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Evaluation of relative resistance in some selected genotypes Mahaleb (*Prunus mahaleb* L.) dwarf to Phytophthora species

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ABSTRACT: The disease Phytophthora crown and root rot consist of the most important problems in cherry cultivation. In this study, the relative resistance of 5 selected genotypes Mahaleb (Prunus mahaleb L) dwarf including 24, 100, 155, 136 and 268 to Phytophthora citrophthora (smith et smith Leonian) and Phytophthora citricola (Sawada) were evaluated by using excised twig assay, excised shoot method and soil inoculation method with perlite infested with Phytophthora mycelium. The Mahaleb genotypes showed different susceptibility to Phytophthora citrophthora. 155 genotypes was the least susceptible and 268 and 24 genotypes were the most susceptible, which suggests that the latter genotypes are unsuitable for orchards in which the conditions are favorable for Phytophthora diseases. 100 and 136 were moderately susceptible. The plants that were inoculated with Ph. citricola in the glasshouse and in vitro showed that 155 were the most resistant, 24 and 268 were the most susceptible, 136 and 100 were moderately resistant. The present results demonstrate 155 genotypes was resistant to Ph.citrophthora and Ph. citricola and P. citricola less virulent than P. citrophthora.

***Keywords*:** Mahaleb(Prunus mahaleb), Phytophthora crown, Relative resistance, Root rot.

INTRODUCTION

Mahaleb, Prunus mahaleb L., is an important seed stock for cherry and sour cherry (Perry, 1987). This stock, due to its deepened roots, establishes a proper confirmation in the soil and also is compatible with most of the soils except humid soils (Christov and Koleva, 1995). This stock is compatible with light and loamy soils and rocky lands and also is compatible with cool and oceanic weather with which cherry is not compatible. Mahaleb roots penetrate deeper than those of mazarrd stock in the soil. This fact demonstrates that Mahaleb is more resistant in dry conditions. Moreover, it can bear zinc deficiency and also the chlorosis caused by iron deficiency in loamy soils (Albertini and Desalvador, 1991; Buman, 1997). Mahaleb roots are resistant to frost and freeze, so it can stand cold winters (Standardi, 1993; Giorgio et al., 1992). But this stock is not proper for heavy and humid soils and is susceptible to Phytophthora rots. Root and crown rots caused by Phytophthora is one of the most important fungal diseases of cherry which causes heavy damages on saplings and trees in the nurseries and gardens. Because of infection with Phytophthora rots, the growth of the tree and twigs diminishes gradually and the appearance of the tree gets weak and withered leading to death after a few years (Ashkan, 1999). Three species of Phytophthora; Ph. cambivora, Ph. megasperma, and Ph. dreschleri cause root and crown rot in California and among these three species, Ph. megasperma and Ph.cambivora are more virulent on Mahaleb stock (Mircetich et al., 1976). So far, species such as Ph.cambivora, Ph. drechsleri , Ph.. cactorum , Ph. megasperma Ph.citrophthora Ph.. syringae , Ph. citricola , Ph. medicaginis, Ph. capsici, and Ph. cryptogea have been isolated from Mahaleb, Prunus avium (mazzard cherry), and cherry, P. cerasus (sour cherry) in different regions of the world (Alizadeh & Agharafee, 1998; Wilcox & Mircetich, 1985; Mircetich et al., 1974). In Iran, according the studies carried out on the fruits of cold

regions five species of Phytophthora have been reported from almond and pear (Ph. cryptogea), apricot, cherry, walnut, and apple (Ph. cactorum), almond and cherry (Ph. citricola), peach (Ph. drechsleri), and plum and almond (Ph. iranica) (Ershad, 1977, 1991 and 1995; Banihashemi, 1986; Behrouzin and Ershad, 1989; Zakii and Ershad, 1994) Alizadeh and Agharafee, (1998) while investigating the causes of death of stone fruit trees in Tehran province, reported three soil-born fungi; Ph. citrophthora, Ph. capsici, and Ph. citricola as the causes of the decline of cherry trees in Tehran province (Karaj region). Exadaktylou and Tomidis, (2005) reported some species of Phytophthora; Ph. citricola, Ph. citrophthora, Ph. cryptogea, Ph. nicotianae, Ph. syringae, and Ph. cactorum as the main causes of crown rot of cherry trees. Carianis and tumidis, (2008) investigated the susceptibility of 30 genotypes of cherry to Ph. citricola, Ph. citrophthora, Ph. cactorum, and Ph. parasitica. The genotypes A4/01 and A11 inoculated with Ph. parasitica and the genotypes A7/01, hybrid 9˚, and D1/99, inoculated with Ph. citrophthora, showed the most extensive necrosis. Whereas, the genotypes A14, D3/99, and D1/99 inoculated with Ph. parasitica and the genotypes B1/98 and D3/99 inoculated with Ph. citrophthora and the genotypes A4/01, A6/01, D4/99, D3/99, and A13 inoculated with Ph. citricola displayed the least extensive necrosis. Therefore, in order to control the crown rot of cherry trees it is suggested to apply resistant stocks, irrigation system modification, and soil drainage, and to decrease nitrogen fertilizer usage. Among mentioned solutions, applying resistant stocks is the most important one. So, this study has been conducted in order to evaluate the susceptibility of five dwarf genotypes of Mahaleb to the species Ph. citricola and Ph. citrophthora in laboratorial and greenhouse conditions.

MATERIALS AND METHODS

The species *Ph. citricola* and *Ph. citrophthora* used in this study were obtained from the fungi collection of Shiraz University (dedicated by Dr. BaniHashemi) and were used in all of the experiments. Among 30 seed-originated dwarf genotypes of Mahaleb, which were better in traits of horticulture features and more compatible with light and loamy soils and rocky land, five genotypes numbered as 268, 136, 155, 100, and 24 were used in this assay. These five genotypes were proliferated through growth proliferation method and their one-year-old samplings were maintained in greenhouse.

Evaluating the relative resistance of young cut twigs and one-year-old woody twigs

These experiments were based on method described by Jeffers et al, (1981). this experiment was carried out as factorial arrangement based on completely randomized experimental design. Factors were Mahaleb genotypes (268, 136, 155, 100, and 24) and fungi species (*Ph. citricola*, *Ph. citrophthora* and control). In order to carry out this experiment 50 conical flasks which could held 250 ml of water were used. Each flask was considered as a replicate and was prepared as following: 1.36 gr of CMA environment was added to each flask along with 60 ml of distilled water and stirred well. After closing the aperture of the flasks using aluminum foil, flasks got sterile in autoclave at 121˚C under 1.5 ATM pressure in 20 min. After cooling the medium (to 45˚C), each antibiotic was added to the flasks as following: pimaricin (10 mg L^{-1}), ampicillin (250 ml L^{-1}), Rifampin (10 mg L^{-1}), and PCNB (10 mg L^{-1}). Then, disks of the mycelium of both fungi (4 mm diameter) were inoculated on the mentioned environments in each flask (control flasks were inoculated with the disks free of fungi). After that, the apertures of the flasks were closed using aluminum foil and maintained in the incubators at 28˚ C in darkness for three weeks. Young and one-year-old twigs were cut in 13-15 cm length from the origin ten-year-old tree of the genotypes 268, 136, 24, 100, and 155 in late march and leached under slow flow of water. Then the twigs of each genotype were disinfected using a hypochlorite solution (4%) and maintained in distilled water for 10 min in order for the disinfection solution to get extracted. Then they were dried using a blotting paper. After that, with a sterile scalpel, 2 cm of the end of each twig was scraped obliquely and five twigs from each genotype were settled in each flask containing the well-grown fungi. Moreover, five disinfected twigs for each genotype were used as the control in the fungus-free CMA media. The flasks were again transferred into the incubators at 28˚C and maintained for 20 days and then for each genotype and each fungus species, the diameter and length of each twig and also type of symptoms were measured. Genotypes' screening was carried out based on method described by Horssfall, (1978).

Evaluating the relative resistance of perennial cut twigs

This experiment was carried out based on method described by Tomidis et al, (2008). According to this method some cut twigs (2 cm diameter, 10 cm length) were prepared. In a sterile environment a layer of the twig bark was separated partially and a disk of each isolates of five-day-old fungi on CMA media was placed under the separated bark. The scars were treated with glue and jelly materials in order to prevent dryness. For each genotype, six cut twigs were taken into consideration. For each fungus species three cut twigs were allocated and also for each genotype one cut twig, for which fungus-free media was applied, were considered as the control. Each cut twig was

considered as one replicate. Cut twigs were maintained in well-disinfected desiccators for 30 days at 25˚ C. Two ends of the twigs were covered with paraffin in order to prevent dryness. Afterward, the rate of symptom progress was recorded and analyzed based on the method described by Horssfall, (1978) using a completely randomized experimental design in a factorial experiment with two factors and four replicates. Evaluating and screening the genotypes was according the Horresfall, (1978) method. Observing the symptoms was done through separating the bark from upper and lower regions of the inoculated part and measuring the length of the discoloration area. Infected parts were cultured on semi-selective media PARP.

Relative resistance of the seedlings in greenhouse

In this experiment fifteen-month-old seedlings of Mahaleb were used. So, the seedlings were placed in the pots containing a soil with similar portion of clay, sand, and sterile humus. Pots were then maintained in the greenhouse at 25˚C for a few weeks. Analyses were carried out through a completely randomized experimental design in factorial experiment (five genotypes of Mahaleb and the control and two species of fungi). In order to inoculate the pots, the soil around each seedling was swept up to 3 cm in depth and then 10 gr of inoculation material of each fungus was placed around the crown and roots of each seedling (Alexander and Stewart, 2001). To determine the active presence of the fungi in the soil, the lower drainage hole of the pots was closed using solid paraffin and the soil of the pots was saturated with water. After 24 hr the drainage hole was opened and the pots' water was gathered in the plates below the pots. Twenty round disks of sour orange leaves (5 mm diameter) were placed in each plate. After 24 hr leaf disks were removed and leached with water flow. After getting dried on blotting paper, they were transmitted to the semi-selective media CMA-PARP (Banihashemi, 2004). Control pots were also inoculated according to the same method but with the perlite containing cannabis extract. After 60 days, seedlings were extracted from the soil charily and after leaching with water, the number of dead seedlings, amount of fungi progress on the roots, height of the seedlings, and the percentage of colonization of each tissue in each genotype was determined and the severity of the disease was evaluated according to the method described by broadbent and Gollnow, (1992). Statistical analysis was made using SAS 9.2 program. Differences between traits means were assessed using LSD Test. Diagrams draw with Excel program.

RESULTS AND DISCUSSION

Applying resistant stocks against pathogens is one of the important and economic strategies in the management of soil-borne diseases. The reaction of various stocks of fruit trees against various species of *Phytophthora* is different. For instance, Mahaleb stocks are significantly more susceptible to some species of Phytophthora in comparison with mazard (Browne et al, 1995; Ogawa et al, 1995; Wilcox, 1985; Wicks, 1989).

Result of variance analysis indicated five genotype had significant difference for susceptibility. Comparisons of mean show that genotypes 268 and 24 were the most susceptible to *Ph. citrophthora* and *Ph. citricola* and the most resistant genotype was 155. there was no significant difference between the two species of Phytophthora in terms of severity and type of symptoms, . But there was significant difference between the two ages of the twigs (young and one-year-old) in terms of the severity of symptoms expression (α=1%). One-year-old twigs expressed more severe symptoms compared whit young twigs. Investigating the reaction of cut perennial twigs of five genotypes of Mahaleb showed that all twigs expressed disease symptoms (rot, discoloring, rot and discoloring), but there was no symptoms seen on control twigs. Results of the comparison of the means showed that the genotypes 268 and 24 expressed the symptoms more than others and there was no significant difference between them (Figure 1). Furthermore, the most and the least rate of symptoms progress was observed on the genotypes 268 and 155. The genotype 155 was the most resistant one against symptom progress of two fungus species through the tissue of cut twigs (Figure 2). On the other hand, *Ph. citrophthora* was the more aggressive species in terms of colonization (Figure 3).

Sixty days after inoculation, infected plants and the controls were extracted from plastic pots and leached meticulously. The severity of the disease was evaluated using seven scales based on the method described by bradbent and Gollnow, (1992). Results indicated that the genotypes 268 and 24 were the most susceptible ones and the genotype 155, expressing the least amount of damage, was the most resistant (Table 2). Results of the present study show that the Iran's native dwarf genotypes are valuable unknown resources suitable for using against Phytophthora rots. Applying these native dwarf stocks, results in more density of cultivation. On the other hand, due to high compatibility to Iran's climate and resistance against root Phytophthora rots, yield and quality of products will increase and costs will decrease.

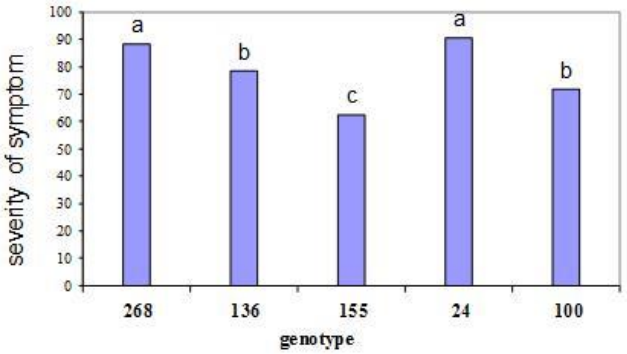

Figure 1. Measuring the effect of genotype on the severity of symptom expression by evaluating the reaction of young and one-year-old twigs against Ph. citrophthora and Ph. Citricola in the laboratory

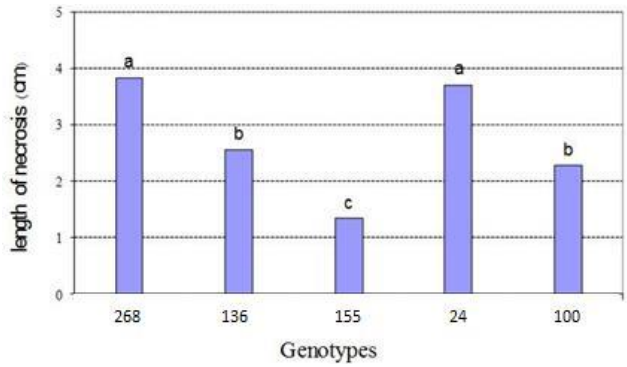

Figure 2. Measuring the effect of genotype by evaluating the reaction of cut perennial twigs of five Mahaleb genotypes against Ph. citrophthora and Ph*. citricola* in the laboratory

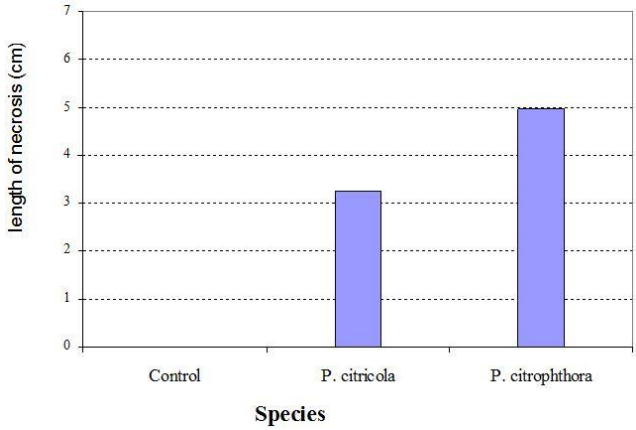

Figure 3. Measuring the effect of fungus species by evaluating the reaction of cut perennial twigs of five Mahaleb genotypes against Ph. citrophthora and Ph. citricola in the laboratory

Table 1. Score for percent of root rot

Severity of symptoms	Percent of root rot
0	0
1	%1-5
2	%6-15
3	%16-25
4	%26-40
5	%41-55
6	%56-75
7	%75-100

Table 2. Comparing the means resulted from evaluating the reaction of three genotypes of Mahaleb planted in pots against Ph. citrophthora and Ph. citricola in greenhouse

Factor		Type of the symptoms	height of the seedling from soil surface before inoculation (cm)	Height of the seedling from soil surface after inoculation (cm)	Number of leaves before inoculation	Number of leaves after inoculation	percentage of root rot
Genotype	268	3.55a	49.67a	79.44a	108.11a	92.00a	53.78a
	136	3.11a	37.83b	46.67b	74.44b	54.336	47.00b
	155	1.55b	42.67ab	57/00b	109.78a	76.67ab	38.44c
Species of fungi	control	.0001c	51.33a	70.83a	127.11a	147.22a	0.00b
	p.citricola	3/00b	30.00b	49.28b	75.78b	46.78b	67.44a
	p.citrophthora	5.22a	48.83a	63.00a	89.44b	29.00b	71.78a

* Values followed by the same letter within the same columns do not differ significantly at p = 5% based on LSD

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