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Phytophthora parsiana, A NEW THREAT TO ALMOND TREES AND ITS HOST RANGE EXPANSION*

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Abstract

The distribution of *Phytophthora parsiana* a high-temperature tolerant newly described species was studied in southern Iran and examined its host range in woody perennial plant species in greenhouse condition under high inoculum potential. Among different plant species examined, almonds were highly susceptible by different isolates. From 24 woody plants inoculated, the disease severity in descending orders were: *Prunus dulcis*, *P. armeniaca*, *Pistacia vera* cv. *Sarakhs*, *Prunus dulcis* var. *fragilis*, *Carica papaya*, *Pistacia khinjuk*, *P. vera* cv. *Badami*, *Mangifera indica, P. mutica*, *Platanus orientalis*, , *Ficus carica* and *Juglans regia*. The pathogen was detected from soil and infected trees only in pistachio orchard in Rafsanjan in Kerman province and adjacent province Yazd.

Keywords: *Prunus* spp., Woody plants, *Pistacia* spp., Perennial plants, Iran.

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Introduction

Phytophthora species are among the most devastating pathogens worldwide affecting various annual and perennial plant species. Over 100 species of *Phytophthora* have been described from different plant species under various climatic conditions that only 13% grow above 35°C (Erwin and Ribeiro 1978; Gallegly and Hong 2008). Among high temperature tolerant species, a newly described *P. parsiana* (Mostowfizadeh-Ghalamfarsa *et al*. 2008) have been previously reported as high temperature *P. cryptogea* from fig in southern Iran (Banihashemi and Ghaisi 1993) and from pistachio in California, USA (MacDoland *et al*. 1992) and Iran (Banihashemi 1993 unpublished data). The distribution of the pathogen and disease it causes in other parts of the world is not documented. Recent study showed that *P. parsiana* is limited to woody plant species and did not infect herbaceous annual crops and weeds (Hajebrahimi and Banihashemi 2011).

The objective of the present study was to detect the pathogen from soil, water stream and orchards in southern provinces of Iran. The reaction of different woody plant species at high inoculum was carried out under greenhouse conditions.

Materials and methods

During 2009 to 2010, pistachio orchards in Kerman and Yazd provinces of Iran showing decline were visited and soil and tissue samples of diseased trees were collected. In Fars and Bushehr provinces, soil samples around different trees and from water streams were also collected. Infected root and crown tissues were washed under running tab water and cut into 2-4 cm segments and plated on PARPH (Massago *et al*., 1977). Soil samples were flooded and baited with citrus leaf disc and incubated over night at room temperature (Banihashemi, 2004). Water samples were assayed the same day collected by using citrus leaf disc method (Banihashemi, 2004) and incubated at room temperature for 48 h. The baits at the specific periods were washed under running tab water, blotted dry and cultured on *Phytophthora* selective medium (Massago *et al*. 1977). The growing colonies from all isolates identified to the genus level by boiled hemp seed method reported earlier (Banihashemi 2004).

Hyphal tip isolates were grown on corn meal agar and used for further studies. Growth rate at various temperatures from 5 to 40°C with 5°C increments was determined on CMA on the basis of mm/day. Sporangia were produced on cleared V-8 agar blocks in distilled water or soil extract under fluorescent illumination (200 lux) at room temperature. Sporangial morphology and dimension was determined. Oospore formation was studied on hemp seed extract agar in the presence of *P. capsici* mating types in the dark at 25°C. The formation of botryose hyphal swellings a characteristic feature of *P. parsiana* (Banihashemi, unpublished data) was used for early screening to discriminate from other identical morphological species. To confirm identification, DNA was extracted from randomly selected isolates and checked with species specific primers using PCR (Mostowfizadeh-Ghalamfarsa, unpublished data).

Inocula of the representative isolates of *P. parsiana* (Table 1) was prepared using vermiculite amended with hemp seed extract (Banihashemi 2004). Seeds of perennial plants requiring cold treatment were treated with benomyl (0.1%) and stratified between moist vermiculite or sand at 4°C for 1-6 months. All seeds were subsequently sown in steam sterilized soil: sand mixture (2:1 v/v) in 20 cm diam pot at 18-35°C under fluorescent illumination. Plants were irrigated as needed. Due to fastest growth of almond seedlings under greenhouse conditions, one-month-old local almond seedlings were used for the initial pathogenicity test. Fifty ml of inocula were placed around the basal stem of each plant and covered with thin layer of soil and flooded. Disease symptoms such as wilting and mortality was recorded daily.

Twenty four perennial plants raised in the pots were inoculated as indicated in the previous section. Five pots each containing four plants were used for each isolate. Four standard isolates of *P. parsiana* were used on all perennial plants (Table 2) and pots were flooded over night. Control pots, received amended vermiculite with no pathogen.

Detached stems of various fruit and ornamental trees were collected during dormant and growing seasons. Stems (20×2 cm) were washed, blotted dry and wiped with cotton impregnated with 95% ethanol and inoculated and incubated at room temperature for 30 days as reported earlier (Hajebrahimi and Banihashemi 2011). Five to six replicates were used for each plant species and isolate.

In host range study, the activity of *P. parsiana*

Table 1. Representative isolates of *Phytophthora parsiana* used in host range study

Year isolated	Location	Source	Host	Isolate code
1992	USA	Z. Banihashemi	Pistachio	PH21.3.08
1991	Iran	Z. Banihashemi	Fig	PH21.5.08
1993	Iran	Z. Banihashemi	Pistachio	PH21.6.08
2008	Iran	A. H. Mohammadi	Pistachio	PH21.13.08

Table 2. Reaction of different woody plants species to isolates of *Phytophthora parsiana* under greenhouse conditions.

Isolates of *Phytophthora parsiana*				Plant species
PH21.13.08	PH21.5.08	PH21.6.08	PH21.3.08	
+	+	+	+	*Carica papaya*
-	-	-	-	*Citrus sinensis*
-	-	-	-	*Cydonia oblonga*
-	-	-	-	*Corylus avellana*
-	-	-	-	*Eriobotrya japonica*
-	-	-	+	*Ficus carica*
-	-	+	-	*Juglans regia*
+	+	+	+	*Mangifera indica*
-	-	-	-	*Olea europea*
+	+	+	+	*Pistacia mutica*
+	+	+	+	*Pistacia khinjuk*
-	-	-	-	*Punica granatum*
+	+	+	+	*Pistacia vera* cv. Badami
+	+	+	+	*Pistacia vera* cv. Sarakhs
-	-	-	-	*Pistacia vera* cv. Ghazvini
-	-	-	-	*Pistacia atlantica*×*Pistacia integrrima*
+	+	+	+	*Prunus armenica*
+	+	+	+	*Prunus dulcis*
+	+	+	+	*Prunus dulcis* var. *fragilis*
+	+	+	+	*Plantago orientalis*
-	-	-	-	*Punica granatum*
-	-	-	-	*Tamarindus indica*
-	-	-	-	*Vitis vinifera*
-	-	-	-	*Zizyphus mauritiana*

+ infected; - non infected

Table 3. Reaction of detached stems of different plant species collected during dormant and growing seasons to different isolates of *Phytophthora parsiana*.

Isolates of *Phytophthora parsiana*								
PH21.13		PH21.6		PH21.5		PH21.3.08		Plant species
G	D	G	D	G	D	G	D	
+	+	+	+	+	+	+	+	*Juglans regia*
-	-	-	-	-	-	-	-	*Magnolia grandiflora*
-	-	-	-	-	-	-	-	*Fraxinus rotundifolia*
+	+	+	+	+	+	+	+	*Malus orientalis*
-	-	-	-	-	-	-	-	*Morus alba*
+	+	+	+	+	+	+	+	*Plantanus orientalis*
+	+	+	+	+	+	+	+	*Prunus dulcis*
-	-	-	-	-	-	-	-	*Prunus persica*
-	-	-	-	-	-	-	-	*Punica granatum*
-	-	-	-	-	-	-	-	*Rosa canina*
-	-	-	+	-	+	-	-	*Ulmus campestris*

D= Dormant stage; G= Growing season
+ tissues discoloration and reisolation; - No tissue discoloration

isolates were monitored in soil during the trials as reported earlier (Banihashemi 2004). Disease symptoms were monitored daily and recorded. The intensity of disease symptoms such as wilting, necrosis and plant mortality was compared in inoculated plants. At the end of the trials, roots and stem basis of all plants were removed and plated on selective medium (Massago *et al*. 1977).Any discoloration beyond the point of inoculation in detached stems was also recorded and re-isolation was attempted on selective medium.

Results and Discussion

Phytophthora parsiana was detected only from pistachio orchards in Rafsanjan in Kerman province and also in Harat in Yazd province. It was not detected in water streams and soil samples in Fars and Bushehr provinces. This is the first incidence of *P. parsiana* in Yazd province. All of the *P. parsiana* isolates produced botryose hyphal swellings, non caducous ovoid to obpyriform non papilate sporangia with internal proliferation. The isolates were heterothallic produced oospores at the presence of opposite mating type. The identity of the species was confirmed using species specific primers (Mostowfizadeh-Ghalamfarsa, unpublished data). The pathogen is a high temperature tolerant species causing severe disease at elevated temperature (Rafiee 2011). Both high and low temperature plant species were found to be host of the pathogen among them almond a temperate tree is the most susceptible species to the pathogen and showed first disease symptoms 8-10 days after inoculation as wilting followed by root rot and crown necrosis

From 24 woody plants inoculated with isolates of *P. parsiana,* all of the *Pistacia* and *Prunus* species,. *Carica papaya*, *Mangifera indica*, *Ficus carica*, *Juglans regia* and *Platanus orientalis* developed disease symptoms (Table 2). In spites of recovery of the pathogen in infested soil during the trials, no disease symptoms appeared in *Vitis vinifera*, *Cydonia oblonga*, *Tamarindus indica*, *Olea europea*, *Corylus avellana*, *Acacia pseudoacacia*, *Eriobotrya japonica*, *Punica granatum*, *Ziziphus mauritiana* and *Pistacia vera* hybrid UCB1 (*P. atlantica*× *P. integrrima*) (Table 2). Detached stem of the above plants with no symptoms also were not infected by the isolates of *P. parsiana.* Among woody plant species, almond

(*Prunus dulcis*) was the most susceptible host which caused mortality 8 days after inoculated by all isolates used. Symptoms on other woody plant species such as *Prunus armenica*, *Pistacia vera* cv. Sarakhs, *P. dulcis* var. *fragilis* and *Carica papaya* showed disease symptoms respectively 10, 15, 18 and 20 days after inoculation. Longer inocubation periods (23 to 43 days) required to express disease symptoms in *P. mutica*, *P. khinjuk*, *P. vera* cv. Badami and *Mangifera indica*. Plane tree showed severe disease symptom two months after inoculation. Inoculated peanut seedlings (an annual herbaceous plant) did not show disease symptoms even after several months. Disease severity by different isolates on the various plant species varied. Some of the pistachio isolates were more aggressive to fig than other isolates.

Inoculated detached stems showed disease symptoms by tissue discoloration and expansion beyond inoculation point. All isolates infected detached stems of almond, walnut, plane tree and apple both on active and dormant stages. Most of ornamental trees, *Prunus persicae* and pomegranate were not infected by the isolates of *P. parsiana* collected during growing season. Some isolates caused infection on some trees only on dormant stage (Table 3).

Previous study by Hajebeahimi and Banihashemi (2011) showed that *P. parsiana* is limited to woody plants and could not infect any herbaceous plant species. *P. drechsleri*, *P. cryptogea* and *P. melonis* which are morphologically identical infect both woody and herbaceous plant species and could be separated by using molecular method. However safflower seedling could only discriminate between *P. melonis* and *P. drechsleri* and *P. cryptogea* (Banihashemi and Mirtalebi 2008). Thus *P. parsiana* and *P. melonis* which are morphologicaly identical could be differentiated by their maximum temperature for growth, presence of botryose hyphal swelling in *P. parsiana* and woody plants as host range. It is therefore necessary to reexamine the identity of *P. cryptogea*, *P. drechsleri* and *P. melonis* previously reported from woody plant species. The recent global warming will be of great environmental factor for the activity of *P. parsiana* under different climatic conditions. Other morphological identical species such as *P. hydropathica* (Hong *et al*. 2010) and *P. irrigata* (Hong *et al*., 2008) has been frequently isolated from water streams and their maximum temperature for growth is above 37°C. Their pathogenicity on woody plant species in not known. *P. parsiana* and *P. irrigata* are close relative to *P. hydropathica* (Hong *et al*., 2010). Some of our *P. parsiana* isolates that have been examined morphologically, molecularly and pathogenic reaction to woody plants might have been close to *P. hydropathica* or even new species to be described (Hong, personal communication by the second author).

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