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Major and minor QTL and epistasis contribute to fatty acid compositions and oil concentration in high-oil maize

Xiaohong Yang · Yuqiu Guo · Jianbing Yan · Jun Zhang · Tongming Song · Torbert Rocheford · Jian-Sheng Li

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Abstract High-oil maize is a useful genetic resource for genomic investigation in plants. To determine the genetic basis of oil concentration and composition in maize grain, a recombinant inbred population derived from a cross between normal line B73 and high-oil line By804 was phenotyped using gas chromatography, and genotyped with 228 molecular markers. A total of 42 individual QTL, associated with fatty acid compositions and oil concentration, were detected in 21 genomic regions. Five major QTL were identified for measured traits, one each of which explained 42.0% of phenotypic variance for palmitic acid, 15.0% for stearic acid, 27.7% for oleic acid, 48.3% for linoleic acid, and 15.7% for oil concentration in the RIL population. Thirty-six loci were involved in 24 molecular marker pairs of epistatic interactions across all traits, which explained phenotypic variances ranging from 0.4 to 6.1%. Seven of 18 mapping candidate genes related to lipid metabolism were localized within or were close to identified individual QTL, explaining 0.7–13.2% of the population variance. These results demonstrated that a few major QTL with large additive effects could play an important role in attending fatty acid compositions and increasing oil concentration in used germplasm. A larger number of minor QTL and a certain number of epistatic QTL, both with additive effects, also contributed to fatty acid compositions and oil concentration.

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X. Yang · Y. Guo · J. Yan · J. Zhang · T. Song · J.-S. Li (✉)
Beijing Key Laboratory of Crop Genetic Improvement, National Maize Improvement Center of China, China Agricultural University, Yuanmingyuan West Road, Haidian, 100193 Beijing, China
e-mail: lijiansheng@cau.edu.cn; lijs@163bj.com

J. Yan
International Maize and Wheat Improvement Center (CIMMYT), Apdo. Postal 6-641, 06600 Mexico, D.F., Mexico

T. Rocheford
Department of Agronomy, Purdue University, West Lafayette, IN 47907, USA

Introduction

High-oil maize developed by artificial selection is a value-added crop since its kernels have advantages as sources of animal feed for higher caloric energy and better protein quality (Han et al. 1987; Benitez et al. 1999; Lambert et al. 2004). Additionally, maize oil is considered as high quality oil for human health due to the high proportion of poly-unsaturated fatty acids (Lambert 2001). The long-term selection experiment for maize oil and protein was initiated with the open-pollinated variety Burr's White in 1896 (Dudley and Lambert 1992, 2004). The oil concentration of Illinois High Oil (IHO) reached about 20% after 100 generations of selection. In the early 1980s, Song et al. began to develop Chinese synthetic populations with increased oil concentration by artificial selection (Song et al. 1999; Lambert et al. 2004). Beijing High Oil (BHO) originated from a normal maize synthetic known as Zhongzong No. 2, which was synthesized with 12 inbred lines of Lancast heterotic group. Its oil concentration had increased from 4.71 to 15.55% after 18 selection cycles (Song and Chen 2004). These materials are unique resources providing an opportunity to examine the genetic basis of high oil concentration in maize.

A number of chromosomal regions and QTL controlling oil concentration were identified using segregating mapping populations through molecular markers in maize (Goldman et al. 1994; Berke and Rocheford 1995; Mangolin et al. 2004; Song et al. 2004; Wassom et al. 2008a; Zhang et al. 2008). Recently, Laurie et al. (2004) estimated the number of QTL for oil concentration to be about 50 in a larger randomly mated population, IHO (70) × ILO (70), with SNP markers, which was consistent with the estimates obtained using traditional quantitative genetic methods (Dudley 1977). Thus, a number of studies suggested that oil concentration was controlled by a large number of genes with individually small effects and mainly additive gene action.

Chemically, maize oil is a mixture, mainly (99%) comprising five fatty acids, viz., palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) acids (Lambert 2001). Sixty-eight loci associated with the five major fatty acids were detected by single-factor ANOVA using 80 RFLP markers in an IHO (90) × ILO (19) population of 200 F_2S_1 lines (Alrefai et al. 1995). Wassom et al. (2008b) identified 15 QTL controlling these fatty acids using the same markers in the same populations described in Wassom et al. (2008a).

Epistasis, the interaction between alleles from two to more genetic loci (Fisher 1918), may play an important role in evolutionary and quantitative variation in crops (Doebley et al. 1995; Yu et al. 1997; Lukens and Doebley 1999; Gadau et al. 2002; Malmberg et al. 2005; Mei et al. 2005; Xu and Jia 2007). QTL mapping is one experimental approach to explore the role of epistasis in the genetic basis of complex traits (Carlborg and Haley 2004). However, there are practical problems in efficiently measuring epistatic genetic variances. One common method is to use multiple interval mapping (MIM; Kao et al. 1999) in the Windows QTL Cartographer software (Wang et al. 2005), but this program only detects epistatic interactions among identified QTL with main effects. Recently, several computer programs were developed that enable genome-wide scans for epistatic effects (Chase et al. 1997; Holland 1998; Manly et al. 2001; Sen and Churchill 2001; Borevitz et al. 2002; Yang et al. 2007). Among these, the mixed linear model presented in QTLNetwork Version 2.0, can integrate multiple QTL, epistasis, QTL-by-environment interactions, and epistasis-by-environment interactions into one mapping system (Yang et al. 2007). Using this software, epistasis contributing to genetic variation in oil concentration was identified in oilseed crops (Zhao et al. 2005, 2006). In maize, a certain number of epistatic interactions affecting oil concentration and fatty acid compositions were identified in segregating populations (Dudley 2008; Wassom et al. 2008a, 2008b). However, similar studies have not been undertaken in recombinant inbred line (RIL) populations.

Biosynthesis of storage oil in plant seeds is a complex metabolic process (Ohlrogge and Browse 1995). Most of the biochemical steps are well known and many of the genes involved in these reactions have been characterized in Arabidopsis (Beisson et al. 2003). However, much less is known in maize, and only a few genes related to lipid metabolism were cloned (Lee and Huang 1994; Egli et al. 1995; Berberich et al. 1998; Schreiber et al. 2000). Some loci for the key enzymes in lipid metabolism associated with QTL for fatty acid compositions were identified in Arabidopsis, sunflower and mustard (Lionneton et al. 2002; Pérez-Vich et al. 2002; Hobbs et al. 2004). In maize, the map positions of two fatty acid desaturases, *fad2* and *fad6*, were compared with the positions of QTL for the ratio of oleic:linoleic acids to determine if they map to the same genomic region (Mikkilineni and Rocheford 2003). The results suggested that comparison of the positions of candidate genes and QTL was a suitable strategy to investigate the molecular basis of quantitative traits. Additionally, the positioned candidate genes can be used to develop functional markers for increasing selection efficiency by marker-assisted selection in plant breeding (Jeppe and Thomas 2003).

In the present study, a RIL population derived from a single cross with a high-oil line was used to: (1) map QTL for fatty acid compositions and oil concentration; (2) estimate the number and magnitude effects of QTL and epistatic interactions underlying fatty acid compositions and oil concentration; and (3) analyze the relationships between candidate genes involved in lipid metabolism and mapped QTL for fatty acid compositions and oil concentration.

Materials and methods

Genetic materials

A RIL population was developed from a cross between B73 (normal inbred) and By804 (high-oil inbred), which was selected from a high-oil population, BHO Cycle 13. Two hundred and forty-five $F_{7:8}$ RILs along with both parents were planted in a randomized complete block design with three replications on the Agronomy Farm, China Agricultural University, Beijing, in 2005 and 2006, respectively. Each family line was grown in a single 5 m row, rows were 0.67 m apart, and planting density was 45,000 plants/ha. In order to avoid xenia effects, more than six plants in each row were pollinated by bulked pollen within the row and pollinated ears were harvested at maturity. Equal amounts of grain from each harvested ear in each row among RIL population were bulked for chemical analysis. Grain samples from two field

replications over 2 years were used for fatty acid compositions and oil concentration analysis.

Genetic linkage and candidate gene mapping

A total of 208 markers, including 202 SSR markers and six IDP markers developed by our laboratory, were used to build a genetic framework map. SSR markers were selected from public maize database (http://www.maizegdb.org). Genomic DNA was extracted from all 245 F_7 RILs and the parents using the modified procedure of Murry and Thompson (1980). A SSR procedure was followed: template DNA 50 ng, primers 0.67 μM, 10× reaction buffer 1×, $MgCl_2$ 2.5 mM, dNTPs 0.2 mM, Taq DNA polymerase 0.5 U, made the final volume of 15 μL with deionized double distilled water. The PCR reaction conditions were set as: denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min, elongation at 72°C for 1.5 min, with final extension at 72°C for 5 min and then stored in refrigerator at 4°C.

In order to analyze the co-locations of QTL for fatty acid compositions and oil concentration with the genes of lipid metabolism, 18 candidate genes (supplementary Table 1) were chosen to develop molecular markers for mapping according to the Arabidopsis lipid metabolism pathway (Beisson et al. 2003; http://www.plantbiology.msu.edu/lipids/genesurvey). Because the cDNA or DNA sequences in maize were unknown, known protein sequences of Arabidopsis were submitted to a tBLASTn (Altschul et al. 1997) search for maize homologues of candidate genes in the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST), and the highest matching hits were chosen for developing candidate gene markers. Gene-specific primers (supplementary Table 1) for developing SNP and IDP markers were designed from gene or EST sequences, using the Primer 5.0 software (Clarke and Gorley 2001) to amplify and sequence parts of the genes. MAPMAKER/EXP 3.0 (Lincoln et al. 1993) was used to construct a genetic linkage map and to integrate the candidate genes into the map. The Kosambi mapping function was used for converting recombination values to map distances.

To amplify the candidate gene fragments for sequencing, DNA was amplified with a PTC-200 Peltier Thermalcycler (Bio-Rad, USA) in a 50-μl reaction volume containing 100 ng genomic DNA, 5 μl of 10× PCR reaction buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 μM of each gene-specific primer, and 1.25 U of Taq polymerase (Tiangen). Thermal cycling conditions were 95°C for 5 min; 35 cycles of 95°C for 45 s, optimal annealing temperature for 45 s, 72°C for 1.5 min; followed by a final extension of 10 min at 72°C. PCR products were analyzed on agarose gels, purified using a multifunctional DNA purification kit (Bioteke Corporation, China), and cloned into pGEM-T easy vector (Promega, USA). Three positive clones were picked and sequenced. The DNAMAN software (Lynnon Biosoft, Canada) was used to search for nucleotide polymorphisms between sequences of the two parents. Subsequently, three types of genotyping, insertion–deletion polymorphisms (IDP, Bhattramakki et al. 2002), cleaved amplified polymorphic sequences (CAPS; Konieczny and Ausubel 1993) and allele-competitive PCR (AC-PCR) SNP (Falque et al. 2005) were employed to develop markers for mapping candidate genes.

Measurements of fatty acid compositions and oil concentration

Fatty acid compositions of 223 lines of the 245 RILs were measured because some RILs did not have enough grain or their kernels did not develop very well. Fifty kernels randomly chosen from the bulked grain of each plot were ground and a HP6890 gas chromatogram (GC) (Agilent Technologies, USA) was employed for fatty acid analysis. All samples were measured with the two sub-samples at the same time. Lipids were extracted as described by Sukhija and Palmquist (1988) with some modifications: 200–300 mg of each sample was weighed and transferred to 15 ml culture tubes. To each tube 4 ml methyl alcohol:acetyl chloride (10:1) were added, followed by 5 ml of internal reference (methyl nonadecanoate, Sigma, USA), with 1 mg/ml in hexane, and after general vortexing, heated for 2 h in a water bath at 80°C. After the contents were cooled to room temperature, 5 ml of 7% K_2CO_3 were added to neutralize extracted fatty acids, and the upper phase was transferred to sample bottles. Samples at 250°C were automatically injected (1 μl) and separated in the GC system equipped with a HP-INNOWAX polyethylene glycol capillary column (30 m × 320 μm × 0.5 μm, Agilent Technologies). The GC was operated at constant flow pressure of 140.9 kPa with an initial oven temperature of 220°C, 13 min isothermal, then oven temperatures were increased by 20°C/min to 240°C, 5 min isothermal. The FID temperature was 250°C and the split ratio of nitrogen was 20:1. Fatty acids were identified by comparison of their retention times with that of the internal reference. All data were acquired on ChemStation software (Agilent Technologies) and normalized to sample weight and to the internal reference. The oil concentration was calculated as the sum of all identified fatty acid concentrations with percentage (%) of kernel weight. Individual fatty acids were expressed as percentage (%) of oil concentration.

Phenotypic data analysis

The variance of fatty acid compositions and oil concentration were estimated using PROC GLM in SAS 8.02

software (SAS Institute 1999). The model for variance analysis was $Y = \mu + \alpha_g + \beta_y + (\alpha\beta)_{gy} + \gamma_{yr} + \varepsilon_{gyr}$, where α_g was the effect of the gth line, β_y was the effect of the yth year, $(\alpha\beta)_{gy}$ was the line × year interaction, γ_{yr} was the year × replicate interaction, and ε_{gyr} was the residual. All effects were considered to be random.

Broad-sense heritability (H^2) was estimated as $H^2 = \sigma_g^2/(\sigma_g^2 + \sigma_{gy}^2/r + \sigma_e^2/\mathrm{yr})$ (Knapp et al. 1985), where σ_g^2 is the genetic variance, σ_{gy}^2 is the interaction of genotype with year, σ_e^2 is the residual error, r is the number of replications, and y is the number of years. All the variances were acquired from variance analysis. To quantify the relationships among measured traits, correlation coefficients were calculated for each trait pair using PROC CORR in SAS 8.02 software (SAS Institute 1999).

QTL and epistasis analysis

As described as Yang et al. 2007, QTLNetwork version 2.0 was employed for QTL and epistasis analysis. A mixed linear model was used to identify QTL at 1-cM intervals with a window size of 10 cM. 10-cM windows were defined to distinguish two adjacent test statistic peaks whether or not they represented two QTL. Two-dimensional (2D) genome scans were used to search for multiple interacting QTL. For each trait, a genome-wide threshold value of the F-statistic ($\alpha = 0.01$) for declaring the presence of a QTL was estimated by 10,000 random permutations (Doerge and Churchill 1996). A Bayesian method with Gibbs sampling was used to estimate QTL effects (Wang et al. 1994). Some marker intervals coming directly from QTLNetwork version 2.0 were modified if the position of adjacent QTL for different traits was less than 10 cM. The sum of individual phenotypic variance explained by each QTL was calculated as the total phenotypic variance explained by all QTL for each trait.

Results

Variation in fatty acid compositions and oil concentration

Eleven fatty acid compositions were determined using GC in RIL, and their sum was tabulated as oil concentration. Data for four major fatty acids, viz. palmitic (16:0), stearic (18:0), oleic (18:1), and linoleic (18:2) acids, and total oil concentration were used for subsequent analysis because the other seven fatty acids were present in only trace analyzed amounts. The means of the RIL population combined over years was close to the mid-parent value for all measured traits (Table 1). A normal distribution with a wide range of variation was observed for each trait (data not shown). Oil concentration was positively correlated with the level of 18:1, but was negatively correlated with levels of 16:0 and 18:2 (Table 2). Oleic acid showed a highly negative correlation with 18:2 ($r = -0.96$) and low or moderate correlations were detected for other fatty acid pairwise comparisons (−0.43 to 0.38). The GLM indicated highly significant effects due to genotype, year and genotype × year interaction for each trait (Table 1). For all traits, broad-sense heritability (H^2) estimates were generally high, ranging from 86.8% for 18:0–97.8% for 18:2. The relatively high heritabilities indicated that much of the phenotypic variance in the RIL population was genetically controlled.

Table 1 Means, range, variances and broad-sense heritability (H^2) for fatty acid compositions and oil concentration

Traits[a]		16:0	18:0	18:1	18:2	Oil
B73	Mean	13.45 ± 0.17	2.30 ± 0.11	25.11 ± 0.82	55.85 ± 0.72	4.08 ± 0.20
By804	Mean	13.42 ± 0.20	2.38 ± 0.21	35.21 ± 0.36	46.82 ± 0.50	11.18 ± 0.58
RIL	Mean	13.47 ± 0.06	2.09 ± 0.01	30.56 ± 0.22	51.29 ± 0.21	6.64 ± 0.04
	Range	9.17–18.42	1.10–3.49	17.45–46.47	37.49–63.41	3.71–10.41
	F values year × replicate	4.39*	20.64**	0.89	4.19	0.09
	Year	214.34**	210.69**	26.66**	1.39**	2.52**
	Genotype	44.00**	19.38**	105.04**	114.21**	24.08**
	Genotype × year	2.00**	2.56**	2.44**	2.57**	1.80**
	Heritability (H^2)[b]	95.4	86.8	97.7	97.8	92.5
	Confidence interval[c]	94.3–96.4	83.3–89.5	97.1–98.2	97.2–98.2	90.6–94.1

*,** Significant at $P < 0.05$ and 0.01, respectively

[a] 16:0, Palmitic acid; 18:0, stearic acid; 18:1, oleic acid; 18:2, linoleic acid

[b] Broad-sense heritability (H^2) of fatty acid compositions and oil concentration

[c] 90% Confidence intervals of broad-sense heritability

Table 2 Pearson correlation coefficients for trait pairs affecting fatty acid compositions and oil concentration in the RIL population

Traits	16:0	18:0	18:1	18:2	Oil
16:0	1				
18:0	−0.33**	1			
18:1	−0.43**	0.38**	1		
18:2	0.16**	−0.40**	−0.96**	1	
Oil	−0.31**	0.07	0.43**	−0.33**	1

** Significant at $P = 0.001$

Table 3 QTL for fatty acid compositions and oil concentration in RIL population

Traits	QTL[a]	Chr	Marker interval	P[b]	A[c]	$h^2(a)$[d] (%)
16:0	*pal1*	1	umc2232-umc1988	134.5	−0.338	2.7
	pal2	2	phi96100-kt2	14.0	−0.185	0.8
	pal4	4	bnlg2162-umc2286	110.1	−0.354	3.0
	pal5	5	umc2373-umc1221	84.3	−0.282	1.9
	pal6	6	nc010-umc1105	63.4	−0.619	9.1
	pal8	8	umc1130-umc1562	65.9	−0.398	3.8
	pal9	9	bnlg1401-umc2213	26.1	1.332	42.0
	pal10	10	umc1367-umc2016	40.2	−0.288	2.0
Subtotal[e]						65.3
18:0	*ste2*	2	phi083-u4	113.6	0.051	1.3
	ste3	3	phi053-sad7004	109.1	−0.162	13.2
	ste5	5	umc1491-umc1253	0.0	0.055	1.5
	ste6-1	6	Q8-nc010	54.3	0.173	15.0
	ste6-2	6	phi299852-umc2059	152	−0.137	9.3
	ste8	8	umc1075-umc1304	17.0	−0.136	9.2
	ste9	9	bnlg1401-umc2213	27.7	−0.088	3.9
Subtotal[e]						53.4
18:1	*ole2-1*	2	phi96100-kt2	16.2	0.459	0.7
	ole2-2	2	bnlg108-u4	114.8	0.633	1.4
	ole4	5	m1-bnlg2291	81.4	1.628	9.0
	ole6-1	6	Q8-nc010	41.5	2.859	27.7
	ole6-2	6	nc010-umc1105	67.4	1.394	6.6
	ole6-3	6	umc1614-acda6001	89.8	1.494	7.6
	ole7	7	dupssr13-CA33	103.4	−0.763	2.0
	ole8	8	umc1130-umc1562	65.9	0.904	2.8
	ole9	9	umc1743-umc1654	49.5	−0.873	2.6
Subtotal[e]						60.4
18:2	*lin1-1*	1	umc2217-bnlg2086	74.8	0.707	1.6
	lin1-2	1	umc1725-phi064	264.0	−0.461	0.7
	lin2	2	bnlg108-u4	114.8	−0.564	1.0
	lin4	4	m1-bnlg2291	81.4	−1.115	4.0
	lin6-1	6	Q8-nc010	42.5	−3.881	48.3
	lin6-2	6	umc1614-acda6001	89.8	−1.400	6.3
	lin6-3	6	phi299852-umc2059	147.0	0.786	2.0
	lin7	7	dupssr13-CA33	104.4	0.592	1.1
	lin9	9	bnlg1401-umc2213	26.7	−0.512	0.8
Subtotal[e]						65.8
oil	*oil1-1*	1	umc2217-bnlg2086	85.0	0.480	15.7
	oil1-2	1	ols1-phi308707	215.2	0.200	2.7
	oil2	2	phi96100-kt2	9.0	0.309	6.5
	oil4	4	dupssr28-bnlg2162	104.8	0.146	1.5
	oil5	5	umc2373-umc1221	90.3	0.275	5.3
	oil6	6	Q8-nc010	45.5	0.351	8.4
	oil8	8	umc1130-umc1562	65.9	0.279	5.3
	oil9	9	bnlg1401-umc2213	22.1	0.233	3.7
	oil10	10	umc1367-umc2016	40.2	0.303	6.2
Subtotal[e]						55.3

[a] Number following the three letters represents the chromosome location of the QTL. Different lowercase numbers following the dash indicate putatively different QTL located on the same chromosome

[b] The QTL location on chromosome was estimated by QTLNetwork

[c] Additive effects estimated by QTLNetwork. Positive (+) indicates that the By804 allele increases trait expression, and negative (−) indicates that the B73 allele increases trait expression, respectively

[d] Percentage of phenotypic variance explained by individual additive effects of the mapped QTL

[e] Total percentage of phenotypic variance explained by all additive effects of the mapped QTL for each trait

Single-locus QTL for fatty acid compositions and oil concentration

Based on a linkage map of 1,675 cM with an average interval of 7.3 cM between adjacent markers, a total of 42 QTL controlling fatty acid compositions and oil concentration were detected, corresponding to 21 genomic regions because some QTL for different traits clustered in the same regions and shared the same molecular markers (Table 3, Supplementary Fig. 1). These loci were distributed across all ten chromosomes. Individual loci for any given trait explained 0.7–48.3% of the phenotypic variance. Among the mapping population, all additive effects of mapped QTL for each trait accounted for a medium proportion of the total phenotypic variance, ranging from 53.4 to 65.8% (Table 3).

For oil concentration, nine QTL distributed across all chromosomes, except chromosome 3 and chromosome 7, were detected. The QTL, *oil1-1*, with the largest effect (15.7% of the phenotypic variance) was located on chromosome 1, flanked by umc2217 and bnlg2086. The By804 allele at this locus had an additive effect of 0.48% for increased oil concentration. Another important QTL for oil concentration, *oil6*, was located in the same region as *ste6-1*, *ole6-1* and *lin6-1*, and explained 8.4% of phenotypic variance with an additive effect of 0.35% on the

chromosome 6. The other minor QTL for oil concentration each explained 1.5–6.5% of the phenotypic variance. Alleles from By804, a high-oil parent, at all of mapped loci had increasing effects for oil concentration.

Eight QTL were associated with 16:0 and explained 65.3% of the total phenotypic variance. One major QTL for 16:0, *pal9*, terminally located on chromosome 9S, and bordered by markers bnlg1401 and umc2213, contributed 42.0% of the explained phenotypic variance. The additive effect of the By804 alleles increased 16:0 by 1.332% absolutely. The remaining seven QTL were located on chromosomes 1, 2, 4–6, 8 and 10 with explained variances ranging from 0.8 to 9.1%; B73 alleles increased 16:0 at these loci.

Seven QTL were involved in 18:0 and accounted for 53.4% of the total phenotypic variance. Two QTL with large effects, *ste6-1*, flanked by the markers Q8 and nc010 on chromosome 6, and *ste3*, flanked by the marker phi053 and sad7004 on chromosome 3, accounted for 15.0 and 13.2%, respectively, of the phenotypic variance. The other QTL explained only 1.3–9.3% of the phenotypic variance. By804 alleles contributed to increased 18:0 at three loci, *ste2*, *ste5* and *ste6-1*, and B73 alleles contributed increases at the other loci.

Nine QTL were significantly associated with 18:1. The strongest QTL, *ole6-1*, located in the same position as *st6-1*, explained 27.7% of the phenotypic variance, and By804 alleles had an additive effect of 2.85% for increasing 18:1. The phenotypic variance explained by the other eight QTL ranged from 0.7 to 9.0%. Alleles of these eight QTL with increasing effects came from By804 with the exception of *ole7* and *ole9*. Collectively, the nine QTL explained 60.4% of the total phenotypic variance.

A total of nine QTL controlling 18:2 explained 65.8% of the total phenotypic variance. One major QTL, *lin6-1*, with a very large effect localized to the same region as *ste6-1*, accounted for 48.3% of the phenotypic variance. B73 alleles at this locus had an additive effect of 3.88% for increased 18:2. Another eight QTL with small effects explained 0.7–6.3% of the phenotypic variance. Among these eight loci, alleles with increasing effects at three loci were contributed by By804 and five came from B73.

Epistatic QTL for fatty acid compositions and oil concentration

Twenty-four pairs of epistatic QTL involving 36 loci were identified for all measured traits (Table 4). The number of epistatic QTL pairs for each trait ranged from two to seven. The proportion of total phenotypic variance explained by all epistatic QTL ranged from 5.2 to 12.6% for each trait. Based on genetic effects, mapped epistatic QTL comprised three types: interactions between two QTL with additive effects (AA), interactions between a QTL with additive effect and a locus without significant additive effect (AN or NA), and interactions between two loci with only epistatic effects (NN). There were both positive and negative epistatic interactions, indicating the combination of alleles at interacting loci with increasing contributions from the same and different parents, respectively.

For 16:0, six pairs of epistatic QTL were detected, explaining 0.4–6.1% of the phenotypic variance in the RIL population. Of these, increased 16:0 from three pairs of loci came from the parental digenic combination, and three came from combinations of alleles from different parents. These QTL were partitioned into four NA interactions and two NN interactions. Five pairs of epistatic QTL (two AN, and three NN interactions) for 18:0 were detected, accounting for 1.5–2.0% of the phenotypic variance. All those with increasing effects came from the parental digenic combination, except for an interaction between one locus flanked by umc2408-bnlg197 on chromosome 3 and another flanked by umc1241-umc1695 on chromosome 7. For 18:1, four pairs of epistatic QTL with explained variance from 0.5 to 2.5% were partitioned into one AA interaction, two NA interactions and one NN interaction. One of four pairs with increasing effect came from combinations of alleles from different parents, whereas the remaining ones were from parental digenic combinations. Seven pairs of epistatic QTL for 18:2 (two AN or NA) interactions and five NN interactions) explained 0.5 to 1.6% of the phenotypic variance. The epistatic effects of these interactions came from different parents except one interaction between a locus flanked by umc1725-phi064 on chromosome 1 and another flanked by umc1367-bnlg1655 on chromosome 10. Two pairs of epistatic QTL were detected for oil concentration. One accounted for 1.3% of the phenotypic variance with an increased epistatic effect from different parents, and another explained 3.8% of phenotypic variance with an increased epistatic effect coming from the parental digenic combination.

Co-location of mapped candidate genes with single-loci QTL

A total of 18 candidate genes related to lipid metabolism, having either InDel or SNP polymorphisms between the parents, were mapped using 20 PCR-based markers corresponding to the candidate genes (Table 5; Fig. 1). Among these markers, 11 were IDP, seven were CAPS and two were AC-PCR SNP. Four markers corresponding to two putative functional genes were located at different loci due to multiple copies. They were *ketoacyl-ACP synthase*II (*KAS*II) on chromosome bins 2.07–2.09 and 7.04, and

Table 4 Epistatic QTL for fatty acid compositions and oil concentration in RIL population

Trait	Chr_i	$Marker\ interval_i$	P_i^a	Chr_j	$Marker\ interval_j$	P_j^a	Interaction[b]	AA[c]	$h^2(aa)^d$ (%)
16:0	2	phi083-phi092	121.1	8	phi119-bnlg2086	40.2	N*N	0.126	0.4
	2	C9_3-mmc027	138.2	8	umc1562-umc1141	80.7	NA	−0.160	0.6
	2	mmc027-kass2	172.3	8	umc1130-umc1562	65.9	NA	0.292	2.0
	2	mmc027-kass2	172.3	8	phi121-bnlg2046	60.9	NA	−0.507	6.1
	6	umc1614-acda6001	100.3	7	phi034-umc1409	44.9	N*A	−0.303	2.2
	6	phi299852-umc2059	146.0	7	phi091-atf2	64.5	N*N	0.236	1.3
Subtotal[e]									12.6
18:0	6	Q8-nc010	54.3	9	bnlg1401-umc2213	27.7	AA	0.054	1.5
	1	bnlg400-umc1955	169.1	9	Q2-umc1170	4.6	NN	0.057	1.6
	2	phi083-u4	113.6	3	bnlg1144-umc1012	34.0	AN	0.057	1.6
	3	phi036-bnlg1638	61.9	8	umc2082-bnlg1863	54.2	NN	0.056	1.6
	3	umc2408-bnlg197	127.3	7	umc1241-umc1695	1.0	NN	−0.064	2.0
Subtotal[e]									8.3
18:1	1	umc2217-bnlg2086	80.0	3	bnlg1144-umc1012	45.0	N*N	0.750	1.9
	1	umc1988-umc1122	152.4	6	umc1614-acda6001	89.8	N*A	0.862	2.5
	1	bnlg400-umc1955	162.2	6	nc010-umc1105	67.4	NA	−0.722	1.8
	2	phi96100-kt2	16.2	9	umc1743-umc1654	49.5	AA	0.382	0.5
Subtotal[e]									6.7
18:2	1	umc2232-umc1988	138.5	5	umc2143-aca	184.0	N*N	−0.704	1.6
	1	bnlg400-umc1955	164.3	9	umc1794-bnlg1525	72.7	NN	−0.398	0.5
	1	umc1725-phi064	264.0	10	umc1367-bnlg1655	40.9	AN*	0.638	1.3
	2	apat5-phi101049	216.3	9	bnlg1401-umc2213	26.7	NA	−0.525	0.9
	3	phi053-sad7004	103.1	5	bnlg1237-f22a	129.7	N*N	−0.543	1.0
	6	phi126-umc1753	10.9	10	umc1337-phi050	23.9	NN*	−0.394	0.5
	8	bnlg2082-bnlg1863	53.8	9	umc1078-umc1471	66.5	NN	−0.77	1.9
Subtotal[e]									7.7
oil	1	umc2217-bnlg2086	85.0	1	ols1-ph308707	215.2	AA	−0.14	1.3
	6	Q8-nc010	45.5	9	bnlg1525-bnlg1904	118.2	AN	0.238	3.9
Subtotal[e]									5.2

* Without significant additive QTL for this trait but with significant additive QTL for other trait in this study

[a] QTL location on chromosome was estimated by QTLNetwork

[b] Types of epistatic interaction. *AA* interactions between two QTL with additive effects, *NA* (AN) interactions between a QTL with additive effects and a locus without significant additive effects, *NN* interaction between two loci with epistatic effects only

[c] AA means the epistatic effects estimated by QTLNetwork. Positive (+) means that parental digenic genotypes increase trait expression, and negative (−) means that recombinant alleles from two parents increase trait expression, respectively

[d] Percentage of phenotypic variance explained by individual epistatic effects of the mapped QTL

[e] Total percentage of phenotypic variance explained by all epistatic effects of the mapped QTL for each trait

Stearoyl-ACP desaturase (*SAD*) on chromosome bins 3.05–3.06 and 8.05–8.06.

Comparing map positions of candidate genes and detected QTL for fatty acid compositions and oil concentration (Fig. 1), seven candidate genes were located in regions containing 18 mapped QTL with explained phenotypic variance ranging from 0.7% (*ole2-1*) to 13.2% (*ste3*) (Table 6). They were *Acetyl-CoA carboxylase* (*ACCase*), *KAS*II, *Ketoacyl-ACP synthase*III (*KAS*III) and *SAD* for fatty acid synthesis, and *Monoacylglycerol acyltransferase* (*MAGAT*), *Acyl-CoA:diacylglycerol acyltransferase* (*DGAT*) and *Oil-body Oleosin 17KD* (*OLE17*) for oil synthesis and storage. *ACCase* (u4) fell within QTL, *ste2*, *ole2-2* and l*in2*, explaining 1.3% of the phenotypic variance for 18:0, 1.4% for 18:1 and 1.0% for 18:2, respectively. *KAS*II (kass5001B), located close to QTL *ole7* and *lin7*, contributed 2.0% of the explained genetic variance for 18:1 and 1.1% for 18:2. *KAS*III (kt2) fell within a cluster of QTL for 16:0, 18:1 and oil concentration with explained variances ranging from 0.7 to 6.5%. *SAD* (sad7004) co-located with the strong QTL, *st3*, accounting for up to 13.2% of the phenotypic variance for 18:0. *MAGAT* (m1)

Table 5 Polymorphisms of candidate genes between two mapping parents and their map positions

Marker type	Enzyme	Marker	InDel/enzyme/SNP	Flanking marker	Bin
IDP	FAD8	fad83	49 bp	umc1403-phi001	1.03
	MCAT	mcat81	310 bp	umc1403-phi001	1.03
	FAD6	f6a	6 bp	umc1556-umc1955	1.07–1.08
	KASIII	kt2	21 bp	umc1265-umc1518	2.02
	PAPase	pap4	28 bp	nc003-umc2373	2.06
	KASII	kass2	14 bp	mmc0271-umc1551	2.07–2.09
	GPAT	gat5	6 bp	dupssr23-umc2408	3.06
	MAGAT	m1	3 bp	umc1299-bnlg2291	4.06
	ACA	aca	62 bp	umc2143	5.08
	TE	atf2	24 bp	phi091-umc1112	7.03
	KASI	kb1	3 bp	phi028	9.00–9.01
CAPS	OLE17	ols1	*Xsp* I	umc2047-phi308707	1.09–1.1
	LPAAT	apat5	*Eco*RI	bnlg1520-phi101049	2.09–2.1
	KASII	kass5001B	*Taq* I	CA33-umc1125	7.04
	SAD	sad7004	*Stu* I	phi053-dupssr23	3.05–3.06
		sad2702	*Bgl* II	umc1149-umc1724	8.06
	DGAT	acda6001	*Pst* I	umc1614-nc013	6.05
	FAE	fae2	*Mun* I	phi065-umc1258	9.03
AC-PCR SNP	ACCase	u4	T/G,G/A	Bnlg108-umc1635	2.04–2.05
	FAD2	f22a	C/G	bnlg1237-phi085	5.05–5.06

was located within two QTL, *ole4* and *lin4*, and accounted for 9.0 and 4.0% of the explained variance, respectively. *DGAT* (acda6001) fell within a locus containing two QTL for 18:1 and 18:2, explaining 7.6 and 6.3% of the variance, respectively. *OLE17* (ols1) was associated with *oil1-2*, accounting for 2.7% of the variance in oil concentration.

Discussion

Comparison of mapped QTL between the BHO and IHO populations

Combined previously mapped QTL for fatty acid compositions and oil concentration (Alrefai et al. 1995; Berke and Rocheford 1995; Laurie et al. 2004; Song et al. 2004; Clark et al. 2006; Wassom et al. 2008a, 2008b; Zhang et al. 2008) with present results, 28 and 56 genomic regions related to oil synthesis and accumulations were identified in the BHO and IHO populations, respectively (Fig. 1). Comparing locations within chromosome bins, 23 loci were located to common chromosome regions, such as the loci on chromosome bins 2.02, 3.05, 5.04 and 6.04 representing 37.7% of all mapped loci in both populations. However, the strongest QTL for oil concentration differed between the populations. *oil1-1* in bin 1.04, making the largest contribution to oil concentration in the present studies as well as in the earlier F_2 and F_3 studies (Song et al. 2004; Zhang et al. 2008), was not identified in the IHO population. Only a small proportion of loci controlling each fatty acid (two loci for 16:0, four for 18:0, two for 18:1 and two for 18:2) were shared between the populations. A notable exception was a locus in bin 6.04 that shared the largest magnitude of genetic effects for 18:0, 18:1 and 18:2 (Alrefai et al. 1995; Wassom et al. 2008b). The largest effective QTL for 16:0 was closely linked to bnlg 4.07 (bin 7.04) in the IHO population, but was located in bin 9.02 in the BHO population. The different locations of mapped QTL between the two populations have two explanations. First, different genetic loci for fatty acid compositions and oil concentration are involved in the different original populations. IHO was developed from an open-pollinated variety Burr's White in US; while BHO was developed from Zhongzong No. 2 synthetic which was synthesized by several inbred lines in China (Dudley and Lambert 1992, 2004; Song and Chen 2004). These two basic populations with different background may contain a few different favorable alleles with different allele frequency. Second, different loci for high oil concentration were selected during the production of the different populations. The changes per generation for oil concentration were different in two populations, 0.15% for IHO and 0.60% for BHO (Dudley and Lambert 2004; Song and Chen 2004). Both explanations suggest it should be possible to accumulate more favorable alleles for enhancing oil concentration in both populations.

Among nine loci controlling QTL for oil concentration, eight were associated with QTL for one to three individual fatty acids (Table 2, Fig. 1). According to standards

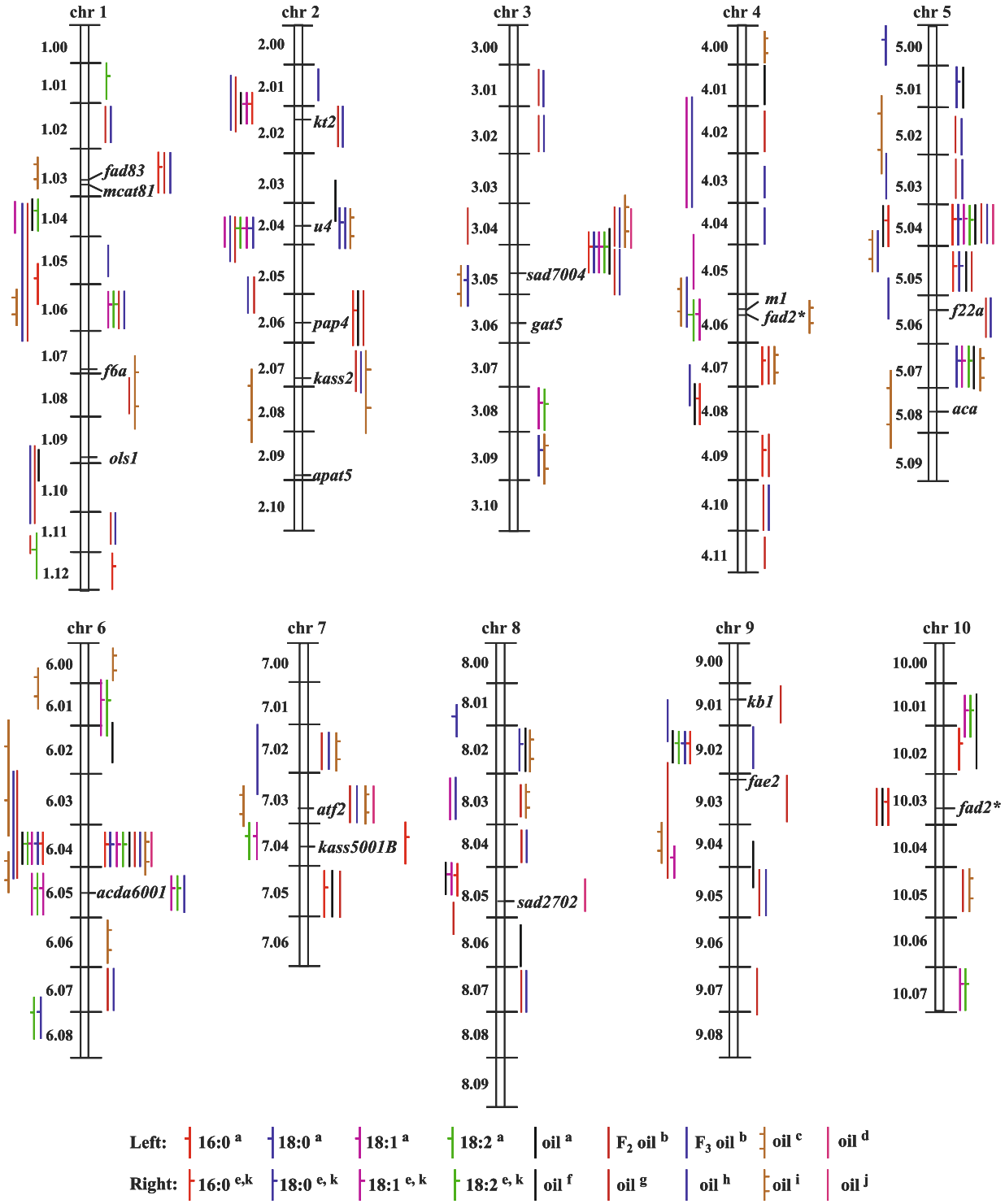

Fig. 1 Distribution of candidate genes involved in lipid metabolism and QTL for fatty acid compositions and oil concentration in this study and previous publications. The strongest QTL located on bin 6.04 and 7.04 in IHO/IHP population, bin 1.04, 6.04 and 9.02 in BHO population, bin 1.06 in tropical population. The italic markers were developed from maize gene orthologs of known lipid metabolic enzymes. QTL to the left of each chromosome indicate studies in BHO and tropical populations and QTL to the right indicate studies in IHO and IHP populations. *Asterisks* the candidate gene position mapped by Mikkilineni and Rocheford (2003); *a* Results from this study; *b* results from Song et al. (2004); *c* results from Mangolin et al. (2004); *d* results from Zhang et al. 2008; *e* results from Alrefai et al. 1995; *f* results from Berke and Rocheford 1995; *g* results identified in inbred lines from Laurie et al. 2004; *h* results identified by SIM in inbred and hybrid lines from Clark et al. 2006; *i* results from Goldman et al. (1994); *j* results from Wassom et al. (2008a); *k* results from Wassom et al. (2008b)

Table 6 Co-locations between candidate genes in lipid metabolism and QTL for fatty acid compositions and oil concentration

Enzyme	Marker	Bin	Flanking marker	QTL	A[a]	$h^2(a)$[b]
OLE17	ols1	1.09–1.1	umc2047-phi308707	oil1-2	0.200	2.7
KASIII	kt2	2.02	umc1265-umc1518	pal2	−0.185	0.8
				ole2-1	0.459	0.7
				oil2	0.309	6.5
ACCase	u4	2.04	bnlg108-umc1635	ste2	0.051	1.3
				ole2-2	0.633	1.4
				lin2	−0.564	1.0
SAD	sad7004	3.05–3.06	phi053-dupssr23	ste3	−0.162	13.2
MAGAT	m1	4.06	umc1299-bnlg2291	ole4	1.628	9.0
				lin4	−1.115	4.0
DGAT	acda6001	6.05	umc1614-nc013	ole6-3	1.494	7.6
				lin6-2	−1.400	6.3
KASII	kass5001B	7.04	CA33-umc1125	ole7	−0.763	2.0
				lin7	0.592	1.1
FAD2*		4.06		ole4	1.628	9.0
				lin4	−1.115	4.0
FAD2*		10.03		pal10	−0.288	2.0
				oil10	0.303	6.2

* Candidate gene position mapped by Mikkilineni and Rocheford (2003)

[a] Additive effects estimated by QTLNetwork. Means of positive (+) and negative (−) signs refer to Table 3

[b] Percentage of phenotypic variance explained by individual additive effects of the mapped QTL

proposed by Groh et al. (1998), the number of common loci controlling oil concentration and fatty acid compositions ranged from two to six (oil and 16:0 shared six; oil and 18:0 two; oil and 18:1 three; oil and 18:2 three). 18:1 was highly correlated with 18:2 ($r = -0.96$), and five loci controlled QTL for both 18:1 and 18:2 simultaneously. For other pairs of fatty acids, one to four loci had joint effects (18:2 and 18:0 shared four QTL; 18:2 and 16:0 one; 18:1 and 18:0 two; 18:1 and 16:0 three; 18:0 and 16:0 one). These results were similar to those reports by Alrefai et al. (1995) for the IHO population. Generally, common genomic regions with QTL for different traits could be explained by pleiotropic effects of single genes, or by close physical linkage of genes controlling different traits. Pleiotropic effects of single genes are probably the more likely reason. Among 26 common loci for two traits, the directions of favorable alleles of 21 loci were the same as those of the phenotypic correlations between them. This suggests that centralized common QTL for target traits could be exploited for molecular breeding by marker-assisted selection (Champoux et al. 1995; Redoňa and Mackill 1998).

Genetic basis of fatty acid compositions and oil concentration in high-oil maize

So far, a large number of QTL associated with different agronomic and economic traits have been identified using molecular markers in crops. For each trait, a single QTL may explain large or small amounts of variation. According to Salvi and Tuberosa (2005), a QTL explaining more than 15% of the phenotypic variance in a primary genetic analysis can be defined as a major QTL. According to this criterion, five major QTL were identified in the present study (Table 7). The phenotypic variance explained by the major QTL for each trait accounted for 24.3–65.7% of the total variance. Several recent studies demonstrated that important polygenic agronomic and domestication-related traits were controlled by a few major QTL with large effects. For example, two major QTL, *fw2.2* and *GS3*, were responsible for fruit size in tomato and grain size in rice, respectively (Grandillo et al. 1999; Fan et al. 2006). Konishi et al. (2006) and Li et al. (2006) identified two major QTL, *qSH1* and *sh4*, associated with seed shattering in rice and accounting for about 70% of the phenotypic variance. Two major QTL, *tb1* and *tga1*, governing morphological differences between maize and teosinte, explained 30–50% of the phenotypic variance (Doebley et al. 1990; Doebley and Stec 1993). It can be concluded that major QTL also play a significant role in oil synthesis and accumulation in high-oil maize, although multiple minor QTL are also present.

Barton and Keightley (2002) pointed out that short-term (<20 generations) selection responses tend to be based on alleles of larger effect whereas sustained responses to long-term selection must be based on many extremely minor effects. In this study, the high-oil parent of the mapping population, By804, was developed from BHO after a relatively low number of generations of selection (cycle-13). The oil concentration of the BHO population reached 15.53% after 18 cycles, nearly the same as the IHO population with 15.29% oil concentration after 65 cycles of

Table 7 Summary of QTL types for fatty acid compositions and oil concentration in RIL population

Trait	QTL action	Number	h^2 (%)		
			Range	Subtotal[a]	% of each trait
16:0	Major	1	42	42.0	53.9
	Minor	7	0.8–9.1	23.3	30.0
	Epistatic	6	0.4–6.1	12.6	16.1
	Total[b]			77.9	
18:0	Major	2	15.0	15	24.3
	Minor	5	1.3–9.3	38.4	62.2
	Epistatic	5	1.5–2.0	8.3	13.5
	Total[b]			61.7	
18:1	Major	1	27.7	27.7	41.3
	Minor	8	0.7–9.0	32.7	48.7
	Epistatic	4	0.5–2.5	6.7	10.0
	Total[b]			67.1	
18:2	Major	1	48.3	48.3	65.7
	Minor	8	0.7–6.3	17.5	23.8
	Epistatic	7	0.5–1.6	7.7	10.5
	Total[b]			73.5	
Oil	Major	1	15.7	15.7	26.0
	Minor	8	1.5–8.4	39.6	65.4
	Epistatic	2	1.3–3.9	5.2	8.6
	Total[b]			60.5	

[a] Total percentage of phenotypic variance explained by major, minor, or epistatic QTL

[b] Total percentage of phenotypic variance explained by all mapped QTL for each trait

selection (Dudley and Lambert 2004; Song and Chen 2004). Therefore, the accumulation of major QTL for oil concentration and fatty acid compositions during population improvement in BHO can explain the magnitude of response to selection after just 18 cycles.

Thirty-seven of the 42 individual QTL mapped in this study belonged to the minor QTL category, and most were mapped in previous studies with different mapping populations (Goldman et al. 1994; Alrefai et al. 1995; Berke and Rocheford 1995; Laurie et al. 2004; Mangolin et al. 2004; Song et al. 2004; Clark et al. 2006; Wassom et al. 2008a, b; Zhang et al. 2008). The total phenotypic variances explained by all minor QTL for each trait varied from 23.8% (16:0) to 65.4% (oil) (Table 7). These results were similar to those from studies in the RM10:S1 population with IHO germplasm (Laurie et al. 2004). Six of 19 loci containing minor QTL for oil concentration identified in the earlier F_2 or F_3 populations of B73 × By804 (Song et al. 2004) were not detected in the RIL population. Such minor QTL may be unstable and easily influenced by environment.

Another notable finding in the present study is that 24 pairs of epistatic QTL were associated with fatty acid compositions and oil concentration. However, they made only limited contributions to increased fatty acid compositions and oil concentration, as the sum of phenotypic variance explained by all epistatic QTL for each trait ranged from 8.6% for oil concentration to 16.1% for 16:0 (Table 7). These results can be contrasted with those obtained in rapeseed using similar statistical methods (Zhao et al. 2005, 2006), where epistatic interactions explained nearly the same proportion of phenotypic variance (30.3%) as additive effects (40–45%). This demonstrated that epistasis could make a substantial contribution to variation in oil concentration in different crops. Among epistatic effects, one type of epistasis occurred between loci with additive effects, the remaining two types occurred between pairs of loci in which at least one QTL had no detectable additive effects, indicating that loci without significant additive effects played important roles in epistatic interactions that indirectly influence regulation of oil synthesis and accumulation.

Oil concentration comprising a mixture of different fatty acids in maize grain was the most complex among the five measured traits, but only two pairs of epistatic interactions were detected. Nine loci contributed to epistatic effects for one trait with non-additive effects but had individual effects for other traits (Table 4). As expected, some mapped candidate genes, such as *TE* (atf2), *LPAAT* (apat5), and *ACA* (aca), were located within QTL without significant additive effects, but they interacted with other loci to influence oil synthesis and accumulation. These results provide additional evidence to support the existence of the epistatic interactions influencing fatty acid compositions and oil concentration.

The magnitudes of individual QTL with additive effects were greater than those of epistatic QTL for all measured traits. The average percentages of total phenotypic variance explained by individual QTL and epistatic QTL for all traits were 60.0 and 8.1%, respectively, indicating that additive effects contributing to variation in fatty acid compositions and oil concentration are predominated in our population. The results of this study lead to a conclusion about the genetic basis of fatty acid compositions and oil concentration in high-oil maize. Whereas a few major QTL with large additive effects may play an important role in increasing fatty acid compositions and oil concentration, many minor and a certain number of epistatic QTL, also with additive effects, additionally contribute to both fatty acid compositions and oil concentration.

Association of candidate genes with QTL for fatty acid compositions and oil concentration

Mapping gene orthologs of known lipid metabolic enzymes provided an opportunity to investigate the co-location of

candidate genes and mapped QTL for fatty acid compositions and oil concentration. In present study, one ACCase isoform, accA, a rate-limited enzyme in the fatty acid biosynthetic pathway (Ohlrogge and Browse 1995), was located within the chromosome 2 locus controlling QTL *ste2*, *ole2-2* and *lin2*. Two genes encoding two condensing enzymes, 3-ketoacyl-ACP synthase II (KASII) and 3-ketoacyl-ACP synthase III (KAS III), were mapped close to clusters with three QTL (*pal2*, *ole2-1*, *oil2*; chromosome 2) and two QTL (*ole7*, *lin7*; chromosome 7), respectively. These locations imply that genes upstream in the lipid metabolic pathway may contribute indirectly to fatty acid compositions and oil concentration in high-oil maize. Stearoyl-ACP desaturase (SAD), a key enzyme to convert stearic acid to oleic acid (Ohlrogge and Browse1995), fell within QTL *ste3* accounting for 13.2% of the variance in 18:0. Two acyltransferase genes, coding monoacylglycerol acyltransferase (MAGAT) and diacylglycerol acyltransferase (DGAT), co-located with loci on chromosomes 4 (4.06) and 6 (6.05) where QTL for 18:1 and for 18:2 were located, respectively. Similarly, a previously mapped locus, *FAD2*, was also located in bin4.06 (Mikkilineni and Rocheford 2003), the same genomic region as *MAGAT*. The gene encoding an oil body-embedded protein, OLE17, was mapped to chromosome 1, in the same region as QTL *oil1-2*. Even though many factors may be involved in the formation of a co-location between a candidate gene and a QTL for measured traits, 21 genomic regions (the total length was 190 cM) were detected to associate with fatty acid compositions and oil concentration in this study, which accounted for 11.34% of the whole genome (1,675 cM); and only 18 candidate genes (20 markers) from oil metabolism pathway were mapped. The probability of co-location caused by chance should be very low. However, the actual functions of such genes should be confirmed by association mapping or gene complementation in future investigations. If confirmed, they can be used to develop functional markers or as means to clone the QTL.

According to the mapping results, only seven (39%) of 18 mapped candidate genes were co-located with mapped QTL with minor effects. One explanation for such a low level is that molecular markers based on candidate genes may not cover all isoforms of the genes since most of the genes involved in lipid metabolism belong to multiple gene families (Ohlrogge et al. 2004). Most of the candidate genes, except *KAS*II and *SAD*, mapped only to one locus, suggesting that other isoforms might also affect oil synthesis and accumulation. One isoform of DGAT, DGAT1-2, recently cloned by Zheng et al. (2008), was identified to control major QTL in Bin 6.04. Another finding supported the viewpoint that two copies of *SAD* mapped to bins 3.05–3.06 and 8.05–8.06, but only the locus on bin 3.05–3.06 was associated with stearic acid. Interestingly, no candidate gene co-located with major QTL in bin 1.04 and 9.02 (Fig. 1). It suggests other genes may also regulate oil synthesis and accumulation, such as genes controlling embryo size, transcription factors, protein kinases and other regulatory factors.

Major QTL application in molecular breeding and genomic research

A notable discovery in the present study was that five major QTL for fatty acid compositions and oil concentration in three genomic regions were detected. This should allow introgression favorable alleles from high-oil maize into high-yielding normal lines using marker-assisted backcrossing. The strongest QTL for oil concentration, *oil1-1*, located on chromosome 1, was stable over different generations (Song et al. 2004; Zhang et al. 2008). The additive effects of the By804 allele at this locus increased oil concentration by 0.52, 0.59 and 0.48% in F_2, F_3 and the RIL population, respectively. The next strongest QTL, *oil6*, with additive effects of 0.35% for oil concentration was located in the same chromosome region as three major QTL, *st6-1*, *ole6-1* and *lin6-1*. Two important chromosome regions, bin 1.04 and bin 6.04, will be available for introgression of the favorable alleles from high-oil lines using markers identified in molecular breeding program.

The major QTL identified in this study also provide opportunities to clone these QTL using genomic techniques. As a vegetable oil, the quality of maize oil was determined by the ratio of saturated and unsaturated fatty acids (Lambert 2001). Palmitic acid is a key intermediate in lipid metabolism, and directly determines the ratio of saturated and unsaturated fatty acid. The major QTL *pal9* with 1.33% of additive effects identified in this study (Table 3) thus has positive effects in oil concentration by regulating the amount of 16:0. Inspection of the maize sequence database in the region of *pal9* revealed that more than 895 genes occurred within this region. Among them, several might encode functional genes, such as transcription factors, protein kinases and other regulatory factors. Thus, data from the maize genome sequencing initiative (http://www.maizesequence.org) will greatly facilitate elucidation of the genomic factors that determine oil synthesis and accumulation in maize grain. Such continuing investigation will be helpful in unraveling the mysteries of oil regulation and accumulation in maize grain.

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References

Alrefai R, Berke TG, Rocheford TR (1995) Quantitative trait locus analysis of fatty acid concentration in maize. Genome 38:894–901

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402

Barton NH, Keightley PD (2002) Understanding quantitative genetic variation. Nat Rev 3:11–21

Beisson F, Koo AJK, Ruuska S, Schwender J, Pollard M, Thelen JJ, Paddock T, Salas JJ, Savage L, Milcamps A, Mhaske VB, Cho Y, Ohlrogge JB (2003) Arabidopsis genes involved in acyl lipid metabolism. A 2003 census of the candidates, a study of the distribution of expressed sequence tags in organs, and a web-based database. Plant Physiol 132:681–697

Benitez JA, Gernat AG, Murillo JG, Araba M (1999) The use of high oil corn in broiler diets. Poult Sci 78:861–865

Berberich T, Harada M, Sugawara K, Kodama H, Iba K, Kusano T (1998) Two maize genes encoding ω-3 fatty acid desaturase and their differential expression to temperature. Plant Mol Biol 36:297–306

Berke T, Rocheford TR (1995) Quantitative trait loci for flowering, plant and ear height, and kernel traits in maize. Crop Sci 35:1542–1549

Bhattramakki D, Dolan M, Hanafey M, Wineland R, Vaske D, Register JC III, Tingey SV, Rafalski A (2002) Insertion-deletion polymorphisms in 3′ regions of maize genes occur frequently and can be used as highly informative genetic markers. Plant Mol Biol 48:539–547

Borevitz JO, Maloof JN, Lutes J, Dabi T, Redfern JL, Trainer GT, Werner JD, Asami T, Berry CC, Weigel D, Chory J (2002) Quantitative trait loci controlling light and hormone response in two accessions of Arabidopsis thaliana. Genetics 160:683–696

Carlborg O, Haley CS (2004) Epistasis: too often neglected in complex trait studies. Nat Rev Genet 5:618–625

Champoux MC, Wang G, Sarkarung S, Mackill DJ, O'Toole JC, Huang N, McCouch SR (1995) Locating genes associated with root morphology and drought avoidance in rice via linkage to molecular markers. Theor Appl Genet 90:969–981

Chase K, Adler FR, Lark KG (1997) Epistat: A computer program for identifying and testing interactions between pairs of quantitative trait loci. Theor Appl Genet 94:724–730

Clark D, Dudley JW, Rocheford TR, Ledeaux JR (2006) Genetic analysis of corn kernel chemical composition in the random mated 10 generation of the cross of generation 70 of IHO × ILO. Crop Sci 46:807–819

Clarke KR, Gorley RN (2001) PRIMER v5: user manual/tutorial. Plymouth: PRIMER-E Ltd., 91

Doebley J, Stec A (1993) Inheritance of the morphological differences between maize and teosinte: comparison of results for two F2 populations. Genetics 134:559–570

Doebley J, Stec A, Wendel J, Edwards M (1990) Genetic and morphological analysis of a maize-teosinte F2 population: implications for the origin of maize. Proc Natl Acad Sci USA 87:9888–9892

Doebley J, Stec A, Gustus C (1995) Teosinte branched 1 and the origin of maize: evidence for epistasis and the evolution of dominance. Genetics 141:333–346

Doerge RW, Churchill GA (1996) Permutation tests for multiple loci affecting a quantitative character. Genetics 142:285–294

Dudley JW (1977) Seventy-six generation of selection for oil and protein percentage in maize. In: Pollak E (ed) Proceedings of international conference on quantitative genetics. Iowa State University Press, Ames, pp 459–473

Dudley JW (2008) Epistatic interactions in crosses of Illinois High Oil × Illinois Low Oil and of Illinois High Protein × Illinois Low Protein corn strains. Crop Sci 48:59–68

Dudley JW, Lambert RJ (1992) Ninety generations of selection for oil and protein in maize. Maydica 37:81–87

Dudley JW, Lambert RJ (2004) 100 generations of selection for oil and protein in corn. Plant Breed Rev 24:79–110

Egli MA, Lutz SM, Somers DA, Gengenbach BG (1995) A maize acetyl-coenzyme a carboxylase cDNA sequence. Plant Physiol 108:1299–1300

Falque M, Décousset L, Dervins D, Jacob AM, Joets J, Martinant JP, Raffoux X, Ribière N, Ridel C, Samson D, Charcosset A, Murigneux A (2005) Linkage mapping of 1454 new maize candidate gene loci. Genetics 170:957–1966

Fan CC, Xing YZ, Mao HL, Lu TT, Han B, Xu CG, Li XH, Zhang QF (2006) GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. Theor Appl Genet 112:1164–1171

Fisher RA (1918) The correlations between relatives on the supposition of Mendelian inheritance. Trans R Soc Edinb 52:399–433

Gadau J, Page RE, Werren JH (2002) The genetic basis of the interspecific differences in wing size in Nasonia (Hymenoptera; Pteromalidae): major quantitative trait loci and epistasis. Genetics 161:673–684

Goldman IL, Rocheford TR, Dudley JW (1994) Molecular markers associated with maize kernel oil concentration in an Illinois High Protein × Illinois Low Protein cross. Crop Sci 34:908–915

Grandillo S, Ku HM, Tanksley SD (1999) Identifying the loci responsible for natural variation in fruit size and shape in tomato. Theor Appl Genet 99:978–987

Groh S, González-de-León D, Khairallah MM, Jiang C, Bergvinson D, Bohn M, Hoisington DA, Melchinger AE (1998) QTL mapping in tropical maize: III. Genomic regions for resistance to *Diatraea* spp. and associated traits in two RIL populations. Crop Sci 38:1062–1072

Han Y, Parsons CM, Alexander DE (1987) Nutritive value of high oil for poultry. Poult Sci 66:103–111

Hobbs DH, Flintham JE, Hills MJ (2004) Genetic control of storage oil synthesis in seeds of Arabidopsis. Plant Physiol 136:3341–3349

Holland JB (1998) Epistacy: a SAS program for detecting two-locus epistatic interactions using genetic marker information. J Hered 89:374–375

Jeppe RA, Thomas L (2003) Functional markers in plants. Trends Plant Sci 8:552–560

Kao CH, Zeng ZB, Teasdale RD (1999) Multiple interval mapping for quantitative trait loci. Genetics 152:1203–1216

Knapp SJ, Stroup WW, Ross WM (1985) Exact confidence intervals for heritability on a progeny mean basis. Crop Sci 25:192–194

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations co-dominant ecotype-specific PCR based markers. Plant J 4:403–410

Konishi S, Izawa T, Lin SY, Ebana K, Fukuta Y, Sasaki T, Yano M (2006) An SNP caused loss of seed shattering during rice domestication. Science 312:1392–1396

Lambert RJ (2001) High-oil corn hybrids. In: Hallau AR (ed) Special corn. CRC Press, Boca Raton, pp 131–153

Lambert RJ, Alexander DE, Mejaya IJ (2004) Single kernel selection for increased grain oil in maize synthetics and high-oil hybrid development. Plant Breed Rev 24:153–175

Laurie CC, Chasalow SD, LeDeaux JR, McCarroll R, Bush D, Hauge B, Lai CQ, Clark D, Rocheford TR, Dudley JW (2004) The genetic architecture of response to long-term artificial selection for oil concentration in the maize kernel. Genetics 168:2141–2155

Lee K, Huang AH (1994) Genes encoding oleosins in maize kernel of inbreds Mo17 and B73. Plant Mol Biol 26:1981–1987

Li CB, Zhou AL, Sang T (2006) Rice domestication by reducing shattering. Science 311:1936–1939

Lincoln SE, Daly MJ, Lander ES (1993) Constructing genetic linkage maps with MAPMAKER/EXP Version 3.0: A tutorial and reference manual. A Whitehead Institute for Biomedical Research Technical Report

Lionneton E, Ravera S, Sanchez L, Aubert G, Delourme R, Ochatt S (2002) Development of an AFLP-based linkage map and localization of QTLs for seed fatty acid content in condiment mustard (Brassica juncea). Genome 45:1203–1215

Lukens LN, Doebley J (1999) Epistatic and environmental interactions for quantitative trait loci involved in maize evolution. Genet Res 74:291–302

Malmberg RL, Held S, Waits A, Mauricio R (2005) Epistasis for fitness-related quantitative traits in *Arabidopsis thaliana* grown in the field. Genetics 171:2013–2027

Mangolin CA, de Souza CL Jr, Garcia AAF, Garcia AF, Sibov ST, de Souza AP (2004) Mapping QTLs for kernel oil content in a tropical maize population. Euphytica 137:251–259

Manly KF, Cudmore RH Jr, Meer JM (2001) Map Manager QTX, cross-platform software for genetic mapping. Mamm Genome 12:930–932

Mei HW, Li ZK, Shu QY, Guo LB, Wang YP, Yu XQ, Ying CS, Luo LJ (2005) Gene actions of QTLs affecting several agronomic traits resolved in a recombinant inbred rice population and two backcross populations. Theor Appl Genet 110:649–659

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

Murry MG, Thompson WF (1980) Rapid isolation of high molecular weight plant DNA. Nucleic Acids Res 8:4321–4325

Ohlrogge J, Browse J (1995) Lipid biosynthesis. Plant Cell 7:957–970

Ohlrogge J, Mhaske VB, Beisson F, Ruuska S (2004) Genomics approaches to lipid biosynthesis. In: Proceedings of the 4th international crop science congress, 26 Sep to 1 Oct 2004, Brisbane, Australia

Pérez-Vich B, Fernández-Martínez JM, Grondona M, Knapp SJ, Berry ST (2002) Stearoyl-ACP and oleoyl-PC desaturase genes cosegregate with quantitative trait loci underlying high stearic and high oleic acid mutant phenotypes in sunflower. Theor Appl Genet 104:338–349

Redoňa ED, Mackill DJ (1998) Quantitative trait locus analysis for rice panicle and grain characteristics. Theor Appl Genet 96:957–963

Salvi S, Tuberosa R (2005) To clone or not to clone plant QTLs: present and future challenges. Trends Plant Sci 10:297–304

SAS Institute (1999) SAS software. Cary, NC

Schreiber L, Skrabs M, Hartmann K, Becker D, Cassagne C, Lessire R (2000) Biochemical and molecular characterization of corn (Zea mays L.) root elongases. Biochem Soc Trans 28:647–649

Sen S, Churchill GA (2001) A statistical framework for quantitative trait mapping. Genetics 159:371–387

Song TM, Chen SJ (2004) Long term selection for oil concentration in five maize populations. Maydica 49:9–14

Song TM, Kong F, Li CJ, Song GH (1999) Eleven cycles of single kernel phenotypic recurrent selection for percent oil in Zhongzong no. 2 maize synthetics. J Genet Breed 53:31–35

Song XF, Song TM, Dai JR, Rocheford TR, Li JS (2004) QTL mapping of kernel oil concentration with high-oil maize by SSR markers. Maydica 49:41–48

Sukhija PS, Palmquist DL (1988) Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. J Agric Food Chem 36:1202–1206

Wang CS, Rutledge JJ, Gianola D (1994) Bayesian analysis of mixed linear models via Gibbs sampling with an application to litter size in Iberian pigs. Genet Sel Evol 26:91–115

Wang SC, Basten CJ, Zeng ZB (2005) Windows QTL Cartographer 2.5 user manual. North Carolina State University, Raleigh

Wassom JJ, Wong JC, Martinez E, King JJ, DeBaene J, Hotchkiss JR, Mikkilineni V, Bohnh MO, Rocheford TR (2008a) QTL associated with maize kernel oil, protein, and starch concentrations; kernel mass; and grain yield in Illinois High Oil × B73 backcross-derived lines. Crop Sci 48:243–252

Wassom JJ, Mikkelineni V, Bohn MO, Rocheford TR (2008b) QTL for fatty acid composition of maize kernel oil in Illinois High Oil × B73 backcross-derived lines. Crop Sci 48:69–78

Xu SZ, Jia ZY (2007) Genome-wide analysis of epistatic effects for quantitative traits in barley. Genetics 175:1955–1963

Yang J, Zhu J, Williams RW (2007) Mapping the genetic architecture of complex traits in experimental populations. Bioinformatics 23:1527–1536

Yu SB, Li JX, Xu CG, Tan YF, Gao YJ, Li XH, Zhang QF, Maroof MAS (1997) Importance of epistasis as the genetic basis of heterosis in an elite rice hybrid. Proc Natl Acad Sci USA 94:9226–9231

Zhang J, Lu XQ, Song XF, Yan JB, Song TM, Dai JR, Rocheford T, Li JS (2008) Mapping quantitative trait loci for oil, starch, and protein concentrations in grain with high-oil maize by SSR markers. Euphytica 162:335–344

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2005) Oil content in a European × Chinese rapeseed population: QTL with additive and epistatic effects and their genotype–environment interactions. Crop Sci 41:51–59

Zhao JY, Becker HC, Zhang DQ, Zhang YF, Ecke W (2006) Conditional QTL mapping of oil content in rapeseed with respect to protein content and traits related to plant development and grain yield. Theor Appl Genet 113:33–38

Zheng PZ, Allen WB, Roesler K, Williams ME, Zhang SR, Li JM, Glassman K, Ranch J, Nubel D, Solawetz W, Bhattramakki D, Llaca V, Deschamps S, Zhong GY, Tarczynski MC, Shen B (2008) A phenylalanine in DGAT is a key determinant of oil content and composition in maize. Nat Genet 40:367–372