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Research Article

Sources of Verticillium wilt resistance in wild olive germplasm from the Golestan province, Northern Iran

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Abstract: Nine wild olive accessions collected from the Golestan province, the North of Iran, were screened under greenhouse conditions for their resistance to Verticillium wilt. Plants of the highly susceptible cv. 'Zard', frequently used as a local cultivar, were also included in this test. Nine-month-old nursery olive plants were inoculated by root-dip method with defoliating (VCG1, D) and non-defoliating (VCG4B, ND) isolates of *Verticillium dahliae*, both obtained from diseased olives in Golestan province. Resistance was evaluated by assessing symptom severity using 0-4 rating scale and estimating the area under disease progress curves. The percentage of plants killed, final mean severity of symptoms, frequency of *V. dahliae* reisolation from olive xylem, dry weight of new green leaves and shoots and total phenol content in root tissues were used as additional parameters. The results showed that seven of the nine wild olive accessions were highly resistant to D and ND isolates of *V. dahliae*. A second group of wild olive accessions (P4 and P7) were classified as moderately resistant and resistant to D and ND isolates of *V. dahliae*, respectively. Phenol content was significantly higher in highly resistant plants and correlation coefficient analyses revealed a negative relation between disease severity and root total phenol contents. Dendrogram of wild olive accessions and 'Zard' cultivar based on all parameters represented two main clusters, major and minor. Minor cluster comprised only two wild olive accessions and 'Zard' cultivar. Major cluster could be divided into two groups, I and II, showed a highly resistance reaction to pathotypes of *V. dahliae*.

Keywords: Wild olive, defoliating and non-defoliating pathotypes, resistance, *Verticillium dahliae*

Introduction

Olive *Olea europaea* L., which is cultivated on large areas in the Mediterranean countries, is one of the most important agricultural crops in Iran, with 10.8% of tropical fruits located on 83941.9 ha (76433.4 hectare irrigated and 7508.5 hectare non-irrigated) (Anonymous, 2015). Olive cultivation has expanded during the last two decades especially in Golestan province, the North of Iran (Fig. 1). In this province nearly 22,000 hectare (15135.3 hectare irrigated and 6583 hectare non-irrigated) of olive orchards are present, which represents about 26% of total national olive area (Anonymous, 2015).

Various diseases and pests have negative effects on the yield and quality of olive. *Verticillium dahliae* Kleb., causing wilt in more than 500 crops (Sanei *et al*., 2008), is one of the most important pathogens of olive

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in Iran and has been reported in all important olive cultivation areas of the world (LÓpez-Escudero and Blánco-LÓpez, 2007). Verticillium wilt of olive (VWO) may limit the production of high-yielding and high quality olive cultivars grown extensively and intensively in different regions (Levin *et al*., 2007). In major areas of production, the pathogen regularly causes the death of many infected trees (Jiménez-Díaz *et al*., 2012; Trapero *et al*., 2013). In a study conducted in Greece, it was determined that the ratio of affected and dead olive trees due to Verticillium wilt reached 2-3% and 1%, respectively, within a tree population of 14 million (Thanassoulopoulos *et al*. 1979).

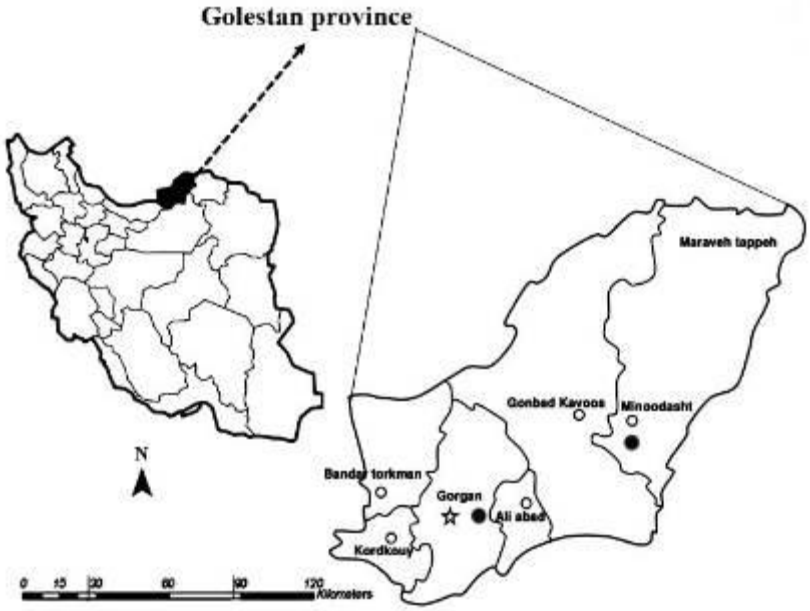

Figure 1 Iran map (left) and regional surveyed of wild olives (Golestan province, right with ●) in this study.

The new olive orchards established in Iran during the last 20 years have increased the importance of Verticillium wilt of olive (Sanei *et al*., 2004). Spread of the disease is linked to planting olive trees in fields previously used for growing susceptible hosts to the pathogen, or in those close to cotton fields infested with *V. dahliae* (Bejarano-Alcázar *et al*., 1996; LÓpez-Escudero and Blánco-LÓpez, 2001). The main factor for the increase in disease incidence and severity has been the establishment of orchards in fields previously cropped with *V. dahliae* host susceptible host especially cotton and vegetables (Sanei *et al*., 2004). Also, propagation of symptomless but infected planting material by nurseries and uncontrolled seedling production and distribution to the other provinces might have contributed to the introduction of Verticillium wilt to newly established orchards (Erten and Yıldız, 2011; Sanei *et al*., 2004).

Tree death and symptom recovery depend on the pathogenesis and ecology of *V. dahliae* (Jiménez-Díaz *et al*., 2012). *V. dahliae* isolates have two distinct pathotypes: defoliating (D) and non-defoliating (ND) (LÓpez-Escudero *et al*., 2004). The D pathotype is generally more aggressive than the ND, and causes faster disease development. Olive trees exhibit a high susceptibility to the D pathotype of pathogen, even at very low inoculum levels (LÓpez-Escudero and Blánco-LÓpez, 2007). In Iran, both pathotypes of *V. dahliae*, D (VCG1) and ND (VCG4B), were found infecting acer,

cotton, tomato and olive especially in the North (Sanei *et al*., 2008).

Many studies focusing on the control of Verticillium wilts of various crops, including olives have been conducted, but several characteristics of *V. dahliae* make its control difficult (Tsror *et al*., 2001). The pathogen survives in the soil for long periods of time (Wilhelm, 1955), attacks many dicotyledonous cultivated plants and weeds (Sanei *et al*., 2010), and chemical compounds are not effective against it (Erten and Yildiz, 2011). *V. dahliae* is generally controlled by a combination of preventive measures; among these is the use of resistant cultivars or root stocks (Markakis *et al*., 2010). Olive has a wide range of genetic variability. Use of resistant olive varieties is the most effective, economically feasible and ecologically sustainable means of control (Cirulli and Montemurro, 1976; Cirulli *et al*., 2008; Tjamos, 1993). For this purpose, a large number of olive cultivars have been evaluated for resistance to Verticillium wilt (Mercado-Blánco *et al*., 2003). In these studies, some cultivars exhibited a high level of resistance against *V. dahliae* pathotypes, showing less disease symptoms and vascular colonization by the pathogen compared with other olive cultivars (LÓpez-Escudero *et al*., 2004).

As grafting is the most common olive propagation technique (Porras Soriano *et al*., 2003), the use of resistant rootstocks could effectively contribute to the control of *V. dahliae*, even if the scion is susceptible. The wild olive germplasm throughout the Golestan province may include valuable sources of new and more effective resistances to *V. dahliae* pathotypes (Sanei and Razavi, 2011), as exemplified by some olive genotypes that can be seen in the East of Golestan province (Sanei *et al*., 2005). The purpose of this work was to screen wild olive with a view to examining their response to D and ND pathotypes of *V. dahliae*. This research describes resistance of wild olive germplasm for potential use as rootstocks or as sources for resistance in future breeding programs.

Materials and Methods

Plant and fungal material

Wild olives growing naturally in non-cultivated districts were considered in this study (Fig. 1). Nine-month-old rooted cuttings of nine wild olive accessions, were used to test the susceptibility to *V. dahliae* infection. In the experiment, nine-month-old olive 'Zard' cultivar was used as susceptible (control) to *V. dahliae* pathotypes (Sanei and Razavi, 2011). Highly virulent isolates of *V. dahliae*, D12 a D type (VCG1), and ND13 a ND type (VCG4B), isolated from olive 'Zard' cultivar in Golestan province were provided from the culture collection of the Plant Pathology Laboratory of the Plant Protection Department, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.

Inoculum production and inoculation

Inoculum was prepared from single-spore cultures of isolates, maintained on potato dextrose agar (PDA) slants at 4 °C. Inoculations were made with a conidial suspension prepared from 7-day-old cultures grown on potato-dextrose-agar (PDA) in Petri dishes at 25 ± 0.5 °C in the dark. The cultures were flooded with sterile distilled water and their surface was gently scraped with a sterile scalpel. The resulting suspension was filtered through cheesecloth to remove mycelial fragments. After filtration, the inoculum concentration was adjusted to 4×10^6 conidia per ml. Plants were inoculated by dipping the bare root system of each plant into the inoculums for 30 min (Colella, *et al*., 2008). The plants were then transplanted individually into sterile plastic pots containing sterile sand:field soil mixture (1: 1) and moved to a glasshouse. The experiments were performed from April to June in the greenhouse with air temperature fluctuating between 20 °C and 30 °C. Non-inoculated control plants were subjected to the same process described above, but treated with distilled sterile water. The plants were arranged on greenhouse benches according to a

randomized block design. Five replications were used per experiment and repeated twice.

Disease assessment

Disease severity was evaluated on a weekly basis for 13 weeks, starting 2 weeks after inoculation. Wilt resistance was assessed on a scale from 0 to 4 based on the percentage of plant tissue affected by chlorosis, leaf and shoot necrosis or defoliation (0 = healthy plant or plant without symptoms; 1 = plant affected at 1%-33%; 2 = 34%-66%; 3 = 67%-99%; 4 = dead plant). The percentage of dead plants, recovery from the disease (LÓpez-Escudero and Blanco-LÓpez, 2001; Wilhelm and Taylor, 1965) and other symptoms such as marginal leaf spots and irregular growth of twigs were also considered to estimate the severity of reactions. The area under the disease progress curve (AUDPC) was estimated based on Campbell and Madden (1990) according the following formula: AUDPC = [*(t*/2 * *(S*2 +2 **S*3+· · ·+ 2*Si*−1 + *Si)*/4 **n*] *100 where, *t* = interval in days between observations; *Si* = final mean severity; 4 = maximum disease rating; *n* = number of observations. Plant infection was confirmed by the isolating the fungus from affected shoots.

Classification of resistance in cultivars

The resistance of each olive cultivar to wilt was categorized according to the criteria of LÓpez - Escudero *et al*. (2004, Table 2).

Colonization index

To determine the colonization index, all leaves of inoculated and non-inoculated (control) cuttings were removed and the stem was disinfected by 1% NaOCl for 10 min, washed, dried and its epidermis peeled. Four segments (5 mm length) from different parts were cut and placed on C'zapeck Dox Agar (supplemented with 100 ppm streptomycin) for 14 days at 25 ± 1 ºC. Once *V. dahliae* was detected the segment was considered as infected. Colonization index (CI) was calculated as follows: CI = (2 × Nb + 6 × Nt)/N, where Nb, is the number of infected segments at the base of the stem- cutting, and Nt is the number of infected segments at the top (10 cm above the base), and N is the total number of tested segments. The number of infected segments from the base and upper parts was multiplied by the coefficient factors of 2, and 6, respectively, resulting in a calculated colonization index in range between 0 and 8. As detection of *V. dahliae* at the upper parts of the stem is less frequent than at the base, detection at the upper parts may reflect a higher level of plant colonization (Tsror *et al*., 2001).

Dry weight (DW)

For evaluating the influence of Verticillium wilt progress on the vegetative growth of olive plants, the tips of all shoots of each plant were marked with an indelible marker at the last node. At the end of the experiments, the fresh weight of new tissues grown from marked points at plant shoot tips were collected separately in paper envelopes and dried in an oven at 70 °C for 48 h, and dry weight of the samples was recorded.

Total phenol content

Biochemical changes in olive plant upon root inoculation were monitored in order to determine the effect of this inducer agent on the contents of total phenol in the plant. The changes in the content of total phenol in roots were estimated at 90 days after inoculation by *V. dahliae*. Colorimetric determination of total phenols was based on the procedure of Folin-Ciocalteau as described by Sofo *et al*. (2004) and results are expressed as milligrams of tannic acid equivalents per gram dry weight. There were two replications per assay of sample.

Statistical analysis

The analysis of variance (ANOVA) of AUDPC, severity of external wilt symptoms 90 days after inoculation and frequency of *V. dahliae* reisolation from olive xylem of reference-cultivars in each experiment were performed to determine the variability among experiments. Cluster analysis of data from all disease parameters using the UPGMA method, was conducted to generate a dendrogram showing relationships between

isolates with regard to their virulence to the wild olive accessions and cultivar 'Zard'. Statistical analysis was performed by R.3.3.1 program. Mean values were compared by the by the Fisher's protected LSD at $P \leq 0.05$.

Results

No Verticillium wilt symptoms were observed in non-inoculated control plants, nor was the pathogen reisolated from any of them. Interactions between wild olive accessions or 'Zard'cultivar and *V. dahliae* isolates were significant for each dependent variable (AUPDC, severity of Verticillium wilt external symptoms 90 days after inoculation and frequency of *V. dahliae* reisolation from olive xylem), and analyses of variance were conducted separately for each *V. dahliae* pathotype (Table 1). It was found that seven out of nine wild olive accessions were highly resistant and two of them were moderately resistant based on their ability to withstand infection by the D pathotype.

Table 1 Mean disease parameters assessed in the wild olive accessions or 'Zard' cultivar inoculated with the defoliate and nondefoliate isolates of *Verticillium dahliae*.

Variability factors	Severity of Verticillium wilt external symptoms		Frequency of *V. dahliae* re-isolation from olive xylem (%)	Resistance level[3]	Percentage of dead plants
	AUDPC[1]	90 dai[2] (0-4 scale)			
P values					
Block	0.0037	0.0350	0.7230		
Wild olive accessions or cultivar	< 0.0001	< 0.0001	< 0.0001		
Pathotype	< 0.0001	< 0.0001	< 0.0001		
Wild olive accessions or cultivar × pathotype	< 0.0001	< 0.0001	< 0.0010		
Mean values of disease parameters for defoliating isolate (VCG1)[4]					
P-1	0.00 e	0.00 e	1.34 de	HR	0
P-2	0.00 e	0.00 e	1.37 de	HR	0
P-3	0.00 e	0.00 e	1.56 cde	HR	0
P-4	44.35 b	2.75 b	3.40 b	MR	0
P-5	0.00 e	0.00 e	1.32 e	HR	0
P-6	0.00 e	0.00 e	1.12 ef	HR	0
P-7	44.60 b	2.87 b	3.27 b	MR	0
P-8	0.00 e	0.00 e	1.44 de	HR	0
P-9	0.00 e	0.00 e	1.50 cde	HR	0
'Zard'	74.52 a	3.62 a	4.50 a	ES	60
Mean values of disease parameters for non-defoliating isolate (VCG4B)					
P-1	0.00 e	0.00 e	0.56 g	HR	0
P-2	0.00 e	0.00 e	0.62 g	HR	0
P-3	0.00 e	0.00 e	0.68 fg	HR	0
P-4	19.97 d	1.37 c	1.50 cde	R	0
P-5	0.00 e	0.00 e	0.68 fg	HR	0
P-6	0.00 e	0.00 e	0.60 g	HR	0
P-7	22.02 d	1.50 c	1.81 cd	R	0
P-8	0.00 e	0.00 e	0.56 g	HR	0
P-9	0.00 e	0.00 e	0.56 g	HR	0
'Zard'	30.10 c	2.73 b	1.97 c	ES	0

1. AUDPC: Area under disease progress curve estimated as the percentage with regard to the maximum potential value.
2. dai = days after inoculation.
3. Resistance level of each cultivar as concluded from the AUDPC, final Severity of Verticillium wilt external symptoms and percentage of dead plants values (Table 2). ES = extremely susceptible; S = susceptible; R = resistant; HR = highly resistant.
4 Values in columns followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

Mean disease parameters assessed showed that seven wild olive accessions were highly resistant in reaction to ND pathotype of *V. dahliae* (Table 1). The percentage of dead plants was zero for all wild olive accessions for reaction to both *V. dahliae* pathotypes, and dead plants were observed only in 'Zard' cultivar (extremely susceptible) to the D pathotype (Table 1).

Table 2 Resistance categories and disease parameters, for reactions of olive cultivars to *Verticillium dahliae* (López-Escudero *et al*., 2007).

Resistance category	AUDPC[a]	FMS[b]	PDP[c]
Highly resistant	0-10	0.0-1.5	0
Resistant	11-30	0.0-1.5	0
Moderately resistant	31-50	1.5-2.5	0-30
Susceptible	51-70	2.5-3.0	31 - 50
Extremely susceptible	71-100	3.0-4.0	51-100

a. AUDPC, Area under disease progress curve.
b. FMS, final mean severity of symptoms.
c. PDP, percentage of dead plants.

Resistance categories, and disease parameters, for reactions of wild olive accession inoculated with the D and ND pathotypes of *V. dahliae* shows that the pathotypes had different effects ($P \leq 0.01$) on AUDPC, final mean severity of Verticillium wilt external symptoms, frequency of *V. dahliae* reisolation from olive xylem, root total phenols and dry weight of new green leaves and shoots 90 days after inoculation (Table 3). Analysis of variance revealed that differences of reduction in the production of vegetative growth in inoculated plants were significant only for D pathotype ($P \leq 0.01$). The reduction was remarkably higher in P4, P7 of wild olive and 'Zard' cultivars about 27.20%, 33.60% and 35.96%, respectively (Fig. 2). For other wild olive accessions, the reduction was between 16.66 and 20.71. Relationship between dry weight of new green leaves and shoot with AUDPC fit polynomial curve with high R^2 only for D pathotype (0.704, Fig. 3) which show that the rate of dry weight of new green leaves and shoot was significantly correlated ($P \leq 0.001$) with the variable AUDPC (Fig. 4).

Quantification of total phenols in inoculated plants revealed that the inoculation significantly induce the accumulation of total phenol content in root. The pathotypes of *V. dahliae* have different effects ($P \leq 0.01$) on phenols accumulation in olive root. Inoculation showed an increase of 1.1-1.8-fold and 1.1-2.22 fold for D and ND pathotypes as compared to the control plants, respectively (Fig. 4). Relationship between root total phenols of wild olive accessions or cultivar with severity of Verticillium wilt external symptoms 90 days after inoculation fit polynomial curves $Y = 0.014X^2-0.832X + 12.13$, $Y = 0.006X^2-0.433X + 7.59$ and $Y = 0.010X^2-0.652X + 9.88$ for D, ND pathotypes and for all data, respectively (Fig. 5). Correlation analysis revealed a negative correlation (r = -0.794 and-0.828, $P \leq 0.001$ for the D and ND pathotypes, respectively) between total phenol content and severity of Verticillium wilt external symptoms 90 days after inoculation.

Table 3 Resistance categories and disease parameters for reactions of wild olive accessions inoculated with the defoliate and non defoliate isolates of *Verticillium dahliae.*

Entries	Control	Defoliating isolate	Nondefoliate isolate
AUDPC[1]	0.00 c	16.23 a	7.13 b
FMS[2]	0.00 c	0.96 a	0.56 b
V. dahliae reisolation[3]	0.00 c	2.08 a	0.96 b
Root total phenols[4]	14.30 c	22.87 a	26.58 b
Dry weight[5]	5.03 a	3.91 b	5.02 a

1 AUDPC: Area under disease progress curve estimated as the percentage with regard to the maximum potential value.
2 FMS, final mean severity of Verticillium wilt external symptoms 90 days after inoculation.
3 Frequency of *V. dahliae* reisolation from olive xylem (%).
4 Root total phenols, milligrams of gallic acid equivalents per gram dry weight of root tissue.
5 Dry weight of new green leaves and shoots (g).
Values in columns followed by the same letter are not significantly different ($P = 0.05$) according to Fisher's protected least significant difference test.

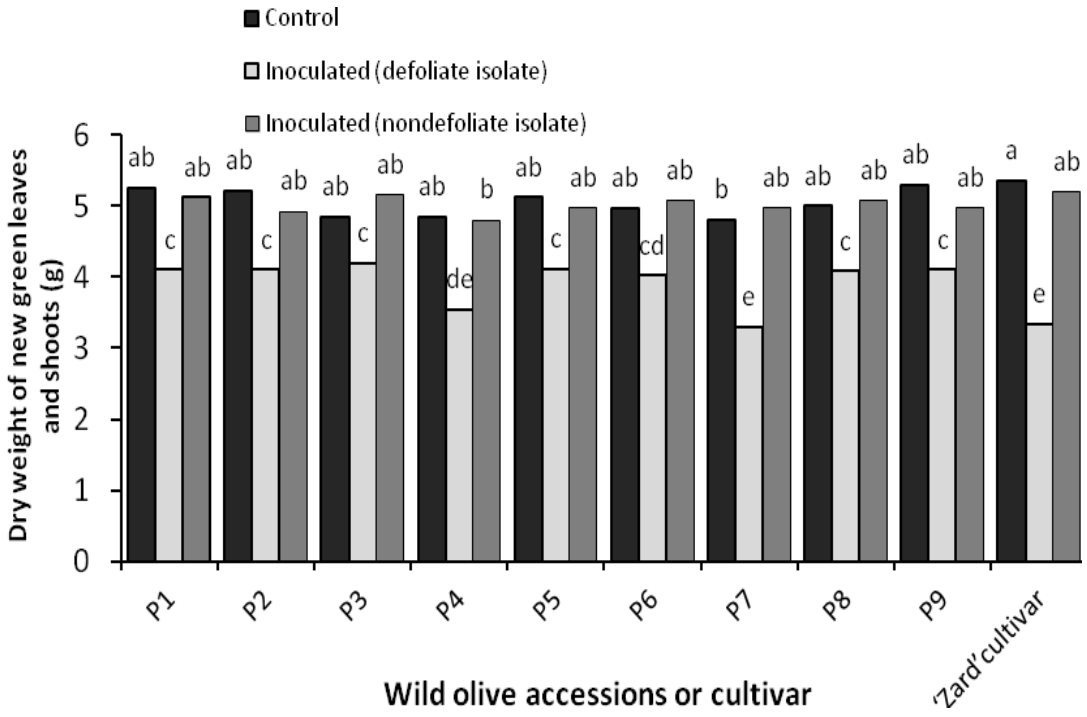

Figure 2 Dry weight of new green leaves and shoots (gram) of wild olive accessions or 'Zard'cultivar in relation to inoculation with defoliating or nondefoliating *Verticillium dahliae* pathotypes. Values followed by the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test.

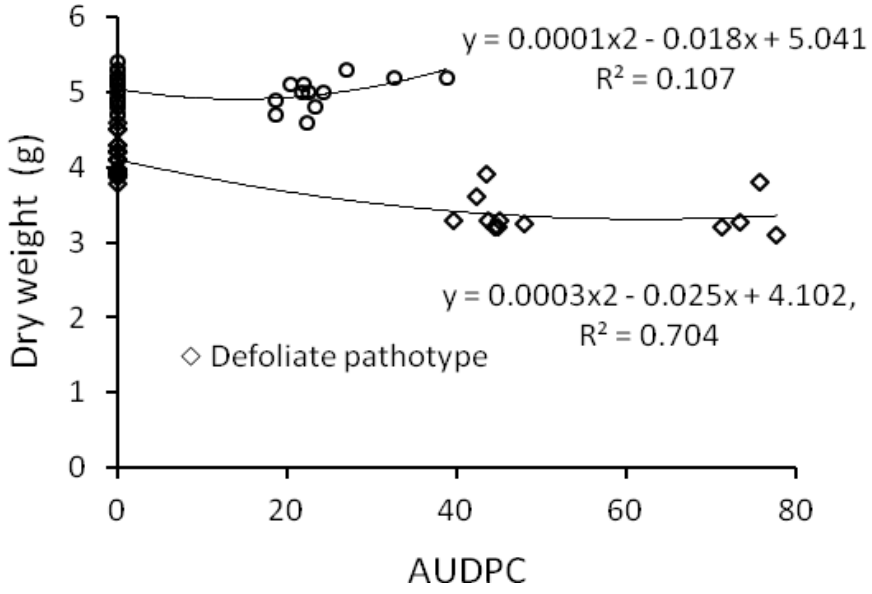

Figure 3 Relationship between dry weight of new green leaves and shoots of wild olive accessions or 'Zard' cultivar with area under the disease progress curve (AUDPC).

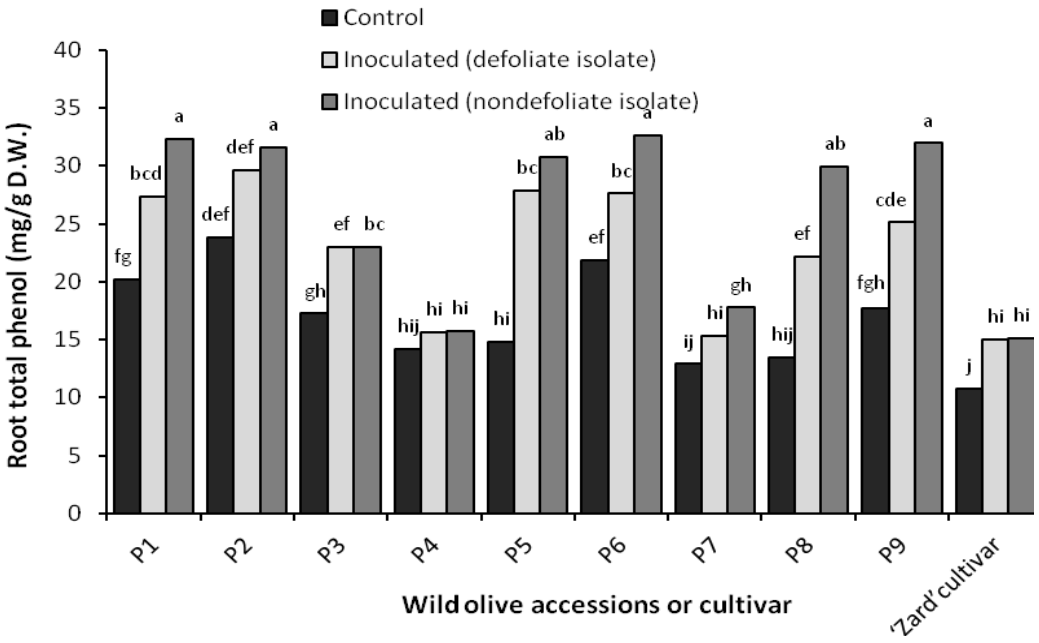

Figure 4 Root total phenols (milligrams of gallic acid equivalents per gram dry weight of root tissue) of wild olive accessions or 'Zard' cultivar in relation to inoculation with defoliating or nondefoliating *Verticillium dahliae* pathotypes. Values followed by the same letter are not significantly different (P = 0.05) according to Fisher's protected least significant difference test.

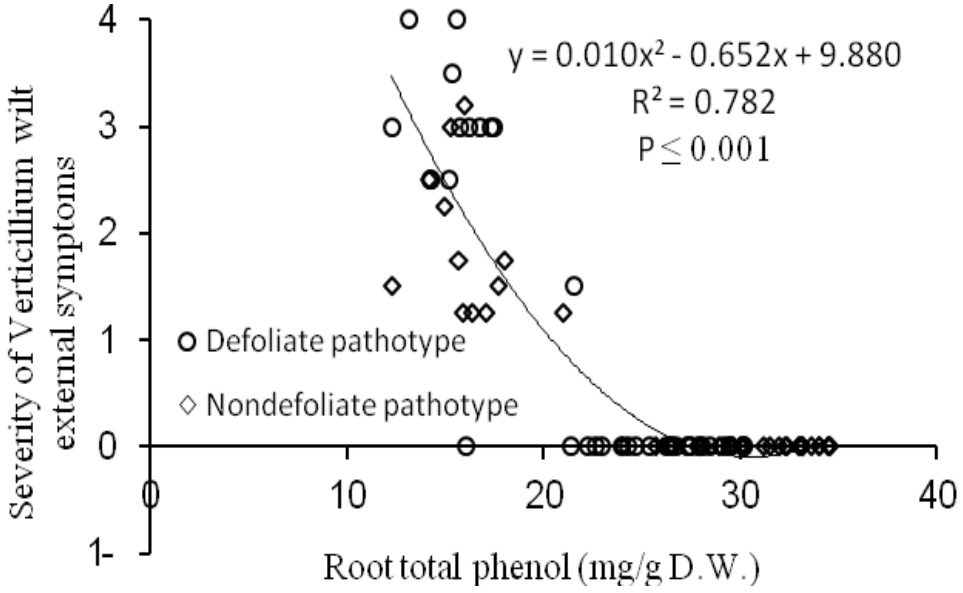

Figure 5 Relationship between root total phenols (milligram of gallic acid equivalents per gram dry weight of root tissue) of wild olive accessions or 'Zard' cultivar with severity of Verticillium wilt external symptoms 90 days after inoculation (0 - 4 scale).

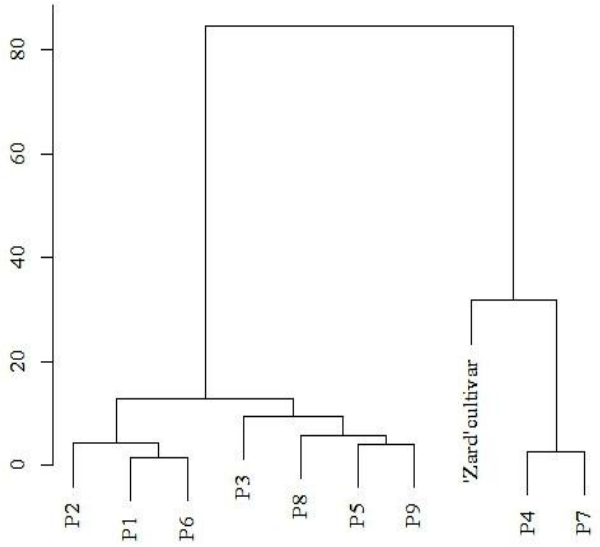

Figure 6 Dendrogram showing differences in reaction of wild olive accessions and 'Zard' cultivar based on their ability to infect with defoliate and nondefoliate isolates of *Verticillium dahliae*.

Dendrogram of wild olive accessions and 'Zard' cultivar based on the AUDPC**,** severity of Verticillium wilt external symptoms 90 days after inoculation, frequency of *V. dahliae* reisolation from olive xylem dry weight of new green leaves and shoots and root total phenols in infected plants with D and ND pathotype of *V. dahliae* are shown in Fig. 6. The dendrogram represented two main clusters, major and minor. Minor cluster comprised only two wild olive accessions and 'Zard' cultivar. Major cluster could be divided into two groups, I and II, all wild type accessions of which showed a highly resistant reaction to both pathotypes of *V. dahliae*.

Discussion

Verticillium wilt caused by *V. dahliae* is the most destructive disease of olive trees worldwide, and because of the lack of a single effective control measure, its management is currently based on an integrated approach (Erten and Yıldız, 2011). Use of resistant olive varieties is the most effective, economically feasible and ecologically sustainable means of control.

Several scion cultivars and rootstocks resistant/tolerant to Verticillium wilt have been identified in olive germplasm. It is shown that cvs 'Frantoio', 'Coratina', 'Frangivento', and 'Kalamon' have good resistance properties while cvs 'Ascolana', Cellina', 'Leccino', 'Manzanillo', 'Chemlalie', 'Konservolia', 'Mission' and 'Picual' are susceptible (Cirulli *et al*., 2008). Olive cultivars 'Frantoio', 'Empeltre' and 'Koroneiki' are considered the most resistant/tolerant to both *V. dahliae* pathotypes among over 120 world cultivars tested (Bubici and Cirulli 2012). Three olive rootstocks, namely 'Oblonga' (Hartmann *et al*., 1971) and 'Berkeley 117' (Wilhelm *et al*., 1974) in California, and two wild olive rootstocks, designated 'OutVert' and 'StopVert' in Italy (Bubici and Cirulli 2012) were identified as resistant only to ND pathotype. Three accessions showed high resistance to both *V. dahliae* pathotypes in wild olive germplasm from the Mediterranean region (Colella *et al*., 2008).

As cultivars tolerant to Verticillium wilt may not meet market requirements or oil quality, grafting of susceptible cultivars onto resistant rootstocks, instead, would combine these features. Resistant rootstocks are widely used in several horticultural and woody crops mainly to control soil-borne fungal, bacterial and viral pathogens, nematodes, insects and abiotic stresses such as salinity and calcareous soils (Levin *et al*., 2007). A variable effectiveness of grafting against Verticillium wilt has been observed in eggplant (Bletsos *et al*., 2003; Ioannou, 2001) and tomato (Paplomatas *et al*. 2002). In olive, Tjamos (1993) and Porras-Soriano *et al*. (2003) have dealt with experiments demonstrating the control of Verticillium wilt by using susceptible cultivars grafted onto resistant rootstocks. While grafting is a common technique in grapevine and stone fruit trees, it is not so in olives, except for southern Italy.

This study demonstrated the high effectiveness of resistant rootstocks in the control of olive Verticillium wilt, as no or low disease external symptoms developed on wild olive accessions. This research also showed that wild olive germplasm from the geographical areas of the North of Iran was very variable in resistance to D and ND pathotypes of *V. dahliae*. Some accessions were found to be highly resistant to Verticillium wilt. The higher value of disease indices with D12 confirmed the aggressiveness of the D pathotype and colonizing colonized the olive plant faster by D pathotype than by the ND one (Rodriguez Jurado *et al*., 1993; Bejarano-Alcázar *et al*., 1996). Because the pathogen was isolated from symptomless plants (Table 1), disease escape (Colella *et al.*, 2008; Wilhelm and Taylor, 1965) could not explain resistant characteristics.

Several physiological alterations may occur in infected plants that influence, among other things, photosynthesis, nutrient translocation, water transport, and/or respiration (Sadras *et al.*, 2000). The dry weights of plants have been reported as the major parameters used to assess the differential vegetative growth in plants with different levels of resistance to abiotic and biotic stress (Birem *et al*., 2016). Several reports showed the reduction in the vegetative growth of various herbaceous hosts due to vascular wilts caused by *Verticillium* spp. or *Fusarium* spp. (Karagiannidis *et al*., 2002). In this study, growth reduction was related to the susceptibility level of the evaluated wild olive accessions and 'Zard' cultivar especially to D pathotype. Conversely, plants of the resistant genotype exhibited few symptoms, and they produced new shoots and leaves after inoculation. This study showed that infections caused by the D isolate of the pathogen reduced the growth of inoculated plants of genotypes, although the reduction was significantly higher in P4, P7 of wild olive and 'Zard' cultivar than the other accessions (Fig. 2). A negative correlation was revealed between dry weight of new green leaves and shoot with AUDPC for D isolate (Fig. 4).

Plant disease resistance can be defined as the ability of the plant to prevent or restrict the pathogen growth and multiplication. The resistance of plant disease can be defined as the ability of the plant to prevent or restrict the pathogen growth and multiplication (Cayuela *et al*., 2006). All plants, whether resistant or susceptible, react to pathogen attack by inducing defense, which is designed to affect pathogen growth and viability. Resistance prior to pathogen infection increase the level of some defense compounds and sensitize the plants after infection to produce rapidly some compounds and thereby, provide protection against the disease (Biswas *et al*., 2012). Several authors have reported the increased accumulation of phenolic compounds in plant tissues after pathogen infection (Markakis *et al*., 2010; Roussos *et al*., 2002). Polyphenols are well known as antifungal, antibacterial and antiviral compounds and play an important role in the resistance of plants to pathogen attack because they belong to the antimicrobial defense reaction (Ryan and Robards, 1998; Von Ropenack *et al*., 1998). It has been shown that some phenolic substances of olive trees may inhibit the growth of *Phytophthora* (Del Rio *et al*., 2003) and *Pseudomonas savastanoi* pv.

savastanoi (Cayuela *et al.*, 2006; Roussos *et al*., 2002). Furthermore, Baidez *et al*. (2007) reported the antifungal activity of oleuropein (an *o*-diphenol present in olive trees) against *V. dahliae in vitro*. In the present study, a higher accumulation of total phenols was observed in the roots of *V. dahliae*-infected wild olive accessions and 'Zard' trees compared with the noninfected plants.

Conclusions

This research identified wild olive accessions with high levels of resistance to Verticillium wilt. The selected accessions in this research could be tested as resistant rootstocks, against *V. dahliae*, especially against D pathotypes for susceptible local olive cultivars. Once characterized by the use of molecular markers, these new sources of resistance could also be propagated for future use in breeding programmes for resistance to Verticillium wilts.

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منابع مقاومت به پژمردگی ورتیسیلیومی در ژرم‌پلاسم زیتون وحشی از استان گلستان

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چکیده: نُه توده زیتون وحشی از استان گرگان به‌منظور بررسی مقاومت به پژمردگی ورتیسیلومی (با عامل *Verticillium dahliae* Kleb.) در شرایط گلخانه مورد بررسی قرار گرفتند. از رقم بومی زرد نیز به‌عنوان شاهد حساس به پژمردگی ورتیسیلومی استفاده شد. نهال‌های نُه ماهه زیتون با جدایه‌های برگ‌ریز (VCG1، D) و غیربرگ‌ریز (VCG4B، ND) از *V. dahliae* جداسازی شده از درختان زیتون آلوده منطقه گرگان، به‌روش غوطه‌ور کردن ریشه مایه‌زنی شدند. مقاومت به پژمردگی از طریق ارزیابی شدت علائم بیماری با استفاده از مقیاس۰ تا چهار محاسبه شد و سطح زیر منحنی پیشرفت بیماری کمّی گردید. درصد گیاهان خشک شده، میانگین شدت علائم نهایی، فراوانی جداسازی *V. dahliae* از بافت آوندی، وزن خشک برگ و شاخه‌های جدید و مقدار فنل کل در بافت ریشه نیز مورد سنجش قرار گرفت. نتایج نشان داد که هفت توده از نُه توده زیتون وحشی مورد بررسی نسبت به جدایه‌های D و ND از *V. dahliae* بسیار مقاوم هستند. گروه دوم از توده‌های زیتون وحشی (P4 و P7) در مقابل جدایه‌های D و ND به‌ترتیب مقاومت متوسط و مقاومت نشان دادند. مقدار فنل در گیاهانی با مقاومت بیش‌تر نسبت به گیاهانی با مقاومت متوسط و بسیار حساس به‌طور معنی‌داری بالاتر بود و تجزیه و تحلیل هم‌بستگی، ارتباط منفی بین شدت علائم و مقدار فنل کل ریشه را نشان داد. دندروگرام توده‌های زیتون وحشی و رقم زرد براساس تمام صفات اندازه‌گیری شده، دو کلاستر شامل کلاستر اصلی و فرعی را مشخص نمود. کلاستر فرعی تنها دو توده از زیتون وحشی و رقم زرد را شامل بود. کلاستر اصلی با مقاومت بالا به پاتوتیپ‌های *V. dahliae*، به دو گروه I و II تقسیم شد.

واژگان کلیدی: زیتون وحشی، پاتوتیپ های برگ‌ریز و غیر برگ‌ریز، مقاومت، *Verticillium dahliae*