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In Vitro Evaluation of Resistance of *Pyrus syriaca*, a Pear-tree Rootstock, to Phytophthora Crown Rot

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ABSTRACT

Phytophthora crown rot, caused by *Phytophthora cactorum*, is a damaging disease of apple, peach and plum in Tunisia. While causing heavy losses to fruit production, no or some effective disease control methods are available. In Tunisia, there are no approved fungicides to control *P. cactorum*. The use of tolerant or resistant rootstocks seems to be the most effective measure for controlling Phytophthora crown rot. *Pyrus syriaca*, a pear-tree rootstock, was tested for resistance to *P. cactorum*, demonstrating high resistance to this pathogen. Indeed, on excised twigs, the necrotic length ranged between 1.22 and 0.67 mm. However, this value varied between 12 and 15 mm for Myrandier 617, known to be sensitive to *P. cactorum*. In *in vitro* experiments, the percentage of necrotic plants varied between 1.8 and 1.9% for the rootstock *P. syriaca*. These values were almost equal to those obtained with GF 667. However the percentage of necrosis *in vitro* plants obtained with Myrandier 617 varied from 86.2 to 95.2%. These results suggest that *P. syriaca* seems to be a potential pear-tree rootstock that can be used in Tunisian orchards where *P. cactorum* causes problems.

Keywords: excised twigs, *in vitro* plants, *Phytophthora cactorum*

INTRODUCTION

Phytophthora crown rot is a serious soil-borne fungal disease of apple and pear trees in Tunisia. It is also a serious disease of peach, plum and apricot. Caused by *Phytophthora cactorum* (Leb. and Cohn) Schroet., this pathogen infests soils in many parts of the world (Jung *et al.* 2000; Jung and Blaschke 2004). Chemical control has been attempted in many areas, but its effectiveness is limited (Utkhede and Smith 1992; Thomodis and Elina 2001). Very little work has been done to develop biological control of Phytophthora crown rot (Janisiewicz and Covey 1983; Utkhede 1983, 1984a, 1984b; Utkhede and Gaunce 1984). Resistant rootstocks would be useful, but at present none is known to be completely resistant. Commercial rootstocks such as M4, M9 and M26 are only moderately resistant to *P. cactorum.* Breeding programs to develop resistant rootstocks and cultivars have not been successful (Elena and Tsipouridis 2000). Most of the efforts in this direction have resulted in the development of screening techniques to identify sources of resistance and to screen commercial rootstocks for resistance to crown rot.

Dormant twig assays have been used to examine differences in resistance caused by host-genotypes and time of year (Jeffers *et al.* 1981; Utkhede 1986; Scott *et al.* 1992; Thomidis *et al.* 2002).

To date, no efforts have been made to screen systematically the Tunisian rootstock collection to identify sources of resistance to *P. cactorum.* This paper describes our attempt to evaluate the resistance of *Pyrus syriaca*, a pear-tree rootstock located in the collection of rootstocks in the Agronomic High Institute of Chott Meriam in Tunisia.

MATERIALS AND METHODS

Isolates of *Phytophthora cactorum*

Two isolates of *P. cactorum* PC1 and PC2 were used in this study. These isolates were originally obtained from naturally infested trees in apple orchards in the region of Kasserine in the central part of Tunisia in 1998. The fungi were isolated on corn meal agar (CMA) amended with antibiotics (100 mg mycostatin, 50 mg polymyxin and 20 mg penicillin per litre of CMA). Isolates were maintained on CMA at 22°C in the culture collection of the Agronomic High Institute of Chott Meriam in Tunisia. Fresh cultures were prepared by transferring an agar disc with mycelium to plates containing CMA. Plates then were placed in an incubator at 23°C for 5 days.

The rootstock

Pyrus syriaca is a pear seedling rootstock that is located in the collection of rootstocks of the genus *Pyrus* in the Agronomic High Institute of Chott Meriam in Tunisia. It is characterised by its tolerance to dryness, to salinity and many diseases such as pear decline (Carraut 1986).

Excised twigs experiment

Excised twigs of the pear rootstocks were inoculated in the laboratory following the methods described by Jeffers *et al.* (1981) and Scott *et al.* (1992).

CMA amended with antibiotics (10 mg primaricin, 250 mg ampicillin and 10 mg rifampicin per litre) was added to Pyrex jars (10 cm diameter and 12 cm high) to give an agar depth of about 1 cm. Two agar plugs containing mycelium from one of the two isolates of *P. cactorum* were transferred to each jar. Jars then were sealed with Parafilm to avoid desiccation and placed in a dark incubator at 23°C until colony growth covered the agar surface. One-year-old shoots were excised from 10-year-old *P. syriaca* plants. Shoots were cut into 70 mm sections and surface disinfec-

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Research Note

ted in domestic chlorine bleach 10% (sodium hypochloride; 4.8%) for 5 min and then rinsed three times with sterile distilled water. A flamed scalpel was used to remove about 1 mm bark from the base of each segment to expose the cambium. Ten of these paired segments were placed upright in each jar of CMA. The jars were then resealed with Parafilm and returned to a dark incubator at 23°C. After 4 days, the twig segments were removed from the jars and stripped of their periderms with a sharp scalpel. The length of necrosis on each twig and the depth of agar in each jar were measured. By subtracting the depth of agar from the total length of necrosis, a value for net necrosis length was obtained.

In this essay shoots of the rootstock GF 677, resistant *to P. cactorum* (Elena and Tsipouridis 2000), and of the rootstock Myrandier 617, sensitive to the same pathogen (Elena and Tsipouridis 2000), treated similarly served as positive and negative control respectively.

All the experiments were conducted twice.

Five jars per elementary treatment were used and variance analysis of the treatment effect on measured data was performed by using the general linear model procedure of SPSS (SPSS 10.0). Experiments were analyzed using standard analysis of variance (ANOVA) with factorial treatment structure and interactions. When F values were significant at p>0.05, differences among the treatments were determined by S-N-K (Student-Newman-Keuls) test.

In vitro plant experiment

Shoot cultures of *P. syriaca* were initiated and propagated on modified Murashige and Skoog (1962) media supplemented with 30 $g.L^{-1}$ of sucrose, 1 mg $g.L^{-1}$ of benzyladenine (BA) and 8 $g.L^{-1}$ of Difco agar.

Micropropagated shoots of *P. syriaca* were inoculated *in vitro* using the method of Scott *et al.* (1992). The culture media CMA amended with the antibiotics (10 mg primaricin, 250 mg ampicillin and 10 mg rifampicin per litre) was added to test tubes to give an agar depth of about 5 cm.

One agar plug containing mycelium from one of the two isolates of *P. cactorum* was transferred to each test tube. Then, tubes were sealed with Parafilm to avoid desiccation and placed in a dark incubator at 23°C until colony growth covered the agar surface.

In vitro uninodal segments were excised from plants obtained from the 11th subculture and 3 cm in height and were placed in each test tube. Tubes were then resealed with Parafilm and transferred to a growth chamber at 23 ± 2°C under a 16 h light / 8 h dark photoperiod with cool white fluorescent light (40 $\mu E.m^{-2}.s^{-1}$).

After 4 days, the percentage of necrotic *in vitro* plants was calculated. Ten *in vitro* plants per elementary treatment were used in this essay.

Statistical analyses were conducted as described above.

RESULTS AND DISCUSSION

Evaluation of the resistance of *Pyrus syriaca* on excised twigs

The margin of necrosis was not always obvious unless the bark was removed. At 4 days after inoculation, the bark was stripped and the brown necrosis of the inner bark was assessed. A distinct margin between healthy and necrotic tissues was clearly visible, especially on the sensitive rootstock (Myrandier 617). On GF 677 or on *P. syriaca* little or no symptoms developed. Results presented in **Fig. 1** show a significant difference between Myrandier 617 and the two other rootstocks (GF 677 and *P. syriaca*). Indeed, the length of necrosis does not exceed 1.3 mm and it ranged between 1.22 and 0.86 mm for GF 677 and between 1.22 and 0.67 for *P. syriaca*. However, this value varied between 12 to 15 mm for the sensitive rootstock (Myrander 617).

Data obtained from this study suggests that while the rootstock GF 677 is known to be resistant to *P. cactorum*, the rootstock *P. syriaca* exhibits the highest tolerance to the two isolates of *P. cactorum*. Indeed, the lowest necrotic length was obtained with the *P. syriaca* rootstock.

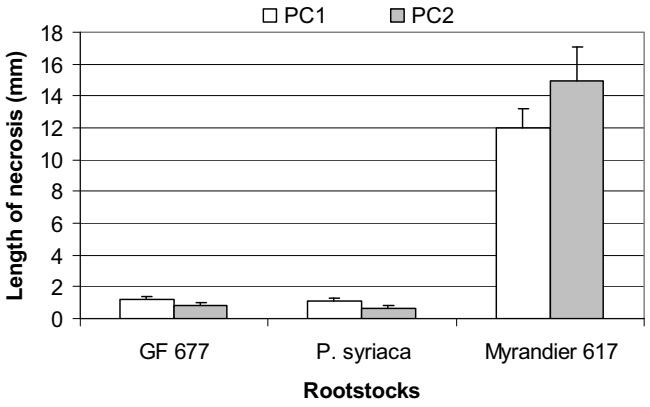

Fig. 1 Length of necrosis (mm) measured on the 3 rootstocks after an incubation period of 4 days at 23°C. PC1 and PC2: The tow isolates pf *Phytophthora cactorum* used in this essay.

Basing on an excised twigs essay, Thomidis *et al.* (2001) showed that GF 677 was moderately resistant to *P. cactorum*. By using the same method (excised twigs) to evaluate the resistance of stone fruit rootstocks to *P. crown* rot, Elena and Tsipouridis (2000) demonstrated that the rootstocks Tsukuba 5 and St. Julien 655/2 were more resistant to *P. catorum* than Myrandier 617. Similar results were obtained by Tsipouridis *et al.* (2005) showing that Myrandier 617, a peach-tree rootstock, inoculated with the isolate *P. cactorum* 1906 had a significantly lower chlorophyll index than the control.

Evaluation of the resistance of *Pyrus syriaca* on *in vitro* plants

In this assay, *in vitro* plants obtained from the 11th subculture were used (maximum multiplication rate and shoot elongation) and the percentage of necrotic *in vitro* plants was calculated.

Statistical analysis did not separate GF 667 (the positive control) from *P. syriaca*; however, there was a significant difference between these two rootstocks and Myrandier 617 (the negative control). Results obtained showed that the percentage of necrosis in *in vitro* plants varied between 1.8 and 1.9% for the rootstock, *P. syriaca*. These results were almost equal to those obtained with GF 667. However, the percentage of necrosis in *in vitro* plants obtained with Myrandier 617 varied from 86.2% with the first isolate of *P. cactorum* to 95.2% with the second isolate of the same pathogen (**Fig. 2**).

We note in this essay that some *in vitro* plants inoculated with *P. cactorum* appear to be healthy with no symp-

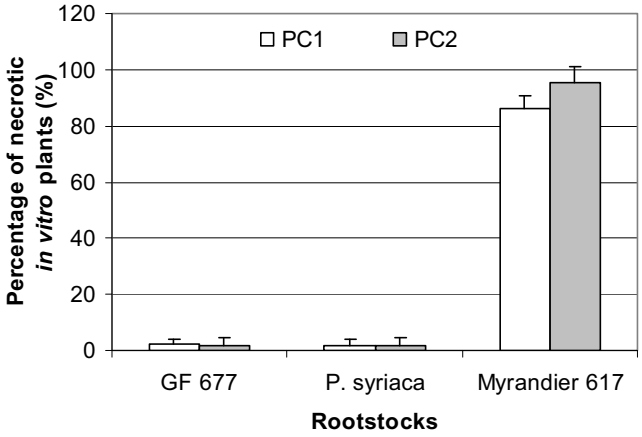

Fig. 2 Percentage of necrotic *in vitro* plants of the 3 rootstocks, inoculated with two isolates of *P. cactorum* (PC1, PC2), obtained after an incubation period of 4 days at 23°C.

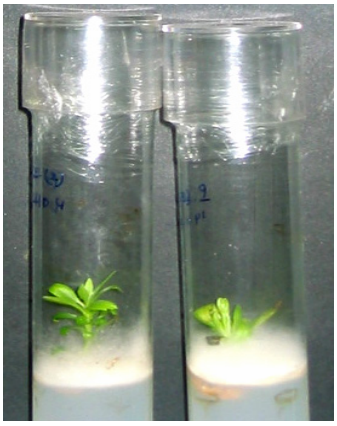

Fig. 3 Aspect of *P. syriaca in vitro* plants growing on a CMA media inoculated with *P. cactorum*. *In vitro* plants appear healthy with no symptoms of necrosis after an incubation period of 4 days at 23°C, despite fungal infection.

toms of necrosis (**Fig. 3**).

Inoculation of *in vitro* plants was used by Eikemo *et al.* (2000) for screening for resistance to Phytophthora crown rot in strawberry cultivars. These authors demonstrated that, four days after inoculation, cv. 'Polka' had 45% more affected leaves than 'Senga Sengana'.

Results of the second inoculation method (*in vitro* plants experiment) were almost similar to those obtained with the excised twigs essay. In fact, inoculated *P. syriaca* plants showed little or no necrosis compared to Myrandier 617 plants. This assay seems to be more efficient for screening resistant rootstocks based on the ability of *in vitro* plants to be more sensitive to *P. cactorum* and for pathogen inoculation in general.

Finally, to be more informative, plant inoculation must be reproduced in pots to select resistant rootstocks.

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