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QTL mapping for fatty acid composition in olive oil using a high-density genetic map based on SNP markers

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Abstract: Olive (*Olea europaea* L.) is an evergreen tree species that grows naturally in regions with Mediterranean climates. Its oil and fruits are commercially valuable. Olive oil contains high levels of omega-9 (oleic acid). Because the high percentage of oleic acid makes olive oil deterioration-resistant, the development of olive varieties containing high oleic acid is one of the major goals of olive breeding programs. Therefore, this study aimed to determine quantitative trait loci (QTL) affecting the fatty acid composition of olive oil. Thus, early selection of olive genotypes with a high oleic acid content can be possible. For the determination of QTLs affecting the fatty acid composition of olive oil, a high-density genetic map was developed using a segregating olive F1 population with 121 progeny and single-nucleotide polymorphism (SNP) markers based on genotyping by sequencing (GBS). The 2892.14 cM genetic map was composed of 3254 SNP markers on 23 chromosomes, with an average distance of 0.93 cM. For QTL analysis, the fatty acid composition of the segregating olive F1 population was determined using gas chromatography in two different years. A total of 31 QTLs were discovered in the first year and 29 in the second year. Common QTLs associated with fatty acid composition in both years have been found on chromosome 1, chromosome 2, and chromosome 10. For oleic acid, 11 QTLs were discovered in the first year and 12 QTLs in the second year. With these results, the QTLs linked to fatty acid synthesis in olive oil can be used as genetic resources for marker-assisted selection (MAS) in olive breeding studies.

Key words: Fatty acids, olive oil, genotyping by sequencing, *Olea europaea* L., QTL, SNP markers

1. Introduction

The olive branch and olive oil have both been revered as symbols of purity and beauty since antiquity. Because of the positive impact of table olives and olive oil on human health, it is one of the most widely cultivated tree species in the Mediterranean region. According to the International Olive Council, approximately 3 million tons of olive oil was produced worldwide in 2021 (IOC, 2021). Table olives and olive oil are the mainstays of Mediterranean diets, and are popular worldwide. Recent studies have shown that olive oil helps to reduce obesity and risk of cardiovascular diseases (Schwingshackl and Hoffmann, 2014; Donat-Vargas et al., 2022).

The oil content of olive fruits varies between 10% and 35% depending on the growing region, genotype, and climatic conditions (Lavee and Wodner, 1991). The major fatty acids in olive oil are 55%–83% oleic acid, 3.5%–21% linoleic acid, 7.5%–20% palmitic acid, 0.5%–5.0% stearic acid, and 0.5%–1.5% linolenic acid (Mataix and Martinez, 1988). There is a negative correlation between oleic acid and linoleic acid contents in olives (León et al., 2004). Being a monounsaturated fatty acid, oleic acid makes olive oil more stable and deterioration-resistant (Gutiérrez et al., 1999). In addition, the effects of olive oil on cardiovascular disease prevention have been linked mostly to its high oleic acid content (Rietjens et al., 2007). Thus, one of the most important goals of olive breeding is to develop olive varieties with high oleic acid. However, selection of olive trees with high oleic acid may take a very long time (10–15 years) due to the long juvenile period of the olive tree (Janick and Moore, 1996; Rao et al., 2009). With the development of molecular markers, it is now

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possible to select particularly important traits such as fruit characteristics and oil content and composition in olives without waiting for trees to bear fruits (Montemurro et al., 2019).

The haploid genome of the olive is around 1.31 GB, spread across 23 chromosomes, and contains 56,349 proteins (Green and Wickens, 1989; Cruz et al., 2016). SNP markers can occur in both coding and noncoding genomic regions and are relatively common in plant genomes (Edward et al., 2008). With the advancement of next-generation sequencing techniques (NGS), it is now possible to identify a high number of segregating SNP markers within a population utilizing techniques such as GBS. Therefore, SNP markers based on GBS present a great advantage for plant breeders by reducing the time for marker development and providing a cost-effective marker system (Elshire et al., 2011; Rowe et al., 2011).

In a recent study utilizing SNP, amplified fragment length polymorphism (AFLP), and simple sequence-repeats (SSR) markers, Kaya et al. (2013) examined the genetic relationship between olive genotypes. The SNP markers identified by Biton et al. (2015) were used to assess the phylogenetic relationship among olive cultivars. In addition, SNP markers developed using NGS have been used to create high-density genetic maps for olive (Domínguez-Garcia et al., 2012; Sadok et al., 2014; Ipek et al., 2016, 2017; Kaya et al., 2016; Marchese et al., 2016; Taranto et al., 2018; Mariotti et al., 2020). Unver et al. (2017) sequenced the whole genome of wild olive and generated a reference genome for use in genomic studies. Recently, QTLs affecting the fatty acid content of olive oil were detected using diversity arrays technology (DArT) and SSR markers (Hernández et al., 2017). The genome-wide association study of five agronomic traits (leaf length, fruit weight, stone weight, and fruit flesh-to-pit ratio) was carried out in olive using the mixed linear model (MLM_K) (Kaya et al., 2019). Mariotti et al. (2020) mapped the incompatibility locus using sequence-tagged site marker (STS) in olive. The purpose of this study was to identify QTLs effecting the fatty acid composition of olive oil using an SNP-based high-density olive genetic map.

2. Materials and methods

2.1. Plant materials and DNA extraction

In this study, 121 F1 progeny from the cross between 'Gemlik' and 'Edincik Su' were used as plant materials. 'Gemlik' was the maternal parent, while 'Edincik Su' was the paternal parent. Gemlik has a high oil content, whereas 'Edincik Su' produces juicy fruits with a low oil content. In addition, these two varieties differ from each other in terms of their fatty acid composition (Gündoğdu, 2018). Therefore, the 'Edincik Su' and 'Gemlik' cross was made to generate a segregating F1 population for fatty acid composition. DNA was extracted from 20 mg of lyophilized young olive leaves using Qiagen's DNeasy Plant Kit (Germany). The quality and quantity of DNA samples in the kit's elution buffer were measured with the QUBIT fluorimeter (Invitrogen, USA), and the concentration of DNA samples was adjusted to 50 ng/µL and stored at –80 °C until use.

2.2. Olive oil extraction and fatty acid analysis

One hundred grams of olive samples from 91 olive trees out of 121 F1 olive trees were collected from the Atatürk Horticultural Central Research Institute, Yalova, Türkiye. There were not enough samples for olive oil extraction from the remaining 30 trees because they did not set fruit due to either a long juvenility period or alternate bearing. After the removal of the pits, the flesh of each sample was crushed into paste with the help of a hand blender. The olive oil was extracted by centrifugation at 6200 × *g* for 10 min. Fatty acids from 0.1 g olive oil samples were isolated using 10 mL of hexane and 0.5 mL of methyl KOH. The fatty acid compositions of each oil sample were analyzed using a GC 2010 (Schimadzu, Japan) according to the method described by Gundogdu and Kaynas (2020).

2.3. SNP discovery

GBS analysis was performed in the Biotechnology Center at University of Wisconsin using a method developed by Elshire et al. (2011). Briefly, DNA samples from Gemlik, Edincik Su, and their 121 F1 progeny were cut with the restriction enzyme, *ApeKI*. Unique barcode nucleotides, and Illumina sequencing adaptors were ligated to DNA fragments. DNA fragments with DNA barcodes were sequenced using the Illumina HiSeq 2500 sequencing system. A total of 123 genotypes were sequenced with a 101-nt read length. The SNP markers for olive segregating in F1 population were identified using the program called TASSEL (Bradbury et al., 2007). Sequenced DNA fragments were aligned to the olive reference genome developed by Unver et al. (2017) using BOWTIE 2.0 (Langmead and Salzberg, 2012). Approximately 225,000 markers were filtered with a minimum heterozygosity of 0.25 (25%), a maximum heterozygosity of 0.75 (75%), and a minimum count of 100 genotypes. The sequencing depth was set to 15, and all DNA fragments with sequencing depths less than 15 were converted to missing data points. SNP markers with more than 10% missing data were excluded from the analysis.

2.4. Linkage map construction

SNP markers were scored according to whether the parents were heterozygous or not. It was scored as "hk x hk" if both parents were heterozygous, "lm x ll" if only the paternal parent was heterozygous, and "np x nn" if only the maternal parent was heterozygous. Linkage maps were

developed using JoinMap 4.0 software according to the procedure of "CP Map Population"[1]. While the markers were grouped with a minimum LOD score of 7, Kosambi's statistic was used for ordering markers. Linkage maps for maternal and paternal parents were developed, and then an olive linkage map was constructed by merging the parental maps using JoinMap 4.0[1]. Maps were visualized using MapChart 2.3 software (Voorrips, 2002).

2.5. QTL mapping

The QTLs for fatty acid traits were identified using MapQTL 5.0 software and the interval mapping procedure[2]. The LOD significance threshold was determined to be 3.0. Thus, QTLs with LOD scores higher than the LOD threshold of 3.0 were deemed to be significant. QTL graphs were visualized using MapChart 2.3 software (Voorrips, 2002).

3. Results

3.1. SNP detection and linkage map construction

For mapping QTLs affecting the fatty acid composition of olive oil, a high-density genetic map was constructed by using GBS-based SNP markers. The average number of GBS raw reads per plant was 8,785,022, and it ranged from 2,120,260 to 35,983,710. A total of 7530 segregating SNP markers were used for developing a high-density linkage map for olive, while 1835 SNP markers were segregating in maternal parents, 1634 SNP markers in paternal parents, and 2089 SNP markers in both parents. We were able to map 3254 SNP markers, and the number of markers mapped to each chromosome ranged from 75 to 325 SNP markers (Table 1; Figure 1). The mean genetic distance between the markers was 0.93 cM, and the length of the olive genome was 2892.14 cM. The longest was chromosome 10 with 210.42 cM, and the shortest was chromosome 9 with 80.17 cM.

3.2. Fatty acid analysis and QTL mapping

3.2.1. Oleic acid (C18:1)

The oleic acid content of olive oil in F1 progeny ranged from 55.11% to 84.18% (SD 5.72) in the first year, and from 51.21 % to 72.81 % (SD 5.61) in the second year (Table 2). According to the first-year results, significant QTLs were detected on chromosomes 2, 7, 8, 9, 10, 11, 12, 13, and 16. The major QTL was discovered on chromosome 9 at 64.019 cM, explained 46% of phenotypic variance, and had a LOD score of 3.79 (Table 3; Figure 2). Using the second-year results, QTLs affecting oleic acid content in olive oil were detected on chromosomes 1, 2, 3, 7, 10, 12, 17, and 20. The major QTL was found on chromosome 20 at 29.366 cM, with a LOD score of 3.34, accounting for 66.4% of the phenotypic variance (Table 3; Figure 3). In addition, a common QTL on chromosome 2 was detected to be linked to the oleic acid content in both years (Table 3; Figure 4).

3.2.2. Palmitic acid (C16:0)

The palmitic acid content of olive oil in F1 progeny differed from 9.35% to 20.7% (SD 1.95) in the first year, and from 12.89 to 20.0% (SD 1.57) in the second year (Table 2). The first year analysis showed that significant QTLs affecting palmitic acid concentration were found on chromosomes 3, 6, 7, 8, 9, 10, 13, 15, 16, and 21. The major QTL was found to range from 36.070 to 37.070 cM on chromosome 8, explained 53.3% of phenotypic variance, and its LOD score was 4.29 (Table 3; Figure 5). According to the second year data, the significant QTLs impacting the amount of palmitic acid in olive oil were revealed on chromosomes 10, 12, 15, 16, and 19. Furthermore, as demonstrated in Table 3 and Figure 6, the major QTL found on chromosome 10 was between 163.702 and 166.885 cM, with an LOD score of 3.69, accounting for 55.9% of phenotypic variance.

3.2.3. Linoleic acid (C18:2)

The linoleic acid content of olive oil in F1 progeny ranged from 3.16% to 21.51% (SD 4.15) in the first year and from 6.36% to 23.00% (SD 3.88) in the second year (Table 2). The first year's results demonstrated that QTLs linked to linoleic acid content in olives were identified on chromosomes 1, 2, 8, 10, 11, 13, and 16. In addition, a major QTL was found on chromosome 1 between 43.956 and 46.951 cM, had a LOD score of 5.18, and explained 54.5% of phenotypic variance (Table 3; Figure 7). In the second year analysis, QTLs influencing the linoleic acid concentration of olive oil were found on chromosomes 1, 2, 3, 7, 10, 12, 18, and 22. A major QTL on chromosome 10 was located at 163.702 cM, had a LOD score of 3.55, and explained 63.5% of phenotypic variance (Table 3; Figure 8). A common QTL on chromosome 2 was also found to be linked with the linoleic acid content in both years (Table 3; Figure 9).

4. Discussion

SNP markers based on NGS have been developed in order to do genome-wide genetic analyses in olive. Kaya et al. (2013) discovered 2987 SNP markers among the five olive genotypes for transcriptome-based sequencing in olive. Biton et al. (2015) developed 145,974 SNP markers for

[1]Van Ooijen JW (2006). JoinMap 4: Software for the calculation of genetic linkage maps in experimental populations. Kyazma B.V., Wageningen, The Netherlands.

[2]Van Ooijen JW (2004). MapQTL 5: Software for the mapping of quantitative trait loci in experimental populations. Kyazma B.V. Wageningen, The Netherlands.

Table 1. An olive genetic map based on SNP markers.

Chromosome number	Chromosome length (cM)	Number of markers	Average distance (cM)
1	141.62	148	0.99
2	117.74	101	1.17
3	115.40	136	0.85
4	109.09	75	1.45
5	92.54	138	0.67
6	156.94	194	0.81
7	122.10	147	0.83
8	94.68	133	0.71
9	80.17	88	0.91
10	210.42	325	0.65
11	170.35	196	0.87
12	163.05	187	0.87
13	157.98	148	1.06
14	120.60	110	1.09
15	115.92	115	1.00
16	96.93	115	0.84
17	138.61	156	0.89
18	123.16	170	0.72
19	153.78	153	1.01
20	91.92	127	0.72
21	108.04	114	0.95
22	106.85	100	1.07
23	104.25	78	1.34
Total	**2892.14**	**3254**	**Mean 0.93**

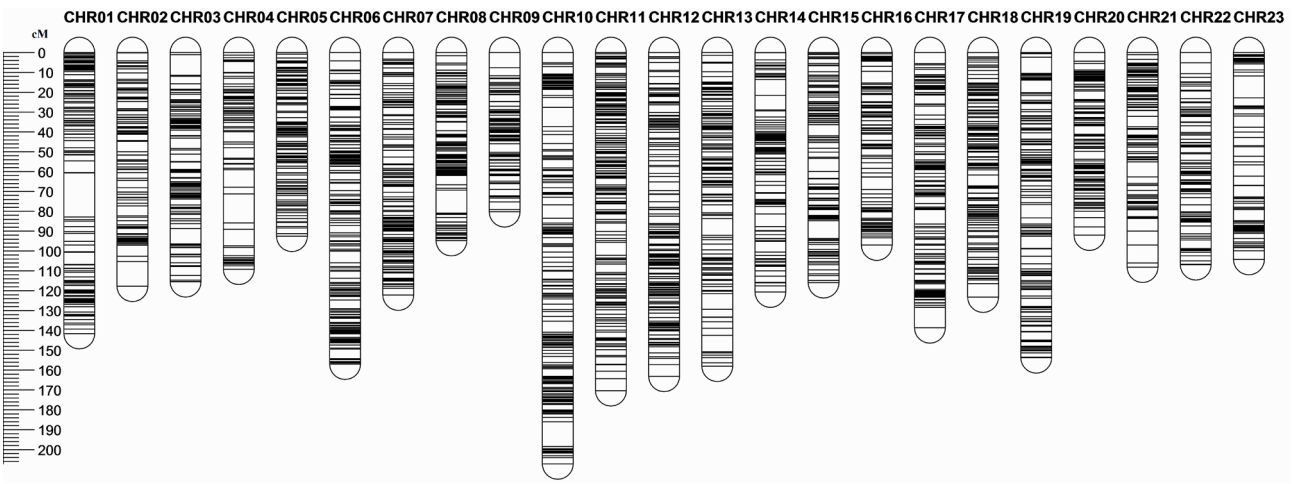

Figure 1. The genetic linkage map of *Olea europaea* L.

assessing phylogenetic relationships between the olive cultivars in the Israeli olive germplasm collection. Ipek et al. (2016) discovered a total of 10,947 SNP markers using GBS in olive ('Gemlik' and 'Edincik Su').

The genetic maps have been developed for different purposes in olive. Marchese et al. (2016) constructed a genetic map for olive using 1597 GBS-based SNP markers. Their genetic map covers 1189.7 cM on 23 linkage groups. Ipek et al. (2017) used 3384 GBS-based SNP markers from expressed regions of olive to develop a genetic map, and this map spans 3340.8 cM of the olive genome and segregates on 23 linkage groups. Mariotti et al. (2020) constructed a genetic map for olive to map incompatibility loci using restriction-associated DNA-

Table 2. Fatty acid composition of olive oil from the F1 progeny resulting from the cross between 'Edincik su' and 'Gemlik'.

Fatty acids	First year			Second year		
	Mean (%)	Range (%)	Standard deviation	Mean (%)	Range (%)	Standard deviation
C16:0	12.68	9.35–20.70	1.95	16.88	12.89–20.00	1.57
C16:1	1.53	0.58–4.21	0.69	2.03	0.03–4.68	1.06
C17:0	0.12	0.01–0.38	0.07	0.37	0.00–3.08	0.59
C17:1	0.28	0.02–1.31	0.18	0.42	0.00–3.08	0.21
C18:0	1.50	0.26–2.75	0.42	3.60	1.91–6.75	1.09
C18:1	71.84	55.11–84.18	5.72	61.27	51.21–72.81	5.61
C18:2	10.65	3.19–21.51	4.15	12.81	6.36–23.00	3.88
C18:3	0.63	0.35–1.03	0.13	1.77	0.83–2.56	0.35

based SNP markers, and the authors were able to identify 16,743 SNP markers, including 7006 in the maternal and 9737 in the paternal parents. These studies demonstrated that NGS enabled the discovery of a large number of SNP markers in olive. Similarly, NGS-based SNP markers were utilized for the development of high-density genetic maps in many other plant species. For example, Temel et al. (2015) identified 420 SNP markers for lentil, and Aldemir et al. (2017) used GBS-based SNP markers to detect QTLs link to iron concentration in lentil seeds. Carrasco et al. (2018) constructed a high-density genetic linkage map for Japanese plum using 1441 high-quality GBS-based SNP markers. In another study, Han et al. (2019) developed a high-density genetic map using 4.801 GBS-based SNP markers in Korean pear. Our results demonstrated that the genetic map developed in this study was a high-density olive linkage map and it possesses a high level of discrimination power.

In this study, fatty acid analysis was carried out at two different harvest years (Table 2). The amounts of palmitic and linoleic acids in olive oil increased while oleic acid content decreased in the second year compared to the first year. The variation in fatty acid contents depending on the harvesting years is probably due to the ecological factors such as temperature and precipitation.

In a previous study, Hernández et al. (2017) found significant negative correlation between the two primary fatty acids contained in olive oil, oleic and linoleic acids. Palmitic acid also had negative correlation with oleic acid. Linolenic acid was shown to be negatively correlated with oleic acid and positively correlated with linoleic acid. In the present study, there was a negative correlation between oleic and linoleic acid contents, whereas a positive correlation was revealed between palmitic acid and linoleic acid contents. In addition, a negative correlation was found between palmitic acid and oleic acid contents (Table 4).

In the first-year analysis, common QTLs affecting oleic acid and linoleic acid synthesis were discovered on chromosomes 2, 8, 11, and 13 (Table 3). In addition, palmitic acid and oleic acid were negatively correlated, and common QTLs were found on the same locus (chromosomes 8, 9, and 16) (Tables 3 and 4). Thus, the gene(s) responsible for variation in the synthesis of linoleic and oleic acids are referred to as the "pleiotropic effect of a single QTL" (Zhao et al., 2008). Similarly, Zhao et al. (2008) found the QTL region on chromosome 06 that are responsible for the synthesis of stearic, oleic, and linoleic acids in *Brassica napus* L.

In the second-year results, the common QTLs were found on chromosomes 1, 2, 3, and 10 (Table 3) for oleic acid and linoleic acid contents. A common QTL for linoleic and palmitic acids was detected on chromosome 10 in both the first-year and second-year analyses (Table 3). The colocalized QTL on chromosomes for the synthesis of oleic acid and palmitic acid was not observed. On the other hand, some QTLs affecting oleic acid, linoleic acid, and palmitic acid contents differ between the first-year and the second-year analyses (Table 3). Furthermore, a common QTL on chromosome 2 was found to be linked to only oleic acid content in both years (Figure 4), while QTLs on chromosomes 1, 10, and 2 were found to be linked with linoleic acid synthesis (Figures 7–9).

The observation of QTLs for fatty acid content of olive oil at different chromosomal regions in different years could be due to the different climatic condition in different years. Hernández et al. (2011) reported that while oleic acid desaturase activity increased under high temperature conditions, it decreased under low temperature conditions. The QTLs linked to oleic acid and linoleic acid contents were detected on chromosome 7 in the second year analysis but not in the first year analysis. However, QTLs linked to only oleic acid content were detected on chromosome 7 in both years. Unver et al. (2017) identified the DNA sequence within a scaffold called "NW.019263883.1" in the reference genome, but the authors were unable to assign it to any chromosome. Our results demonstrated that this scaffold is a part of chromosome 7. According to our map,

Table 3. QTLs linked to fatty acid contents of olive oil.

First year				Second year			
CHR	**QTL position (cM)**	**Max. LOD**	**Exp. (%)**	**CHR**	**QTL position (cM)**	**Max. LOD**	**Exp. (%)**
Oleic acid							
				01	47.351	3.29	31.1
02	62.491	3.70	21.1	02	43.250[a], 50.996, 64.447	4.88	25.2
				03	17.770, 61.354	3.42	48.2
07	114.619	3.87	37.5	07	89.710, 91.714	3.16	22.6
08	21.105[a], 39.461, 85.486	4.05	42.4				
09	64.019[b]	3.79	46.0				
10	66.470	2.99	36.9	10	147.518	3.50	60.8
11	75.286	3.40	17.6				
12	139.490	3.28	32.9	12	149.297	3.17	63.3
13	15.529	2.93	33.0	17	124.290	3.19	52.0
16	2.930	3.52	40.4	20	29.366[b]	3.34	66.4
Palmitic acid							
03	103.882	4.27	50.5				
06	1.000	3.80	26.5				
07	33.956	3.29	26.2				
08	21.105, 37.070[ab], 47.004	4.29	53.3				
09	64.019	3.37	47.4				
10	136.792	3.20	26.2	10	163.702[ab]	3.69	55.9
13	68.734	3.24	36.1	12	26.304, 126.544	3.36	15.4
15	36.299	2.94	15.4	15	Unmapped 53.00	3.06	14.2
16	3.271	2.98	39.7	16	84.116	3.09	21.3
21	1.000	3.06	15.1	19	55.240	3.55	38.0
Linoleic acid							
01	45.351[ab]	5.18	54.5	01	46.351	3.07	36.7
02	64.447	4.15	22.1	02	42.250, 53.99, 64.447[a]	5.43	27.0
				03	61.354	3.16	60.4
				07	6.081	3.86	45.2
08	85.346	3.10	15.7				
10	135.309, 145.157	3.53	17.5	10	147.518, 163.702[b]	3.55	63.5
11	75.286	3.19	16.7				
13	15.529	3.44	29.9	12	41.142	4.25	46.2
16	20.530	3.09	16.3	18	18.834	3.04	16.3
				22	60.419	2.94	60.5

CHR, chromosome
Max. LOD, maximum logarithm of odds.
Exp. (%), explained of genetic variance.
[a], position of the maximum LOD score.
[b], the highest position explained of genetic variance and the major QTL.

the QTL affecting oleic acid synthesis was linked to SNP marker at the 53,355 bp of "NW.019263883.1" scaffold. In the chromosomal region where this QTL is located, there is a gene called "*Olea europaea* var. s*ylvestris 3-ketoacyl-CoA synthase* 4-like (*KASII*) (LOC111389048). The palmitic acid content of seed oil is decreased and the stearic acid content is increased when *KASII* is overexpressed in *Brassica napus* L. (Gupta et al., 2012). In addition, another QTL affecting oleic acid synthesis was located at 358,661 bp of "NW.019263883.1". Similarly, in the chromosomal region where QTL is located, there is another gene called "*Olea europaea* subsp. *europaea chloroplastic delta12 fatty acids desaturase* (FAD6) gene". *FAD6* converts monounsaturated oleic acid to polyunsaturated linoleic

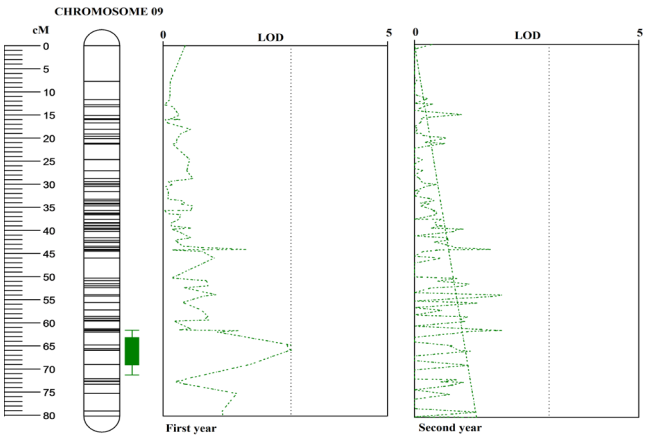

Figure 2. The major QTL chromosome 09 linked to oleic acid content in the first year.

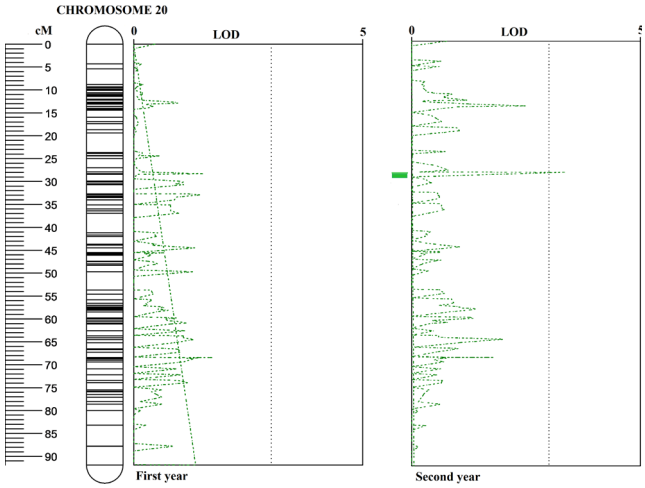

Figure 3. The major QTL on chromosome 20 affecting oleic acid content in the second year.

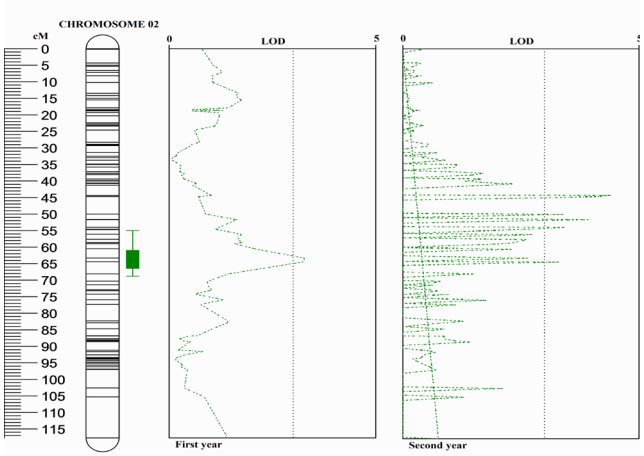

Figure 4. The common QTL on chromosome 02 linked to oleic acid content in both years.

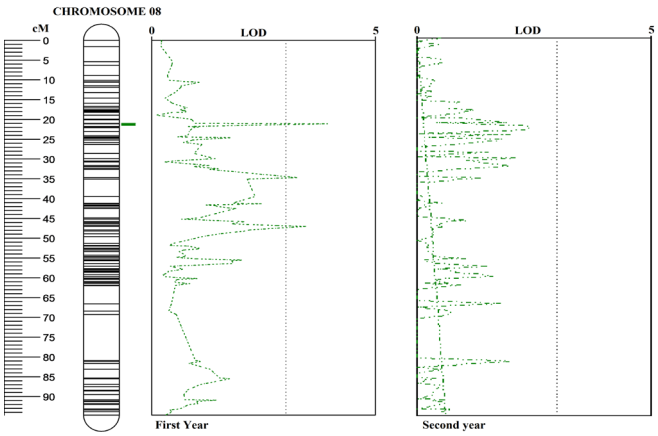

Figure 5. The major QTL on chromosome 08 affecting palmitic acid content of olive oil in the first year.

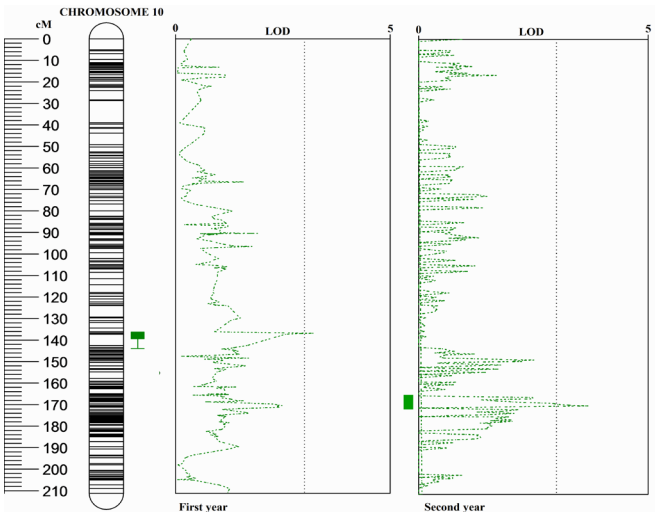

Figure 6. The major QTL on chromosome 10 linked to palmitic acid content in the second year.

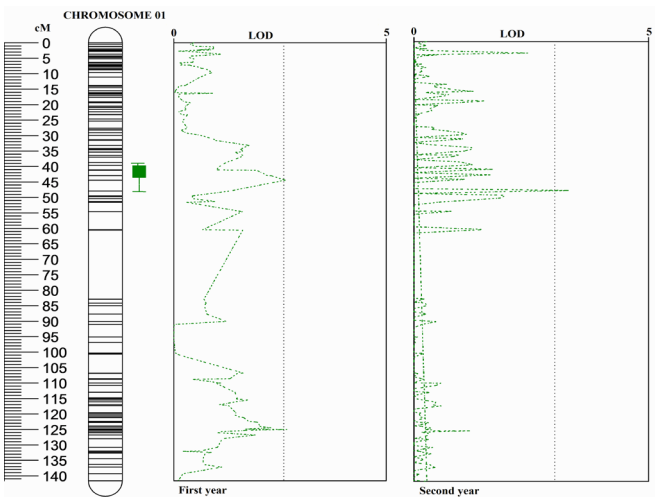

Figure 7. The major QTLs on chromosome 01 linked to linoleic acid content in the first year and common QTL in both years.

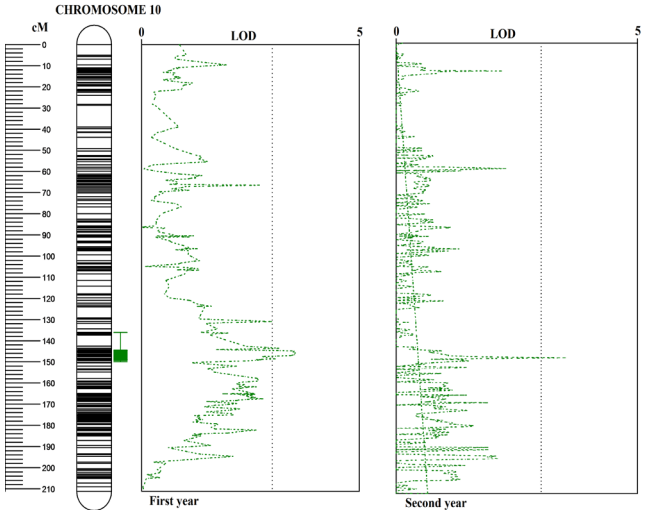

Figure 8. The major QTL on chromosome 10 linked to linoleic acid content in the second year and common QTL in both years.

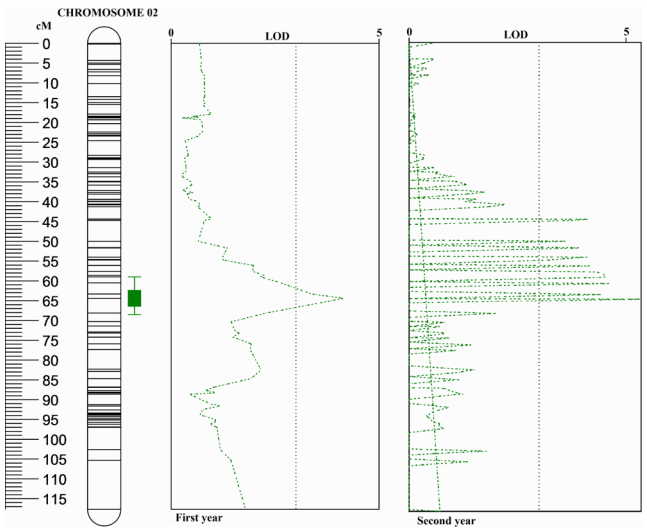

Figure 9. The common QTL on chromosome 02 linked to linoleic acid content in both years.

acid (Banilas et al., 2005; Hernandez et al., 2008). The conversion of oleic acid to linoleic acid may have caused a decrease in the amount of oleic acid in the second-year fatty acid analysis (Table 2).

There is another scaffold called "NW 019240091.1" identified by Unver et al. (2017) also mapped on chromosome 7 in our map. The QTL linked to linoleic acid synthesis was detected in this DNA sequence at 127.924 bp. In this QTL region, there is a "PREDICTED: *Olea europaea* var. *sylvestris* uncharacterized (LOC111369964) transcript variant X3, mRNA". When this uncharacterized DNA sequence was translated, its putative amino acid sequences matched with 78.8% similarity to amino acid sequences of "*Cytochrome P450 93A3-like*, A0A8S0VDE4·*Olea europaea* subsp. *europaea*" (UniProt Consortium, 2023). *Cytochrome P450s* catalyzes the conversion of hydroperoxyoctadeca-9,11,15-trienoate to 12-oxo-dodec-9-enoate and cis-3-hexenal in arabidopsis (Bate et al., 1998). A metabolite of both linolenic and linoleic acids, 12-oxo-cis-dodec-9-enoic acid is a C12, omega-oxo fatty acid with a double bond at position 9 (Madeira et al., 2022). In a previous study, it was indicated that *fatty acid desaturase II (FADII)* encodes *delta 12 fatty acid desaturase* and transforms oleic acid to linoleic acid (Okuley et al., 1994; Shanklin et al., 1998). In another study, Kumar et al. (2015) found that

Table 4. Pearson's correlation coefficients between the fatty acid contents of olive oil. The upper half pertains to the first-year fatty acid analysis, while the lower half pertains to the second-year fatty acid analysis.

	C16:0	**C16:1**	**C17:0**	**C17:1**	**C18:0**	**C18:1**	**C18:2**	**C18:3**
C16:0	-	0.706	–0.039	–0.131	0.167	–0.631	0.366	0.240
C16:1	0.636	-	–0.150	0.171	–0.105	–0.413	0.079	0.227
C17:0	–0.051	–0.497	-	0.453	0.094	–0.123	0.236	0.241
C17:1	0.117	0.151	–0.049	-	–0.083	–0.131	0.053	0.316
C18:0	0.168	0.039	–0.224	0.288	-	–0.169	0.076	–0.005
C18:1	–0.727	–0.424	0.152	–0.285	–0.370	-	–0.869	–0.566
C18:2	0.385	0.120	–0.126	0.151	0.111	–0.867	-	0.570
C18:3	0.349	0.208	–0.134	0.290	0.360	–0.577	0.426	-

fatty acid reductase, *HXXX-type acyl transferase* family protein, *Myb-like transcription factor family protein*, and *Acyl CoA N-acyl transferases (NAT) superfamily protein* which converts oleic acid to linoleic in flax. Our results demonstrated that mapping QTLs using high-density genetic maps based on NGS-based SNP markers can help to identify the gene or genes affecting economically important traits.

5. Conclusion

In conclusion, a high-density genetic linkage map based on GBS-based SNP markers was developed. On this genetic map, QTLs affecting the fatty acid composition of olive oil were determined, and these QTL markers could be used in olive breeding studies. In addition, the QTL map developed in this study can be used to localize nucleotide sequences for the genes affecting fatty acid biosynthesis in olive.

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