Quantitative trait loci (QTL) that control seed oil content and fatty acid composition were studied using a recombinant inbred population derived from a cross between the Arabidopsis ecotypes Landsberg erecta and Cape Verdi Islands. Multiple QTL model mapping identified two major and two minor QTL that account for 43% of the variation in oil content in the population. The most significant QTL is at the bottom of chromosome 2 and accounts for 17% of the genetic variation. Two other significant QTL, located on the upper and lower arms of chromosome 1, account for a further 19% of the genetic variation. A QTL near to the top of chromosome 3 is epistatic to that on the upper arm of chromosome 1. There are strong QTL for linoleic (18:2) and linolenic (18:3) acids contents that colocate with the FAD3 locus, another for oleic acid (18:1) that colocates with FAD2 and other less significant QTL for palmitic (16:0), stearic (18:0), and eicosaenoic (20:1) acids. The presence of the QTL for seed oil content on chromosome 2 was confirmed by the generation of lines that contain a 22-cM region of Landsberg erecta DNA at the bottom of chromosome 2 in a background containing Cape Verdi Islands in other regions of the genome that had been shown to influence oil content in the QTL analysis.

**Seed oils, which are composed mainly of triacylglycerols (TAG), are an important source of fatty acids for human nutrition and hydrocarbon chains for industrial products. Developing an understanding of the control of TAG synthesis in seeds is an important challenge if yields are to be increased. Storage lipid is synthesized in two stages in developing seeds, firstly through the production of acyl chains by the plastids, followed by their sequential incorporation into glycerolipids by the acyltransferases of the endoplasmic reticulum (Ohlrogge and Browse, 1995). Most of the biochemical steps are known and many of the genes involved have been identified (Beisson et al., 2003). Evidence from microarray expression experiments shows that many genes involved in the conversion of sugar to oil are regulated in a coordinated fashion during Arabidopsis seed development (Ruuska et al., 2002). This suggests that one or more regulatory factors are involved in controlling expression of many of the genes of the pathway. To substantially change flux through the oil synthesis pathway, the identification of these regulatory factors is likely to be important. Genetic approaches to investigating the regulation of oil content have so far met with limited success. Screens of mutant Arabidopsis populations have identified the *tracylglycerol biosynthesis defect 1* (**tag1**) and *wrinkled 1* (**wr1**) loci as causing reduced seed oil content (Katavic et al., 1995; Focks and Benning, 1998). **TAG1** encodes DGAT1 (Zou et al., 1999), and **WRI1** is suspected to be involved with the regulation of multiple steps of the sugar metabolism pathways that lead to the generation of acetyl-CoA, the plastidial precursor of fatty acid synthesis, and enzymes involved in lipid synthesis itself (Focks and Benning, 1998; Ruuska et al., 2002). No other mutants specific to storage oil content have been described, and other sources of genetic variation in this trait are required if we are to make further progress.

Plant yield and agronomic performance traits are typically quantitatively inherited ( Tanksley, 1993). With the advent of molecular markers and the development of quantitative trait loci (QTL) mapping procedures, genes that control storage lipid synthesis can be identified and marker-assisted selection used to move beneficial QTL alleles into elite agricultural genotypes in oilseed breeding programs (Lande and Thompson, 1990). QTL analyses of oil content have been made in a number of crops, including oilseed rape (*Brassica napus*; Ecke et al., 1995; Burns et al., 2003), soybean (*Glycine max*; Lee et al., 1996; Csanadi et al., 2001), maize (*Zea mays*; Alrefai et al., 1995), and sunflower (*Helianthus annuus*; Mokrani et al., 2002; Leon et al., 2003). An analysis of great oil content in oats revealed the presence of a small number of QTLs and an association between the major QTL and the plastidic acetyl-CoA carboxylase gene (Kianian et al., 1999). It should be noted that the major factors influencing seed oil content may well be maternally controlled as demonstrated by reciprocal crosses using high and low oil lines of soybean, sunflower, and oilseed rape (Brim et al., 1968; Pawlowski, 1964; Grami and Stefansson, 1977; Thompson et al., 1979).
The fatty acid composition of seed oil varies considerably both between species and within species, with fatty acids varying in both chain length and degrees of desaturation. In Arabidopsis, mutagenesis experiments facilitated the identification of genes responsible for fatty acid elongation and desaturation (James and Dooner, 1990; Lemieux et al., 1990; Aronnel et al., 1993; Okuley et al., 1994; James et al., 1995). Since then, QTL affecting fatty acid composition that co-locate with loci for fatty acid elongase and fatty acid desaturase genes have been identified in Brassica species, sunflower, and maize (Alrefai et al., 1995; Eckle et al., 1995; Jourdren et al., 1996; Cheung et al., 1998; Fourmann et al., 1998; Lionneton et al., 2002; Perez-Vich et al., 2002; Burns et al., 2003; Mikkilineni and Rocheford, 2003).

Recent surveys have shown large variations in content and fatty acid composition of seed oil of Arabidopsis, suggesting populations derived from selected crosses will be useful for investigating these traits (Millar and Kunst, 1999; O’Neill et al., 2003). The Arabidopsis recombinant inbred line (RIL) population derived from a cross between the Cape Verdi Islands (Cvi) accession and the Landsberg erecta (Ler) laboratory strain has been an important tool for the analysis of a number of quantitative traits of Arabidopsis and in particular aspects of seed growth and physiology (Alonso-Blanco et al., 1998a, 1999, 2003; Swarup et al., 1999; Bentsink et al., 2001; El-Assal et al., 2001; Kleibenstein et al., 2001; Borevitz et al., 2002). In this paper we describe mapping of QTL for seed oil content and composition in the Ler/Cvi RIL population and show that it should be possible to use a map-based approach to clone at least one of the loci involved in controlling seed oil content.

RESULTS

Seed Oil Content of Ler/Cvi Recombinant Inbred Population

Plants of the Ler/Cvi RILs were grown in a randomized array in a glasshouse. The oil content of the seed harvested from these plants was determined using NMR spectroscopy and the fatty acid composition of the oil was measured as fatty acid methyl esters using gas chromatography. Seeds from the parental lines Cvi and Ler showed a statistically significant difference in oil content; 39.3% (±1.8; n = 5) and 43.4% (±0.9; n = 5), respectively (mean oil mass as percentage of mature seed mass ± s.e.s for n samples, each sample comprising 200 mg from pooled seed harvested from a single pot of five plants). The mean seed oil content for all 162 of the Ler/Cvi RILs combined was approximately equal to the mid-parent value (41.8%), and the trait expressed transgressive segregation in both directions with a minimum seed oil content of 32.0% (±0.8) and a maximum seed oil content of 46.3% (±1.3; Fig. 1). The seed oil content of the population showed a normal distribution with 71.6% of the individuals having values within one SD of mid-parental values. Estimates of the effect of the environment on seed oil content were determined using the Expected Means Squares to calculate the components of variation. The results showed that 33% of the variation in seed oil content was a consequence of environmental and technical variation; thus, 67% of the variation observed was due to genetic factors. The population was derived from progeny obtained from reciprocal crosses between Cvi and Ler (Alonso-Blanco et al., 1998b), thus the influence of the cytoplasm from each parent on oil content can be determined. In general, there was little cytoplasmic effect on oil synthesis (Fig. 1) with an even distribution of Ler and Cvi cytoplasm throughout the range of seed oil contents. The exceptions to this were the 15 lines with the lowest oil contents since all except two of them had Cvi cytoplasm.

To examine possible maternal effects, oil content was determined in seeds derived from reciprocal crosses between Cvi and Ler and from plants that were self fertilized using the crossing method. The seed oil contents in this experiment were lower than in the RIL population experiment since these plants were grown at another time of year and the difference is likely to be due to environmental factors. However, the relative values remain between the experiments in that Cvi is always low and Ler is always high. Seed oil content of the F1 seed from the Ler × Cvi cross, where Ler was the maternal line, at 33.5% (±0.3; n = 5) was statistically not significantly different from the Ler self-fertilized seed (33.7% ± 1.6; n = 5), demonstrating a strong maternal effect. The seed oil content from the Cvi × Ler cross, where Cvi was the maternal line, at 19.4% (±1.8; n = 5), was actually lower than in the Cvi self fertilized seed (25.2% ± 0.4; n = 5). Again this shows a strong maternal influence on seed oil content combined with additional negative interactions between the genomes. The environmental component of the variation in oil content in this experiment was 16%. This is much less than the environmental component in the QTL analysis (33%) because for the analysis

![Figure 1. Frequency distribution of the mean seed oil content of the Ler/Cvi recombinant inbred population (n = 3). The arrows depict the mean values of the parental lines (n = 3). The gray shading of the bars refers to the numbers of lines containing Ler cytoplasm and the black refers to those containing Cvi cytoplasm.](https://www.plantphysiol.org/doi/fig/10.1104/pp.033650)
of maternal effects, just a few plants were grown in a small area of glasshouse bench, thus minimizing differences in growing conditions within the experiment. It is possible that the maternal effect on seed oil contents is due to presence of different proportions of maternal tissue in seeds of different size since Cvi and Ler differ markedly in this respect (Alonso-Blanco et al., 1999). However, in the RIL population, the seed oil content was found to be almost independent of seed mass (Fig. 2), except for those few lines that had been shown to contain very low seed oil (these lines are not included in Fig. 2). A doubling in seed mass correlated with a decrease of only 0.6% in seed oil content.

Mapping QTLs for Seed Oil Content

To identify the genetic loci controlling seed oil synthesis the data for seed oil content of the 162 RILs were used for QTL analysis. The linkage map was created using the marker data previously reported (Alonso-Blanco et al., 1998b). Preliminary QTL mapping revealed a significant QTL at the bottom of chromosome 2 that transcended the last amplified fragment-length polymorphism (AFLP) marker on that linkage group. Further markers were developed and mapped to increase the marker density between those few markers that had been shown to contain very low seed oil (these lines are not included in Fig. 2). A doubling in seed mass correlated with a decrease of only 0.6% in seed oil content.

![Figure 2](image)

**Figure 2.** Relationship of seed oil content (percent w/w) and hundred seed weight (milligrams) for the Ler/Cvi recombinant inbred population. Each point represents the mean seed oil content (n = 3) for an individual recombinant inbred line.

Table 1. QTLs for % seed oil content

<table>
<thead>
<tr>
<th></th>
<th>MQM</th>
<th>QTL1t</th>
<th>QTL1b</th>
<th>QTL2</th>
<th>QTL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak LOD score and position (cM)</td>
<td>7.06 (20)</td>
<td>2.81 (108.5)</td>
<td>7.29 (90.4)</td>
<td>4.43 (8.7)</td>
<td></td>
</tr>
<tr>
<td>Flanking markers</td>
<td>EC.480C-GD.143L-Col</td>
<td>GH.157L-Col-CC.318C</td>
<td>vpmh13-vpmh14</td>
<td>GAPC-GD.248C-249</td>
<td></td>
</tr>
<tr>
<td>2 LOD (cM)</td>
<td>15–21</td>
<td>108–114</td>
<td>85–96</td>
<td>0–19</td>
<td></td>
</tr>
<tr>
<td>Additive effect (%)</td>
<td>1.0</td>
<td>0.5</td>
<td>1.3</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Percent of variance</td>
<td>14.6</td>
<td>4.5</td>
<td>16.7</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

Control of Seed Oil Content in Arabidopsis
Further Characterization of QTL2

The significance of the QTL for seed oil content on chromosome 2 was demonstrated by the development of lines carrying Ler alleles at the bottom of chromosome 2 in a genetic background carrying Cvi alleles in the other regions of the genome that the QTL analysis had shown to be important in controlling oil. Line N22161 was selected from the RIL population as having the required genotype at the oil content QTLs, backcrossed to Cvi, and three lines (Cvi32, Cvi41, and Cvi5) were selected for further study by genotyping (Fig. 4). The seed oil content of the lines Cvi32 (33.5%) and Cvi41 (34.2%) was not significantly different from the Ler parent (32.1%; Fig. 4). This demonstrates that the presence of Ler genotype at the bottom of chromosome 2 has a positive effect on seed oil content. The seed oil content of line Cvi5 in which the Ler alleles at the lowest two markers on chromosome 2 (vpmh13 and vpmh14) had been replaced with Cvi through recombination, was 27.7%, which was not significantly different from the Cvi parent (28.6%; Fig. 4). The environmental component of the variation in oil content in this experiment was 30%, which is close to the environmental component in the QTL analysis (33%).

Mapping QTL for Fatty Acid Composition of Seed Oil from the Ler/Cvi RIL Population

To map QTLs associated with the fatty acid composition of seed lipids, a base set of 50 RILs was selected as described by the Arabidopsis Stock Centre (http://nasc.nott.ac.uk/). The seed oil content of the selected RILs shows a normal distribution and was representative of the data for all 162 RILs (Fig. 1). The fatty acids in the seed were converted to their methyl ester derivatives and resolved by gas chromatography. Nine fatty acids were identified of which 14:0, 20:0, 22:0, and 22:1 were only present in trace amounts. ANOVA showed there were significant differences in the content of certain fatty acids (16:0 and 18:0 at *P* < 0.05, and 18:1 and 18:2 at *P* < 0.001) between the two parents. For the Ler/Cvi RIL population the fatty acids were present at a range of proportions with 16:0, 18:0, 18:1, and 20:1, showing a normal distribution. All of the fatty acids showed some transgressive segregation (Fig. 5, A–F). Oleic acid was positively correlated with oil content but negatively correlated (*P* < 0.01) with 16:0 and 18:3 (Table II). Linolenic acid showed a positive correlation with 16:0 and a highly significant negative correlation with 18:2.

Interval mapping identified QTL for each of the fatty acids present in significant amounts in the seed (Table III). A QTL for 16:0 was located at the top of chromosome 3 at marker CH.322C, explaining 36% of the variance. One QTL for 18:0 was located at the marker PVV4, at the upper arm of chromosome 1, accounting for 28% of the variation. One QTL for 18:1 content...
mapped close to the marker CH.322C on chromosome 3 and explained 47% of the variance. The QTL for 18:1 mapped to the same position as the QTL for 16:0 and while the QTL had a negative effect for 16:0, it had a positive effect for 18:1. The content of 18:2 and 18:3 appears to be regulated by a major QTL around the marker GH.247L on chromosome 2. For the 18:2 dataset, the QTL explained 59% of the variation of this fatty acid, and the Cvi genotype had a positive effect at this locus. For 18:3 content, the QTL around GH.247L explained 74% of the variation, but in this case the Ler genotype had a positive effect. A second QTL that explained 32% of the variance of 18:3 (LOD 4.05) was also identified, mapping to the top of chromosome 3 at marker DF.77C, and the Cvi genotype at this locus had a positive effect on the trait. One minor QTL for 20:1 (LOD 3.1) explaining 25% of the variation was detected on chromosome 3 at the marker GD.106C. MQM mapping of the 18:2 dataset, using the QTL on chromosome 2 as a cofactor, revealed another QTL on chromosome 3 around the marker GH.226C, 14.9 cM from the top of the chromosome. The QTL had a LOD of 4.92 and explained 13.9% of the variance. A similar analysis of the data was carried out for the 18:3 dataset but no additional QTLs were revealed.

DISCUSSION

Analysis of the Ler/Cvi RIL population reveals variation in seed oil content that is determined by genetic components though environmental effects also influence seed oil content. The range of seed oil content within the Ler/Cvi RIL population, in the maternal effects experiment, and in isolating QTL2 show that while environmental factors affect seed oil content the relative differences between the lines remain and that the phenotypic variation is affected mainly by genetic factors in each experiment. Four QTL for seed oil content were identified and the strongest QTL is located between 85 and 96 cM on chromosome 2. The increased oil content in seed from back-crossed lines carrying Ler alleles at the bottom of chromosome 2 in a genetic background carrying Cvi alleles in the other regions of the genome that the QTL analysis had shown to be important in controlling oil (Fig. 4) confirms the positive influence of the Ler genotype at this location. It is interesting that a preliminary study of a F2 population derived from a cross between the accessions Kondara and Br-0 revealed an association between seed oil content and a marker toward the bottom of chromosome 2 (O’Neill et al., 2003). In light of the results from the reciprocal crosses, it must be considered that genes controlling oil content may also be acting maternally through controlling the

| Table II. Spearman rank-order correlation among fatty acids and oil content in the Ler/Cvi population, used to determine the phenotypic correlations among the fatty acid and oil content |
|-----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Oil  | 16:0 | 18:0 | 18:1 | 18:2 | 18:3 |
| 16:0 | −0.12 | 0.12 | 0.25 | 0.54<sup>a</sup> | −0.38<sup>b</sup> | 0.18 |
| 18:0 | 0.12 | 0.25 | 0.05 | 0.05 | 0.08 | 0.15 |
| 18:1 | −0.06 | 0.41<sup>b</sup> | 0.12 | −0.36<sup>b</sup> | −0.57<sup>a</sup> |
| 18:2 | 0.27 | −0.03 | −0.14 | −0.06 | −0.07 | 0.24 |

<sup>a</sup> Significant at P < 0.001 level.  
<sup>b</sup> Significant at P < 0.01 level.
Hobbs et al.

Table III. QTL detected by interval mapping for fatty acid content in Ler/Cvi RIL population

<table>
<thead>
<tr>
<th>Trait</th>
<th>Marker</th>
<th>Linkage Group</th>
<th>2-LOD</th>
<th>LOD</th>
<th>Additive Effect (%)</th>
<th>Percent of Variance</th>
</tr>
</thead>
<tbody>
<tr>
<td>16:0</td>
<td>CH.322C</td>
<td>3</td>
<td>9–15</td>
<td>3.4</td>
<td>−0.3</td>
<td>36.3</td>
</tr>
<tr>
<td>18:0</td>
<td>PVV4</td>
<td>1</td>
<td>5–11</td>
<td>5.9</td>
<td>0.2</td>
<td>47.9</td>
</tr>
<tr>
<td>18:1</td>
<td>CH.322C</td>
<td>3</td>
<td>0–22</td>
<td>5.5</td>
<td>1.7</td>
<td>46.8</td>
</tr>
<tr>
<td>18:2</td>
<td>GH.247L</td>
<td>2</td>
<td>50–73</td>
<td>9.6</td>
<td>−2.4</td>
<td>59.4</td>
</tr>
<tr>
<td>18:3a</td>
<td>GH.247L</td>
<td>2</td>
<td>45–73</td>
<td>14.2</td>
<td>2.8</td>
<td>73.6</td>
</tr>
<tr>
<td>18:3b</td>
<td>DE.77C</td>
<td>3</td>
<td>0–4</td>
<td>4.1</td>
<td>−1.9</td>
<td>31.7</td>
</tr>
<tr>
<td>20:1</td>
<td>GD.106C</td>
<td>3</td>
<td>82–83</td>
<td>3.1</td>
<td>0.4</td>
<td>25.1</td>
</tr>
</tbody>
</table>

The closest marker to each QTL is shown and the linkage group number followed by its 2-LOD position indicates its map position. For each QTL, the maximum LOD score, additive effect, and percentage variance are shown. Positive additive effects indicate that the Ler allele increases the phenotype.

The supply of carbon and/or regulatory factors to the seed. Certainly, strong maternal effects on seed oil content have been observed for a number of oilseed crops including spring oilseed rape (Grami and Stefansson, 1977), sunflower (Pawlowski, 1964; Thompson et al., 1979), and soybean (Brim et al., 1968) but not safflower (Yermanos et al., 1967). However, it is not possible to suggest which genes might be involved in maternal control given that it is not well understood at present. On the other hand, inspection of the Arabidopsis sequence database in the region underlying QTL2 revealed two genes that may be involved in lipid metabolism within the embryo (http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm), but of these only stearoyl-ACP desaturase (fatty acid biosynthesis 2 [FAB2]; At2g43710) that converts 18:0-ACP to 18:1-ACP is known to be directly involved in fatty acid synthesis. Since the QTL for 18:0 and 18:1 fatty acids are associated with chromosomes 1 and 3 it is unlikely that the FAB2 gene controls oil content in the absence of a strong effect on these fatty acids. A long chain acyl-CoA synthetase (At2g47240) also lies in this region, but whether it plays a role in lipid and oil synthesis has not been demonstrated. There are also many genes encoding transcription factors, F-box proteins, protein kinases, and phosphatases, among others, that must be considered as candidates for regulating the process of oil synthesis. However, in the absence of functional characterization little comment on these candidates can be made at this stage. The oil content QTL on chromosome 2 colocalizes with a small effect QTL for Suc content of seeds that is independent of the major QTL for overall oligosaccharide composition (Bentsink et al., 2000). The presence of Ler alleles in this region has a negative effect on Suc concentration (Bentsink et al., 2000), whereas we show here that Ler alleles have a positive effect on oil content. Since Suc is the main source of carbon for fatty acid synthesis, it is possible there is a causal link between the two QTL. It has been shown that the wrf1 and tag1 mutations in Arabidopsis that result in reduced accumulation of seed oil both lead to a 2- to 4-fold increase in Suc content (Focks and Benning, 1998; Lu and Hills, 2002).

Cvi alleles have a positive effect on oil content at the locus responsible for the QTL at the top of chromosome 1 (QTL1t). Although QTL1t maps below QTLs for a number of seed traits such as seed mass, seed length, and ovule number per fruit (Alonso-Blanco et al., 1999) and between QTLs for seed dormancy (Alonso-Blanco et al., 2003), the possibility that oil content is influenced by one or more of these maternally controlled seed related QTLs cannot be ruled out due to their close proximity as mentioned above. Cvi alleles under QTL3 have a negative effect on oil content only in the presence of Ler at QTL1t. One explanation is that these two regions contain “weak” alleles of complementary genes encoding proteins with the same function that give rise to low oil when both are present. A BLAST comparison of the approximately 1,000 genes underlying QTL1t and QTL3 reveals several that might encode the same biochemical function, including kinases, transcription factors, and other potential regulatory proteins as mentioned above. An alternative explanation of the epistasis is that genes underlying the two QTLs encode enzymes involved in the same biochemical pathway. It is interesting that genes encoding enzymes that putatively catalyze the first and third steps of the glycerolipid synthesis pathway (endoplasmic reticulum localized glycerol-3-phosphate acyltransferase (At3g11430) and phosphatidic acid phosphatase (At1g15080) underlie QTL3 and QTL1t, respectively. However, the presence of Cvi cytoplasm in all but one of the LerQTL1t/CviQTL3 lines suggests the explanation is likely to be more complicated, involving interactions with the mitochondria and/or plastids.

The parental lines had significantly different fatty acid profiles, and transgressive segregation in the RIL population showed it is likely that control of fatty acid

<table>
<thead>
<tr>
<th>Chromosome</th>
<th>Marker</th>
<th>Size of PCR Product</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>vpmh54</td>
<td>231</td>
<td>att tgt ggc tga aag aca ag</td>
<td>tga gtt tgt cat gag tgt tgt tgt</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vpmh57</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vpmh3</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>vpmh4</td>
<td>210</td>
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<tr>
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<td>vpmh26</td>
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<td></td>
<td>vpmh13</td>
<td>208</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>vpmh14</td>
<td>207</td>
</tr>
</tbody>
</table>

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composition is regulated at several points in the pathway. Interval mapping identified several QTL associated with fatty acid composition. A QTL at the top of chromosome 3 explains 34% of the variation in 16:0. Genes in this region that are candidates for the regulation of 16:0 trait include acyl carrier protein 1 (At3g05020), which has been shown to be up-regulated in developing seeds (Bonaventure and Ohlrogge, 2002), a glycerol-3-phosphate acyltransferase homolog (At3g11430) that is strongly expressed in seeds based on relative abundance of expressed sequence tags (http://www.plantbiology.msu.edu/lipids/genesurvey/index.htm), and a lysophosphatidic acid acyltransferase homolog (At3g18850). A QTL for 18:0 at the proximal end of chromosome 1 explains almost one-half of the variation in this fatty acid. The gene encoding FatB (At1g08510) that catalyzes the hydrolysis of saturated fatty acids such as 18:0 from ACP (Bonaventure et al., 2003) is positioned close to the QTL and is thus a good candidate for regulating this trait. However, it lies just outside the 2-LOD range for the QTL and so assignment should be treated cautiously at this stage. A small QTL for the elongation of 18:1 to 20:1 was identified at the bottom of chromosome 3 that explained 25% of the variance. The fatty acid elongase 1 (FAE1) gene lies on chromosome 4 (At4g34520) and was not polymorphic between the parents in our study. A FAE1 homolog (At3g52160) lies close to the region of the QTL at the bottom of chromosome 3 and may play a role in this case. The highly significant negative correlation between 18:2 and 18:3 suggests the linoleate desaturase is important in determining the extent of the conversion of 18:2 to 18:3 in this population. QTLs for 18:2 and 18:3 map to the fatty acid desaturase 3 (FAD3) locus (At2g29980) that encodes linoleate desaturase as expected from studies of the fatty acid synthesis mutants (Wallis and Browse, 2002). It is interesting to note that the second QTL affecting 18:3 content maps close to the regulator of fatty acid composition loci (RFC2, RFC3, and RFC4; Horiguchi et al., 2001). The QTL for 18:1 is located at the proximal end of chromosome 3 and as predicted from the mutant studies maps around the FAD2 locus (At3g12120) that codes for oleate desaturase (Okuley et al., 1994). This contrasts with Brassica juncea where two QTLs for 18:1 together accounted for 32% and 62% of the trait variance, respectively (Sharma et al., 2002; Lionnet et al., 2002). These QTLs collocated with the FAE1 loci that control elongation to 22:1. This is to be expected since B. juncea converts a much larger proportion of 18:1 to elongated fatty acids than does Arabidopsis, which predominately desaturates it to 18:2 and 18:3.

CONCLUSION

We have shown that natural variation for seed oil between the ecotypes Lr and Cvi can be used to investigate the genetic control of oil content as an alternative approach to screening for mutants. A number of QTLs that control both oil quality and quantity have been identified. The presence of four QTL for oil content provides us with new targets for investigating the molecular basis for the control of this important yield trait. Given the number of candidate genes under each QTL, finer mapping of each region is required before direct experiments can be carried out. Several of the QTLs controlling fatty acid composition are likely to be explained by the structural genes identified by studies of mutants, but these studies open the way to investigating the molecular basis for the control of fatty acid composition in the natural situation.

MATERIALS AND METHODS

Plant Material

RILs from reciprocal crosses between the Lr and Cvi ecotypes were developed by Alonso-Blanco et al. (1998b), who also determined their genotype using a number of molecular markers. Seed from the set of 162 RILs (N22000) and the parents Lr (N8581) and Cvi (N8580) were obtained from the Nottingham Arabidopsis Stock Centre (http://nasc.nott.ac.uk/). Seed from reciprocal crosses was obtained by emasculating the Lr and Cvi flowers and hand pollinating with pollen from the other genotype. Control, parental seed was obtained by the same method but using pollen from a sister plant.

Further lines were generated from the progeny of a cross between Cvi and N22161 to introgress the Lr genotype into the region of the QTL at the bottom of chromosome 2 in a background that has the Cvi genotype underlying each of the other QTLs. This was facilitated since the Cvi genotype was inherited from the RIL N22161 for the QTL1t and QTL3. For QTL1b, markers vpmh54 and vpmh57 were developed to select for the desired Cvi genotype. The genotype of the lines was determined using microsatellite, cleaved amplified polymorphic sequences (CAPS) and simple sequence length polymorphism (SSLP) markers as described below. Three lines, Cvi5, Cvi32, and Cvi41, were selected for further study.

Growth Conditions

Seeds were imbibed and cold treated at 4°C for 4 d to break dormancy and promote uniform germination. Seedlings were pricked out to give five plants in each of five pots and pots placed in the glasshouse using a randomized block design. The plants were maintained under long-day conditions in air-conditioned greenhouses supplemented with additional light, using a 16-h day at a daytime temperature of 18°C and nighttime temperature of 15°C. The plants were bagged with cellophane bags after the terminal bud of the main florescence had flowered and seed harvested after the fruits were mature and plants had undergone senescence. The plants for the reciprocal crosses for investigation of maternal effects and Cvi × N22161 crosses for Mendelization of the QTL on chromosome 2 were grown at a different time of year and Dooner, 1990). The fatty acid methyl esters were resolved by gas-liquid chromatography on a 50-m × 0.25-mm i.d. capillary column coated with CP-Sil 88 (Varian, Shepperton, UK) as previously described (O’Neill et al., 2003). For determining the oil content of seed from the reciprocal crosses, the seeds harvested from individual siliques were counted and weighed before meth-
ylation and analysis of lipids as described above including an appropriate amount of triheptadecanoin as an internal standard.

The seed oil content (percent w/w) for the complete set of RILs, Ler, and Cvi parents and lines Cvi5, Cvi32, and Cvi41 was measured using NMR spectroscopy. Approximately 200 mg of seed was weighed accurately into a 10-mm diameter NMR tube and the oil content was determined using a benchtop QP20+ NMR (Oxford Instruments, Oxford) and was performed in general accordance with the guideline ISO 10565 (International Standardization Organization, 1993). The standard curve was produced using canola standards supplied by the Canadian Grain Council. Data are presented as means ± se. To validate the calibration for working with Arabidopsis seeds, the oil content of 12 of the RILs was also measured by methylation of the seed oil fatty acids and resolution by gas chromatography using the method described by Garces and Mancha (1995). This showed that there was a strong linear relationship between seed oil contents determined by the two methods ($r^2 = 0.96$).

**Statistical Analysis**

Analysis of the seed oil content data was performed using Minitab release 13.1 (Minitab, Birmingham, UK). The percentage oil trait data are approximately normally distributed. The seed oil content was analyzed using the one-way ANOVA was carried out and the expected mean squared values used to determine the components of variation. Spearman’s rank-order correlation coefficient was used to determine the phenotypic correlations among the fatty acid and oil content.

The original linkage map was derived using the genotypic data of Alonso-Blanco et al. (1998b). For further genotyping of the genomic regions covered by the oil content QTL, CAPS markers g2395, m235, and m323, microsatellite marker nga168 and a series of SSLP markers (Table IV) were used and linkage maps constructed using the software package JOINMAP version 3 (Van Ooijen and Voorrips, 2001). The map position of each marker was estimated using the Kosambi mapping function (Kosambi, 1944) and a LOD threshold of 3.0. The computer program MapQTL version 4 (Van Ooijen et al., 2002) was used to identify and locate QTLs linked to the markers using both interval mapping and multiple QTL model mapping. A LOD score of 2.8 was set to determine the presence of a QTL using the method described by Van Ooijen (1999). This value represents a genome-wide significance of 5%. Markers covered by a QTL with a LOD score of >2.8 from the interval mapping were used as cofactors during subsequent rounds of MQM mapping. If the LOD value for a QTL linked with a cofactor dropped below 2.8 during the MQM mapping the cofactor was removed and analysis repeated. This procedure was repeated until the cofactor list remained stable. The estimated additive effect and the percentage of variance explained by each QTL were obtained from the final MQM mapping round. Interaction between QTL1 and QTL3 was analyzed by treating them as Mendelian loci. Each RIL was assigned genotypes at QTL1 and QTL3 based on DNA markers in the region of the QTL peak LOD, heterozygous or indeterminate lines were excluded from the analysis ($n = 139$). Comparisons of segregation ratios, means, and variances of the resulting four classes of RILs were made using $x^2$, $t$, and F-tests, respectively.

**Genomic DNA Preparation and PCR**

DNA was prepared from leaf material using the simple DNA preparation method described on the University of Wisconsin Biotechnology Center Web site (http://www.biotech.wisc.edu/NewServicesAndResearch/Arabidopsis/ FindingYourPlant.asp): fresh leaf material was ground in a microfuge tube and 250 µL extraction buffer (0.2 N Tris-HCl, pH 9.0, 0.4% LiCl, 25 mM EDTA, and 1% (w/v) SDS) was added. The material was regrind and the tube spun in a microfuge for 5 min. The DNA was precipitated by adding 175 µL of the supernatant to an equal volume of isopropanol and pellet centrifugation. This DNA pellet was dried and resuspended in 100 µL of 10 mM Tris-HCl, pH 8, and 1 mM EDTA.

Each PCR reaction contained 10 ng DNA as template. The amplification reactions contained 2 units of Taq DNA polymerase (Amersham, Chalfont St. Giles, UK), 2 µL 10 × PCR buffer (Amersham), 0.1% Triton X-100, 250 µM of each dNTP, and 0.75 µM primer. The DNA amplification protocol was 30 s at 96°C, followed by 35 cycles of 55 s at 94°C, 1 min at 63°C and 1 min at 72°C, and a final 30 s cycle at 72°C.

A series of SSLP markers were designed using small insertions/deletions identified between Ler and Col (Jander et al., 2002) and primers designed using Primer3 internet based primer design program (Rozen and Skaltsky, 2000), and those that were useful for the Ler/Cvi population were used. The SSLP markers were used to assist the genotyping of QTL identified by interval mapping associated with seed oil content. Markers vpmh54 and vpmh57 map to the bottom of chromosome 1; vpmh5, vpmh6, vpmh13, and vpmh14 map to the bottom of chromosome 2. The primers for these markers are presented in Table IV and all of the markers in this series will be published on the TAIR database.

CAPS markers were analyzed as previously described (Baumbusch et al., 2001) using the DNA extractions described above. Microsatellite marker nga168 was analyzed in the Ler/Cvi population according to (Bell and Ecker, 1994).

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**LITERATURE CITED**


controlling light and hormone response in two accessions of Arabidopsis thaliana. Genetics 160: 683–696


Mikkilineni V, Rocheford TR (2003) Sequence variation and genomic organization of fatty acid desaturase-2 (fad2) and fatty acid desaturase-6 (fad6) cDNAs in maize. Theor Appl Genet 106: 1326–1332


Pawloski SH (1964) Seed genotype and oil percentage relationship between seeds of a sunflower. Can J Genet Cytol 6: 293–297


