Preliminary evaluation of *Prunus* spp. germplasm for resistance to white root rot (*Rosellinia necatrix*) disease

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Abstract

*Rosellinia necatrix* (Prill.) is one of the most important soil-borne pathogens affecting fruit trees in Iran. A preliminary selection of open-pollinated seedlings of 13 *Prunus* spp. genotypes, including ‘Early Golden’, ‘Obilnaja’, ‘Mirabolano’, ‘Blue Free’, ‘Tanagol’, ‘Mandchurica’, ‘Saint Julien’, ‘Greengage’, ‘Cadaman’, GF-677, K-R.T-02, K-R.T-01, and K-R.T-41, for resistance to white root rot disease was carried out through artificial inoculation in the greenhouse as part of plum and prune rootstocks breeding programme. In the first experiment, among 559 seedlings belonging to the *Prunus* population, 282 plants survived 120 days after inoculation. The survivors were exposed to twofold higher concentration of inoculum. The successful establishment of the pathogen on roots of ‘Early Golden’, ‘Obilnaja’, ‘Greengage, K-R.T-41, ‘Blue Free’, and ‘Tanasgol’ led to the destruction of almost all plants. The resistance level of ‘Mirabolano’, ‘Saint Julien’, ‘Blue Free’, and K-R.T-02 did not change with the amount of inoculum, and they were defined as moderately resistant in both experiments. Among all studied *Prunus* spp. germplasm, the resistance level of the seedlings of K-R.T-01 was determined as resistant regardless of inoculum density. Although a large number of hybrid GF-677 seedlings died with exposure to a low level of inoculum, the remaining plants were able to survive even at high fungal density. Similarly, the plants of ‘Mandchurica’ and ‘Cadaman’ showed resistant responses with a high level of inoculum.

It was a preliminary test for the screening of *Prunus* spp. population against *R. necatrix*. The selected seedlings that survived after two fungal inoculation trials are currently being evaluated for reproductive performance and will be screened for precise resistance to white root rot disease in the near future.

Keywords: plum and prune, resistant rootstocks, *Rosellinia necatrix*, white root rot.

Introduction

Fruit crops belonging to the genus of *Prunus* including cherries, apricots, plums, peaches, nectarines, and almonds are among the most important economic products in Iran. According to the FAOSTAT (2021), Iran with approximately 389,000 tonnes of production is the fifth largest producer of plums in the world. Soil-borne pathogens have been reported to be a limiting biotic factor of fruit trees production worldwide (Arjona-Lopez et al., 2020). White root rot has been recognised as a destructive disease caused by *Rosellinia necatrix* Prill. (anamorph: *Dematophora necatrix* Hartig), which has a wide host range between herbaceous and woody plants. It can infect more than 340 plant species in 160 genera and is widely distributed throughout the world (Pasini et al., 2016; Fungal Databases, 2022). In Iran, most of the economic fruit species including almond, walnut, pistachio, apple, pear, cherry, sweet cherry, apricot, peach, plum, nectarine, fig, and grape are susceptible to this disease. The disease is a common problem, especially in the north and northwest regions of Iran with rainy weather.

The first report of the pathogen in Iranian plum orchards dates to 1966 (Behdad, 1975). The pathogen is destructive both in nurseries and orchards. Heavy soils, humid conditions, and soil temperature are the main influencing factors for pathogen growth and subsequently infection of orchards.

The invasive fungus causes root rot in trees, especially in tropical and temperate climates. Common symptoms of the disease are yellowing foliage, early leaf fall, poor branch growth, small and shriveled fruits, and dieback. Greyish-white and cottony mycelium mats
appear on infected roots or under the bark. *Rosellinia* infection is characterised by the development of white mycelial fans between the bark and wood. The infected trees show different degrees of decline depending on their age as well as severity of infection. Trees will eventually die within a few growing seasons.

The life cycle of the pathogen takes place in the soil. Once the disease establishes in an orchard, the infestation can spread by the growth of mycelial strands through soil or transferred by infected roots within the orchard (Kulshrestha et al., 2014; Pasini et al., 2016; Dafny-Yelin et al., 2018; Pal et al., 2020). Along ago study in Iran showed that the infected 9-year-old cherry trees could produce only 14% of fruit in comparison with the healthy ones (Behdad, 1975).

The ability of the pathogen to survive in acidic soils and remain active on the residues of susceptible hosts for many years as well as having a wide host range make its control difficult (Kulshrestha et al., 2014; Pasini et al., 2016). Using agricultural practices (Martínez-Ferri et al., 2019), biological agents (Kulshrestha et al., 2014; Pasini et al., 2016; Arjona-Girona, López-Herrera, 2018), soil disinfection and solarisation (Kulshrestha et al., 2014), and chemical treatments (Arjona-López et al., 2020) are expensive and usually insufficient to control the disease successfully. Contamination of new orchards commonly occurred by transferring infested plant material from nurseries. Accordingly, white root rot disease can mostly be managed by using healthy propagating material and establishing new plantations in non-infested soils (Kulshrestha et al., 2014; Pasini et al., 2016). The most effective and sustainable method to solve the problem is using of healthy tolerant rootstocks. To control the disease more effectively, many efforts have been made to identify sources of resistance in fruit crops (Lee et al., 2000; Mansoori, Dorostkar, 2008; Pinochet, 2010; Tutmus et al., 2012; Sharma et al., 2013; Arjona-López et al., 2020; Kumar et al., 2020; Choi et al., 2021).

The plum rootstocks breeding and development programmes have been operating in Iran since 2010, selecting indigenous germplasm to develop new cultivars and rootstocks with suitable horticultural traits. In such programmes, a unique germplasm resource is accessible that allows breeders to achieve the project goals. Resistance to *R. necatrix* is also of the priority objectives of stone fruit rootstock breeding programmes (Pirkhezri, 2021).

The purpose of this study was an initial screening of open-pollinated *Prunus* spp. seedlings for resistance to *R. necatrix*.

### Material and methods

**Identification of white root rot (Rosellinia necatrix) from plum roots.** In spring 2016, the wilting of plum trees was observed in an orchard in Shahriyar, Tehran Province, Iran. The infected plants were 8–10 years old, growing in the soil with the texture of clay loam and irrigated using traditional methods. Diseased plum trees were primarily identified by the aerial symptoms including progressive weakness, chlorosis, reduced shoot growth, wilting, premature defoliation, dieback, and death of young shoots. The development of white strand mycelia on the root surface of the infected trees, which occasionally was extended under the bark, confirmed the presence of the pathogen in symptomatic trees. The samples were collected from naturally root-infected tissues carrying white cottony sheets under the bark. The fungus of *Rosellinia* was isolated from the samples by the method of Pasini et al. (2016). For this, pieces of diseased root tissues (approximately 5–7 cm long) were carefully washed with running tap water, surface sterilised for 1 min in 75% ethanol and 1 min in sodium hypochlorite 1% solution, rinsed three times with sterile distilled water, and then incubated in a humid chamber at 22 ± 1°C in the dark to induce the development of white cottony mycelium. One week after incubation, growing aerial mycelia were placed on the potato dextrose agar (PDA) medium and incubated at 24 ± 1°C in the dark for 10–12 days. To obtain pure isolates, hyphal tips of developing fungi were transferred to corn meal agar (CMA) and used for further research. The isolates were grown on the oatmeal agar (OMA) medium containing pieces of sterilised McIntosh apple twigs to induce synnemata development. *R. necatrix* was identified by cultural features (such as vegetative characteristics on PDA and CMA media) as well as microscopic traits (such as synnemata, conidia, and pear-shaped hyphal swellings) (Takemoto et al., 2009; Pliego et al., 2012; Castro et al., 2013; Wittstein et al., 2020).

**Preparation of inoculum.** The artificial inoculum was prepared by inoculating soil according to Lee et al. (2000) method with some modifications. Briefly, young twigs of McIntosh apple trees were collected during the dormancy stage in January. McIntosh apple sticks (30–40 mm in length, 5–10 mm in diameter) were washed with tap water and kept at 75–80°C for 20 hours. Four to six sterilised apple sticks were used as host plants, placed around the growing fungal colonies on CMA and incubated at 25 ± 1°C in darkness. After four weeks, the sticks were covered with fungal mycelia. Six to eight infected sticks were inserted into each box (40 × 35 × 25 cm) containing sterile soil, perlite, and peat moss (10:1:1 v/v). The boxes were incubated in humid conditions under a plastic cover at 25 ± 1°C temperature and 75–80% humidity. After 35–37 days, soil inoculum was ready for use (Figure 1). The sterile apple sticks were used to inoculate the soil as a negative control.

**Evaluation of virulence of R. necatrix isolates.** The virulence of two *R. necatrix* isolates (SH401 and SH402) was determined on 2-year-old dormant M9 and MM106 apple rootstocks by adding and mixing the inoculum soil to the pots (1:10 v/v), as described by Lee et al. (2000). The isolate KH400, which was obtained from the Fruit Pathology Laboratory of the Iranian Research Institute of Plant Protection, was also included in the experiment. This isolate was collected from the infected roots of stone fruit trees in Isfahan, Iran (Table 1). Five plants were tested for each isolate. The experimental unit was one plant per pot. The inoculated...
and non-inoculated (control) plants were observed weekly for the development of disease symptoms. Three months after inoculation, the length of new shoot growth and disease symptoms were recorded.

Evaluation of Prunus spp. for resistance to \textit{R. necatrix}. Open-pollinated seedlings of 13 \textit{Prunus} spp. including ‘Early Golden’, ‘Mandchurica’, ‘Obilnaja’, ‘Mirabolano’, ‘Saint Julien’, ‘Blue Free’, ‘Cadaman’, GF-677, ‘Tanasgol’, ‘Greengage’, K-R.T-01, K-R.T-02, and K-R.T-41 were tested for resistance to \textit{R. necatrix} under greenhouse conditions in 2018. To produce experimental plant materials, 70–130 healthy seeds were harvested from fruits of each open-pollinated \textit{Prunus} spp. genotype from June to September 2017. Each population of seeds was placed on sterile perlite and stored at 0–4°C for 70–90 days to break seed dormancy. The seeds of each genotype were planted in plastic pots (51 × 38 × 38 cm) containing cocopeat, soil, peat moss, and perlite (7:2:2:3) and kept outside the greenhouse until germination. The seedlings were ready for inoculation 80–90 days after seeding (Figure 2).

\textbf{Figure 1.} Growth of \textit{Rosellinia necatrix} isolate SH402 on ‘McIntosh’ apple sticks (A); transferring of infected sticks on soil (B); boxes with soil after inoculation by infected sticks (C); infected soil 10 days (D) and 37 days (E) after inoculation

\textbf{Figure 2.} \textit{Prunus} spp. cultivars GF-677 (left) and ‘Cadaman’ (right) young seedlings prepared for inoculation in May 2018
Inoculation was conducted on 18 May 2018. For this, 1 litre of infested soil was added evenly on the surface of 10 litres of soil in each pot and placed in the plant rhizosphere. The pots were placed in large trays and transferred to the greenhouse at a temperature of 25 ± 1°C. At least 14 plants were tested for each genotype (Table 2). Treated plants were watered regularly by adding water to trays and observed for symptom appearance. For the control plants, non-infested soil was used as inoculum. Observations including wilting, leaf chlorosis, aerial damage, and mortality were recorded weekly for 120 days. The studied Prunus spp. genotypes were classified into three different susceptibility groups according to Lee et al. (2000) as follows: R – resistant, 0–30% seedlings with necrotic symptoms; M – moderately resistant, 31–70% seedlings with necrotic symptoms; S – susceptible, 71–100% seedlings with necrotic symptoms.

The seedlings that survived the first evaluation (with a low inoculum, 1:10 v/v) were tested at further disease pressure caused by twice the inoculum level of the first evaluation (2:10 v/v). During the experiment, the plants were observed for the symptoms 90 days after inoculation. To confirm root colonisation of treated seedlings by R. necatrix, the re-isolation of the fungus from the infected roots was performed using PDA.

Results

Identification of white root rot (Rosellinia necatrix) from plum roots. The fungal mycelium was observed on the surface of the roots and under the bark of trees creating mycelium strands and fans. In total, two fungal isolates (SH401 and SH402) were isolated from five infected 8–10-year-old plum trees. The identification of isolates was performed on the basis of cultural and microscopic characteristics. The fungus initially produced a white and cottony mycelia on the CMA medium. Inside the agar, brownish-cream pigments gradually were generated, and the colour of the colony on the lower surface of the Petri dish changed (Figure 3).

![Figure 3](image1.png)

Figure 3. Under (on the left in both photos) and upper (on the right in both photos) image of two Rosellinia necatrix isolates 7 days after growing on the CMA medium

After cultivation on PDA and CMA media at 24 ± 1°C temperature in the dark for 7 days, the average diameter of the fungal colony was 7.3 and 9.0 cm, respectively. Synnemata (average length 1.2 mm) and conidia were produced on a 25–30-day-old OMA medium. Conidia were unicellular, hyaline, round to slightly oval in shape, 3.5–4.5 × 1.5–2.5 µm, which closely matched (i.e., 3–5 × 2.5–3 µm) the dimensions reported by Pliego et al. (2012). The particular morphological feature, generally used to identify R. necatrix, is the presence of pyriform swellings immediately above the hyphal septum, which usually has a diameter up to 13 µm (Pliego et al., 2012). In this study, the average diameter of pear-shaped hyphal swellings was 11 µm, approximately 5 folds of the hyphal diameter (Figure 4).

![Figure 4](image2.png)

Figure 4. Typical pear-shaped swelling above the septum of fungal mycelium (left); conidia and conidiophore of Rosellinia necatrix (right)
**Evaluation of virulence of *R. necatrix* isolates.**

The results of virulence of three *R. necatrix* isolates on 2-year-old M9 and MM106 apple rootstocks are presented in Table 1. The first symptoms were observed 21 days after inoculation on M9 plants inoculated with isolates KH400 and SH402 as leaf necrosis, chlorosis, followed by wilting and finally the death of plants 35–60 days after inoculation. Later, symptoms on MM106 rootstocks developed at a slower rate. The plants inoculated with isolate SH401 began to wilt six weeks after inoculation. Root evaluation of treated plants confirmed the presence of the white cottony mycelium pathogen on the root surface. With the isolate SH401, the root growth was reduced in both M9 and MM106 rootstocks, but only two M9 plants died approximately three months after pathogen inoculation. Inoculation of apple rootstocks with the isolate SH401 reduced the growth length of new shoots compared to the control plants. According to the results, the pathogenicity of isolates KH400 and SH402 was similar, so their inoculum mixture was used for further experiments. None of the control plants showed the symptoms.

**Table 1.** Virulence of *Rosellinia necatrix* isolates on 2-year-old M9 and MM106 rootstocks

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>Location and host</th>
<th>Rate of dead plants to total plants tested</th>
<th>Length of new shoot growth after inoculation cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M9</td>
<td>MM106</td>
</tr>
<tr>
<td>KH400</td>
<td>Esfahan-stone fruits</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>SH402</td>
<td>Shahriyar-plum</td>
<td>5/5</td>
<td>3/5</td>
</tr>
<tr>
<td>SH401</td>
<td>Shahriyar-plum</td>
<td>2/5</td>
<td>0/5</td>
</tr>
<tr>
<td>non-inoculated</td>
<td>(control)</td>
<td>0/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

Note.1 – shoot length was not possible to measure due to the death of all inoculated plants; different letters indicate a significant difference at a 5% level of probability (Duncan’s multiple range test) between non-inoculated plants and MM106 plants treated with isolate SH402 and M9 plants treated with isolate SH401.

**Assessment of Prunus spp. resistance to *R. necatrix*.** Seedlings for all 13 *Prunus* species were successfully grown. The seed germination rate varied from 13% to 63% with the lowest value assigned to ‘Saint Julien’, ‘Obilnaja’, ‘Early Golden’, and ‘Blue Free’. The first symptoms of the disease were observed on GF-677 and ‘Cadaman’ rootstocks 17 and 27 days after inoculation with yellowing and wilting. Disease development in *Prunus* spp. after inoculation with the combination of two *R. necatrix* isolates (KH400 and SH402) is shown in Figure 5.

According to the results of the experiment, the white root rot pathogen first colonised the roots and then produced different types of symptoms on the above-ground plant parts including chlorosis, wilting, leaf curling, and defoliation. Based on the results from the 559

**Figure 5.** Disease progress in *Prunus* spp. 120 and 90 days after inoculation with low and high level of *Rosellinia necatrix* inoculum
Preliminary evaluation of Prunus spp. germplasm for resistance to white root rot (Rosellinia necatrix) disease

Seedling populations belonging to 13 different species of Prunus, 282 plants indicated necrotic symptoms during the first evaluation, when a low amount of inoculum was used. The percent of infected seedlings varied from 22% (in ‘Tanasgol’) to 91% (in GF-677) (Table 2).

Seedlings of ‘Tanasgol’ and ‘Early Golden’ did not show any aerial symptoms until the 90th day after inoculation. The infection rate was lower than 30% for the seedlings originating from these two cultivars. However, at a high level of inoculum, almost all seedlings of ‘Tanasgol’ and ‘Early Golden’ were susceptible to faster disease development. The maximum delay in the expression of disease symptoms was in ‘Obilnaja’ and K-R.T-01. Symptoms appearance in these two genotypes delayed until the 83rd and 78th day after inoculation, respectively. Due to a low germination rate of ‘Obilnaja’ and ‘Blue Free’ and an insufficient number of seedlings, any conclusions regarding their resistance should be made with caution. During two evaluation tests, 22 seedlings of ‘Obilnaja’ (out of 22) and 26 of ‘Blue Free’ (out of 27) plants were infected. Only one ‘Blue Free’ plant was resistant, even at high concentrations of inoculum (Table 2).

Table 2: Response of open-pollinated seedlings of Prunus spp. to Rosellinia necatrix

<table>
<thead>
<tr>
<th>Prunus spp. genotypes / cultivars</th>
<th>Plant species</th>
<th>Origin</th>
<th>Low inoculum</th>
<th>High inoculum</th>
<th>No. of survived plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>No. of tested seedlings</td>
<td>% of infected seedlings</td>
<td>Resistance level</td>
</tr>
<tr>
<td>Early Golden</td>
<td>P. salicina</td>
<td>China</td>
<td>23</td>
<td>26.1</td>
<td>R</td>
</tr>
<tr>
<td>Mandchurica</td>
<td>P. mandchurica</td>
<td>China</td>
<td>61</td>
<td>36.1</td>
<td>M</td>
</tr>
<tr>
<td>Obilnaja</td>
<td>P. salicina</td>
<td>Ukraine</td>
<td>22</td>
<td>40.9</td>
<td>M</td>
</tr>
<tr>
<td>Mirabolano</td>
<td>P. domestica</td>
<td>Italy</td>
<td>40</td>
<td>60.0</td>
<td>M</td>
</tr>
<tr>
<td>Saint Julien</td>
<td>P. insititia</td>
<td>Italy</td>
<td>14</td>
<td>64.3</td>
<td>M</td>
</tr>
<tr>
<td>Blue Free</td>
<td>P. domestica</td>
<td>Italy</td>
<td>27</td>
<td>77.8</td>
<td>S</td>
</tr>
<tr>
<td>Cadaman</td>
<td>P. persica × P. davidiana</td>
<td>Italy</td>
<td>60</td>
<td>46.7</td>
<td>M</td>
</tr>
<tr>
<td>GF-677</td>
<td>P. dulcis × P. persica</td>
<td>Italy</td>
<td>66</td>
<td>90.9</td>
<td>S</td>
</tr>
<tr>
<td>Tanasgol</td>
<td>P. domestica × P. armeniaca</td>
<td>Iran</td>
<td>32</td>
<td>21.9</td>
<td>R</td>
</tr>
<tr>
<td>Greengage</td>
<td>P. ceracifera</td>
<td>Iran</td>
<td>61</td>
<td>49.2</td>
<td>M</td>
</tr>
<tr>
<td>K-R.T-01</td>
<td>Prunus spp.</td>
<td>Iran</td>
<td>67</td>
<td>28.4</td>
<td>R</td>
</tr>
<tr>
<td>K-R.T-02</td>
<td>Prunus spp.</td>
<td>Iran</td>
<td>45</td>
<td>51.1</td>
<td>M</td>
</tr>
<tr>
<td>K-R.T-41</td>
<td>Prunus spp.</td>
<td>Iran</td>
<td>41</td>
<td>58.5</td>
<td>M</td>
</tr>
</tbody>
</table>

Determined: 1 – 120 days after inoculation; 2 – 90 days after inoculation; 3 – R – resistant (0-30% seedlings with necrotic symptoms), M – moderately resistant (31-70%), S – susceptible (71-100%) of ‘Tanasgol’ and ‘Early Golden’ were susceptible to faster disease development. The maximum delay in the expression of disease symptoms was in ‘Obilnaja’ and K-R.T-01. Symptoms appearance in these two genotypes delayed until the 83rd and 78th day after inoculation, respectively. Due to a low germination rate of ‘Obilnaja’ and ‘Blue Free’ and an insufficient number of seedlings, any conclusions regarding their resistance should be made with caution. During two evaluation tests, 22 seedlings of ‘Obilnaja’ (out of 22) and 26 of ‘Blue Free’ (out of 27) plants were infected. Only one ‘Blue Free’ plant was resistant, even at high concentrations of inoculum (Table 2).

Seedlings of ‘Cadaman’, ‘Mirabolano’, ‘Mandchurica’, ‘Saint Julien’, ‘Greengage’, K-R.T-02, and K-R.T-41 that showed symptoms 30–40 days after inoculation were classified as moderately resistant during the first evaluation. In this case, the plants showed different susceptibility response in the second experiment when exposed to twofold concentration of inoculum. Although the most infected seedlings were observed in the GF-677 population with 91%, all six GF-677 plants that successfully passed the first evaluation, survived during the second evaluation when exposed to a high level of inoculum. Subsequently, the lowest mortality was measured for ‘Cadaman’ (21.9%) followed by ‘Mandchurica’ (28.2%) and K-R.T-01 (29.2%) (Table 2). Twenty-five (out of 32) seedlings of ‘Cadaman’ that were grouped as moderately resistant in the first evaluation showed to be resistant to a high amount of inoculum. Seedlings of genotype K-R.T-01 that showed the first symptoms 11 weeks after inoculation were resistant in both low and high inoculation tests.

Based on the results of the first experiment, a total of 277 seedlings that had been classified into different susceptibility groups were exposed to a high level of inoculum, and 118 plants (belonging to 9 Prunus species) survived (Table 2). In this case, the highest seedling mortality rate was found in ‘Early Golden’, ‘Obilnaja’, ‘Greengage’, and K-R.T-41, as none of their plants survived. The root surfaces of symptomatic seedlings were covered with white cottony mycelial strands, as described by Lee et al. (2000) and Hartley et al. (2022) (Figure 6).
Discussion

*Rosellinia necatrix* is a soil-borne ascomycete pathogen that causes white root rot disease in many plant species including fruit trees. As the pathogen inhabits soil, it attacks the underground parts of the host plants causing tissue decay, and aerial symptoms will be observed after a long time of infection. White root rot can develop during rainy seasons and would intensify in poor soils and disease susceptible cultivars (Kumar et al., 2020). In this study, the wilting and curling of leaves were observed as the first symptoms produced by *R. necatrix* on above-ground parts of the plants.

In controlled greenhouse experiments under conditions highly favourable to *R. necatrix* infection, the tested *Prunus* spp. germplasm showed various responses to *R. necatrix*, but none of them was immune. The delay period associated with disease expression was longer at low inoculum densities for more studied species. The symptoms of the disease appeared on the 17th day. According to the results of Gupta and Verma (1978), *R. necatrix* produces toxic metabolites which cause the symptoms of plant necrosis. The maximum production was determined from the 7th to the 11th day of fungal growth (Gupta, Gohain, 1982). The response of host plants to the pathogen would be associated with their ability to overcome the toxic effects of the metabolites produced by the fungus (Sharma et al., 2013). Therefore, based on the results of the first experiment, the delayed appearance of the symptoms in different species may be related to the tolerance of the host species.
‘Early Golden’ and ‘Tansagol’ were found to possess better tolerance than the other species and took a longer time (90 days) for the external symptoms expression and plant death. Although the delayed appearance of symptoms as well as the number of infected seedlings allowed classifying them as resistant plants, most of the selected resistant seedlings did not survive, when a high level of inoculum was used (Table 2). Also, the seedlings of ‘Obilnaja’, ‘Greengage’, and K-R-T-41 died when exposed to high fungal density. Therefore, the seedlings originating from all these five cultivars/genotypes possess tolerance to white root rot only under lower disease pressure.

The existence of a shallow and weak root system may have forced the seedlings to show the highest disease severity when exposed to a high amount of inoculum. A low resistance level and shallow root system have been reported as the main factors in the development of wilting symptoms by *R. necatrix* in apple rootstocks (Kumar et al., 2020). Therefore, using such rootstocks in sandy soils that have a poor water holding capacity should be done with caution. The resistance level of ‘Mirabolano’, ‘Saint Julien’, ‘Blue Free’, and K-R-T-02 did not change with the amount of inoculum. Among all studied *Prunus* spp. germplasm, the genotype K-R-T-01 was found to be resistant regardless of inoculum density. The pathogen caused mild wilt symptoms on genotype K-R-T-01 seedlings at low and high fungal densities. The mortality rates were 28.4% and 29.2% at low and high test doses, respectively (Table 2). The presence of a long root system in genotype K-R-T-01 appears to contribute to its resistance against the pathogen. The peach-almond hybrid GF-677 has been reported to be susceptible rootstock to the white root rot in peach orchards in Spain (Pinolch, 2010). In this study, a large number of hybrid GF-677 seedlings died with exposure to a low level of inoculum; however, the remaining plants were able to survive even at high fungal density. Similarly, the plants of ‘Mandchurica’ and ‘Cadaman’ exhibited resistant response with a high level of inoculum.

The present study revealed that the percentage of infested seedlings was affected by both the inoculum density and host genotypes. These observations are in agreement with the findings of other studies (Lee et al., 2000; Sharma et al., 2013; Kumar et al., 2020). The appearance of symptoms as well as disease development were faster in all studied genotypes, when the plants were exposed to high fungal density. The response of cultivars to the pathogen under greenhouse conditions could be stimulated by the appropriate disease pressure occurring near the rhizosphere as well as the type of inoculum. However, under field conditions, the symptoms are exhibited for a longer time compared to those tested in the greenhouse (Sharma et al., 2013). According to a study of Gupta and Verma (1978), some of the apple rootstocks susceptible in pot treatments disappeared in the field even after 500 days and were classified as field resistant.

Due to the special features of the white root rot pathogen, agricultural and chemical control of the disease is difficult and expensive. Therefore, host plant resistance to the main soil-borne pathogens has always been considered to be one of the objectives of fruit tree breeding programmes (Pliego et al., 2012; Choi et al., 2021; Arjona-López et al., 2022). Breeding for resistance to *R. necatrix* in some fruit trees has generally been successful. The study of Choi et al. (2021) showed that there is little difference among G, CG, and M series apple rootstocks resistance to *R. necatrix*. Various studies have reported the influence of the rootstock on the resistance of apple trees to white root rot disease (Lee et al., 2000; Sharma et al., 2013; Kumar et al., 2020; Choi et al., 2021). Although currently there are no commercial avocado rootstocks resistant to *R. necatrix*, some disease-tolerant rootstock candidates have been reported for the avocado rootstock selection programme in Spain (Barceló-Muñoz et al., 2007). Arjona-López et al. (2022) studied the susceptibility of some promising citrus rootstocks to white root rot, of which B11R5T64 and B11R5T60 showed the highest tolerance response. For stone fruits, ‘Replantpac’ is a plum-almond hybrid that has been proposed for transplanting as resistant to *R. necatrix* (Pinolch, 2010).

Further research on plant-pathogen interaction is needed to improve the white root rot management options in orchards. However, based on the results of the experiment, genotype K-R-T-01, whose seedlings showed delayed disease development and were classified as resistant in both tests, would be a good candidate to manage the disease in an integrated management system of white root rot endemic areas.

To our knowledge, this is the first preliminary report of *Prunus* spp. rootstocks investigation for resistance to *R. necatrix* in Iran. The plant materials that have survived after two fungal inoculation tests would be used as a primary source of the *R. necatrix*-resistant *Prunus* spp. rootstocks in future breeding programmes. These selected materials are now indentified as single-plant stool populations. The focus is on propagation properties including root development, rooting capacity, and protection against burrknots. The number of rootstocks that have performed well will be increased in order to allow replicated tests and produce a reliable and accurate assessment of resistance to various biotic and abiotic stresses that the plants will encounter in orchards. They will be also studied for some important horticultural traits such as tree vigour, initial adaptation to calcareous soils, scion compatibility with commercial cultivars, dwarfing, and yield efficiency.
**Conclusion**

The successful establishment of white root rot (Rosellinia necatrix) on roots revealed that the inoculation method can be used for the primary screening of Prunus spp. in the greenhouse. Almost all plants of the cultivars ‘Early Golden’, ‘Obilnaja’, ‘Greengage’, K-R.T-41, ‘Blue Free’, and ‘Tanasgol’ died when exposed to a high level of R. necatrix inoculum; therefore, they should not be suitable for white root rot endemic areas. The resistance level of ‘Mirabolano’, ‘Saint Julien’, ‘Blue Free’, and K-R.T-02 did not change with different amounts of inoculum. The resistance level of the seedlings of cultivar K-R.T-01 was defined as resistant regardless of inoculum density. The plants of ‘Mandchurica’ and ‘Cadaman’ exhibited resistant response to a high level of inoculum. These plant materials would be used as sources of early resistance in the future breeding programmes of R. necatrix-resistant Prunus spp. rootstocks.

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