Evaluation of early vigor traits in wild olive germplasm

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Abstract

The control of the olive tree vigor is a critical factor to ensure adequate production and longevity of high-density olive cultivation. To deal with this problem, we intend to take advantage of the genetic variability present in the wild subspecies of *Olea europaea* to identify genotypes of reduced vigor with optimal adaptability to diverse soils and adverse environmental conditions. In this work, early vigor traits have been characterized in different wild olive subspecies that could potentially be used as rootstocks to control the vigor of grafted cultivars. Significant differences between genotypes were obtained for all vigor traits and high values of broad sense heritability were obtained for most of them, indicating strong genetic effect and therefore good possibilities for selection. Compared to ‘Arbequina’ and ‘Picual’ cultivars, some of the wild genotypes evaluated showed a

clear lower vigor at the end of the experimental period. These genotypes could be tested as dwarfing rootstocks for high-density olive plantations.

Keywords: crop wild relatives; heritability; *Olea europaea*; rootstock; variability

Introduction

Crop wild relatives could represent interesting sources of variability for agronomic traits difficult to find in cultivated materials, such as resistance to pest and diseases and abiotic stresses, yield and quality traits. Thus, in the last years, the use of crop wild relatives germplasm has been extensively promoted in breeding programs of several fruit trees and nut crops (Aradhya et al., 2015).

Olea europaea subsp. *europaea* var. *europaea* and var. *sylvestris* constitute the botanical varieties of cultivated and wild olives respectively, both of them widely spread throughout the whole Mediterranean basin. Besides the subsp. *europaea*, five other subspecies are included within the *Olea europaea* species, based on morphology and geographical distribution, including subspp. *cuspidata* and *laperrinei* spread in tropical and subtropical regions, subspp. *guanchica*, only recognized in the Canary Islands as well as the only two polyploid subspp. *maroccana* (6n) and *cerasiformis* (4n) present in southwestern Morocco; and Madeira archipelago, respectively (Green 2002; Besnard et al., 2013).

The potential use of olive wild genetic resources also known as oleasters as a source of genetic variability for important agronomic traits has been suggested, particularly regarding resistance to specific adverse biotic and abiotic environmental conditions (Lavee and Zohary, 2011, Hernandez-Santana et al., 2019). Agronomic evaluation of

progenies involving wild parents showed higher vigor, shorter juvenile period and more abundant flowering than progenies from cultivated materials (Klepo et al., 2013). Improved oil composition for some important quality components has been also reported (Hannachi et al., 2009; León et al., 2018). However, wild olives also transmit undesirable fruit traits to its descendants (particularly lower fruit size and oil content), which indicates the need of additional back-cross generations and, therefore, slow down the selection process (Klepo et al., 2014). The need for a longer selection period respect to the cultivated germplasm has limited the potential use of wild relatives as parents in olive breeding programs up to now.

Rootstock selection allows a straightforward way of valorizing wild genetic resources (Warschefsky et al., 2016). In fact, grafting on wild materials was extensively used in the antiquity as a propagation technique in olive. Thus, the analysis of ancient olive trees in several countries reveals that grafting by using seedling, pre-selected clonal rootstocks or oleasters has been a practice widely used in the Middle East (Barazani et al., 2014) and at a lower extent in central (Lazović et al., 2016) and western part (Ninot et al., 2018) of Mediterranean Basin.

However, it should be noted that olive trees are currently mainly grown on their own roots and rootstocks are exceptionally used in olive compared to other fruit tree species. The use of rootstocks has been recommended to cope with adverse biotic factors such as soilborne disease (Porrás-Soriano et al., 2003; Bubici and Cirulli, 2012) and abiotic environmental conditions such as preventing frost injury (Panelli et al., 2002) or iron chlorosis caused by Fe deficiency (Alcantara et al., 2003). The use of rootstocks to control the vigor of the grafted cultivar has also been tested in several works (Baldoni and Fontanazza, 1990; Panelli et al., 2002; Romero et al., 2014; Rugini et al., 2016),

particularly due to the spreading of high density hedgerows olive orchards in recent years and the need for increasing the number of cultivars suitable for this new growing system (Rallo et al., 2013).

The use of wild genetic resources as rootstocks may represent a new source of variability for important agronomic traits. In olive, it has been only studied as a way to improve resistance to *Verticillium* wilt of susceptible cultivars (Arias-Calderón et al., 2015; Jiménez-Fernández et al., 2016). However, to the best of our knowledge, a comprehensive evaluation of vigor traits in olive wild materials and the potential use of wild genetic resources as rootstocks for vigor control has not been attempted so far in olive.

The aim of this work was the characterization of early vigor traits in different wild olive subspecies representing a wide range of genetic variability of the species *Olea europaea* in order to study the variability and heritability for these traits and the relationships between them, and to identify genotypes that could be potentially used as rootstocks to optimize intensive olive cultivation.

Materials and Methods

Early vigor parameters were studied in 43 olive wild genotypes from different subspecies and areas of origin:

- *Olea europaea* subsp. *europaea* var. *sylvestris*: 17 genotypes from open pollination of two trees coming from different provenances in Andalusia (11 from Cádiz (CAD) province (South-West of the region) and 6 from Jaén (JAE) province (East of the region)), and 7 from open pollination of one tree of the area of Marrakech (MAR) in Morocco.

- *Olea europaea* subsp. *guanchica* (GUA): 9 genotypes from open pollination of one tree of Canary Island

- *Olea europaea* subsp. *cuspidata* (CUS): 1 genotype from open pollination of one tree of South Africa

- Hybrids *O. europaea* subsp. *cuspidata* x *europaea* var. *sylvestris* (CEH): 9 genotypes from crosses.

Homogeneous plants of each genotype were initially obtained from *in-vitro* seedlings germinated in 100% strength Rugini medium (Rugini, 1984) without hormones. Uninodal segments were further micropropagated in 100% strength Rugini medium (Rugini, 1984) supplemented with 1 mg/l zeatin. *In vitro* explants were incubated in a room chamber under 16 h light photoperiod (34 μ M intensity with light-emitting diode (LED) 70% red plus 30% blue) at $25 \pm 2^\circ\text{C}$. Explants were grown up to 7-8 cm height and transferred for 21 days to rooting medium, consisting on 50% strength Rugini medium supplemented with α -naphthalacetic acid (0.8 mg / l). Rooted seedlings were acclimatized *ex-vitro* for 3 weeks, transplanted to 1L pots and grown under greenhouse conditions.

Afterwards plants were established in the field at the experimental farm of IFAPA Centre “Alameda del Obispo” in Cordoba (Spain) in May 2015 at 4×2 m spacing in a randomized design with 6 replicates per genotype. Plants of ‘Arbequina’ and ‘Picual’ cultivars obtained by standard vegetative propagation of semi-hardwood stem cutting were included for comparison. Standard cultural practices were followed, with no pruning to allow free development of plants and irrigation by in-line drip to avoid water stress of plants.

Vigor traits measurements were taken in Summer (July) and Winter (January). In Summer 2015 (S15) the following traits were measured: plant height (PH), diameter of the main axis at different heights (DG, DL, DC measured at ground level, 50 cm and 100 cm from

ground, respectively), maximum internode length (IM), total number of nodes (NN) and number of branches (NB), and length of the longest branch (BL). Plant height, diameter of the main axis at different heights and length of the longest branch measurements were repeated in Winter 2016 (W16), Summer 2016 (S16), Winter 2017 (W17) and Winter 2018 (W18). Finally, canopy volume (CV) was also calculated in Winter 2018 from measurements of canopy height and width for final categorization of plant vigor.

Descriptive statistics and variability box and whisker plots were obtained for the evaluated agronomic traits. Analysis of variance was carried out to test differences between genotypes and broad sense heritability was estimated as $h^2 = \sigma^2_G / (\sigma^2_G + \sigma^2_E)$, where σ^2_G is the variance between genotypes and σ^2_E is the residual variance. Spearman's rank correlation between characters was calculated from average values per genotype.

Results

Overall, a wide variability was observed for all vigor traits measured at different time points. This variability was maintained throughout the evaluation period for some of them such as PH, or gradually increased for others such as DG (Figure 1). Average PH increased from 94 cm PH-S15 to 199 cm PH-W18, while DG increased from 6 mm DG-S15 to 39 mm TDG-W18.

Figure 1.- Box and whisker plots for diameter of the main axis at ground level (DG) and plant height (PH) measured sequentially during the evaluation period (from left to right: Summer 2015, Winter 2016, Summer 2016, Winter 2017 and Winter 2018).

Significant differences between genotypes were obtained for all vigor traits, being the variance component between genotypes higher than the error variance for most of them (Table 1). As a consequence, high values of broad sense heritability were obtained for most of the evaluated vigor traits. It should be noted that the highest heritability values were obtained at the beginning of the evaluation period just after planting in the field, but heritability values decreased throughout the evaluation period for those traits measured sequentially due to a higher proportional increase of error variance as the plants grew older. For instance, heritability values for PH decreased from 0.93 in S15 to a half 0.47 in W18.

Table 1.- Variance components and heritability of vigor traits measured during the evaluation period.

	PH-S15	PH-W16	PH-S16	PH-W17	PH-W18
Genotype	1017,05	697,88	649,20	764,21	762,25
Error	72,39	255,47	404,22	571,94	846,73
h ²	0,93	0,73	0,62	0,57	0,47
	BL-S15	BL-W16	BL-S16	BL-W17	
Genotype	58,73	214,85	278,26	356,11	
Error	38,35	204,11	333,89	469,62	
h ²	0,60	0,51	0,45	0,43	
	DG-S15	DG-W16	DG-S16	DG-W17	DG-W18
Genotype	1,45	5,77	13,39	25,07	80,98
Error	0,47	3,50	8,70	23,84	73,65
h ²	0,75	0,62	0,61	0,51	0,52
		DL-W16	DL-S16	DL-W17	DL-W18
Genotype		4,30	7,72	16,92	44,58
Error		2,26	5,26	15,20	44,18
h ²		0,66	0,59	0,53	0,50
			DC-S16	DC-W17	DC-W18
Genotype			7,56	13,70	43,04
Error			4,59	11,92	46,48
h ²			0,62	0,53	0,48
	IM-S15				
Genotype	76,90				
Error	36,34				
h ²	0,68				
	NN-S15				
Genotype	113,04				
Error	20,46				
h ²	0,85				
	NB-S15				
Genotype	99,88				
Error	40,85				
h ²	0,71				

Plant height (PH), length of the longest branch (BL), diameter of the main axis at different heights (DG, DL, DC measured at ground level, 50 cm and 100 cm from ground, respectively), Maximum internode length (IM), total number of nodes (NN), number of branches (NB). Summer 2015 (S15), Winter 2016 (W16), Summer 2016 (S16), Winter 2017 (W17) and Winter 2018 (W18).

Average data per genotype indicated also a high variability for vigor traits among genotypes and a general reduction on the differences between genotypes as the plants grew older (Table 2). As an example, coefficient of variation values for PH decreased from 34.2 % in S15 to 15.1 % in W18. Only for diameter of the main axis measured at ground level (DG) variability among genotypes remained similar during the evaluation period, with coefficients of variation from 21.3 to 26.5 %. In any case, a general wide variability and, therefore, possibilities for selection according to vigor characterization could be inferred for all the evaluated traits and periods.

Table 2.- Mean, minimum, maximum and coefficient of variation for vigor traits (average values per genotype) measured during the evaluation period on 43 wild genotypes.

	PH-S15	PH-W16	PH-S16	PH-W17	PH-W18
Mean	93,8	130,9	153,1	179,3	199,5
Min	41,8	79,2	97,5	115,8	150,8
Max	163,7	197,5	219,5	253,8	270,0
CV (%)	34,2	20,7	17,5	16,3	15,1
	BL-S15	BL-W16	BL-S16	BL-W17	
Mean	16,9	40,9	57,0	82,2	
Min	4,5	13,8	19,7	28,6	
Max	43,2	88,2	103,8	117,8	
CV (%)	47,6	38,8	32,6	25,5	
	DG-S15	DG-W16	DG-S16	DG-W17	DG-W18
Mean	5,8	10,2	14,5	20,3	39,4
Min	3,5	5,9	8,1	9,9	18,7
Max	9,2	16,6	25,9	37,4	67,6
CV (%)	21,3	24,8	26,5	26,5	24,5
		DL-W16	DL-S16	DL-W17	DL-W18
Mean		7,3	10,4	15,1	30,1
Min		3,4	4,6	7,1	15,5
Max		11,5	16,6	26,5	44,6
CV (%)		29,7	28,3	29,2	23,8
			DC-S16	DC-W17	DC-W18
Mean			6,4	9,5	22,6
Min			1,3	2,3	8,4

Max	12,8	20,3	41,5
CV (%)	47,3	42,4	31,3
IM-S15			
Mean	37,9		
Min	27,0		
Max	63,8		
CV (%)	23,9		
NN-S15			
Mean	39,0		
Min	20,2		
Max	68,3		
CV (%)	27,7		
NB-S15			
Mean	23,7		
Min	3,8		
Max	48,7		
CV (%)	43,9		

Plant height (PH), length of the longest branch (BL), diameter of the main axis at different heights (DG, DL, DC measured at ground level, 50 cm and 100 cm from ground, respectively), Maximum internode length (IM), total number of nodes (NN), number of branches (NB). Summer 2015 (S15), Winter 2016 (W16), Summer 2016 (S16), Winter 2017 (W17) and Winter 2018 (W18).

Spearman's rank analysis showed significant correlations among the different vigor parameters (Figure 2). In general, correlation values were higher between different vigor traits measured at the same timepoint or between common vigor traits measured at consecutive timepoints. However, some traits such as NB-S15 and PH-W18 showed always slight correlations with the other evaluated vigor parameters ($r < 0.60$). Canopy volume (CV), calculated in Winter 2018 showed significant correlation with all the evaluated vigor traits, the highest values (> 0.80) been observed with traits measured from Summer 2016, i.e. one year after planting in field. Among them, CV showed the highest correlation with diameter of the main axis measured at 50 cm from ground in Winter 2018 (DL-W18, $r = 0.901$).

Figure 2.- Spearman's rank correlations between average vigor traits per genotype measured during the evaluation period. Plant height (PH), length of the longest branch (BL), diameter of the main axis at different heights (TDG, TDL, TDC measured at ground level, 50 cm and 100 cm from ground, respectively), Maximum internode length (IM), total number of nodes (NN), number of branches (NB). Summer 2015 (S15), Winter 2016 (W16), Summer 2016 (S16), Winter 2017 (W17) and Winter 2018 (W18).

Final classification of genotypes according to values on CV-W18 and TDL-W18 showed some clear differences regarding the origin of the plant materials (Figure 3). Thus, the lowest vigor was obtained in *sylvestris* genotypes from Jaen (Spain) and Marrakech (Morocco) provenances, while the highest vigor was obtained in *sylvestris* genotypes from Cadiz (Spain) and genotypes from crosses *cuspidata* x *sylvestris*. However, a wide variability was obtained in all cases, particularly in genotypes from *guanchica* origin. Compared to ‘Arbequina’ and ‘Picual’ cultivars, included as controls for comparison,

some of the wild genotypes evaluated showed a clear lower vigor at the end of the experimental period.

Figure 3.- Scatter plot of average data per genotype for diameter of the main axis at 50 cm from ground and canopy volume measured at the end of the experimental period in Winter 2018, TDL-W18 and CV-W18, respectively. Data are grouped according to the origin of the genotypes, including ‘Arbequina’ and ‘Picual’ as controls for comparison. Data were normalized by the mean and SD for each trait.

Discussion

High density hedgerows olive orchards have spread in the last decades in many olive producing countries taking advantages of the higher initial productivity and the

possibilities for labor mechanization, particularly continuous harvesting by straddle harvesting machines (Rallo et al., 2013). However, the difficulties for canopy size control allowing adequate long-term management of plantations mean that only a very limited number of cultivars have been successfully adapted for this system, contrarily to traditional olive growing in which large number of cultivars are available for cultivation. Therefore, only a few early-bearing, highly productive, low vigor and weeping habit cultivars such as ‘Arbequina’ and ‘Arbosana’ are widely planted in hedgerow systems (De la Rosa et al., 2007).

Among the potential techniques to control the vigor of fruit trees, the use of dwarfing rootstocks has been widely used in many fruit species (Gregory et al., 2013). It is well known that full range of tree size control, from dwarfing to highly vigorous, could be achieved in several fruit tree species, particularly apple, by using adequate rootstocks, which allows design of specific growing systems and planting density in each case (Cummins and Aldwinckle, 1983). In olive, the use of rootstocks as a vigor control tool has been attempted in several works by using different plant materials including cultivars and breeding selections obtained by classical cross-breeding or mutagenesis (Baldoni and Fontanazza, 1990; Romero et al 2014; Rugini et al., 2016).

A wide intraspecific variability for vigor traits has been reported from the evaluation in cultivar collections (Del Río et al., 2005; Tous et al., 2005). A quite similar variability has been found in this work in genotypes from different subspecies and area of origin. Thus, for instance, a coefficient of variation of 24.8 % was obtained from the analysis of trunk diameter ten years after planting in 61 cultivars evaluated at the World Olive Germplasm of IFAPA in Córdoba compared to 24.5 % obtained in 43 olive wild genotypes in this work. A high heritability and strong genetic effect could be also inferred

from the evaluation of vigor traits in these cultivar collections. However, significant environmental influence has also been observed for some cultivars, which underlined the need of extensive testing in different environments for adequate selection. In fact, a high correlation has been reported between olive tree vigor and different soil and landscape characteristics such as thickness of the root zone, clay and cation exchange capacity and distance to summit (Gálvez et al., 2004).

The whole results of vigor evaluation should be considered in terms of variability, heritability and correlation among them. A high correlation between 17 different vigor parameters was also observed in the characterization of olive progenies from crosses between different cultivars, which allows the selection of a group of them as the best growth descriptors based on their influence by parent genotype (Hammami et al., 2011). Moreover, a high influence of plant age on growth habit traits was also determined and early characterization at the time of planting in the field was suggested the most appropriate age for evaluating seedling growth parameters (Hammami et al., 2011). Early vigor measurements at the seedling stage before planting in the field was also proposed in olive progenies as a tool for selection for short juvenile period (De la Rosa et al., 2006). In this work however, measurements taken early after planting showed high heritability and variability among genotypes, but lower correlation with final characterization of the genotypes. The results obtained suggest that measurements taken from one year after planting could be considered more efficient for selection.

Among the wild genotypes evaluated in this work, some of them showed a clear lower vigor than ‘Arbequina’ at the end of the experimental period and could be considered potentially interesting dwarfing rootstocks. Future works should be carried out to test whether the differential vigor in the evaluated wild materials is extensible to the grafted

cultivars of interest. Previous works testing single cultivars grafted on a range of potential interesting rootstocks indicate that the intrinsic vigor of some rootstock is transferred to the grafted cultivar. However, opposite results were also found in some cases, although it should be noted that explicit evaluation of vigor of the rootstock used was not carried out but based on previous literature (Troncoso et al., 1990; Romero et al., 2014). Similarly, different vigor control ability has been obtained for some rootstocks depending on the cultivar grafted, while a general dwarfing effect was observed for some others (Panelli et al., 2002). These partly contradictory results suggest that further research is still necessary to guarantee adequate choice of rootstocks. Again, the environmental conditions should be also carefully considered in testing. For instance, water availability showed a marked effect on growth and production parameters that obscure the potential rootstock effect in experiments with reciprocal scion/rootstock combination of three olive cultivars (Lavee and Schachtel, 1999; Hernandez-Santana et al., 2019). Moreover, the effect of grafting itself has been showed to control vigor by reducing plant size of self-grafted genotypes (Rugini et al., 2016), although no effect of self-grafting has also been reported for some cultivars (Panelli et al., 2002).

Several mechanisms have been suggested as responsible of the dwarfing potential of olive rootstock, mainly related to the hydraulic architecture of the different scion/rootstock combinations (Nardini et al., 2006), as also reported for other fruit tree species (Cohen et al., 2007). The hydraulic characterization of the wild plant materials showing contrasting dwarf effect in our work could provide valuable information in future.

Conclusions

A wide variability for early vigor traits has been obtained from the evaluation of wild olive germplasms from different origins. In particular, low vigor and potentially interesting dwarfing effect was obtained in some *sylvestris* genotypes from Jaen (Spain) and Marrakech (Morocco) provenances and some genotypes from *guanchica* origin. Future works should be carried out to test whether the differences in growth traits between the studied plant materials are translated into vigor control of grafted materials and, moreover, whether it represents a general effect or it is limited to particular scion/rootstock combinations. Moreover, the potential effect of the rootstock on other important agronomic traits of the grafted cultivar, such as earliness of bearing, productivity fruit and oil traits, should be also evaluated to guarantee adequate choice of rootstock. The use of rootstocks as a tool to control vigor in olive could open new possibilities for future olive growing, mainly regarding high-density hedgerows olive orchards where only a very limited number of cultivars have been successfully adapted up to now.

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