Euphytica (2015) 206:619–629
DOI 10.1007/s10681-015-1463-7

Evaluation of *Verticillium* wilt resistance in selections from olive breeding crosses

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Received: 11 February 2015 / Accepted: 6 May 2015 / Published online: 12 May 2015

Abstract *Verticillium* wilt (VW) resistance was evaluated in genotypes from olive crosses including resistant cultivars as parents. Thirty-eight genotypes from three crosses were evaluated: 'Changlot Real' × 'Dolce Agogia' (16), 'Frantoio' × 'Arbosana' (13) and 'Koroneiki' × 'Empeltre' (9). These genotypes were previously selected for several agronomic traits from wider initial progenies populations. Several disease severity and plant colonization parameters were evaluated in inoculation experiments under controlled conditions by dipping roots cutting in a conidial suspension of a highly virulent defoliating isolate of *Verticillium dahliae*. Significant differences among the evaluated genotypes, including parents and selections from crosses, were obtained for all the disease parameters assessed. A wide variability in disease parameters was observed in the three cross combinations tested. Genotypes with lower relative susceptible index values than both parents were found in the three progenies tested and 10 out of 38 genotypes (26 %) were finally classified as resistant. The level of resistance of these genotypes will be confirmed in future studies under field conditions.

Keywords Defoliating pathotype · Inheritance · *Olea europaea* L. · Plant colonization · *Verticillium dahliae*

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Introduction

Verticillium wilt (VW), a vascular disease caused by the soilborne fungus *Verticillium dahliae* Kleb, is one of the main problems for olive (*Olea europaea* L.) growing in many areas worldwide. Currently, there are no available control measures which are sufficiently effective when applied singly. Therefore, an integrated disease management strategy, including different control methods before and after planting (López-Escudero and Mercado-Blanco 2011; Jiménez-Díaz et al. 2012), has been recommended. In this sense, the use of resistant plant materials is considered as the most economic and efficient control measure for the disease. Several sources of resistance to VW have been identified in olive, including both cultivated and

wild germplasm. Considerable levels of resistance to development of symptoms caused by the highly virulent defoliating (D) pathotype of *V. dahliae* have been reported for a limited number of traditional cultivars such as 'Frantoio', 'Changlot Real' and 'Empeltre', both upon artificial inoculations and in naturally-infested soils (López-Escudero et al. 2004; Trapero et al. 2013). However, due to some disadvantages such as late bearing and excessive vigour, these cultivars may not represent the best agronomical choice for a modern olive growing and are not suitable for high-density orchards. Screening for *Verticillium* wilt resistance has also been carried out in wild olive materials, which allowed the selection of new sources of resistance to the disease (Mercado-Blanco et al. 2003; Colella et al. 2008; Jiménez-Díaz et al. 2012). The use of resistant materials as rootstocks for grafting of VW-susceptible olive cultivars has been successfully reported, providing significant reduction on the severity of the attacks (Porras-Soriano et al. 2003; Bubici and Cirulli 2012). However, no source of total resistance has been found and all plant materials tested up to now have been infected by the pathogen, so that complete control of VW of olive by means of resistant rootstock cannot be guaranteed, at least in soils heavily infested with *V. dahliae*. In olive, the mechanisms of infection and colonization by *V. dahliae* and plant defense response of the plant are not fully understood yet.

Resistant genotypes can be used as parents in breeding programs to obtain new cultivars combining both high disease resistance and appropriated agronomic characters. In olive, the wide dispersion of the disease and the increase importance of crop losses associated with the rapid extend of fungal pathotypes highly virulent, together with the reduced number of traditional cultivars showing a significant level of resistance to VW, have promoted the development of breeding programs to select new cultivars with improved resistance levels to the disease (Erten and Yildiz 2011; Trapero et al. 2013; Arias-Calderón et al. 2015). However, even in progenies from crosses between cultivars of known merit, most of the seedlings obtained show poor agronomic performance and should be discarded as soon as possible. Thus, the selection process in conventional fruit breeding programs involves several selection steps in which the most promising individuals are retained for further clonal testing (León et al. 2015). Previous results in our olive breeding program selection indicate that selection, at the seedling stage, for early yield, fruit size and oil content can be efficiently performed in the cross progenies, while still retaining high variability for characters not previously considered (León et al. 2015). Therefore, screening of VW resistance after preselection for important agronomic traits at the seedling stage would increase the chances of final selection of genotypes combining both high disease resistance and appropriated agronomic characters. This strategy of multi-stage selection could be a superior alternative particularly in cross-fertilized crops for characters under polygenic control. For instance, multiple-trait selection for VW resistance and agronomic traits resulted in cultivars of balanced commercial utility in strawberry (Shaw et al. 2010) or cotton (Zhou et al. 2014).

In the present work, the resistance to *Verticillium* wilt was evaluated in 38 genotypes from three crosses including at least one parent cultivar previously categorized as resistant to the disease. These genotypes were previously selected for important agronomic traits from wider progenies populations. The aims of this work were to study the inheritance of *Verticillium* wilt resistance and to select the most outstanding genotypes displaying high levels of disease resistance and good agronomic characteristics for further evaluation tests. For that purpose, several disease and plant colonization parameters were assessed in inoculation experiments under controlled conditions.

Materials and methods

Plant materials

VW resistance was evaluated in genotypes from three different crosses: 'Changlot Real' × 'Dolce Agogia', 'Frantoio' × 'Arbosana' and 'Koroneiki' × 'Empeltre'. Parents included some of the cultivars of the World Olive Collection of IFAPA Córdoba (WOGB, CAP-UCO-IFAPA), Spain, that have showed higher resistance response in previous works ('Changlot Real', 'Dolce Agogia', 'Empeltre' and 'Frantoio') as well as cultivars cataloged previously as moderately susceptible ('Koroneiki') and susceptible ('Arbosana') (López-Escudero et al. 2004; Martos-Moreno et al. 2006; Arias-Calderón et al. 2015).

Crosses, germination of seeds and forced growth of seedlings plants in a greenhouse were carried out according to the standard procedures used in the olive breeding program (Santos-Antunes et al. 2005). Afterwards, seedlings plants were transplanted into the field at 4 m × 1.5 m spacing. Drip irrigation and standard cultural practices were followed in the orchard to ensure adequate tree growth. The three progenies were evaluated for length of the juvenile period and fruit characters (oil content and fruit size). As a result, 38 genotypes were selected from the initial populations: 16 from the cross 'Changlot Real' × 'Dolce Agogia', 13 from 'Frantoio' × 'Arbosana' and 9 from 'Koroneiki' × 'Empeltre'.

Plants of the selected genotypes and the cultivars used as parents were obtained by vegetative propagation of semi-hardwood stem cuttings to provide material for screening to VW resistance. 'Picual' and 'Frantoio' cultivars were also propagated as controls of known reaction, susceptible and resistant respectively.

Inoculation procedure and growth conditions

The applied monosporic *V. dahliae* isolate comes from affected olive trees in southern Spain and was characterized in previous study as highly virulent D pathotype (Rodríguez-Jurado et al. 2008). For inoculum preparation, cultures were grown on potato dextrose agar for 7–14 days at 24 °C. Then it was translated to flasks containing 100 ml of potato dextrose broth. Fungal culture incubated for 7 days at 24 °C on an orbital shaker at 125 rpm in the darkness and it was filtered through double cheesecloth. The resultant suspension was assessed using a haemocytometer and it was adjusted to 10^7 conidia ml^{-1} with sterile distilled water.

Eight to twenty plants of six-month-old of each genotype and cultivar were inoculated by dipping the root system slightly trimmed in the conidial suspension for 15 min according to Rodríguez-Jurado et al. (2007). Six to ten non-inoculated control plants of each genotype and cultivar were immersed in sterile distilled water for the same time. Following inoculation, plants were transplanted to individual plastic pots with twice autoclaved soil (lime: peat, 2:1, v/v, 75 min at 121 °C) and kept in a controlled growth chamber at 22 ± 2 °C, 45–85 % relative humidity and 14 h photoperiod light. Plants were watered as required and fertilized weekly with Hoaglands nutrient solution.

Plant infection and symptom assessment

Symptoms severity was periodically assessed for 112 days according to the percentage of aerial part affected by chlorosis, curling leaves, necrosis, green defoliation and/or death of the plant using a severity scale from 0 to 4 (0 = absence of symptoms; 1 = 1–33 % aerial part affected; 2 = 34–66 % aerial part affected; 3 = 67–100 % aerial part affected; 4 = dead plant) (Rodríguez-Jurado et al. 1993). Several disease parameters were calculated:

Standardized area under the disease progress curve (SAUDPC) that was implemented according to Campbell and Madden (1990) (1):

$$SAUDPC = \left[\sum_{i=1}^{n}((S_i + S_{i-1})/2)\Delta t\right][100/(S_{max}T)] \quad (1)$$

where S_i = mean severity of the experimental unit in the observation i; Δt = the number of days between observation; S_{max} = maximum value of severity (= 4); T = experimental period in days; n = observation numbers.

Disease intensity index (DII) was calculated for each observation as shown in Eq. (2):

$$DII = \sum_{x=1}^{n}((S_x \times N_x)/(4N_t))\ 100 \quad (2)$$

where S_x = severity in an individual plant, N_x = number of plants with symptoms of severity S_x and N_t = total number of plants for each experimental unit. Disease intensity index (DII) at the end of the evaluations is called FDII.

Others additional disease parameters were calculated: final disease incidence (FDI) and final dead plants incidence (FDPI) that represent the percentage of plants with disease symptoms and the percentage of dead plants at the end of the experiment, respectively; disease-free period (DFP) estimated as the number of days without appearance symptoms. All parameters were calculated for each experimental unit.

A relative susceptibility index (RSI) was calculated summarizing all previous disease parameters weighted

according the following coefficients (Arias-Calderón et al. 2015) (3):

$$RSI = [(0.3\text{SAUDPC} + 0.3\text{FDPI} + 0.2\text{FDII} + 0.05\text{FDI} + 0.15(100 - RDFP))/SP]100 \quad (3)$$

where SP = average susceptibility in the reference susceptible cultivar 'Picual', i.e. the value of numerator calculated for this cultivar; RDFP = Relative disease-free period estimated as the number of days without appearance symptoms and expressed as percentage to the total experimental period in days.

Plant colonization by the fungus was also assessed in root system and aerial part of each plant at the end of experiments (symptomatic and asymptomatic) or at dead plants by reisolation of the fungus on chlortetracycline water agar. Ten stem's pieces and ten from roots previously cleaned and disinfected, were plated onto the medium and incubated at 24 °C in the darkness for 9 (stem) to 21 (root) days (Rodríguez-Jurado 1993). Plant colonization by *V. dahliae* was microscopically characterized by the presence of hyaline conidiophores and conidia from the tips of phialides borne in whorls of erect conidiophores (namely verticils) and by the formation of typical melanized, resistant resting structures called microsclerotia (López-Escudero and Mercado-Blanco 2011). The results from root and stem isolations of the pathogen were used to calculate a root and stem colonization index (RCI and SCI, respectively) for each experimental unit, as root and stem pieces of which the fungus was isolated relative to number total of root and stem pieces sampled, respectively.

Data analysis

Cultivars used as parents and the selected 38 genotypes were evaluated in two separate experiments that always included 'Picual' (susceptible) and 'Frantoio' (resistant) cultivars as disease reference controls. Initial analyses found no significant differences ($P > 0.05$) among experiments for the various disease parameters assessed in the two control cultivars. Therefore data were pooled over two experiments. Each experiment was arranged according to a complete randomized block design with 8–20 inoculated plants per genotype distributed in four blocks with 2–5 plants per block, as well as 6–10 mock inoculated plants per genotype also distributed in the four blocks. ANOVAs were performed to test for significant differences among genotypes in disease parameters. Means were compared between each genotype and those of susceptible 'Picual' and resistant 'Frantoio' reference controls by the Dunnetts test at $P = 0.05$. Parameters expressed as percentages were subjected to angular transformation before being statistically analysed. Chi square-test (χ^2) was used to compare the distribution of genotypes among crosses according to the resistance categories established by RSI. Pearson's correlation coefficient was calculated among colonization parameters (RCI and SCI) and RSI, in the different crosses. All analyses were carried out using Statistix 9.0 software (Analytical Software, Tallahassee, Florida, USA).

Results

First foliar wilt symptoms after inoculation appeared on the susceptible control 'Picual' 14 days post inoculation (dpi), increased until 77 dpi up to a final 90.3 % DII (Fig. 1). No visual symptoms were observed on the resistant control 'Frantoio' until 28 dpi. Disease symptoms increased in this cultivar until 46 dpi and then remained steady for a final DII of 23.1 %. Among the genotypes evaluated, a wide variability in symptom progress was observed, irrespective of the progeny (Fig. 1).

Significant differences ($P < 0.05$) among the evaluated genotypes, including parents and selections from their respective crosses, were obtained for all the

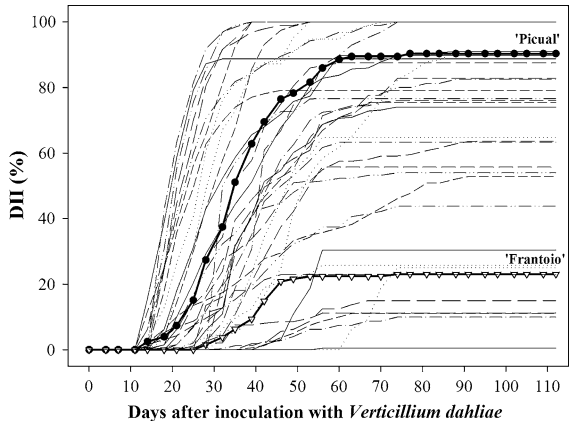


Fig. 1 Progress of the disease intensity index (DII) in olive genotypes from crosses. Reference control cultivars 'Picual' (susceptible) and 'Frantoio' (resistant) are indicated in *bold*

disease parameters evaluated (Table 1). All disease parameters except DFP were highly correlated (data not shown), even though all of them did not provide equal grouping of genotypes when compared to the 'Picual' and 'Frantoio' reference controls according to Dunnetťs test. For instance, SAUDPC, FDPI and FDII, but not FDI and principally DFP, similarly grouped all the evaluated genotypes. Thus, three of the evaluated genotypes (CRxDA-11, FrxAr-11 and FrxAr-6) showed significant differences from control 'Picual' for all disease parameters.

To integrate the information of the different disease parameters, the relative susceptibility index (RSI) was applied for final classification of genotypes according to their levels of resistance to *Verticillium* wilt (Table 1). Among the selections from crosses, 22 (58 %) of them were classified as Susceptible (S, significantly different from 'Frantoio'), 6 (16 %) as Moderately Susceptible (MS, non-significantly different from both 'Picual' and 'Frantoio') and 10 (26 %) as Resistant (R, significantly different from 'Picual'). All parent cultivars were classified as R except 'Arbosana' (S).

A wide variability was obtained in all cross combinations: RSI values ranged from 13.41 to 102.39 in 'Frantoio' × 'Arbosana', 16.02 to 113.77 in 'Changlot Real' × 'Dolce Agogia' and 19.24 to 104.17 in 'Koroneiki' × 'Empeltre' (Table 1). In fact, genotypes with lower RSI values than both parents were found in the three progenies. However, the pattern of distribution of genotypes according to resistance categories established by RSI was significantly different among crosses (Fig. 2; Table 2). This was mainly due to 'Changlot Real' × 'Dolce Agogia' cross, in which a higher proportion of S genotypes was obtained and none of the genotypes evaluated was classified as MS.

Scatter plots of RSI versus colonization by the fungus in stem (SCI) and root (RCI) are shown in Fig. 3. The relationships between these parameters varied according to the different crosses and tissues. The highest correlation was found in 'Changlot Real' × 'Dolce Agogia' for SCI ($r = 0.84$, $P < 0.001$), with all genotypes categorized as R showing low values of SCI that ranged from 2 to 9 %. All genotypes categorized as resistant according to RSI showed low values of colonization in both root and stem tissues, with maximum values of 14 and 25 % for RCI and SCI respectively. However, similar values of colonization was also found in some genotypes showing higher RSI values and, therefore, classified as MS or even S.

Discussion

Verticillium dahliae exhibits a broad host range including many economically-important herbaceous and woody crop species (Bhat and Subbarao 1999; Pegg and Brady 2002; López-Escudero and Mercado-Blanco 2011). For some of them, the importance of the disease and crop losses induced by this fungus has promoted the development of breeding programs to select new cultivars with improved resistance levels to the disease. Considering the limited number of traditional olive cultivars which have displayed a high level of VW resistance, development of olive breeding programs to obtain new cultivars with enhance resistance levels could be of paramount importance for a better control strategy of the disease (López-Escudero and Mercado-Blanco 2011; Jiménez-Díaz et al. 2012). In this work, artificial inoculation experiments under controlled conditions were carried out to evaluate the resistance to VW in seedlings from three olive breeding crosses and their respective parents. Some of the cultivars currently known to show higher levels of resistance to VW were used as parents. The assessment of the resistance to VW in the seedlings was preceded by their evaluation and selection for important agronomic traits as the general objective was to identify new genotypes combining good agronomic behavior with high level of VW resistance.

The disease reaction obtained for the cultivars used as parent of the crosses was similar than previously reported under similar experimental procedures (López-Escudero et al. 2004; Martos-Moreno et al. 2006; Arias-Calderón et al. 2015). Thus, R response was observed for 'Changlot Real', 'Dolce Agogia', 'Empeltre' and 'Frantoio' and S for 'Arbosana'. Slight difference was observed for 'Koroneiki', previously cataloged as MS ussing different inoculation methods (dipping root, soil infested and stem puncture) (López-Escudero et al. 2007; Markakis et al. 2010; Arias-Calderón et al. 2015) but categorized as R in this work. It should be noted that 'Koroneiki' cultivar has also showed high level of resistance under natural field conditions (Trapero et al. 2013).

Table 1 Disease parameters calculated in genotypes from three olive crosses

Cultivar/cross[a]	Disease parameter[b]						Resistance category[c]
	SAUDPC	FDPI	FDII	FDI	DFP	RSI	
'Picual'	**62.12#d**	**88.3#**	**90.32#**	**100#**	**24.11#**	**100#**	**S**
CRxDA-9	78.54#	100#	100#	100#	20.2#	113.77#	S
CRxDA-4	79.87#	100#	100#	100#	32.95#	111.87#	S
CRxDA-10	76.08#	100#	100#	100#	32.01#	110.62#	S
CRxDA-5	81.79#	100#	100#	100#	59.4	107.61#	S
CRxDA-1	74.96#	100#	100#	100#	77.01*	101.71#	S
CRxDA-3	80.99#	100#	100#	100#	93.01*	100.97#	S
CRxDA-12	60.83#	100#	100#	100#	61.39	99.33#	S
CRxDA-6	60.64#	87.5#	87.5#	87.5#	17.36#	98.92#	S
CRxDA-8	72.37#	100#	100#	100#	99.16*	96.56#	S
CRxDA-7	60.54#	90#	90.94#	95#	52.98	94.44#	S
CRxDA-2	62.59#	79.17#	79.17#	79.17	86.98*	80.77#	S
CRxDA-13	49.91#	75#	76.04#	91.67#	61.07	79.3#	S
CRxDA-14	47.58#	67.5#	74.06#	78.75	50.24	76.33#	S
'Dolce Agogia'	20.88*	30*	30.63*	40*	82.54*	32.68*	R
'Changlot Real'	15.93*	25*	25.31*	30*	58.3	3 1.54*	R
CRxDA-15	16.68*	20.83*	25.78*	62.5	81.38*	28.06*	R
CRxDA-16	6.15*	10*	11.09*	21.25*	47.97	20.02*	R
CRxDA-11	7.47*	8.33*	11.2*	29.17*	71.3*	16.02*	R
FrxAr-12	73#	100#	100#	100#	69.49	102.39#	S
FrxAr-1	73.71#	88.75#	88.75#	88.75#	31.95#	101.96#	S
FrxAr-9	51.41#	81.25#	82.81#	100#	23.06#	91.62#	S
FrxAr-2	47.32#	90#	90.94#	95#	52.83	89.49#	S
FrxAr-8	63.65#	87.5#	89.84#	100#	87.17*	88.27#	S
'Arbosana'	49.14#	70#	75.31#	90#	66.47	75.82#	S
FrxAr-7	41.01	55	63.59	82.5#	43.15	68.08	MS
FrxAr-4	38.55	45.83	54.04	91.67#	65.05	57.74	MS
FrxAr-3	31.79	49.58	52.89	79.58#	70.31	54.57	MS
FrxAr-13	28.06*	42.5	43.75*	52.5*	53.69	49.62*	R
FrxAr-5	16.45*	22.92*	22.92*	22.92*	57.94	29.97*	R
'Frantoio'	**14.89***	**19.3***	**23.08***	**34.67***	**70.94***	**26.35***	**R**
FrxAr-11	10*	25*	25*	25*	83.89*	24.08*	R
FrxAr-10	0.27*	0*	0.52*	8.33*	22.93#	15.28*	R
FrxAr-6	5.36*	10*	10*	10*	76.25*	13.41*	R
KoxEm-1	79.41#	100#	100#	100#	72.86*	104.17#	S
KoxEm-3	53.74#	75#	76.56#	100#	46.13	84.22#	S
KoxEm-2	51.86#	80#	82.5#	95#	61.84	83.61#	S
KoxEm-9	48.23#	70.83#	75.52#	100#	52.23	79.16#	S
KoxEm-8	40.61	62.5	64.84	75	37.17#	71.73	MS
KoxEm-6	34.76	54.17	55.73	62.5	25.04#	65.59	MS
KoxEm-5	41.37	62.5	63.28	75	92.19*	61.25	MS
'Koroneiki'	14.24*	20.83*	22.79*	35.42*	23.4#	35.62*	R
'Empeltre'	19.4*	25*	26.56*	37.5*	50.11	35.18*	R

Table 1 continued

Cultivar/cross[a]	Disease parameter[b]						Resistance category[c]
	SAUDPC	FDPI	FDII	FDI	DFP	RSI	
KoxEm-7	16.72*	25*	30.47*	62.5	82.81*	30.55*	R
KoxEm-4	8.27*	15*	15*	15*	69.49	19.24*	R

Genotypes and parents are sorted in descendant order for RSI by crosses. Reference control cultivars 'Picual' (susceptible) and 'Frantoio' (resistant) are indicated in bold

[a] *CRxDA* 'Changlot Real' × 'Dolce Agogia', *FrxAr* 'Frantoio' × 'Arbosana', *KoxEm* 'Koroneiki' × 'Empeltre'

[b] *SAUDPC* standardized area under the disease progress curve, *FDPI* final dead plants incidence, *FDII* final disease intensity index, *FDI* final disease incidence, *DFP* disease-free period, *RSI* relative susceptibility index

[c] Evaluated genotypes were classified by RSI values in three resistance categories to *Verticillium* wilt: resistant (R), significantly different from 'Picual'; susceptible (S), significantly different from 'Frantoio'; and moderately susceptible (MS), non-significantly different from 'Picual' and 'Frantoio'

[d] * significantly different from 'Picual'; # significantly different from 'Frantoio'; without symbol = non significantly different from 'Picual' and 'Frantoio' according to Dunnetts test at $P = 0.05$

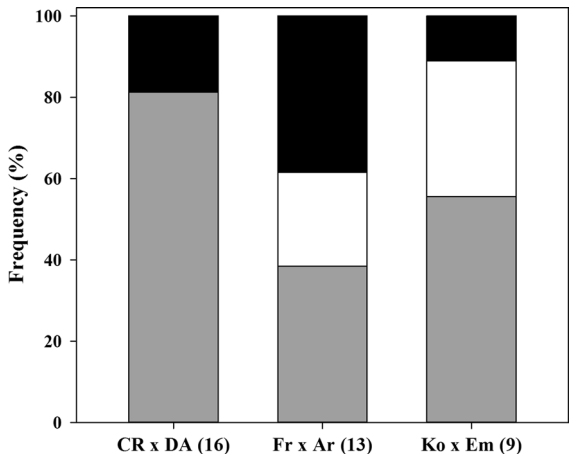
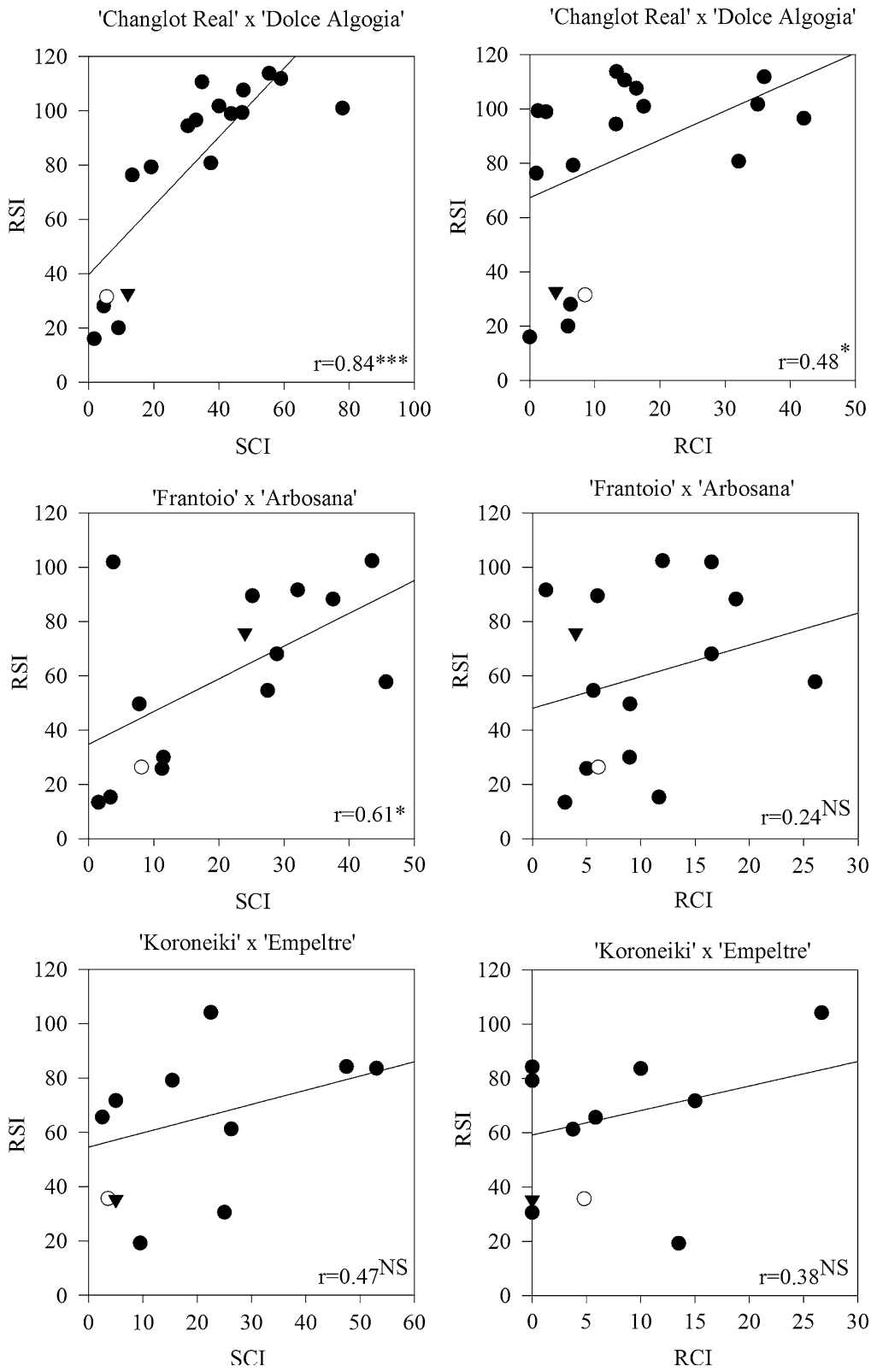


Fig. 2 Frequency distribution of genotypes (%) by cross for resistance category classification according to relative susceptibility index (RSI): resistant (*black*). susceptible (*grey*) and moderately susceptible (*white*). In *brackets* the total number of evaluated genotypes by cross

A wide variability in the values of the different disease parameters calculated was observed in the three cross combinations tested and 10 out of 38 evaluated genotypes (26 %) were finally classified as R according to RSI values. These results represent a higher proportion of resistant genotypes than previously reported in olive progenies from open pollination, although resistant seedlings were selected in progenies of different cultivars including susceptible parents such as 'Arbequina', 'Manzanillo', 'Mission' and 'Picudo' (Wilhelm and Taylor 1965; Arias-Calderón et al. 2015). Trapero et al. (2015) found approximately half the proportion of R genotypes obtained in our work in the evaluation of a high number of genotypes derived from open pollination and crosses involving different olive cultivars, wild olive genotypes and other *Olea* genus. In our case, however, a different strategy was followed as the seedling progenies were first evaluated for agronomic traits and only those genotypes showing good agronomic performance were included in the inoculation tests.

The proportion of genotypes classified in different resistance categories varied among crosses according χ^2 comparison. In this sense, 'Frantoio' × 'Arbosana' showed the highest proportion of R genotypes (38 %) while Changlot Real' × 'Dolce Agogia' showed the highest proportion of S genotypes (81 %). These results are in concordance with previous studies (Wilhelm and Taylor 1965; Trapero et al. 2015), which identified higher proportion of R genotypes in progenies from free pollination and different cross combinations including 'Frantoio' as parent. Different inheritance patterns against VW have been reported in other species such as cotton with no consensus on the genetic basis of resistance (Zhang et al. 2014). Several studies in cotton seems to indicate a quantitative inheritance of the resistance under polygenic control, predominantly due to additive genetic factors and, therefore, highly heritable (Aguado et al. 2008; Yang et al. 2008; Zhou et al. 2014). The high variability obtained from different crosses in our work seems to


'Changlot Real' x 'Dolce Algogia'
RSI
SCI
r=0.84***
'Changlot Real' x 'Dolce Algogia'
RSI
RCI
r=0.48*
'Frantoio' x 'Arbosana'
RSI
SCI
r=0.61*
'Frantoio' x 'Arbosana'
RSI
RCI
r=0.24NS
'Koroneiki' x 'Empeltre'
RSI
SCI
r=0.47NS
'Koroneiki' x 'Empeltre'
RSI
RCI
r=0.38NS

◀**Fig. 3** Scatter plots of relative susceptibility index (RSI) versus root colonization index (RCI) and stem colonization index (SCI) in 38 genotypes selected from three progenies. For each cross, *white circle* and *triangle* represent values for female and male parents, respectively

indicate similar pattern of quantitative inheritance under polygenic control. However, the higher proportion of R genotypes in seedlings from 'Frantoio' × 'Arbosana' cross (the only one involving one S parent) suggests that other than additive effects could be of major importance, indicating a likely complex mode of genetic control of VW resistance in olive. Therefore, searching for specific cross combinations producing high percentage of R genotypes could be the best strategy in olive breeding programs for this trait. New breeding cycles, involving as parents some of the R genotypes identified in this work, are currently underway. This will be useful to test whether a selection strategy based on increased level of parental resistance may result in increased percentage of resistant genotypes, as previously demonstrated in other species (Shaw et al. 2010).

In olive, previous studies have demonstrated that plant defense mechanisms against VW involve the activation of different physical and biochemical barriers to hamper the progress of *V. dahliae* from root to stem (Baídez et al. 2007; Markakis et al. 2010). In 'Arbequina' plants, early and profuse colonization of the root surface have been observed, followed by inter and intracellular hyphae growth within the root and rapid colonization of the plant by hyphae and conidia (Prieto et al. 2009). Plant colonization has been rarely compared to the level of resistance of olive cultivars. Mercado-Blanco et al. (2003) found a correlation of the level of susceptibility to VW of three cultivars ('Picual' > 'Arbequina' > 'Acebuche-L') with the amount of pathogen DNA quantified in roots and stems. However, in Greek cultivars with different susceptibility levels ('Amfissis' > 'Kalamon' and 'Koroneiki'), this correlation was clear in stems but not in roots (Markakis et al. 2009). In the present work, a high significant correlation between RSI and SCI and also between RSI and RCI were observed in the cross 'Changlot Real' × 'Dolce Agogia'. Similarly, significant correlation between RSI and SCI was observed in 'Frantoio' × 'Arbosana', the cross with the highest number of genotypes categorized as R. This might indicate the occurrence of a resistance mechanism to vascular level of the plant involved in the disease response. On the contrary, no correlation between RSI and plant colonization parameters was observed in 'Koroneiki' × 'Empeltre'. Thus, some genotypes with similar values of colonization (SCI, RCI) but different disease response (RSI) were found in this work, as previously reported also in genotypes from open pollination progenies (Arias-Calderón et al. 2015). All the above maybe suggests the existence of a tolerance or resistant response mechanism depending on the olive genotype (Robb 2007), as previously reported in inoculation experiments in tomato isolines with different fungal isolates (Chen et al. 2004). Further investigations are needed to really elucidate the resistance or tolerance mechanisms operating in stems and roots on different olive cultivars/*V. dahliae* interactions.

Conclusion

The assessment of the resistance to VW in olive genotypes from crosses, which have previously displayed good agronomic performance, was carried

Table 2 Chi square comparison between crosses for resistance category classification according to relative susceptibility index (RSI)

Comparison	df	χ^2	p
'Changlot Real' × 'Dolce Agogia' versus 'Frantoio' × 'Arbosana'	2	6.82	0.033
'Frantoio' × 'Arbosana' versus 'Koroneiki' × 'Empeltre'	2	0.69	0.707
'Changlot Real' × 'Dolce Agogia' versus 'Koroneiki' × 'Empeltre'	2	6.52	0.038
Overall	4	8.49	0.075

out under controlled conditions in the present work. This procedure may represent some advantages compared to selection at the seedling stage previously reported (Wilhelm and Taylor 1965; Trapero et al. 2015). On the one hand, the possibility of replication of plant materials, instead of one single plant per genotype, allows more accurate evaluation for disease resistance. Moreover, a balanced commercial utility could be expected in the genotypes finally selected. Among the evaluated genotypes, ten of them were classified as resistant, although future trials to confirm the disease reaction of these genotypes under field conditions are also needed. Also, the genotypes evaluated in the present study represent valuable material for future studies about pattern plant colonisation by the fungus and the resistance/tolerance mechanisms operating on different olive cultivars. Finally, the confirmation of the effect of the cultivar 'Frantoio' in conferring high VW resistance to its progeny, in addition to similar and previously reported effects in conferring high resistance to fungal aerial disease caused by *Spilocea oleagina* and *Colletotrichum acutatum* (Moral et al. 2015), reinforce its utility as a valuable parent in olive breeding programs for disease resistance.

Acknowledgments This work has been supported by research projects RTA2013-00019-00-00 from the Spanish National Institute for Agricultural and Food Research and Technology (INIA), and PEI.PEI2011.1 from Andalusian Institute of Agricultural Research and Training (IFAPA), partially funded by European Regional Development Fund (ERDF). R. Arias-Calderón thanks Research Staff Training (FPI) Grants funding by Subprogram FPI-INIA of the Spanish Ministry of Economy and Competitiveness.

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