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1 **Mapping quantitative trait loci controlling fatty acid composition in olive**

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3 M. L. Hernández[1], A. Belaj[2], M. D. Sicardo[1], L. León[2], R. de la Rosa[2*], A. Martín[3], J. M. Martínez-
4 Rivas[1], and S. G. Atienza[3]

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6 *[1]Department of Biochemistry and Molecular Biology of Plant Products, Instituto de la Grasa (CSIC).*
7 *Campus Universidad Pablo de Olavide, Building 46. Ctra. Utrera, Km 1, E-41013 Sevilla, Spain.*
8 *[2]Área Mejora y Biotecnología, IFAPA-Centro Alameda del Obispo. Avda. Menendez Pidal s/n 14080,*
9 *Córdoba, Spain*
10 *[3]Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, 14004, Córdoba, Spain*

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12 *Corresponding author:
13 Raúl de la Rosa:
14 e-mail: raul.rosa@juntadeandalucia.es phone: ++34671532738
15 fax: ++34957016043

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Abstract

Fatty acids are the main component 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 due to the difficulties in analyzed 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 the selection for them in breeding programs of this species. In the present work, the first identification of QTLs for fatty acids on olive oil is reported. They have been located in a linkage map of a 'Picual' x '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

41 **Introduction**

42 *Olea europaea* L. (2n = 2x = 46) is one of the most economically important trees in the Mediterranean 43 basin with over 98% of the 2.8 MTm of virgin olive oil (VOO) harvested in the world (FAOSTAT 2013). 44 VOO is the main source of fat in the Mediterranean diet. This oil is obtained as a fruit juice, i.e., directly 45 from the crushing of olive fruits and its consumption has been widely associated with positive health 46 benefits (Covas 2008; Schwingshackl and Hoffmann 2014). Fatty acids, the main components of the olive 47 oil, are considered directly implicated in the health benefits of the olive oil (Di Bella et al. 2007; 48 Quintero-Florez et al. 2015). In particular, the role of VOO in the protection against cardiovascular 49 disease has been mostly attributed to its high oleic acid content (Rietjens et al. 2007). In contrast, elevated 50 linoleic acid content may cause a negative impact in the nutritional properties of olive oil, since recent 51 studies using seed oils characterized by high linoleic acid content indicates that an excessive consumption 52 of this fatty acid in the diet is associated with a higher risk of hypertension and cardiovascular and 53 carcinogenic diseases (Bonow and Eckel 2003; Vos 2003). Besides, the oleic/linoleic ratio has also 54 important consequences in the technological properties of the olive oil, with high linoleic acid content 55 affecting negatively its oxidative stability (Gutiérrez et al. 1999). In addition, the levels of individual fatty 56 acids are also important at the regulatory level. According to European Commission regulation 702/2007 57 (EC 2007), the contents of oleic acid must range from 55 to 83%, while linoleic acid must account for 3.5 58 to 21% and linolenic acid for less than 1%.

59 The fatty acid biosynthesis pathway is well known in plants including olive. In vascular plants, 60 the fatty acid biosynthesis starts in the plastids, yielding primarily palmitoyl-acyl carrier protein (ACP) 61 and stearoyl-ACP by successive addition of two carbon atoms from acetyl-CoA (Harwood 2005). Still in 62 the plastid, most of the stearoyl-ACP is desaturated by the action of a soluble Δ9 stearoyl-ACP desaturase 63 producing oleoyl-ACP, which is the main product of the plastidial fatty acid biosynthesis. The oleic acid 64 is then incorporated into glycerolipids inside or outside plastids, and it can be further desaturated to 65 linoleic, and then to α-linolenic acid by the consecutive action of Δ12 and Δ15 desaturases. Two sets of 66 these enzymes are present in plant cells, which differ in their cellular localization (Shanklin and Cahoon 67 1998). The microsomal oleate desaturase (FAD2) and linoleate desaturase (FAD3) are located in the 68 endoplasmic reticulum (ER), whereas the plastidial oleate desaturase (FAD6) and linoleate desaturase 69 (FAD7/8) are located in the chloroplast.

70 The fatty acid composition of olive oil is influenced by pedoclimatic conditions, olive growing
71 practices (Jimenez Herrera et al. 2012; Dabbou et al. 2015) and the cultivar (Rondanini et al. 2011). In
72 fact, high variability for fatty acid composition has been observed in cultivar collections (Rotondi et al.
73 2013; Uceda et al. 2005). However, most of the current olive cultivars are very ancient and have been
74 obtained by the empiric selection of the growers mainly on the basis of their productivity, oil content and
75 fruit size, but not on oil composition (Barranco et al. 2010; Bracci et al. 2011). Besides, none of the few
76 cultivars obtained by systematic breeding, such as 'Barnea' (Lavee et al. 1986), 'Maalot' (Lavee et al.
77 1999), 'Askal' (Lavee et al. 2003), 'Fs-17' (Bellini et al. 2002) or 'Sikitita' (Rallo et al. 2008) has been
78 specifically selected for having a superior oil composition. This is mainly due to the fact that the
79 evaluation of oil quality traits, including fatty acids, is a very time consuming and costly task. Initially,
80 seedlings have to overcome the juvenile period and then to reach a significant size in order to bear enough
81 amount of fruits to allow oil extraction (De la Rosa et al. 2006). Then, to extract and analyze oil from the
82 large progenies usually obtained in breeding programs represents a very complicated and difficult task.
83 The fact that the content of some oil components is not affected by the oil extraction process and can be
84 directly measured in fruit without the need of oil extraction, could partly overcome this problem (Garces
85 and Mancha 1993; Velasco et al. 2014). Although some studies suggested high heritability for fatty acid
86 composition (Dabbou et al. 2010; De la Rosa et al. 2016), there is little knowledge on the genetic control
87 of its variability among olive cultivars.
88 In this context, the use of molecular markers could be helpful to investigate the genetic control of
89 important traits and for the identification of beneficial alleles through the development of linkage maps
90 and marker-trait associations as QTL analysis (El-Soda et al. 2014). Actually, few QTL analyses have
91 been performed in olive including flowering-related traits (Ben Sadok et al. 2013) using a 'Olivière ×
92 'Arbequina' progeny. Thus, the objective of this work was the identification of QTLs associated to the
93 fatty acid profile in a segregation progeny of 'Picual' × 'Arbequina' where molecular markers associated
94 with fruit-related traits and oil content has been previously found (Atienza et al. 2014). This cross has
95 been very successful in olive breeding, showing high variability for fatty acid composition (León et al.
96 2004b) and producing the first olive cultivar registered in Spain, 'Sikitita' (Rallo et al. 2008).
97

98 **Material and methods**

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100 Plant material

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102 A progeny coming from the cross of 'Picual'×'Arbequina' performed in spring 2001 were used in the
103 present study. Seedlings were planted in open field in September 2003, at 4×1.5 m of spacing at the
104 experimental orchard of IFAPA, Centre "Alameda del Obispo", Córdoba, Spain. Trees were trained to
105 form the canopy at 1.6 m height and then develop freely, as suggested in previous experiments (Santos-
106 Antunes et al. 2005) and yearly irrigated with 2,000 m^3/ha of water. This progeny comes from the
107 cooperative breeding program of the University of Cordoba and IFAPA, Spain. The oils of the two
108 parents are known to have contrasting fatty acid composition (Hernández et al. 2009).

109

110 Fatty acid analysis

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112 Sixty genotypes which showed enough crop for oil extraction were selected for fatty acid analyses
113 during the 2008/2009 season. . A random sample of 1000 g of olives was hand-collected per seedling.
114 Samples were collected when most fruits were at maturity index 2,5 (Frías et al. 1991). VOO was
115 extracted using an Abencor analyzer (Comercial Abengoa, S.A., Seville, Spain) that simulates the
116 industrial process of VOO production at lab scale (Martinez-Suarez et al. 1975). Milling of whole olive
117 fruits was performed using a stainless steel hammer mill operating at 3000 rpm provided with a 5 mm
118 sieve. Malaxation was carried out for 30 min with the Abencor thermo-beater operated at 30 °C according
119 to industry recommendations. Centrifugation of the kneaded paste was performed in a basket centrifuge at
120 3500 rpm for 1 min. After centrifugation, the oils were decanted and paper filtered. Oils were stored
121 under nitrogen at -20 ºC until analysis.

122 Fatty acid composition of the different olive oils was determined using the one-step method of
123 (Garcés and Mancha 1993). After the addition of 2 ml of methanol-toluene-H_2SO_4 (80:20: 2, vol/vol/vol)
124 to 50 mg of olive oil, the mixture was incubated for 1 h at 80 ºC. After cooling, 2 ml heptane and 5 ml
125 Na_2SO_4 were added, and the upper phase containing the fatty acid methyl esters was analysed by gas-
126 liquid chromatography using a 7890A (Agilent, Santa Clara, CA USA) fitted with a capillary column (30-
127 m length; 0.32-mm inner diameter; 0.2-µm film thickness) of fused silica (Supelco, Bellafonte, PA, USA)

128 and a FID detector. Hydrogen was used as a carrier gas with a linear rate of 1.34 ml min^{-1} and split ratio
129 of 1/50. The injector and detector temperature was 220 °C and the oven temperature was 170 °C. Results
130 were obtained in mol % of the different fatty acids and expressed as means of three independent
131 determinations.

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

138

139 QTL analyses

140

141 The genetic map for 'Picual'×'Arbequina' progeny (Dominguez-Garcia et al. 2012) was used for QTL
142 analysis. Two independent QTL analyses (one for each parental map) were performed using MAPQTL
143 5.0 package (Van Ooijen 2004). First, the non-parametric Kruskal-Wallis (KW) test was performed to
144 identify association between markers and traits individually, without considering the map information.
145 After this, interval mapping (IM) analyses were performed (Lander and Botstein 1989; van Ooijen 1992).
146 An initial set of cofactors was selected from KW and IM results and a backwards elimination procedure
147 was applied to select significant markers as implemented in MapQTL 5.0. Only significant markers at
148 $P<0.1$ were used as cofactors in the multiple QTL methods (rMQM and MQM) (Jansen 1993, 1994;
149 Jansen and Stam 1994) analyses. A mapping step size of 1 cM was used for IM and MQM analyses. The
150 significance thresholds for accepting the presence of potential QTLs were empirically determined using a
151 permutation analysis (500 permutations) (Churchill and Doerge 1994) as implemented in MapQTL 5.0.
152 An estimation of the total variance explained at the position with the highest LOD score was given by
153 MapQTL® 5.0. The QTL positions were estimated as the position with the maximum LOD score on a
154 linkage group. Uncertainty of the map position was indicated by a 1-LOD support interval (Conneally et
155 al. 1985; van Ooijen 1992). MapChart software (Voorrips 2002) was used to indicate location of the QTL
156 for fruit traits in the 'Picual' and 'Arbequina' maps. For each QTL, the difference in the alleles effect was
157 determined using the Knott et al. (1997) method ((Atienza et al. 2003a, b; Sewell et al. 2000).In a CP

population, a QTL can segregate for four different alleles in a cross between two heterozygous parents (‘CP’ population in Joinmap). 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 material 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

187 Pearson correlations were calculated including not only individual fatty acids and quality indexes, but
188 also the previously reported oil content in fruit on dry weight basis (OCFDW) (Atienza et al. 2014)
189 (Table 2). Although they do not determine the cause-and-effect relationships between the phenotypic
190 traits, they estimate the strength of association between them, which is useful for breeding and mapping
191 purposes.

192 The highest correlation was found between the two main fatty acids of olive oil, oleic and
193 linoleic acids, which indicates that any increase in one of them will imply a decrease in the other. This is
194 to be expected since linoleic acid is directly formed by desaturation of oleic acid, which is catalysed by
195 the oleate desaturase activity (Shanklin and Cahoon 1998). In fact, this negative correlation seems to be
196 general in olive (León et al., 2004a; Dabbou et al., 2012; Sabetta et al. 2013) and in other oil crops such
197 as sunflower (Pérez-Vich et al. 2004), sesame (Were et al. 2006), maize (Wassom et al. 2008), Jatropha
198 (Liu et al. 2011), rice (Ying et al. 2012), almond (Font i Forcada et al. 2012) and oil palm (Montoya et al.
199 2013; Montoya et al. 2014).

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

212 Linolenic acid was inversely associated with oleic acid and positively correlated with linoleic
213 acid, as previously described in maize (Wassom et al. 2008), rice (Ying et al. 2012), and oil palm
214 (Montoya et al. 2013; Montoya et al. 2014). Interestingly, the correlation between linoleic and linolenic
215 acids was moderate, despite the fact that the second fatty acid is directly synthesized by desaturation of

216 the first, as a result of the linoleate desaturase activity (Shanklin and Cahoon 1998). This result has also
217 been observed in an olive collection (Sabetta et al. 2013).
218 As mentioned in the introduction, a high content of oleic acid and low on linoleic, linolenic and
219 palmitic is considered very relevant in the health properties of the olive oil (Di Bella et al. 2007;
220 Quintero-Florez et al. 2015). Therefore, the reported negative correlations of oleic acid with the rest of the
221 mentioned fatty acids content might be of interest for breeding programs aimed at improving the oil fatty
222 acid composition.
223 Stearic and linolenic acids, the two in the lower proportion in the olive oil, were the ones were
224 weakest correlations with the four quality indexes calculated. None of the fatty acids showed a strong
225 correlation with the oil content (OCFDW).
226
227 QTLs involved in fatty acid composition
228
229 QTL analyses were independently performed in each parental map ('Picual' and 'Arbequina') (Table 3,
230 Fig. 1) as usually performed in mapping populations derived from two heterozygous parents (Grattapaglia
231 et al. 1995; Sewell et al. 2000; Atienza et al. 2003b; Socquet-Juglard et al. 2013; Atienza et al. 2014).
232 Two QTLs were detected in 'Picual' map whereas eight QTLs were found in 'Arbequina' map. More
233 QTLs were also detected for fruit traits in the 'Arbequina' than in 'Picual' map in a previous work of our
234 group (Atienza et al. 2014). This is likely influenced by the shorter genetic distance covered in 'Picual'
235 map compared to the one of 'Arbequina' (Dominguez-Garcia et al. 2012).
236 A single QTL for oleic acid was identified on linkage group 20 in 'Arbequina' (Arb_20) map
237 (Table 3, Fig. 1). It accounted for 41% of the phenotypic variance and it has an allele effect of -8.8 which
238 indicates that the allele increasing oleic content is inherited from 'Picual'. Similarly, a QTL for linoleic
239 acid was located in the same position (Table 3, Fig. 1). It explained 69.7% of the phenotypic variation and
240 it shows an allele effect of 7.9, i.e., the allele increasing the content is inherited from 'Arbequina'. This is
241 concordant with the fact that 'Arbequina' has higher linoleic acid and lower oleic acid content than the
242 other parent 'Picual'. The co-localization of both QTLs and the different sign of the allele effect (Table 3,
243 Fig. 1) are in agreement with the high negative correlation between both fatty acids (Table 2).
244 Furthermore, the co-localization in the same region of QTL for monounsaturated and polyunsaturated
245 fatty acids as well as for the ratio oleic/linoleic trait, reinforces the importance of this region for the

246 determination of the fatty acid profile in olive oil. Whether there is a single segregating locus controlling
247 the biosynthesis of oleic and linoleic acids or clusters of linked QTLs independently affecting the
248 biosynthesis of both fatty acids cannot be discerned. Fine-mapping of this QTL region and the analysis of
249 future genomic sequence data could allow the discrimination between both hypotheses.

250 The fact that QTLs for oleic and linoleic acids, as well as for monounsaturated and
251 polyunsaturated fatty acids, and for the oleic/linoleic ratio were co-localized in the same linkage group of
252 'Arbequina' cultivar is significant considering that the proportions of these fatty acids have a important
253 effect on olive oil quality (Gutiérrez et al. 1999). Regarding their metabolic origin, oleate desaturases
254 catalyze the desaturation of oleic acid to produce linoleic acid. Two genes encoding microsomal oleate
255 desaturases (*OepFAD2-1* and *OepFAD2-2*) have been described in olive (Hernandez et al. 2005), whereas
256 only one gene corresponding to the chloroplast oleate desaturase (*OeFAD6*) has been reported (Banilas et
257 al. 2005; Hernández et al. 2011). Expression analysis of these genes revealed that *OepFAD2-2* is the gene
258 mainly responsible for the linoleic acid content in the olive fruit mesocarp and, therefore, in VOO
259 (Hernandez et al. 2009). Hence, the gene *OepFAD2-2* seems to be a good candidate underlined by the co-
260 localized QTLs for oleic and linoleic acids, as well as for monounsaturated and polyunsaturated fatty
261 acids, and for the oleic/linoleic ratio in linkage group 20 of 'Arbequina' cultivar (Arb20). Interestingly,
262 the presence of at least two copies of the *OepFAD2-2* gene in the olive genome has been reported
263 (Hernandez et al. 2005).

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

274 On the other hand, four QTLs were identified for linolenic acid, two in 'Picual' map (linkage
275 groups 5 and 15) and two in 'Arbequina' map (linkage groups 14 and 19) (Table 3). QTL detected in

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

283 Linolenic acid content has also an important effect on the VOO quality. In particular, this ω3
284 fatty acid participates in the proportion of ω3/ω6 fatty acids which has been reported to be very important
285 in terms of nutritional characteristics of edible oils. In addition, it has been demonstrated that the low
286 levels of linolenic acid are essential for aroma biogenesis during the milling and malaxation processes to
287 obtain VOO (Olías et al. 1993). The synthesis of linolenic acid is catalyzed by two different linoleate
288 desaturases. The microsomal enzyme (FAD3) is located in the endoplasmic reticulum, while the plastidial
289 linoleate desaturase (FAD7/8) is located in the plastids. Two *FAD3* genes, designated *FAD3A* (Banilas et
290 al. 2007) and *FAD3B* (Hernández et al. 2016), and two *FAD7* genes, named *FAD7-1* (Poghosyan et al.
291 1999; Sabetta et al. 2013) and *FAD7-2* (Hernández et al. 2016) encoding linoleate desaturases have been
292 isolated and characterised in olive. In contrast to oilseeds, where *FAD3* genes are the main responsible for
293 the linolenic acid content of TAG, in olive fruit mesocarp *FAD7* could be responsible for the synthesis of
294 the linolenic acid present in triacylglycerols (Hernandez et al. 2008; Hernández et al. 2016). Hence, the
295 *FAD7* gene is a good candidate to explain the QTL of linolenic acid detected in ‘Arbequina’ and ‘Picual’
296 in future studies.

297 Finally, a QTL for palmitoleic acid explaining 22.5% of the phenotypic variance was identified
298 on LG13 (Arbequina map). A QTL for palmitoleic acid content was also found in an almond progeny,
299 explaining a similar percentage of variance (Font i Forcada et al. 2012). This monoenoic fatty acid is
300 found in small amounts in most plant oils (Gunstone, 1992). The stability and low melting point of
301 palmitoleic acid makes oils rich in this fatty acid, good lubricants at low temperatures. Additionally, some
302 studies have attributed antitumor activity to palmitoleic acid (Hayatsu et al. 1988), as well as positive
303 effects in the treatment of hyperlipidemia (Maedler et al. 2001). Looking at the pathway for plant fatty
304 acid biosynthesis, palmitoleic acid is produced in the plastid from palmitoyl-ACP by the enzymatic
305 activity of the stearoyl-ACP desaturase, which exhibits low specificity for palmitoyl moieties (Cahoon et

306 al. 1998; Gibson 1993). In olive, one gene encoding stearoyl-ACP desaturase has been isolated and
307 characterized up to date, being its expression temporally and developmentally regulated in olive fruit
308 (Haralampidis et al. 1998).

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

314 The relatively small population size used in this study may have resulted in underestimates of the
315 number of QTL since it is known that the number of QTL increases with population size (Li et al. 2006;
316 Vales et al. 2005). However, QTL with large effect can be identified even with small populations (Vales
317 et al. 2005). Thus, QTLs identified in this work are likely the best targets for breeding since they have the
318 largest effect. Similarly, the amount of phenotypic variance explained by the QTL may be overestimated
319 since this parameter increases as the population size decreases (Vales et al. 2005). In any case, small
320 population sizes have been successfully used for the identification of QTL associated with fatty acid
321 composition in perennial species like oil palm (Singh et al. 2009) and almond (Font i Forcada et al. 2012).
322

323 **Conclusions**

324

325 The present study represents the first detection of QTL underlying the variability of fatty acid
326 composition in olive oil. The current results are based in data from a single season and thus they require
327 further validation. Nevertheless the co-localization of QTLs for oleic, linoleic and three quality indices in
328 one linkage group (Arb_20), indicates that this region could be important for determining the relative
329 proportions of oleic and linoleic acids in olive oil. In particular, it could be useful to increase the
330 efficiency of breeding programs aimed at selecting new cultivars with high oleic acid content, giving the
331 long juvenile period of olive. This could be important in order to enhance the nutritional properties and
332 oxidative stability of the corresponding VOO. Furthermore, these QTLs are independent of the QTL for
333 OCFDW previously reported, and, thus, simultaneous selection for both total oil content and fatty acid
334 profile seems to be feasible, at least under the genetic background here reported.
335

336

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

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

	Palmitic	Palmitoleic	Stearic	Oleic	Linoleic	Linolenic	Saturated	Unsat	Monounsat	Polyunsat
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***			
Monounsaturated	-0.82***	-0.31*	-0.12	0.99***	-0.96***	-0.45***	-0.84***	0.83***		
Polyunsaturated	0.64***	0.11	0.09	-0.93***	1.00***	0.49***	0.65***	-0.65***	-0.96***	
OCFDW[a]	0.29*	0.03	0.10	-0.22	0.18	-0.31*	0.30*	-0.31*	-0.23	0.17

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

[a]OCFDW, Oil content fruit dry weight

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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
Monounsaturated	'Arbequina'	20	5.08	13.8	41.1	3.07	0.000	-8.4
Polyunsaturated	'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' (-)

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349 **Figure captions**

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

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

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greyscale figure 1

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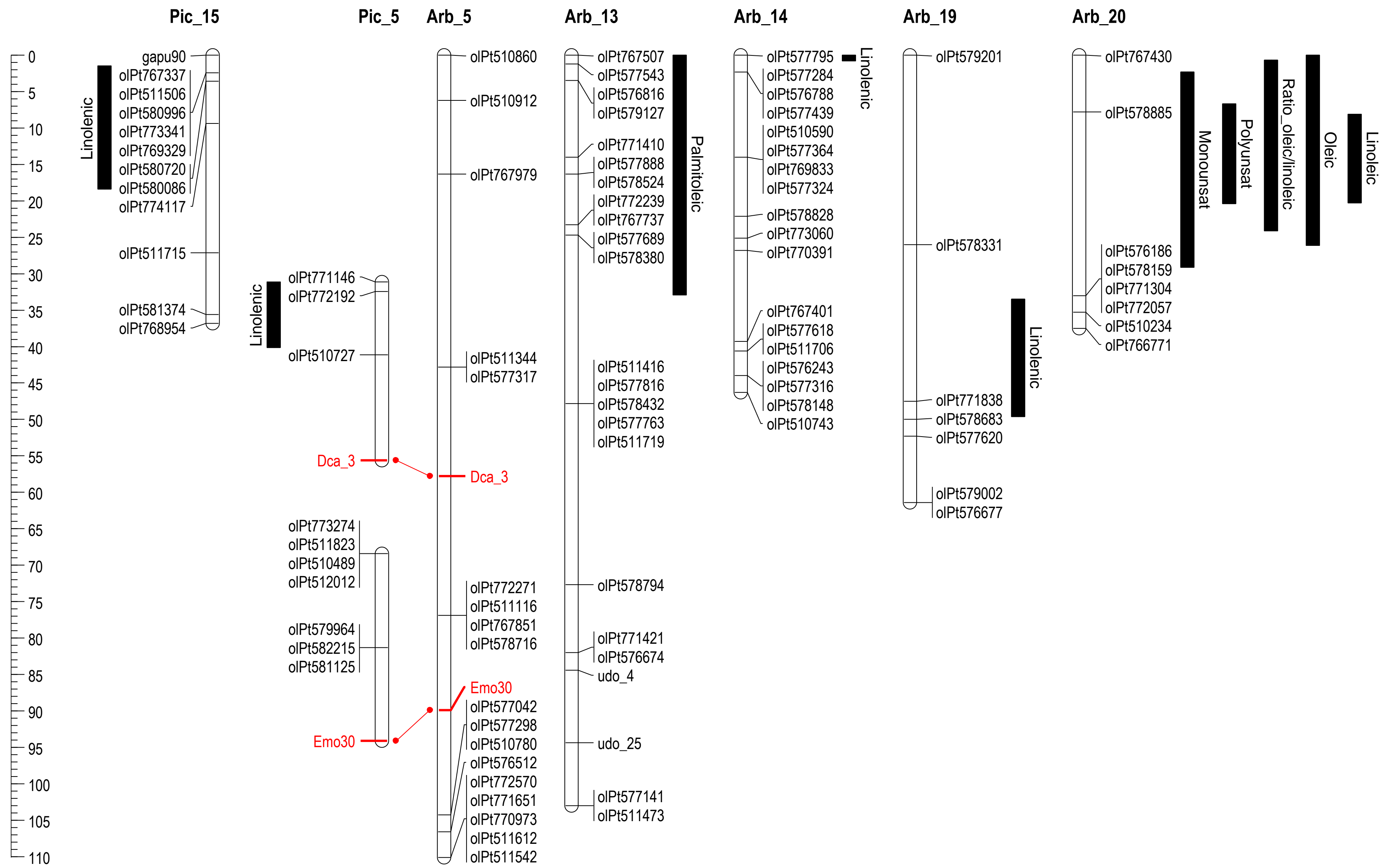

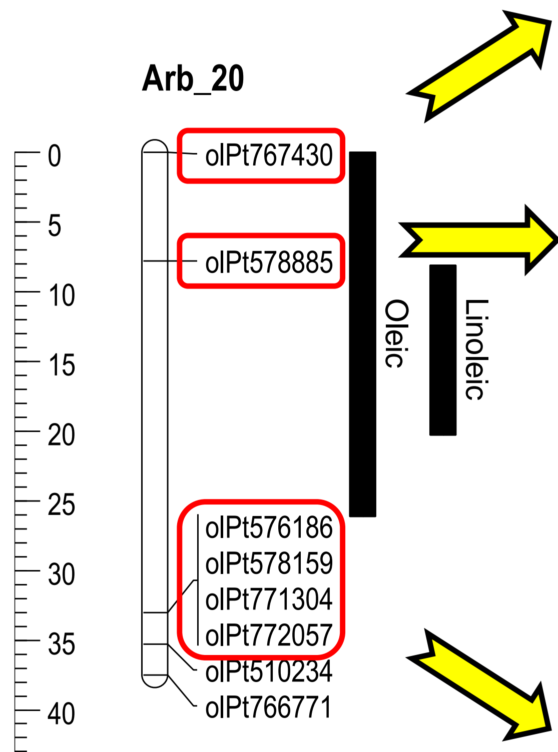
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