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Resistance and vegetative growth analysis of some olive cultivars in response to a defoliating pathotype of *Verticillium dahliae* Kleb

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Abstract
Verticillium dahliae Kleb. the causal agent of vascular wilt is an important pathogen of olive trees in growing areas of the world. Nine-month-old nursery olives including 'Bladi', 'Conservalia', 'Kalamon', 'Koroneiki', 'Manzanilla', 'Mission', 'Marry', 'Picual', 'Rowghani', 'Sevillana' and 'Zard' cultivars were root-dip inoculated with a defoliating (VCG1) isolate of *V. dahliae* obtained from diseased olives. 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 and final mean severity of symptoms were used as additional parameters for classifying the cultivars. Most of the tested cultivars were susceptible or highly susceptible to Verticillium wilt. However, two genotypes 'Kalamon' and 'Koroneiki' exhibited high resistance or resistance to this disease. Vegetative growth was reduced in inoculated plants due to infections caused by *V. dahliae*, although the reduction was remarkably higher in susceptible than in resistant cultivars. Correlation coefficient analyses revealed a negative relation between disease severity and vegetative growth. Dendrogram of olive cultivars based on all parameters represented two main clusters, major and minor. Minor cluster comprised only 'Kalamon' and 'Koroneiki' cultivars as resistant to *V. dahliae*. The major cluster could be divided into two groups, exhibiting extremely susceptible or susceptible reaction to *V. dahliae*.

Keywords: Defoliating pathotype, olive, resistance, *Verticillium dahliae*, vegetative growth.

Introduction

Olive (*Olea europaea* L.) is considered as one of the most important tree species cultivated throughout the Mediterranean basin due to its high nutritive value as well as its antioxidant and curative properties (Colella et al., 2008**)**. In Iran, olive as a traditional woody crop cultivated over large areas about 16000 hectares in 2001 to 83941.9 hectares in 2015 (76433.4 hectares irrigated and 7508.5 hectares non-irrigated) (Anonymous, 2015). Olive cultivation has expanded during the last two decades especially in Golestan province, in the northern part of Iran. In this province, nearly 22,000 hectares (15135.3 hectares irrigated and 6583 hectares non-irrigated) of olive orchards are present, which represents about 26% of the total national olive area (Anonymous, 2015). In the last decade most of the new plantations in this region established with 'Rowghani', 'Zard' and 'Marry' cultivars, which are the native olive cultivars of Iran (Sanei et al., 2004). Commercial cultivars of olive are also planted in Iran (Saremi et al., 2006).

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Olive is cultivated in a wide variety of soils and can tolerate a broad range of physicochemical conditions. However, the olive crop is threatened by many pest and diseases that could notably affect the yield and quality of olive oil. Verticillium wilt of olive (VWO) caused by the soil-borne fungi *Verticillium dahliae* Kleb., is one of the most diseases affecting olive trees (López-Escudero and Blánco-López, 2007). Particularly, the disease has been described as a major problem in soils infested with highly virulent defoliating isolates of the pathogen in Andalucıa (southern Spain) (López-Escudero et al., 2007), Turkey (Erten and Yıldız, 2011) and Iran (Sanei et al., 2005a). VWO may limit the production of high-yielding and high-quality olive cultivars which are grown extensively and intensively in different regions (Levin et al., 2003, 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). However, Verticillium wilt usually develops into a chronic syndrome on trees older than 20-25 years, whereas can develop into an acute syndrome in young trees, which eventually die within a relatively short time (Rodríguez et al., 2008; Sanei et al., 2004). Thus, the disease incidence and severity are usually highest in young trees (Bubici and Cirulli, 2012). Spread of the disease is linked to planting olive trees in fields previously used for growing susceptible hosts to the pathogen (Blanco-López et al., 1984), or in those close to fields infested by *V. dahliae* (Bejarano-Alcázar et al., 1996; López-Escudero and Blánco-López, 2001; Sanei and Razavi, 2011). VWO is currently the most threatening disease for olive crops in Iran. In major areas of production, such as Golestan province, the pathogen regularly causes the death of many infected trees (Sanei et al., 2004; Saremi et al., 2006). The main factor for the increase in disease incidence and severity has been the establishment of orchards in fields previously cropped with *V. dahliae* susceptible host such as cotton and vegetables in the case of Golestan region (Sanei et al., 2010; Sanei and Razavi, 2011).

Several characteristics of *V. dahliae* make control of Verticillium wilt difficult (Tsror et al., 2001). The pathogen can survive in the soil for long periods of time (Wilhelm, 1955), attack many dicotyledonous cultivated plants and weeds (Sanei et al., 2010), and chemical compounds are not effective (Erten and Yildiz, 2011). Like other vascular wilt diseases, planting resistant cultivars is the most effective tool for reducing spread of this disease (Antoniou et al., 2008; CalderÓn et al., 2014; Colella et al., 2008; Bubici and Cirulli, 2011; Jiménez-Díaz et al., 2012).

Olive provides a wide genetic resource because more than 800 olive cultivars are introduced in collections around the world (Caballero and Del Rıo, 2008). Unfortunately, major cultivars from Spain, Italy, and other important olive oil-producing countries are susceptible to this pathogen (Cirulli and Montemurro, 1976; López -Escudero et al., 2004; Martos-Moreno et al., 2006). Efforts have been focused on the assessment of olive resistance to Verticillium wilt, aimed at selecting resistant cultivars for breeding or rootstock selection programs or for replanting trees killed by wilt in established olive orchards (Rodríguez et al., 2008).

Knowledge of the changes in some physiological parameters during disease progression would help us to better understand the resistance mechanisms in plants, but the research is only limited to vegetative growth in plants of resistant and susceptible olive cultivars such as 'Picual' and 'Frantoio', which infected by *V. dahliae* (Birem et al., 2016). The interaction between isolates of *V. dahliae* with different pathotypes and Iranian olive cultivars show that the defoliate pathotype causes higher disease severity index and stem colonization (Sanei and Razavi, 2011). The spread of the defoliating pathotype of *V. dahliae* in Iran (Sanei et al., 2005a, 2008) and its presence

in commercial olive orchards (Sanei et al., 2004, 2008) make it necessary to determine which Iranian and commercial olive cultivars have higher resistance to *V. dahliae* and the influence of disease on vegetative growth.

The objective of this work was to evaluate the resistance of Iranian and commercial olive cultivars to defoliating pathotype of *V. dahliae*, so that resistant cultivars can be identified and used for replanting, as rootstocks or as sources for resistance in future breeding programs. This research was also aimed to assess the progress of vegetative growth in resistant and susceptible olive cultivars infected by *V. dahliae*.

Materials and methods

Plant and fungal material

In the experiment, nine-month-old olive cultivars ('Bladi', 'Conservalia', 'Kalamon', 'Koroneiki', 'Manzanilla', 'Mission', 'Marry', 'Picual', 'Rowghani', 'Sevillana' and 'Zard'), propagated as rooted cuttings, were used to analyze the susceptibility to *V. dahliae* infection. Monoconidial *V. dahliae* isolate D12 representatives of the defoliate pathotype (VCG1), was used in this study. The isolate was originated from diseased olive plants ('Zard' cultivar) in Golestan province, was 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 (Sanei and Razavi, 2017).

Pathogen inoculum and inoculation

The inoculum was prepared from 7-day-old cultures grown on potato dextrose agar (PDA) on Petri dishes at 25±1°C in the dark. A conidial suspension was obtained by flooding the colonies with sterile distilled water and filtering the suspension through cheesecloth to remove mycelial fragments. The concentration of conidia was adjusted with sterile distilled water to 4×106 conidia per ml. Inoculations were performed by dipping the bare root system of each one into the inoculum for 30 min (Colella et al., 2008). Roots of control plants (noninoculated) were dipped in distilled sterile water. The plants were transplanted individually into sterile plastic pots containing sterile soil (1:1, sand:field soil) in sterile pots and placed in a glasshouse with air temperature fluctuating between 20 °C and 30 °C. The plants were arranged in the greenhouse benches as a randomized block design with five replications.

Disease assessment

Disease severity was assessed weekly from 2 till 13 weeks after the inoculation in both experiments. Disease symptoms were evaluated using a severity scale from 0 (healthy plant or plant without symptoms) to 4 (dead plant) based on the percentage of plant tissue affected by chlorosis, leaf and shoot necrosis or defoliation (LÓpez-Escudero et al., 2004). The percentage of dead plants (LÓpez-Escudero and Blanco-LÓpez, 2001; Hiemstra and Harris 1998; Wilhelm and Taylor, 1965) and other symptoms such as marginal spots of leaves and irregular growth of twigs were also considered to estimate the severity of reactions. The area under the disease progress curve (AUDPC) was estimated for each cultivar considering its percentage regarding the maximum possible value that could be reached in the period of assessment, based on the calculation formula of Campbell and Madden (1990): AUDPC= [(t/2 *(S2 +2 *S3+· · ·+ 2Si−1 + Si)/4 *n] *100; where t = days between observations; S = final mean severity; 4 = maximum disease rating; and n = number of observations. Plant infection was verified by the isolation of the fungus from affected shoots or leaf petioles of affected plants during the experiments.

Classification of cultivars according to resistance

To classify the reactions of the cultivars, resistance to VWO was categorized considering the AUDPC, final mean

severity and percentage of dead plants values according to LÓpez-Escudero et al. (2004, Table 1).

Colonization index

Colonization index was assessed at 13 weeks after the inoculation by culturing of different parts of stems on C'zapeck Dox Agar medium (supplemented with 100 ppm streptomycin) for 14 days at 25±1 °C. Once *V. dahliae* was detected the segment was considered as infected. Colonization index (CI) was estimated based on the calculation formula of Tsror et al. (2001): CI= (2*Nb + 6*Nt) /N, where Nb, is the number of infected segments at the base of the stem- cutting, 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 of the base and upper parts was multiplied by the coefficient factors of 2 and 6 respectively, resulting in a calculated colonization index in the range between 0 and 8 (Tsror et al., 2001).

Dry weight (DW)

For evaluating the influence of the VWO progress on the vegetative growth of olive plants inoculated with *V. dahliae* compared with the controls, the tips of all shoots of each plant were marked with an indelible marker at the last node. During inoculation, while plant roots were naked and released from the soil for dipping, the fresh weight of the whole plants was recorded using a balance. At the end of experiments, thirteen weeks after inoculation, the fresh weight of new tissues grown from marked points at plant shoot tips, the roots, and the rest of tissues of each plant were separately recorded. Thereafter, these parts of each plant were separately collected in paper envelopes and dried in an oven at 70˚C for 48 h, and subsequently, the dried weight of the samples was recorded.

Statistical analysis

The analysis of variance (ANOVA) of AUDPC, CI and DW for reference-cultivars in each experiment was performed to determine the variability among experiments. In experiments where reactions of cultivars were statistically different, values of AUDPC CI and DW of cultivars included in these experiments were corrected regarding the percentage of difference between the values of AUDPC for reference-cultivars in significant and non-significant experiments. Cluster analysis of data from all disease parameters using the UPGMA method was conducted to generate a dendrogram showing relationships between cultivars regarding their reaction to *V. dahliae*. Statistical analysis performed by the R.3.3.1 program. Mean values were compared by the Fisher's protected LSD at $P \leq 0.05$.

Results

Defoliate isolates of *V. dahliae* from olive which tested in this experiment were pathogenic to all olive cultivars. The first symptoms developed three weeks after inoculation. Chlorosis in inoculated was associated with cultivars showing a certain level of resistance. Defoliation was frequently observed. It occurred, in the absence of chlorosis, in all susceptible cultivars, starting at 4-5 weeks after inoculation and intensifying from the seventh week after inoculation. The maximum severity of symptoms ranged from 0.77 to 3.69 in 2015 and from 0.83 to 3.67 in 2016 (Table 2). The fungal isolate caused between 50% and 60% of dead plants in five out of the 11 cultivars inoculated, whereas mortality was not observed in resistant or highly resistant cultivars ('Kalamon' and 'Koroneiki', Table 2). Cultivars were classified into susceptible and resistance groups as shown in Tables 2. Most of the evaluated cultivars were susceptible to defoliate isolate of *V. dahliae*. The results in Tables 2 showed that 'Bladi', 'Conservalia', 'Marry' and 'Rowghani' were extremely susceptible and 'Kalamon' and 'Koroneiki' were resistant. Linear regression analysis of plants with foliar symptoms of wilt over time

Table 1. Resistance groups and disease parameters for reactions of olive cultivars to the defoliating isolate D12 of *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 suscetibile	71-100	3.0-4.0	51-100

[a] AUDPC: Area under the disease progress curve.
[b] FMS: Final mean severity of symptoms.
[c] PDP: Percentage of dead plants.

Table 2. Mean disease parameters assessed in the olive cultivars inoculated with the defoliating isolate of *Verticillium dahliae*.

Variability factors	Severity of Verticillium wilt external symptoms		Frequency of *V. dahliae* reisolation from olive xylem (%)	Resistance level[c]	Dead plants (%)
	AUDPC[a]	90 dai[b] (0-4 scale)			
Experiment I (2015)					
P values					
Block	0.535	0.406	0.0188		
Cultivars	<0.001	<0.001	<0.001		
'Bladi'	81.1 a[d]	3.60 a	2.23 a	ES	60
'Conservalia'	79.5 a	3.55 a	2.10 ab	ES	40
'Kalamon'	21.7 e	1.15 d	0.72 e	R	0
'Koroneiki'	11.2 f	0.77 e	0.71 e	R	0
'Manzanilla'	61.6 c	3.20 b	1.58 cd	S	30
'Marry'	62.2 c	3.53 a	1.64 cd	S	50
'Mission'	64.2 c	3.48 ab	1.58 d	S	50
'Picual'	50.4 d	3.51 ab	1.61 cd	S	40
'Rowghani'	72.9 b	3.69 a	1.85 bc	ES	60
'Sevillana'	52.7 d	2.62 c	1.81 cd	S	30
'Zard'	78.3 ab	3.59 a	2.23 a	ES	60
Experiment II (2016)					
P values					
Block	0.443	0.928	0.252		
Cultivars	<0.001	<0.001	<0.001		
'Bladi'	76.60 a	3.40 ab	2.15 ab	ES	60
'Conservalia'	76.60 a	3.40 ab	1.95 abc	ES	40
'Kalamon'	19.70 e	1.02 d	0.83 f	R	0
'Koroneiki'	9.60 f	0.83 d	0.79 f	HR	0
'Manzanilla'	60.10 c	3.16 b	1.66 cde	S	30
'Marry'	65.50 bc	3.42 ab	1.34 e	S	50
'Mission'	60.90 c	3.54 ab	1.55 de	S	40
'Picual'	47.90 d	3.49 ab	1.54 de	S	40
'Rowghani'	70.40 ab	3.67 a	1.74 cd	ES	60
'Sevillana'	50.20 d	2.50 c	1.86 bcd	S	20
'Zard'	74.90 a	3.57 a	2.21 a	ES	50

[a] AUDPC: Area under the disease progress curve estimated as the percentage with regard to the maximum potential value.
[b] dai = days after inoculation
[c] 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; MS = moderately susceptible; R = resistant; HR = highly resistant.
[d] Values in columns followed by the same letter are not significantly different ($P \leq 0.05$) according to Fisher's protected least significant difference test.

Table 3. Linear regression analysis of plants with foliar symptoms of Verticillium wilt over time in the olive cultivars in Experiment I and II.

Olive cultivars	Regression equation[a]	r^{2b}
'Bladi'	y = 0.313x + 0.198	0.862
'Conservalia'	y = 0.291x - 0.257	0.962
'Kalamon'	y = 0.153x - 0.324	0.900
'Koroneiki'	y = 0.065x - 0.224	0.851
'Manzanilla'	y = 0.240x - 0.203	0.938
'Marry'	y = 0.256x - 0.338	0.853
'Mission'	y = 0.254x - 0.294	0.967
'Picual'	y = 0.280x - 0.276	0.964
'Rowghani'	y = 0.280x - 0.204	0.956
'Sevillana'	y = 0.223x - 0.262	0.954
'Zard'	y = 0.275x - 0.488	0.952

[a] *y* = Disease severity (0 = healthy plant or plant without symptoms; 1 = affected plant in 1-33%; 2 = 34–66%; 3 = 67-99%; 4 = dead plant).
[b] r^2= coefficient of determination for each regression line ($P \leq 0.001$).

Table 4. Dry weight of tissues of olive plants after 13 weeks post-inoculation with a defoliating isolate of *Verticillium dahlia* in Experiment I (2015).

Cultivars	Dry weight (g)			
	New green leaves[b]	Roots	New shoots[b]	New shoots:Roots
'Bladi' C[a]	2.512 ab[c]	6.883 abcd	2.888 ab	0.80 bc
'Conservalia' C	2.474 abc	6.829 bcd	2.902 ab	0.80 bc
'Kalamon' C	2.388 c	7.466 a	2.771 abc	0.76 bcd
'Koroneiki' C	2.424 abc	7.349 abc	2.760 abc	0.74 bcd
'Manzanilla' C	2.430 abc	7.383 ab	2.745 abc	0.75 bcd
'Marry' C	2.392 abc	5.417 e	2.789 abc	0.97 a
'Mission' C	2.486 abc	6.808 abcd	2.891 ab	0.79 bc
'Picual' C	2.465 abc	7.409 ab	2.839 abc	0.77 bcd
'Rowghani' C	2.501 abc	6.734 cd	2.951 a	0.84 ab
'Sevillana' C	2.437 abc	7.358 abc	2.764 abc	0.81 bc
'Zard' C	2.449 abc	6.847 cd	2.848 abc	0.81 bc
'Bladi' I	1.389 e	3.949 f	1.131 g	0.63 d
'Conservalia' I	1.419 e	4.134 f	1.416 ef	0.68 cd
'Kalamon' I	2.505 abc	6.810 bcd	2.776 abc	0.70 bcd
'Koroneiki' I	2.514 a	7.006 abcd	2.918 ab	0.73 bcd
'Manzanilla' I	2.477 abc	6.909 abcd	2.812 abc	0.73 bcd
'Marry' I	1.413 e	3.234 g	1.318 fg	0.84 ab
'Mission' I	1.777 d	5.257 e	1.763 d	0.67 cd
'Picual' I	2.474 abc	6.949 abcd	2.704 bc	0.69 cd
'Rowghani' I	1.309 ef	3.957 f	1.578 de	0.73 bcd
'Sevillana' I	2.441 abc	6.529 d	2.670 c	0.69 cd
'Zard' I	1.259 f	3.688 fg	1.109 g	0.63 d

[a] C = non-inoculated control; I = inoculated plants.
[b] Leaves and tissues produced after inoculation during the incubation period in the controlled chamber.
[c] Values in columns followed by the same letter were not significantly different at $P \leq 0.05$ according to Fisher's protected LSD test.

Table 5. Dry weight of tissues of olive plants after 13 weeks post-inoculation with a defoliating isolate of *Verticillium dahlia* in Experiment II (2016).

Cultivars	Dry weight (g)			
	New green leaves[b]	Roots	New shoots[b]	New shoots:Roots
'Bladi' C[a]	2.475 ab[c]	6.843 a	2.94 a	0.81 b
'Conservalia' C	2.433 ab	6.901 a	2.85 ab	0.78 bc
'Kalamon' C	2.505 ab	6.927 a	2.91 a	0.78 bc
'Koroneiki' C	2.519 a	6.942 a	2.97 a	0.78 bc
'Manzanilla' C	2.479 ab	6.986 a	2.95 a	0.77 bc
'Marry' C	2.392 ab	5.546 b	2.82 ab	0.95 a
'Mission' C	2.376 ab	6.866 a	2.78 abc	0.76 bc
'Picual' C	2.474 ab	6.780 a	2.78 abc	0.77 bc
'Rowghani' C	2.359 b	6.756 a	2.88 a	0.79 bc
'Sevillana' C	2.441 ab	6.520 a	2.77 abc	0.81 b
'Zard' C	2.407 ab	6.723 a	2.78 abc	0.79 bc
'Bladi' I	1.389 d	3.921 cd	1.22 e	0.67 c
'Conservalia' I	1.398 d	4.270 c	1.53 e	0.69 bc
'Kalamon' I	2.429 ab	6.794 a	2.54 bc	0.75 bc
'Koroneiki' I	2.398 ab	6.862 a	2.77 abc	0.78 bc
'Manzanilla' I	2.413 ab	6.828 a	2.78 abc	0.78 bc
'Marry' I	1.312 d	3.463 d	1.39 e	0.78 bc
'Mission' I	1.800 c	5.252 a	1.92 d	0.70 bc
'Picual' I	2.37 b	6.698 a	2.68 abc	0.79 bc
'Rowghani' I	1.322 d	3.931 cd	1.41 e	0.70 bc
'Sevillana' I	2.362 b	6.495 a	2.48 c	0.76 bc
'Zard' I	1.293 d	3.648 cd	1.28 e	0.72 bc

[a] C = non-inoculated control; I = inoculated plants.
[b] Leaves and tissues produced after inoculation during the incubation period in the controlled chamber.
[c] Values in columns followed by the same letter were not significantly different at $P \leq 0.05$ according to Fisher's protected LSD test.

in the olive cultivars in Experiment I and II are shown in Table 3 which shows the rate of disease progress was significantly correlated ($P \leq 0.05$) with the variables time. Slop values and coefficients of determination (r2) of the linear regression analysis were 0.065 to 0.313 and 0.851 to 0.967, respectively for resistant and susceptible olive cultivars (Table 3) which indicated a different pattern of disease development among them.

Analysis of variance revealed that differences in vegetative growth reduction in inoculated plants were significant ($p \leq 0.05$, Tables 4 and 5). The reduction was remarkably higher in 'Bladi', 'Conservalia', 'Marry' and 'Rowghani' than other cultivars (Tables 4 and 5). This reduction occurred in all the examined parts of the plants. The reduction in the new green leaves and shoots dry weight of complete inoculated plants were not significant for resistant cultivars compared with that of the corresponding non-infected control plants. The reduction accounted for about 40-47% and 52-60% for susceptible cultivars, respectively (Tables 4 and 5). The root growth of infected plants of this susceptible cultivar was 32-47% of the value recorded in control plants (Tables 4 and 5). The relationship between dry weight of new green leaves and shoot and root with (AUDPC) fit polynomial curves (Fig. 1) which show the rate of the dry weight of new green leaves and shoot was significantly correlated ($P < 0.001$) with the variables AUDPC.

Dendrogram of olive cultivars based on the AUDPC, the final severity of Verticillium wilt external symptoms, the frequency of *V. dahliae* re-isolation from olive xylem and dry weight of new green leaves and shoots are shown in Fig. 2. The

dendrogram represented two main clusters, major cluster and minor cluster. Minor cluster comprised only two ʻKalamonʼ and ʻKoroneikiʼ cultivars as resistant to *V. dahliae*. The major cluster could be divided into two groups, I and II, showed extremely susceptible or susceptible reaction to *V. dahliae*.

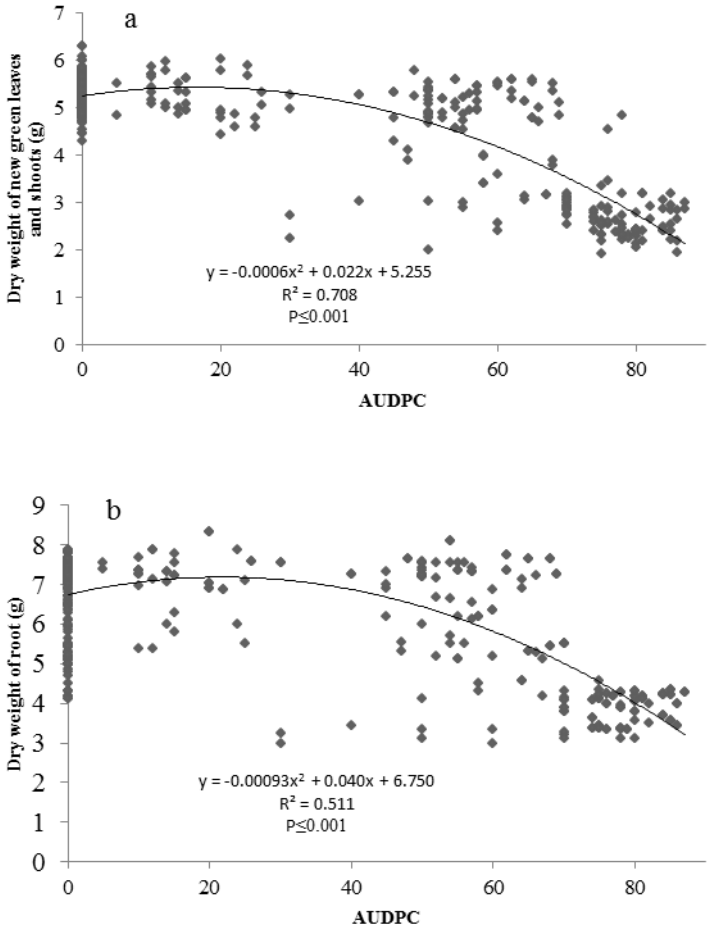

Fig. 1. Relationship between dry weights of new green leaves and shoots (a) and root (b) with area under the disease progress curve (AUDPC).

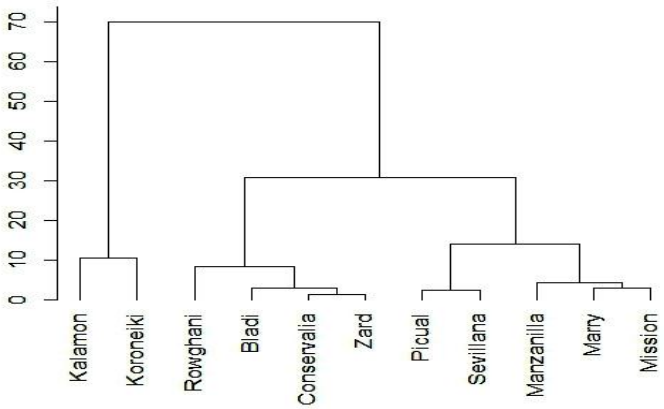

Fig. 2. Cluster analyses of the reaction of olive cultivars to *Verticillium dahliae* infection based on disease parameters and dry weight of new green leaves, shoots and root.

Discussion

Root dip inoculation has been the most common technique for evaluating resistance in olive because it reliably reproduces the infection process induced by *V. dahliae* (Blánco-LÓpez et al., 1998; LÓpez-Escudero et al., 2004). Nevertheless, other studies have revealed that stem puncture inoculation can also be a useful technique for assessing olive resistance to *V. dahliae* (LÓpez-Escudero et al., 2007). The inoculation method of root dipping in a semisolid mass of culture medium containing mycelium and conidia of the pathogen has been effective in developing consistent infections and symptoms on inoculated plants. As previously reported by Garcí-Ruiz et al. (2014), using this methodology, the inoculum mass probably provides continuous infections in the roots over several days; the fungus propagules remain viable allowing successful and effective inoculation.

Olive cultivars show a broad range of genetic variability for a large number of agronomic traits (Owen et al., 2005), including resistance to Verticillium wilt. Resistance/tolerance to Verticillium wilt has been found in several rootstocks and commercial cultivars of olives. Olive cultivars 'Frantoio', 'Empeltre', 'Koroneiki' are considered the most resistant/tolerant to both V. dahliae pathotypes among over 120 world cultivars tested (Bubici and Cirulli, 2012). Research in Italy and other countries indicated that cultivars 'Coratina', 'Frantoio', 'Frangivento', 'Oblonga' and 'Kalamon' have useful resistance characteristics to the non-defoliating Verticillium pathotype (Barranco et al., 2000; Cirulli and Montemurro, 1976; LÓpez-Escudero et al., 2004; Rodriguez Jurado et al., 1993), while 'Ascolana', 'Cellino', 'Leccino', 'Manzanillo', 'Chemlalie', 'Konservolia', 'Mission' and 'Picual' are susceptible to the disease (Cirulli and Montemurro, 1976; Rodriguez-Jurado et al., 1993; LÓpez-Escudero et al., 2004). However, the most important Spanish cultivars, 'Picual', 'Hojiblanca', 'Cornicabra', and 'Arbequina', are susceptible or extremely susceptible to defoliating and nondefoliating V. dahliae isolates (LÓpez-Escudero et al., 2004). One clone of 'Arbequina' named 'Allegra' was also found to be highly resistant to the non-defoliating pathotype of V. dahliae (Tjamos, 1993). The use of resistant rootstocks could effectively contribute to the control of V. dahliae, even if the scion is susceptible (Porras-Soriano et al., 2003; Sanei et al., 2005b). Twenty-six of the 33 cultivars were found to be susceptible or extremely susceptible to the defoliating V. dahliae isolate in Martos-Moreno et al. (2006) experiment. 'Cipresino', 'Koroneiki', 'Oblonga', and 'Sevillenca' cultivars were classified as moderately resistant and 'Changlot Real', 'Empeltre' and 'Frantoio' were resistant (Martos-Moreno et al., 2006). The resistance of 42 Spanish olive cultivars to Verticillium dahliae was evaluated by Garcí-Ruiz et al. (2015). Most of the tested cultivars were susceptible to Verticillium wilt. However, eight genotypes ('Cornezuelo de Jaén', 'Verdial de Badajoz', 'Jaropo', 'Negrillo de Estepa', 'Jabaluna', 'Ocal de Alburquerque', 'Asnal' and 'Racimal') exhibited resistance to the disease (Garcí-Ruiz et al., 2015). The difference of the resistance to VWO under field conditions is strongly influenced by a number of factors, including the environment, inoculum density (ID), pathogen virulence, and management practices (LÓpez-Escudero and Blánco-LÓpez, 2007). The interaction between isolates of V. dahliae with different pathotypes and olive cultivars show that the defoliate pathotype causes higher disease severity index and stem colonization. 'Bladi', were susceptible to both pathotypes of V. dahliae and 'Kalamon' and 'Koroneiki' were resistant to both pathotypes of the pathogen (Sanei and Razavi, 2011). This

research showed that Iranian olive cultivars in Golestan province were variable in resistance/susceptibility to defoliating pathotype of V. dahliae. Almost all the evaluated cultivars have been categorized as susceptible or extremely susceptible to both pathogenic variants of V. dahliae, including the most important Iranian cultivars, 'Rowghani', 'Zard', 'Marry' and 'Mission' (Table 2). Some commercial cultivars were found to be highly resistant to verticillium wilt, especially 'Kalamon' and 'Koroneiki' cultivars since they proved to be resistant to highly pathogenic variants of V. dahliae (Table 1 and 2). These results also confirmed previous studies for susceptibility of 'Picual' (Birem et al., 2016; LÓpez-Escudero et al., 2004) and useful resistance characteristics of 'Kalamon' (Barranco et al., 2000) and 'Koroneiki' (Bubici and Cirulli, 2012), so that they could be used for replanting or as rootstocks for other susceptible cultivars. although, the productivity, oil quality, early fruit maturation and easy mechanical harvesting and some disadvantages for commercial use of the cultivars, especially problems of fruit set and susceptibility to frost injury and other diseases or pests must be studied in this region.

Several physiological alterations may occur in infected plants that influence photosynthesis, nutrient translocation, water transport, and/or respiration (Sadras et al., 2000). The plant dry weight was the main parameter 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* sp. or *Fusarium* sp. (Karagiannidis et al., 2002). In this study, growth reduction was related to the susceptibility level of the evaluated olive variety of the species, which confirm with data for susceptibility level and growth reduction in sunflower (Sadras et al., 2000), pepper (Goicoechea, 2006) and olive (Birem et al., 2016) infected by *V. dahliae*. Infections caused by the defoliating isolate of the pathogen reduced the growth of inoculated olives, although the reduction was significantly higher in susceptible cultivars (Table 5 and 6). 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 defoliating isolate of the pathogen reduced the growth of inoculated plants of genotypes, although the reduction was significantly higher in 'Bladi' and 'Conservalia' than other cultivars (Table 5 and 6).

In conclusion, 'Kalamon and 'Koroneiki' cultivars identified with high levels of resistance to Verticillium wilt in the present study. Moreover, this is the first report showing susceptibility of 'Picual' and 'Marry' germplasms to the defoliating pathotype of *V. dahlia* in Iran. The results also showed the influence of symptom progress on the vegetative growth.

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