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Mapping quantitative trait loci controlling fatty acid composition in olive

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Abstract Fatty acids are the main components of the olive oil and their composition has a critical influence on the oil quality. However, oil quality evaluation has not been frequently included in the selection of new bred cultivars. This can be due to the difficulties in analyzing oil quality in large set of genotypes and also to the long juvenile period of olive seedlings. Therefore, the identification of molecular markers associated to olive oil quality traits could facilitate their selection in breeding programs of this species. In the present work, the identification of the first QTLs for fatty acids on olive oil is reported. They have been located in a linkage map of a 'Picual' × 'Arbequina' progeny of the olive breeding program of Córdoba. Correlations among fatty acids are in agreement with previous reports of breeding progenies. QTLs found for oleic and linoleic acids explained 41.1 and 69.7% of the total variability, respectively, and were co-localized in the same linkage groups. In the same region, QTLs for monounsaturated, polyunsaturated and oleic/linoleic ratio were also identified. In other linkage groups, three QTLs for linolenic and one for palmitoleic acid were also located explaining 15.0–28.0% of the total variability. These results could be useful to increase the efficiency of breeding programs aimed at selecting new cultivars with high oleic acid content, and, therefore, with enhanced nutritional properties and oxidative stability of the olive oil.

Keywords *Olea europaea* L. · Breeding · QTL · Olive oil · Oleic acid

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Introduction

Olea europaea L. (2n = 2x = 46) is one of the most economically important trees in the Mediterranean basin with over 98% of the 2.8 MTm of virgin olive oil (VOO) produced in the world (FAOSTAT 2013). VOO is the main source of fat in the Mediterranean diet. This oil is obtained as a fruit juice, i.e., directly from the crushing of olive fruits and its consumption has been widely associated with positive health benefits (Covas 2008; Schwingshackl and Hoffmann

2014). Fatty acids, the main components of the olive oil, are considered directly implicated in the health benefits of the olive oil (Di Bella et al. 2007; Quintero-Florez et al. 2015). In particular, the role of VOO in the protection against cardiovascular disease has been mostly attributed to its high oleic acid content (Rietjens et al. 2007). In contrast, elevated linoleic acid content may cause a negative impact in the nutritional properties of olive oil, since recent studies using seed oils characterized by high linoleic acid content indicates that an excessive consumption of this fatty acid in the diet is associated with a higher risk of hypertension and cardiovascular and carcinogenic diseases (Bonow and Eckel 2003; Vos 2003). Besides, the oleic/linoleic ratio has also important consequences in the technological properties of the olive oil, with high linoleic acid content affecting negatively its oxidative stability (Gutiérrez et al. 1999). In addition, the levels of individual fatty acids are also important at the regulatory level. According to European Commission regulation 702/2007 (EC 2007), the contents of oleic acid must range from 55 to 83%, while linoleic acid must account for 3.5–21% and linolenic acid for less than 1%.

The fatty acid biosynthesis pathway is well known in plants including olive. In vascular plants, the fatty acid biosynthesis starts in the plastids, yielding primarily palmitoyl-acyl carrier protein (ACP) and stearoyl-ACP by successive addition of two carbon atoms from acetyl-CoA (Harwood 2005). Still in the plastid, most of the stearoyl-ACP is desaturated by the action of a soluble Δ9 stearoyl-ACP desaturase producing oleoyl-ACP, which is the main product of the plastidial fatty acid biosynthesis. The oleic acid is then incorporated into glycerolipids inside or outside plastids, and it can be further desaturated to linoleic, and then to α-linolenic acid by the consecutive action of Δ12 and Δ15 desaturases. Two sets of these enzymes are present in plant cells, which differ in their cellular localization (Shanklin and Cahoon 1998). The microsomal oleate desaturase (FAD2) and linoleate desaturase (FAD3) are located in the endoplasmic reticulum (ER), whereas the plastidial oleate desaturase (FAD6) and linoleate desaturase (FAD7/8) are located in the chloroplast.

The fatty acid composition of olive oil is influenced by pedoclimatic conditions, olive growing practices (Jimenez Herrera et al. 2012; Dabbou et al. 2015) and the cultivar (Rondanini et al. 2011). In fact, high variability for fatty acid composition has been observed in cultivar collections (Rotondi et al. 2013; Uceda et al. 2005). However, most of the current olive cultivars are very ancient and have been obtained by the empiric selection of the growers mainly on the basis of their productivity, oil content and fruit size, but not on oil composition (Barranco et al. 2010; Bracci et al. 2011). Besides, none of the few cultivars obtained by systematic breeding, such as 'Barnea' (Lavee et al. 1986), 'Maalot' (Lavee et al. 1999), 'Askal' (Lavee et al. 2003), 'Fs-17' (Bellini et al. 2002) or 'Sikitita' (Rallo et al. 2008) has been specifically selected for having a superior oil composition. This is mainly due to the fact that the evaluation of oil quality traits, including fatty acids, is a very time consuming and costly task. Initially, seedlings have to overcome the juvenile period and then to reach a significant size in order to bear enough amount of fruits to allow oil extraction (De la Rosa et al. 2006). Then, to extract and analyze oil from the large progenies usually obtained in breeding programs represents a very complicated and difficult task. The fact that the content of some oil components is not affected by the oil extraction process and can be directly measured in fruit without the need of oil extraction, could partly overcome this problem (Garces and Mancha 1993; Velasco et al. 2014). Although some studies suggested high heritability for fatty acid composition (Dabbou et al. 2010; De la Rosa et al. 2016), there is little knowledge on the genetic control of its variability among olive cultivars.

In this context, the use of molecular markers could be helpful to investigate the genetic control of important traits and for the identification of beneficial alleles through the development of linkage maps and marker-trait associations as QTL analysis (El-Soda et al. 2014). Actually, few QTL analyses have been performed in olive including flowering-related traits (Ben Sadok et al. 2013) using a 'Olivière × 'Arbequina' progeny. Thus, the objective of this work was the identification of QTLs associated to the fatty acid profile in a segregation progeny of 'Picual' × 'Arbequina' where molecular markers associated with fruit-related traits and oil content has been previously found (Atienza et al. 2014). This cross has been very successful in olive breeding, showing high variability for fatty acid composition (León et al. 2004b) and producing the first olive cultivar registered in Spain, 'Sikitita' (Rallo et al. 2008).

Materials and methods

Plant material

A progeny coming from the cross of 'Picual' × 'Arbequina' performed in spring 2001 were used in the present study. Seedlings were planted in open field in September 2003, at 4 × 1.5 m of spacing at the experimental orchard of IFAPA, Centre "Alameda del Obispo", Córdoba, Spain. Trees were trained to form the canopy at 1.6 m height and then develop freely, as suggested in previous experiments (Santos-Antunes et al. 2005) and yearly irrigated with 2000 m^3/ha of water. This progeny comes from the cooperative breeding program of the University of Cordoba and IFAPA, Spain. The oils of the two parents are known to have contrasting fatty acid composition (Hernández et al. 2009).

Fatty acid analysis

Sixty genotypes which showed enough crop for oil extraction were selected for fatty acid analyses during the 2008/2009 season. A random sample of 1000 g of olives was hand-collected per seedling. Samples were collected when most fruits were at maturity index 2, 5 (Frías et al. 1991). VOO was extracted using an Abencor analyzer (Commercial Abengoa, S.A., Seville, Spain) that simulates the industrial process of VOO production at lab scale (Martinez-Suarez et al. 1975). Milling of whole olive fruits was performed using a stainless steel hammer mill operating at 3000 rpm provided with a 5 mm sieve. Malaxation was carried out for 30 min with the Abencor thermobeater operated at 30 °C according to industry recommendations. Centrifugation of the kneaded paste was performed in a basket centrifuge at 3500 rpm for 1 min. After centrifugation, the oils were decanted and paper filtered. Oils were stored under nitrogen at −20 °C until analysis.

Fatty acid composition of the different olive oils was determined using the one-step method of (Garcés and Mancha 1993). After the addition of 2 ml of methanol-toluene-H_2SO_4 (80:20: 2, vol/vol/vol) to 50 mg of olive oil, the mixture was incubated for 1 h at 80 °C. After cooling, 2 ml heptane and 5 ml Na_2SO_4 were added, and the upper phase containing the fatty acid methyl esters was analysed by gas–liquid chromatography using a 7890A (Agilent, Santa Clara, CA USA) fitted with a capillary column (30-m length; 0.32-mm inner diameter; 0.2-μm film thickness) of fused silica (Supelco, Bellefonte, PA, USA) and a FID detector. Hydrogen was used as a carrier gas with a linear rate of 1.34 ml min^{-1} and split ratio of 1/50. The injector and detector temperature was 220 °C and the oven temperature was 170 °C. Results were obtained in mol % of the different fatty acids and expressed as means of three independent determinations.

The following traits were considered for QTL analyses. Individual fatty acids (% over total oil content): palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. Quality indices: total saturated (stearic + palmitic); total unsaturated (oleic + linoleic + linolenic + palmitoleic), total monounsaturated (oleic + palmitoleic) and total polyunsaturated (linoleic + linolenic) were also considered. Ratio oleic/linoleic was considered as an additional trait. Pearson correlation coefficients among traits were calculated using IBM SPSS Statistics 20.

QTL analyses

The genetic map, developed by means of DArT and SSR markers, for 'Picual' × 'Arbequina' progeny (Dominguez-Garcia et al. 2012) was used for QTL analysis. Two independent QTL analyses (one for each parental map) were performed using MAPQTL 5.0 package (Van Ooijen 2004). First, the non-parametric Kruskal–Wallis (KW) test was performed to identify association between markers and traits individually, without considering the map information. After this, interval mapping (IM) analyses were performed (Lander and Botstein 1989; van Ooijen 1992). An initial set of cofactors was selected from KW and IM results and a backwards elimination procedure was applied to select significant markers as implemented in MapQTL 5.0. Only significant markers at $P < 0.1$ were used as cofactors in the multiple QTL methods (rMQM and MQM) (Jansen 1993, 1994; Jansen and Stam 1994) analyses. A mapping step size of 1 cM was used for IM and MQM analyses. The significance thresholds for accepting the presence of potential QTLs were empirically determined using a permutation analysis (500 permutations) (Churchill and Doerge 1994) as implemented in MapQTL 5.0. An estimation of the total variance explained at the position with the highest LOD score was given by

Table 1 Basic statistics for the fatty acids, the quality indices, and the olive oil content in the progeny and parent cultivars

Trait[a]	Picual	Arbequina	Population			
			Mean	CV(%)[b]	Min	Max
Oleic	76.95	61.55	71.0	9.7	50.6	81.9
Palmitic	14.05	18.35	14.9	15.4	10.2	21.9
Linoleic	4.17	14.77	8.8	54.5	2.9	23.1
Stearic	2.15	1.74	1.8	27.8	1.0	3.6
Palmitoleic	1.77	2.83	2.5	32.0	1.2	4.2
Linolenic	0.91	0.76	1.0	20.0	0.7	1.5
Saturated	16.20	20.09	16.8	13.7	12.4	23.6
Unsaturated	83.80	79.91	83.2	2.8	76.4	87.6
Monounsaturated	78.72	64.38	73.5	9.0	54.3	83.3
Polyunsaturated	5.08	15.53	9.8	49.0	3.7	24.6
Ratio Sat/unsaturated	0.19	0.25	0.2	0.0	0.1	0.3
Ratio oleic/linoleic	18.5	4.20	11.2	59.8	2.2	27.9

[a] All values but the ratios are expressed as mol%. Saturated = Stearic + Palmitic; Unsaturated = Oleic + Linoleic + Linolenic + Palmitoleic; Monounsaturated = Oleic + Palmitoleic; Polyunsaturated = Linoleic + Linolenic

[b] Coefficient of variation

MapQTL® 5.0. The QTL positions were estimated as the position with the maximum LOD score on a linkage group. Uncertainty of the map position was indicated by a 1-LOD support interval (Conneally et al. 1985; van Ooijen 1992). MapChart software (Voorrips 2002) was used to indicate location of the QTL for fruit traits in the 'Picual' and 'Arbequina' maps. For each QTL, the difference in the alleles effect was determined using the Knott et al. (1997) method (Atienza et al. 2003a, 2003b; Sewell et al. 2000). In a cross between two heterozygous parents ('CP' population in Joinmap) a QTL can segregate for four different alleles. Thus, four different genotypic classes can be obtained 'ac', 'ad', 'bc', 'bd' from the parental mating type ab × cd. Since the pseudo-testcross strategy was used for map construction, 'Picual' markers are genotyped as 'lm × ll' and thus 'ac' ≡ 'ad' ≡ 'll'; 'bc' ≡ 'bd' ≡ 'lm' and the difference in effect of the alleles from 'Picual' (P_{Pic}) = 'bc'−'ac' = 'lm'−'ll'. Similarly, 'Arbequina' markers are genotyped as 'nn × np' and thus, 'ac' ≡ 'bc' ≡ 'nn'; 'ad' ≡ 'bd' ≡ 'np' and the difference in effect of the alleles from 'Arbequina' (P_{Arb}) = 'ad'−'ac' = 'np'−'nn'.

Results and discussion

Phenotypic variation in fatty acid composition

Six fatty acids were quantified including oleic, palmitic, linoleic, palmitoleic, stearic and linolenic acids, although other fatty acids were also found in trace amounts, such as arachidic or eicosenoic acids. Basic statistics were calculated for these compounds and the quality indexes described in the Materials and methods section (Table 1), while their distributions are shown in Supplementary Material 1.

Oleic acid was the main constituent of the fatty acid profile of the progeny with a mean value of 71.0% followed by palmitic (14.9%) and linoleic acid (8.8%) (Table 1). The remaining fatty acids only constituted the 5.3% of the total fatty acid composition. As far as the parents, 'Picual' showed a higher oleic content (76.95%) than 'Arbequina' (61.55%) while 'Arbequina' oil was characterized by higher palmitic (18.35%) and linoleic (14.77%) contents than 'Picual' (Table 1), in total agreement with previous reports (Uceda et al. 1999; Leon et al. 2008). Considerable variability was observed for all the fatty acids content, as previously reported in other progeny of the same cross (León et al. 2004b). This high variability together with the high genotypic effect previously found for this character (De la Rosa et al. 2016) indicates that the cross between 'Picual' and 'Arbequina' is very convenient for breeding programs aimed at producing new cultivars with high percentage of oleic acid in their oils.

Correlation analysis of oil content and fatty acids

Pearson correlations were calculated including not only individual fatty acids and quality indexes, but also the previously reported oil content in fruit on dry

Table 2 Pearson coefficient among fatty acids and oil content in the 'Picual' × 'Arbequina' olive population

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Saturated	Unsat	Mono unsat	Poly unsat
Palmitoleic	0.71***									
Stearic	−0.03	−0.27*								
Oleic	−0.87***	−0.42**	−0.09							
Linoleic	0.64***	0.11	0.10	−0.93***						
Linolenic	0.29*	0.14	−0.15	−0.45***	0.47***					
Saturated	0.98***	0.65***	0.17	−0.87***	0.65***	0.26*				
Unsaturated	−0.98***	−0.65***	−0.17	0.87***	−0.65***	−0.25	−1.00***			
Mono unsaturated	−0.82***	−0.31*	−0.12	0.99***	−0.96***	−0.45***	−0.84***	0.83***		
Poly unsaturated	0.64***	0.11	0.09	−0.93***	1.00***	0.49***	0.65***	−0.65***	−0.96***	
OCFDW	0.29*	0.03	0.10	−0.22	0.18	−0.31*	0.30*	−0.31*	−0.23	0.17

OCFDW Oil content fruit dry weight

Significant correlations are indicated by * $P \leq 0.05$, ** $P \leq 0.0005$; *** $P \leq 0.0001$

weight basis (OCFDW) (Atienza et al. 2014) (Table 2). Although they do not determine the cause-and-effect relationships between the phenotypic traits, they estimate the strength of association between them, which is useful for breeding and mapping purposes.

The highest correlation was found between the two main fatty acids of olive oil, oleic and linoleic acids, which indicates that any increase in one of them will imply a decrease in the other. This is to be expected since linoleic acid is directly formed by desaturation of oleic acid, which is catalysed by the oleate desaturase activity (Shanklin and Cahoon 1998). In fact, this negative correlation seems to be general in olive (León et al. 2004a; Dabbou et al. 2012; Sabetta et al. 2013) and in other oil crops such as sunflower (Pérez-Vich et al. 2004), sesame (Were et al. 2006), maize (Wassom et al. 2008), Jatropha (Liu et al. 2011), rice (Ying et al. 2012), almond (Font i Forcada et al. 2012) and oil palm (Montoya et al. 2013, 2014).

Palmitic acid was negatively correlated with oleic acid (Table 2). This observation agrees with various reports on olive (León et al. 2004a; Dabbou et al. 2012), sesame (Were et al. 2006), rapeseed (Zhao et al. 2008), oil palm (Singh et al. 2009; Montoya et al. 2013, 2014), rice (Ying et al. 2012) and almond (Font i Forcada et al. 2012). The biosynthesis of C18 fatty acids proceeds via an elongation step of C16 acyl chains, followed by desaturation (Voelker and Kinney 2001). The elongation step plays an important role in regulating the relative amounts of palmitic acid and C18 fatty acids (Carlsson et al. 2002). On the contrary, palmitic acid showed a positive correlation with linoleic acid, as it was previously reported in olive (León et al. 2004a) and almond (Font i Forcada et al. 2012). Particularly interesting is the lack of correlation between palmitic and stearic acids despite the fact that the later fatty acid is directly synthesized from the first. On the other hand, palmitoleic acid, which is directly synthesized from palmitic acid by a single desaturation step, is positively correlated with palmitic acid, but inversely associated with oleic acid.

Linolenic acid was inversely associated with oleic acid and positively correlated with linoleic acid, as previously described in maize (Wassom et al. 2008), rice (Ying et al. 2012), and oil palm (Montoya et al. 2013, 2014). Interestingly, the correlation between linoleic and linolenic acids was moderate, despite the fact that the second fatty acid is directly synthesized by desaturation of the first, as a result of the linoleate desaturase activity (Shanklin and Cahoon 1998). This result has also been observed in an olive collection (Sabetta et al. 2013).

As mentioned in the introduction, a high content of oleic acid and low on linoleic, linolenic and palmitic is considered very relevant in the health properties of the olive oil (Di Bella et al. 2007; Quintero-Florez et al. 2015). Therefore, the reported negative correlations of

Table 3 QTLs identified for fatty acid composition in the 'Picual' × 'Arbequina' olive population

Trait	Map	LG	LOD	Peak[a]	Exp %	Threshold[b]	P[c]	Allele effect[d]
Oleic	'Arbequina'	20	3.92	10.8	41.1	2.81	0.004	−8.8
Linoleic	'Arbequina'	20	8.15	10.8	69.7	2.9	0.000	7.9
Mono unsaturated	'Arbequina'	20	5.08	13.8	41.1	3.07	0.000	−8.4
Poly unsaturated	'Arbequina'	20	7.98	10.8	69.0	3	0.000	8.0
Ratio oleic/linoleic	'Arbequina'	20	6.31	10.8	57.6	2.9	0.000	−10.1
Linolenic	'Picual'	5	4.03	0	28.0	2.58	0.003	0.17
Linolenic	'Picual'	15	3.84	9.4	24.1	2.58	0.006	−0.15
Linolenic	'Arbequina'	19	3.21	47.5	15.4	2.84	0.020	0.1
Linolenic	'Arbequina'	14	3.13	0	15.0	2.84	0.021	0.1
Palmitoleic	'Arbequina'	13	3.32	1.2	22.5	3.1	0.038	0.8

[a] Position of the maximum LOD Score

[b] Genome wide threshold determined by 500 permutations

[c] QTL probability determined from 500 permutations

[d] The allele increasing the value are derived from 'Arbequina' (+) or 'Picual' (−)

oleic acid with the rest of the mentioned fatty acids content might be of interest for breeding programs aimed at improving the oil fatty acid composition.

Stearic and linolenic acids, both found in the lowest proportion in the olive oil, were the ones that displayed the weakest correlations with the four quality indexes calculated. None of the fatty acids quantified showed a strong correlation with the oil content (OCFDW).

QTLs involved in fatty acid composition

QTL analyses were independently performed in each parental map ('Picual' and 'Arbequina') (Table 3; Fig. 1) as usually performed in mapping populations derived from two heterozygous parents (Grattapaglia et al. 1995; Sewell et al. 2000; Atienza et al. 2003b, 2014; Socquet-Juglard et al. 2013). Two QTLs were detected in 'Picual' map whereas eight QTLs were found in 'Arbequina' map. More QTLs were also detected for fruit traits in the 'Arbequina' than in 'Picual' map in a previous work of our group (Atienza et al. 2014). This is likely influenced by the shorter genetic distance covered in 'Picual' map compared to the one of 'Arbequina' (Dominguez-Garcia et al. 2012).

A single QTL for oleic acid was identified on linkage group 20 in 'Arbequina' (Arb_20) map (Table 3; Fig. 1). It accounted for 41% of the phenotypic variance and it has an allele effect of −8.8 which indicates that the allele increasing oleic content is inherited from 'Picual'. Similarly, a QTL for linoleic acid was located in the same position (Table 3; Fig. 1). It explained 69.7% of the phenotypic variation and it shows an allele effect of 7.9, i.e., the allele increasing the content is inherited from 'Arbequina'. This is concordant with the fact that 'Arbequina' has higher linoleic acid and lower oleic acid content than the other parent 'Picual'. The co-localization of both QTLs and the different sign of the allele effect (Table 3; Fig. 1) are in agreement with the high negative correlation between both fatty acids (Table 2). Furthermore, the co-localization in the same region of QTL for monounsaturated and polyunsaturated fatty acids as well as for the ratio oleic/linoleic trait, reinforces the importance of this region for the determination of the fatty acid profile in olive oil. Whether there is a single segregating locus controlling the biosynthesis of oleic and linoleic acids or clusters of linked QTLs independently affecting the biosynthesis of both fatty acids cannot be discerned. Fine-mapping of this QTL region and the analysis of future genomic sequence data could allow the discrimination between both hypotheses.

The fact that QTLs for oleic and linoleic acids, as well as for monounsaturated and polyunsaturated fatty acids, and for the oleic/linoleic ratio were co-localized in the same linkage group of 'Arbequina' cultivar is significant considering that the proportions of these fatty acids have a important effect on olive oil quality (Gutiérrez et al. 1999). Regarding their metabolic

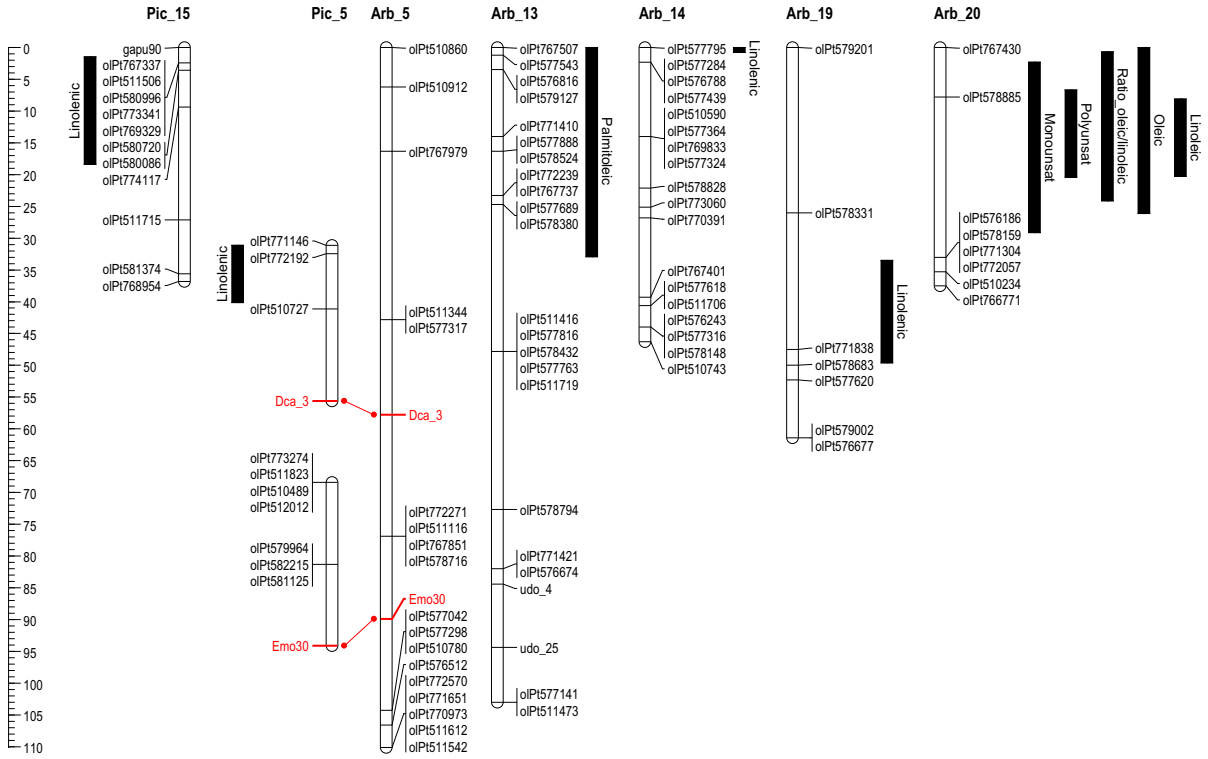

Fig. 1 QTL localization for fatty acid and quality traits in the olive progeny derived from 'Picual' × 'Arbequina'. The map was constructed using a pseudo-testcross strategy. Linkage groups from 'Picual' and 'Arbequina' maps are coded (Pic) and (Arb) respectively. QTL locations are shown as 1-LOD support intervals

origin, oleate desaturases catalyze the desaturation of oleic acid to produce linoleic acid. Two genes encoding microsomal oleate desaturases (*OepFAD2-1* and *OepFAD2-2*) have been described in olive (Hernandez et al. 2005), whereas only one gene corresponding to the chloroplast oleate desaturase (*OeFAD6*) has been reported (Banilas et al. 2005; Hernández et al. 2011). Expression analysis of these genes revealed that the gene *OepFAD2-2* is mainly responsible for the linoleic acid content in the olive fruit mesocarp and, therefore, in VOO (Hernandez et al. 2009). Hence, *OepFAD2-2* seems to be a good candidate gene underlined by the co-localized QTLs for oleic and linoleic acids, as well as for monounsaturated and polyunsaturated fatty acids, and for the oleic/linoleic ratio in linkage group 20 of 'Arbequina' cultivar (Arb20). Interestingly, the presence of at least two copies of the *OepFAD2-2* gene in the olive genome has been reported (Hernandez et al. 2005).

Further analysis was conducted in Arb20 to identify the best genotypes for oleic and linoleic production within the interval of confidence of the QTLs for oleic and linoleic acid (Fig. 2). These QTLs are located within the markers olPt-767430 and a group of four identical markers (olPt-578159, olPt576186, olPt-771304 and olPt772057). At each marker, the mean values for oleic and linoleic content were calculated for both genotypes (np and nn) (Fig. 2). As shown by this figure, the best haplotype for increasing oleic content would be np–np–nn, at each of the three loci respectively (Fig. 2). On the contrary, nn–nn–np would be the best combination if we are interested in raising linoleic content. The change in the amount of one fatty acid affecting the levels of other associated fatty acids was reported earlier (Pérez et al. 2014). In particular, the co-localization of a QTL for oleic and linoleic acid has been also reported for almond (Font i Forcada et al. 2012) and oil palm (Montoya et al. 2014).

On the other hand, four QTLs were identified for linolenic acid, two in 'Picual' map (linkage groups 5 and 15) and two in 'Arbequina' map (linkage groups

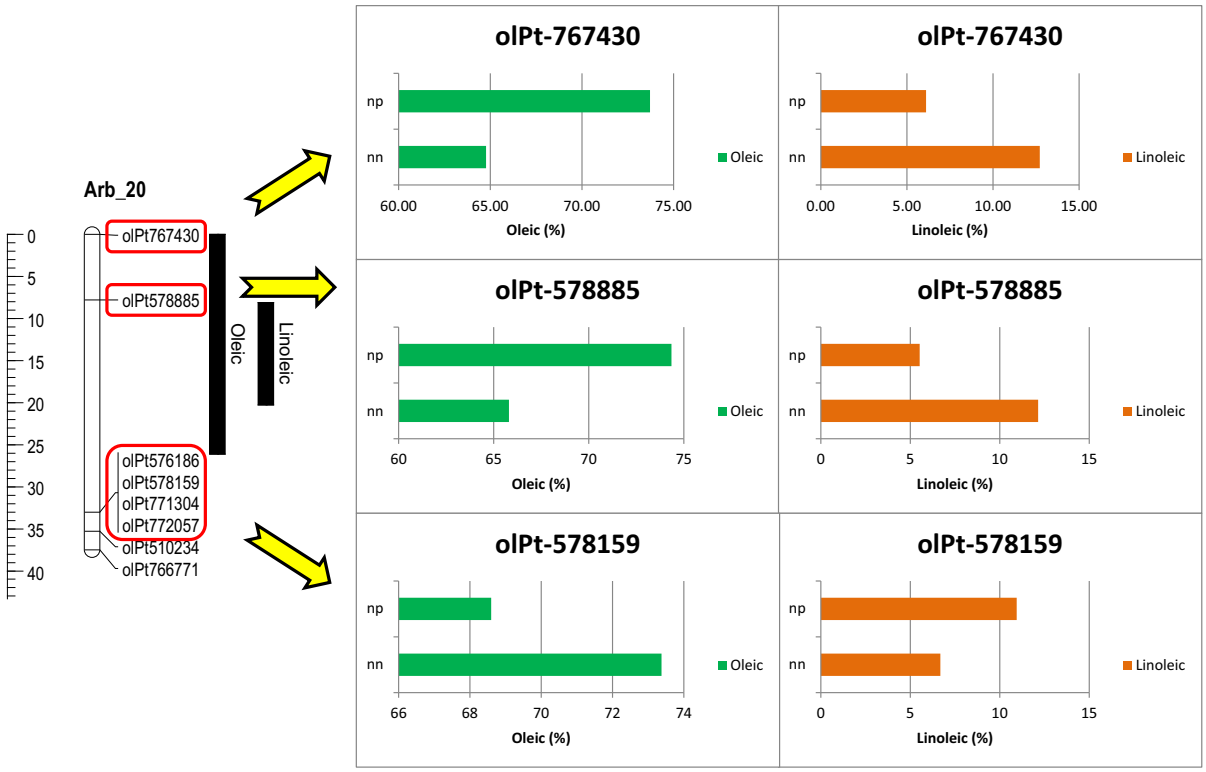

Fig. 2 Identification of the best olive genotypes for oleic and linoleic content in the QTLs located in Arb20. For each marker, the mean values for oleic and linoleic contents for each genotype ('nn' and 'np') were calculated

14 and 19) (Table 3). QTL detected in 'Picual' show opposite allele effects. The QTL on linkage group 5 (LG5) has an allele effect of 0.17, which means that the allele derived from 'Picual' increases the content of linolenic acid. On the contrary, the QTL on LG15 has an allele effect of −0.15 which indicates that the allele from 'Arbequina' increases the content at this QTL. Both QTL detected in 'Arbequina' had a similar allele effect (0.1) and each of them explained around 15% of the phenotypic variation. It is peculiar that the allele increasing the content was derived from 'Arbequina' in three out of four QTLs, despite it has lower linolenic content than 'Picual' (Table 1).

Linolenic acid content has also an important effect on the VOO quality. In particular, this ω3 fatty acid participates in the proportion of ω3/ω6 fatty acids which has been reported to be very important in terms of nutritional characteristics of edible oils. In addition, it has been demonstrated that the low levels of linolenic acid are essential for aroma biogenesis during the milling and malaxation processes to obtain VOO (Olías et al. 1993). The synthesis of linolenic acid is catalyzed by two different linoleate desaturases. The microsomal enzyme (FAD3) is located in the endoplasmic reticulum, while the plastidial linoleate desaturase (FAD7/8) is located in the plastids. Two *FAD3* genes, designated *FAD3A* (Banilas et al. 2007) and *FAD3B* (Hernández et al. 2016), and two *FAD7* genes, named *FAD7-1* (Poghosyan et al. 1999; Sabetta et al. 2013) and *FAD7-2* (Hernández et al. 2016) encoding linoleate desaturases have been isolated and characterised in olive. In contrast to oilseeds, where *FAD3* genes are the main responsible for the linolenic acid content of TAG, in olive fruit mesocarp *FAD7* could be responsible for the synthesis of the linolenic acid present in triacylglycerols (Hernandez et al. 2008; Hernández et al. 2016). Hence, the *FAD7* gene is a good candidate to explain the QTL of linolenic acid detected in 'Arbequina' and 'Picual' in future studies.

Finally, a QTL for palmitoleic acid explaining 22.5% of the phenotypic variance was identified on LG13 (Arbequina map). A QTL for palmitoleic acid content was also found in an almond progeny,

explaining a similar percentage of variance (Font i Forcada et al. 2012). This monoenoic fatty acid is found in small amounts in most plant oils (Gunstone 1992). The stability and low melting point of palmitoleic acid makes oils rich in this fatty acid, good lubricants at low temperatures. Additionally, some studies have attributed antitumor activity to palmitoleic acid (Hayatsu et al. 1988), as well as positive effects in the treatment of hyperlipidemia (Maedler et al. 2001). Looking at the pathway for plant fatty acid biosynthesis, palmitoleic acid is produced in the plastid from palmitoyl-ACP by the enzymatic activity of the stearoyl-ACP desaturase, which exhibits low specificity for palmitoyl moieties (Cahoon et al. 1998; Gibson 1993). In olive, one gene encoding stearoyl-ACP desaturase has been isolated and characterized up to date, being its expression temporally and developmentally regulated in olive fruit (Haralampidis et al. 1998).

It is remarkable that none of the QTLs identified in this work co-localizes with the QTL for OCFDW (Oil content fruit dry weight) previously reported (Atienza et al. 2014). This together with the almost lack of correlation between oil content and fatty acid composition may indicate that these traits are independent, opening thus the possibility of simultaneous breeding selection for both total oil content and fatty acid profiles.

The relatively small population size used in this study may have resulted in underestimates of the number of QTL since it is known that the number of QTL increases with population size (Li et al. 2006; Vales et al. 2005). However, QTL with large effect can be identified even with small populations (Vales et al. 2005). Thus, QTLs identified in this work are likely the best targets for breeding since they have the largest effect. Similarly, the amount of phenotypic variance explained by the QTL may be overestimated since this parameter increases as the population size decreases (Vales et al. 2005). In any case, small population sizes have been successfully used for the identification of QTL associated with fatty acid composition in perennial species like oil palm (Singh et al. 2009) and almond (Font i Forcada et al. 2012).

Conclusions

The present study represents the first detection of QTL underlying the variability of fatty acid composition in olive oil. The current results are based in data from a single season and thus they require further validation. Nevertheless the co-localization of QTLs for oleic, linoleic and three quality indices in one linkage group (Arb_20), indicates that this region could be important for determining the relative proportions of oleic and linoleic acids in olive oil. In particular, it could be useful to increase the efficiency of breeding programs aimed at selecting new cultivars with high oleic acid content, giving the long juvenile period of olive. This could be important in order to enhance the nutritional properties and oxidative stability of the corresponding VOO. Furthermore, these QTLs are independent of the QTL for OCFDW previously reported, and, thus, simultaneous selection for both total oil content and fatty acid profile seems to be feasible, at least under the genetic background here reported.

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