem localised fluorescence was clear and typical of phytoplasma infection. It was generally weak in the samples of the four European varieties. Only in one single plum tree of the cv President the reaction was as strong as that seen in the Japanese varieties. The DAPI reaction was positive also in the Ozark Premier and Shiro symptomatic controls; it was negative in the top-grafted Ozark Premier asymptomatic scions and in the healthy controls.

DNA amplification

The results of PCR applied to all the different trees and graftings tested are reported in Table 1. There was a strong correlation between the symptom expression,

the PCR results and the DAPI staining of the European plum trees top-grafted with the Japanese scions. The correlation between DAPI staining and PCR tests for the European plum trees is not so strong however, with some trees showing positive by PCR testing but negative by DAPI staining.

Discussion and conclusion

The observations carried out during 13 years in the experimental field of Gemona confirmed the high susceptibility to PLN of the Japanese cvs Ozark Premier and Shiro. In fact 100% of the 60 plants of the two Japanese varieties became naturally infected within a seven year period. Of the two varieties, Ozark Premier was the more sensitive to PLN showing severe and progressive necroses and a high percentage of mortality. The Shiro cv, in spite of the clear PLN symptoms, survived and produced an almost normal yield.

On the other hand none of the 120 trees of the European cvs developed symptoms attributable to phytoplasma infection during the 13 years of surveys. In addition, the fruit production of these trees was regular.

Based on the typical PLN symptoms developed by 50% of the Ozark Premier scions top-grafted onto the symptomless European plum trees, we assume that the same percentage of European plums were infected by PLN in the Gemona experimental field. This was confirmed by DAPI and PCR tests performed on samples collected directly from the European plums. As a consequence we can also state that the four cvs of European plums are really tolerant to PLN (symptomless infectable cultivars) but not resistant. Comparing the three tests used in this experiment, we observed a strong correlation between symptom expression and the results of the tests performed.

Considering the epidemiology of PLN, the results achieved indicate the presence of active vectors in the study area and a high and stable rate of natural infections of the phytoplasma. In an experiment conducted in the past in northeastern Italy (Carraro et al., 1992) the possibility of transmitting PLN by grafting from *Prunus cerasifera* Ehrh. (Myrobalan) to *P. salicina* was noted. Consequently, and taking into account historical observations, it can be hypothesised that in the investigated area the early natural infections were passed from wild plums to commercial cvs of *P. domestica,* and later from this to *P. salicina*. This means that in plum tree cultivated areas, *P. domestica* can play an important role in the conservation and diffusion of PLN.

During the course of this work it has become evident that there is a range of different responses shown by the different species and cultivars studied to infection by the PLN phytoplasma. This range of responses can be summarised as follows:
P. salicina cv Ozark Premier (up to 100% of symptomatically infected trees with serious necrosis and high mortality); *P. armeniaca* (50% of the plants of different varieties of apricot exposed to natural infections were demonstrated by PCR, DAPI and observations of symptoms to became infected and decline, R. Osler, unpubl.); *P. salicina* cv Shiro (up to 100% of symptomatically infected trees, no bark necrosis or mortality but yield almost normal); *P. domestica* cv Susina di Dro (according to the results obtained by Poggi Pollini et al.(1995) up to 40% of the trees in an open area can be infected; among these 1-5% can be symptomatic with diffuse necrosis and progressive death of the trees); *P. cerasifera* Myrobalan (often symptomatically infected, no decline and no bark necrosis); *P. domestica* cvs President, Stanley, Ruth Gerstetter and Blue Free (highly infectable but asymptomatic).

In conclusion, the occurrence of healthy looking European plums growing adjacent to infected Japanese plum trees is now understood. In this work we have shown that the European plum trees are able to be infected with the PLN phytoplasma, but do not show any symptoms. They can therefore be described as highly tolerant to the PLN phytoplasma. The research is continuing with the main aims of identifying the PLN vectors, ascertaining wild and cultivated alternative hosts of PLN and clarifying the natural recovery often observed in declined trees of apricot.

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